Remediation of PCB-polluted soil using biochar: the uptake of PCBs in earthworms, plants and passive samplers

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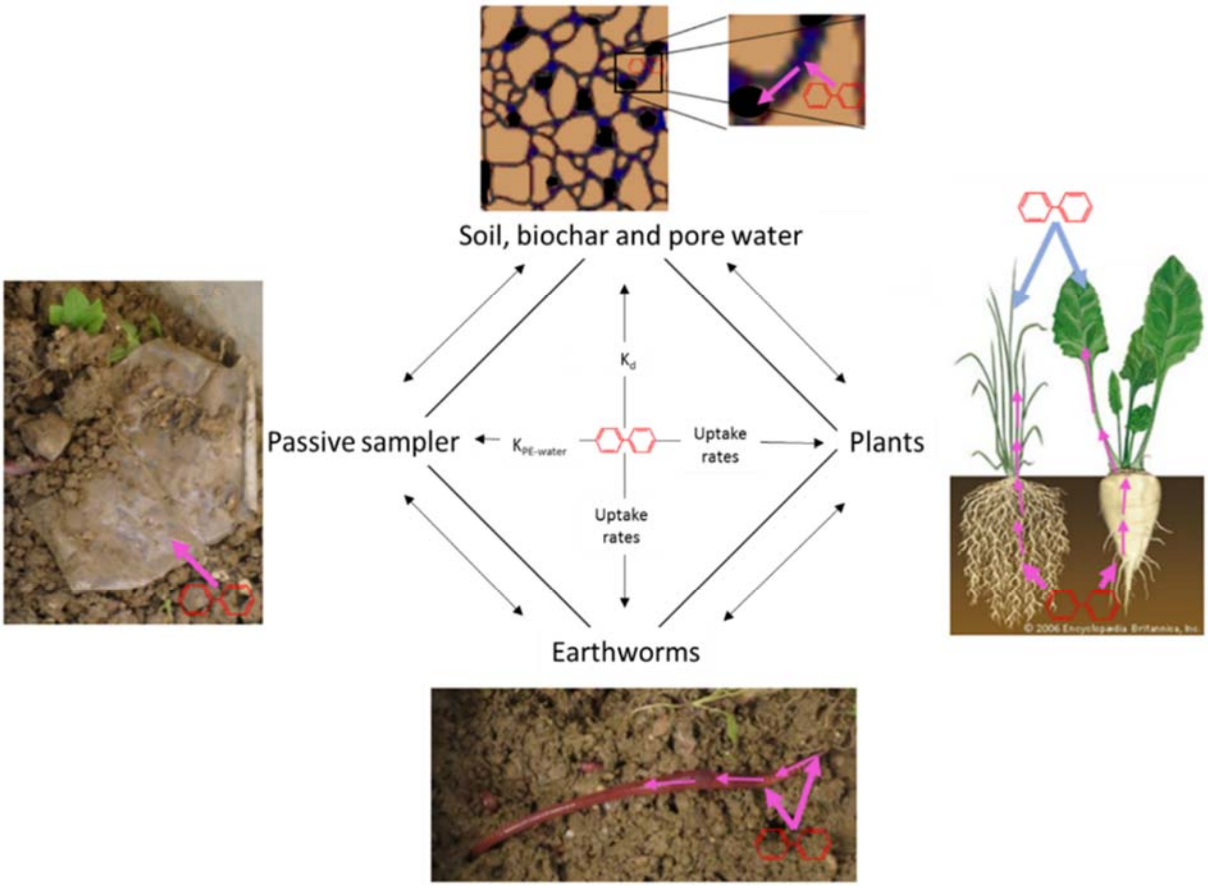
Remediation of PCB-polluted soil using biochar: the uptake of PCBs in earthworms, plants and passive samplers
– a pot experiment



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Abstract

The current move towards a more circular economy seeks to treat contaminated soil as a resource rather than as a waste. The remediation of contaminated soil in order to avoid unnecessary landfilling will help to achieve this aim. In recent years the popularity of biochar as a soil amendment has substantially increased. When added to contaminated soils in small amounts, biochar is able to sequester pollutants and make them unavailable for organisms and the surrounding soil. In addition to this property, producing biochar from biomass waste can contribute positively to waste handling issues, as well as the fact that the amendment of biochar to soil improves soil quality. Another large advantage with the use of biochar is that the production and use process results in a sequestration of carbon, thus a positive impact on climate change. Biochar represents a sustainable material for sorbent amendment and from a life cycle assessment perspective, biochar has been found to have lower energy demand and global warming potential impact than other amendment materials (such as activated carbon), and if engineered correctly be at least as effective as other materials for a lower cost.

This thesis provides additional knowledge in the area of pollutant immobilization in agricultural soils following biochar amendments. A pot experiment was conducted using aged spiked PCB polluted agricultural soil that was amended with two different biochar types; mixed wood shavings biochar and rice husk biochar at two different doses 1% and 4%. The uptake of PCBs to two plants; ryegrass (*Lolium perenne*) and turnip (*Brassica rapa* ssp. *rapa*), to the earthworm species *Eisenia fetida* and passive samplers (polyethylene, PE) was assessed with and without biochar amendment.

The main findings from the work can be summarised as follows. The earthworms showed a preference for the presence of biochar and did not seem to be affected by the presence of PCBs. PCB uptake to earthworms was both dependant on PCB congener and biochar type, with rice husk biochar giving highest reduction in PCB-concentrations (up to 90% reduction). There was no effect of biochar dose suggesting that the remediation of PCB polluted soil with biochar could be effectively achieved with a small biochar addition. Ryegrass yield increased with the presence of both biochars, but was lower in the presence of PCBs. The turnip yield was inconclusive, but did not seem affected by the presence of the PCBs. Low concentrations of

PCBs were detected in both plants with some difference between the PCB congeners. Turnip might be exercising phytoremediation and caution must be exercised if turnips are grown in PCB polluted, biochar amended soil with the intention of human consumption. Plant uptake was generally not affected by either type or dose of biochar. PE passive samplers sorbed PCBs and the uptake was PCB congener specific. Biochar reduced the uptake of PCBs to PE passive samplers and there were no real effects of biochar type or dose, however rice husk biochar seemed to perform better with respect to reduced PCB concentrations (up to 86% reduction) than mixed wood biochar. The rice husk biochar made using an uncontrolled low-technology method, had a higher sorption capacity than the mixed wood biochar produced in a controlled manner. There was a correlation between the uptake of PCBs by PE passive samplers and by earthworms. However there was no correlation between the uptake of PCBs by PE passive samplers and by plants. This suggests that the accumulation of PCBs in PE passive samplers is a good proxy for the accumulation of PCBs in earthworms.

Foreword

This experiment is a part of a Klimaforsk Research project called "Biochar as an adaptation strategy for climate change", funded by the Norwegian Research Council (project number 243789), and coordinated by Sarah Hale, NGI, with partners from Indonesia and Norway.

The practical work of this thesis was done at the lab of the Department for Environmental Engineering at the Norwegian Geotechnical Institute (NGI, Norway), at the Soil Laboratory of the Norwegian University of Life Sciences (NMBU, Norway), at Fytotronen, Department for Biosciences, University of Oslo (UiO, Norway), and at The Research Centre for Toxic Compounds in the Environment (RECETOX, Czech Republic) between November 2014 and August 2017.

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Finally a huge thank you to Sarah Hale, my supervisor at NGI, for sharing her exceptional knowledge and helping me staying tuned the whole way.

Abbreviations

AC: activated carbon

AMAP: Arctic Monitoring and Assessment Programme

AOM: amorphous organic matter

BAF: bioaccumulation factor

BC: black carbon

BSAF: biota to soil accumulation factor

C: carbon

CG: carbonaceous geosorbents

CLRTAP: The Convention on Long-Range Transboundary Air Pollution

C_W: bioavailable pollutant concentration

DDE: dichlorodiphenyldichloroethene

DDT: dichlorodiphenyltrichloroethane

DEHP: di-(2-ethylhexyl)phthalate

dw: dry weight

EMEP: European Monitoring and Evaluation Programme

GC-MS: gas chromatography – mass spectrometry

GPC: gel permeation chromatography

HOC: hydrophobic organic contaminant

K_{AW}; the distribution coefficient between air and water (Henry's law constant)

K_d: the distribution coefficient between soil and soil pore water

K_{OM}: the distribution coefficient between organic carbon and water

K_{OW}: the distribution coefficient between octanol and water

K_{PEW}: the distribution coefficient between PE passive sampler and water

LDPE: low density polyethylene

LOD: limit of detection

LRTP: long-range transport potential

LCA: life cycle analysis

N: nitrogen

NPK: nitrogen, phorsphorous and potassium

OC: organic carbon

OECD: Organisation for Economic Co-operation and Development

OM: organic matter

PAH: polycyclic aromatic hydrocarbon

PBT: persistent, bioaccumulative and toxic

PCB: polychlorinated biphenyl

PCM: pyrogenic carbonaceous materials

PDMS: polydimethylsiloxane

PE: polyethylene

POM: polyoxymethylene

POP: persistent organic pollutant

PRC: performance reference compound

PSAF: phase to soil accumulation factor

REACH: European Regulation on Registrations, Evaluation, Authoristation and Restriction of Chemicals

SA: surface area

SEC: size exclusion chromatography

SOM: soil organic matter

SPMD: semipermeable membrane device

"The Dutch Seven": ∑PCB-7 (BCB-congeners 28, 52, 101, 118, 138, 153 and 180)

UNECE: United Nations Economic Commission for Europe

USEPA: The United States Environmental Protection Agency

WHC: water holding capacity

wt%: weight precentage

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1 Introduction

Biochars ability to sorb contaminants and thus remediate soils presents one novel environmental use. This thesis investigates the suitability of biochar as a remediation strategy for a polychlorinated biphenyl (PCB) contaminated soil. In order to assess remediation efficiency, the following end points were used; two plant species (ryegrass (*Lolium Perenne*) and turnip *(Brassica rapa* ssp*. rapa)*; an earthworm species (*Eisenia fetida*) and a polyethylene (PE) passive sampler. Uptake of PCBs with and without biochar amendment will be measured in these phases. Two different biochars (made from mixed wood shavings and rice husk) will be added at two different doses (1 and 4 %) in order to assess differences in remediation performance for different feedstocks and application rates. Figure 1 shows the most important environmental processes that take place in the system.

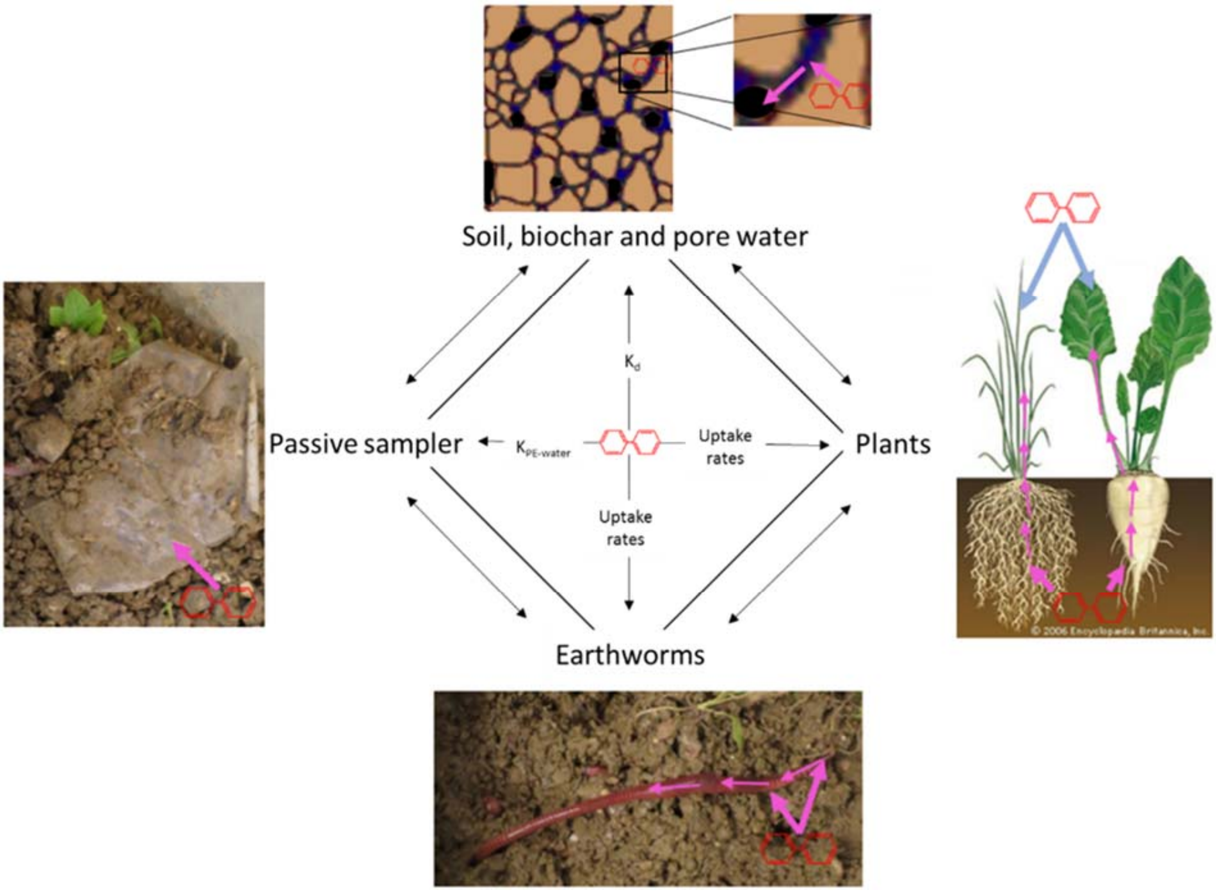


Figure 1 The pathways (pink arrows) for a polychlorinated biphenyl (PCB) molecule (red in figure) in a polluted soil via the soil pore water to biochar particles (black in figure), plants, earthworms and passive samplers

Figure 1 shows a PCB molecule at the centre of the diagram and the phases used in the experimental work around (plant, earthworm, PE passive sampler and soil-biochar system (including pore water)). The partitioning of PCBs to the different phases is dependent on the physical-chemical properties of PCBs themselves that include aqueous solubility, molecular size, and octanol-water partitioning coefficient (amongst others). The uptake of PCBs to a soil-biochar system can be measured using the soil-water portioning coefficient K_d, the uptake of PCBs to PE passive samplers can be measured with the PE-water partitioning coefficient $K_{PE\text{-}water}$, the uptake in plants and worms can be determined with uptake rates. In order for the PCBs to be available for uptake in any of the phases, it must be available in the soil pore water (illustrated by the red PCB in the soil phase in the photographs).

1.1 Persistent organic pollutants

Persistent organic pollutants (POPs) are organic compounds (synthetic or natural) that, to a varying degree, resist photolytic, biological and chemical degradation (Ritter et al., 1995). POPs are, with some exceptions, typically hydrophobic and lipophilic, which leads to a strong partitioning to organic matter (OM) in soils. POPs also partition into lipids in organisms, becoming stored in fatty tissue (Jones and de Voogt, 1999).

Many POPs can be classified as persistent, bioaccumulative and toxic (PBT). These properties were first noted in the 1960s where PCBs were found to accumulate in the food chain and concentrations were found in the top predator white-tailed eagles from the Swedish marine ecosystem (Jensen et al., 1969). In addition, Rachel Carson's book "Silent Spring" placed focus on the environmental and human dangers of use of the DDT (Carson, 1962), another PBT compound.

POPs that are stable and persist in the environment, can have long half-lives in soils, sediments and biota. The half-lives can be several days in the atmosphere and years or decades in soils and sediments (Jones and de Voogt, 1999). In addition, many POPs have a high long-range transport potential (LRTP) and can represent a potential hazard introduced to remote regions. However not all compounds with LRTP possess PBT-properties (Zarfl et al., 2012).

The presence of PCBs, DDTs and other PBT chemicals in the environment, has resulted in a great deal of chemical and ecotoxicological research on these chemicals and it is often them that drive the regulation of chemicals. The United Nations "Stockholm Convention" and Europe's REACH regulation (Registration, Evaluation, Authorization of Chemicals) both intend to prevent emissions of PBT-compounds to the environment (Reemtsma et al., 2016). The Stockholm convention names twelve compounds as the dirty dozen and include; Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene (pesticides) and PCB, polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (industrial chemicals and by-products) (Stockholm Convention, 2008). The convention on Long-Range Transboundary air pollution (CLRTAP) was signed in 1979 and entered force in 1983. CLRTAP is intended to protect, gradually reduce and prevent the human environment against air pollution (United Nations Economic Commission for Europe, 2013). The 1998 the Aarhus Protocol on POPs under the 1979 Geneva Convention on Long-Range Transboundary Air Pollution (UNECE, 1998) came into effect due to the POPs long range transport properties as well as noted harmful effects on living organisms. The long term goal of the protocol is to eliminate any discharges, emissions and losses of POPs to the environment (Breivik et al., 2004).

Although POPs have low water solubility, monitoring of pollutants in the aquatic environment is necessary to ensure that water-quality standards are maintained. The United States Environmental Protection Agency (US EPA) and the EU Water framework Directive set requirements, directives and legislative frameworks that must be followed (Vrana et al., 2005). The United States Environmental Protection Agency (US EPA) has listed PCBs under the Toxic Substances Control Act of 1976 and have the authority to require users to keep records, carry out testing and place restrictions on the use of these chemicals (United States Environmental Protection Agency, 2016).

The focus of this thesis is on PCBs.

1.1.1 Polychlorinated biphenyls (PCBs)

History

Polychlorinated biphenyls (PCBs) were discovered in 1865 as a byproduct of coal tar processing and was first synthesized in 1881 (Myers, 2007). Production of PCBs has been confined to ten different countries in the world, and started in 1929s in the United States. The primary use was as dielectric fluid in electrical equipment (De Voogt and Brinkman, 1989). PCBs were further used in a wide range of products including hydraulic-, cutting- and lubricating fluids, ink solvents, plasticizers in paint, heat transfer fluids and flame retardants. PCBs were banned or severely restricted in many countries during the 1970s and 1980s, and production in Russia was terminated between 1987 and 1993 (AMAP (Arctic Monitoring and Assessment Programme), 2000). Production of PCBs were prohibited from 2005 through the Stockholm Convention, and electrical equipment with high concentrations of PCBs is set to be eliminated by 2025 (Myers, 2007).

Physical-chemical properties

PCBs are synthetic chemicals that do not occur naturally (Myers, 2007), they are highly stable xenobiotic compounds, and are ubiquitous in the environment due to their physical-chemical properties. These properties include chemical stability, hydrophobicity, and lipophilicity which allows them to be bioaccumulated and biomagnified in higher trophic levels of the food chain (Safe, 1994). The physical-chemical properties of the hydrophobic compounds (HOC) PCBs varies with chemical structure and degree of chlorination.

Chemical structure

Biphenyl molecules consist of two benzene rings linked by a single bond formed between two carbons that each have lost their hydrogen atom. When biphenyl reacts with Cl_2 in the presence of a ferric chloride catalyst, the chlorine atoms replace some of its hydrogen atoms. The more chlorine initially present and the longer the reaction is allowed to proceed, the greater the extent of chlorination of the biphenyl molecule. The products thus become polychlorinated (Baird and Cann, 2012). The resulting PCB-molecule is illustrated in Figure 2.

There are 209 congeners in the PCB family. PCBs can be divided into homologue groups, where monochlorobiphenyls (one hydrogen atom replaced by a chlorine atom) are the simplest and decachlorobiphenyl (PCB-209 with 12 chlorine atoms) has the greatest number of chlorine substitutions and a more complex chemical structure. The carbon positions are numbered 1 to 6 on one phenyl ring, and 1' to 6' on the other ring. Positions 2, 2', 6 and 6' are called ortho positions. If none or only one of the ortho-positions have chlorine atoms the PCB-molecule has a planar "flat" configuration, while if more than one of the ortho positions have chlorine atoms, the PCB molecule is non-planar (Lindell, 2012).

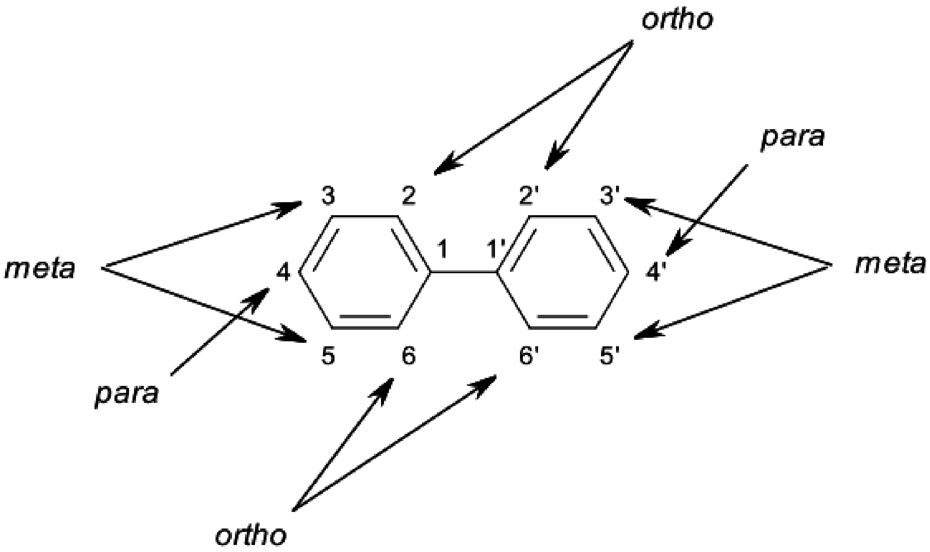


Figure 2 A general chemical structure of a PCB molecule, with the carbon positions numbered 1 -6 and 1' to 6'. Ortho, meta and para are used to describe the positioning of chlorine atoms in the PCB-molecule. From (Lindell, 2012)

Behavior in the environment

PCBs are found in soils, sediments, water, plants, fish, wildlife and human tissues (Bush et al., 1986; Safe et al., 1986). When released into the environment, PCBs persist for long periods of time because of their resistance to breakdown by chemical or biological agents. Volatilized PCBs are redeposited on to land or in water where they partition to soil organic matter or water suspended particulate matter. PCBs have low water solubility and a high lipophilicity. PCBs can be leached to water, however owing to the low water solubility, they are more often sequestered by soils and sediments. PCBs are transported worldwide and can be found in remote polar regions and in bottom ocean environments (Baird and Cann, 2012). The distribution and mobility of PCBs in the environment, as well as their uptake in plants and animals, differs markedly among the different PCB congeners and is dependent on their physical-chemical properties, which itself is governed by the number and position of chlorine substitutions (Zeeb et al., 2006). The persistency and solubility of PCBs in fatty tissues allows PCBs to be biomagnified in food chains (Baird and Cann, 2012). Figure 3 summarizes environmental processes that can occur once PCB is present in the environment.

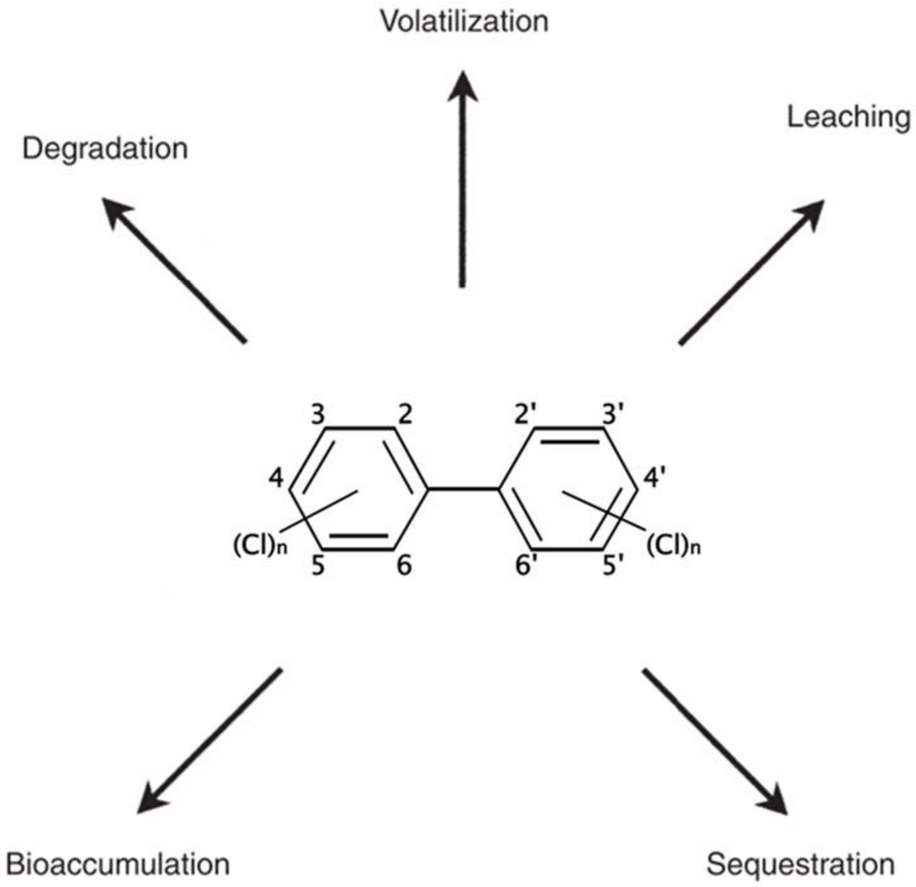


Figure 3 Fate and behaviour of a PCB-molecule in the environment. Image from (Semple et al., 2003) *modified*

Often reported term; sum of PCBs, refers to the sum of seven individual PCB congeners called the "Dutch Seven". These are commonly reported as sum PCB-7 and include PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180 (Kakareka and Kukharchyk, 2005).

Table 1 summarizes some physical-chemical properties of the different PCB congeners of the "Dutch Seven" and gives information about half life in air water and soil (Mackay et al., 1992; ten Hulscher et al., 2006; Lindell, 2012).

Table 1 Summary of selected physical-chemical properties and half life information for 7 PCB congeners

PCB congener	28	52	101	118	138	153	180
Chemical name	2,4,4' trichloro-biphenyl	2,2',5,5' tetrachloro-biphenyl	2,2',4,5,5' pentachloro-biphenyl	2,3',4,4',5 pentachloro-biphenyl	2,2',3',4,4',5 hexachloro-biphenyl	2,2',4,4',5,5' hexachloro-biphenyl	2,2',3,4,4',5,5' heptachloro-biphenyl
Molecular formula	$C_{12}H_7Cl_3$	$C_{12}H_6Cl_4$	$C_{12}H_5Cl_5$	$C_{12}H_5Cl_5$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_3Cl_7$
Homologue group	tri	tetra	penta	penta	hexa	hexa	hepta
Substitution	mono-ortho	di-ortho	di-ortho	mono-ortho	di-ortho	di-ortho	di-ortho
Planar or non-planar room orientation	planar	non-planar	non-planar	planar	non-planar	non-planar	non-planar
Molecular weight (g/mol)	257.5	292	326.4	326.4	360.9	360.9	395.3
Melting point (°C)	57	87	76.5	107	80	103	110
Molar volume (cm^3/mol)	247.3	268.2	289.1	289.1	310	310	330.9
Total surface area ($Å^2$)	243.6	259.6	275.2	269.2	283.3	290.8	298.9
Water solubility (µg/L at 20 - 25°C)	160	30	10	13.4	15.9	0.9	0.2
Henrys law constant (Pa m^3/mol at 20 °C)	28.1 ± 1.9	24 ± 4	35.5	7.8 ± 2.3	9 ± 5	15 ± 7	2.2 ± 1
Log K_{OW}	5.8	6.1	6.4	6.7	7.2	6.9	7.2
Mean half-life in air (months)	1	2			8		
Mean half-life in water (years)	2	6					
Mean half-life in soil (years)	6						

From Mackay (1991)
From Lindell (2012)
From Hulscher (2006)

1.2 The pollution of soils with chemicals

Soil is the upper weathered layer of the earth's crust in which life exists. Soil includes surface layers of plant litter, living organisms, plant roots and other underground parts of plants (Taylor and Pohlen, 1962). Soil is considered to be a renewable resource over a long time scale. Some soils were created hundreds of thousands of years ago, while others are more recently formed. Rocks, the unweathered material of the earth's crust, are the original source materials of most soils. When these rocks are broken down into smaller particles they become the parent materials of soil (Plaster, 2009). Soil is a tremendously heterogeneous environmental matrix with varying spatial and temporal gradients of organic carbon, pH, and particle size distribution (Lanno et al., 2004).

Soil is an essential component of the terrestrial ecosystem and has an important ecological function in biogeochemical cycling of resources needed for plant growth. An individual plant depends on soil for anchorage, water, oxygen and nutrients. Most soil matrixes consist of solid particles of mineral matter, with about 1-10% organic matter (OM). Voids, so called pore spaces take up about 50% of the soil volume and are filled with air and water (Plaster, 2009).

The chemical pollution of soil, along with degradation processes such as erosion, combined with increased urbanization, pose a threat to the sustainability of soil resources (Harrison, 2016). Human use of chemicals and the resultant environmental burden leads to anthropogenic contamination of soils. Contaminated soils and sediments are a significant worldwide environmental problem. The Norwegian Environment Agency state that most known contaminated land sites (over 5000) are contaminated as a result of earlier industrial and mining activities or from closed landfills containing hazardous waste on the sites (The Norwegian Environment Agency, 2017). Contaminated sites pose an environmental and human health hazard. Populations located near contaminated soil sites can be exposed to pollutants via ingestion and/or inhalation of contaminated dust or soil particles, consumption of crops produced on these sites as well as skin contact (Janus et al., 2015).

Soil pollution is also costly for society as a whole as remediation and risk reduction measures are often needed. This will be discussed below.

1.2.1 Aging of pollutants in soil

Aging takes place over time and as a result, pollutants become recalcitrant and less chemically and biologically available (Hatzinger and Alexander, 1995). Alexander (2000) state that once soils are polluted with chemicals, the process of aging, whereby pollutants become less available over time following ad- and absorbing to soil particles over time, can take place. Laboratory tests have confirmed that pollutants in aged soils are less available to microorganisms than unaged compounds (Hatzinger and Alexander, 1995; Alexander, 2000). Soil spiked in the laboratory can resemble natively polluted soils in the field if they are aged for long enough. As the soil-pollution contact time increases, the association strength between the sorbate-sorbent can get stronger and result in a non-labile pollutant fraction (also called non-extractable fraction) (Reid et al., 2000). The non-labile pollutant fraction is both less available and therefore less toxic to biota as it is less available for uptake, but it is also less susceptible to microbial degradation processes and solvent extraction. Aging has been cited as the primary obstacle to pollutant remediation of many sites (White et al., 2005).

Figure 4 provides the influence of contact time between the soil and contaminants during aging schematically.

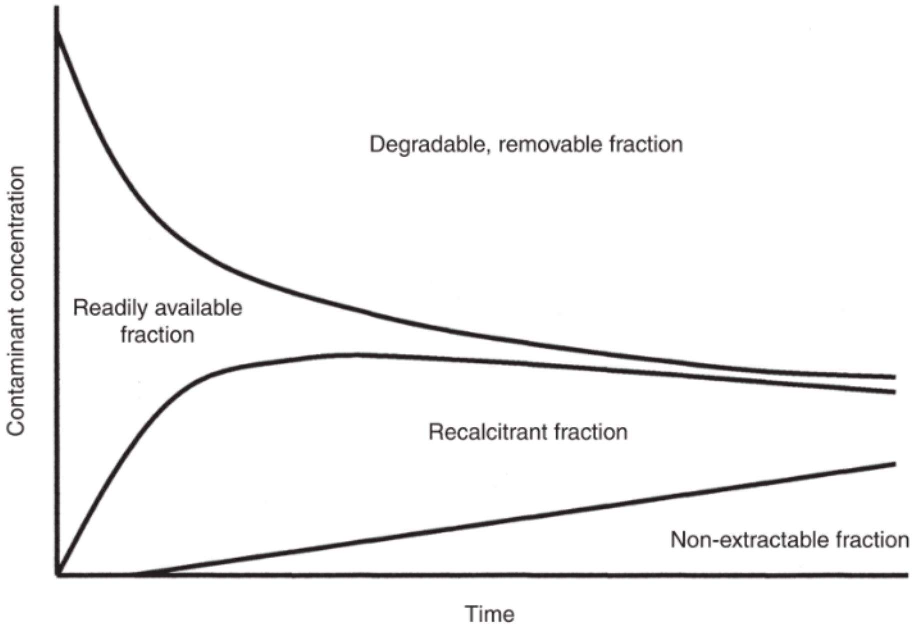


Figure 4 The influence of contact time on aging (Semple et al., 2003)

During aging, it is highly likely that molecules slowly move into sites within the soil matrix that are not readily accessed by even the smallest of microorganisms. The phase that sorbs hydrophobic compounds (HOCs) to the greatest degree is soil organic matter, but minute pores or voids may also play a role (Alexander, 2000). Pores with diameters of 0.3 – 1.0 nm are abundant in soils, and these are also in the size range of many pollutants of toxicological importance (White et al., 1998).

1.2.2 Partitioning of organic pollutants between soil, soil pore water and air

The partitioning of pollutants between soil and soil pore water (or water) is described by K_d, the distribution coefficient between the soil and the soil pore water (or water). Organic pollutants are principally sorbed to the organic fractions of the solid phase of soil. This means that K_d can also be related to the soil organic carbon content of the soil as; $K_d = K_{OC} \times f_{OC}$, where K_{OC} is organic carbon to water partitioning coefficient and f_{OC} is the fraction of organic carbon. K_{OC} is readily calculated and linearly related to K_{OW} (octanol to water partitioning coefficient) with the equation $\text{Log } K_{OC} = a \times \text{Log } K_{OW} + b$, where the constants a and b vary with the type of compound and type of organic matter. For chlorinated hydrocarbons like PCBs, constants reported by Karichoff, where a = 0.989 and b = -0.346 can be used to calculate Log K_{OC}. The strength of sorption of organic pollutants to soil increases with increasing K_{OW} (Karickhoff, 1981).

The partitioning of organic pollutants between air and water is approximated by K_{AW}; the air to water partitioning coefficient (Henry's law constant). If the dimensionless Henry's laws constant is greater than 10^{-4} the compound will partition to the air phase (Collins et al., 2006).

1.2.3 The bioavailable pollutant concentration

The bioavailable pollutant concentration is defined as the concentration of a compound that is freely available to cross an organisms (microorganism, fungi, plants, invertebrates and higher animals) cell membrane, from the matrix the organism inhabits. Once this transfer has occurred, storage, assimilation and degradation processes can take place within the organism (Semple et al., 2004).

Bioavailability can be considered in terms of chemical accessibility and chemical activity (Reichenberg and Mayer, 2006). Bioaccessible compounds are those that are immediately available to cross an organism’s cellular membrane when the organism has direct access to the chemical, or compounds that can become available over time (Semple et al., 2004). The energetic state of the chemical determines the potential for spontaneous physical-chemical processes such as diffusion and partitioning and the bioconcentration potential of pollutants in biota in soil (Reichenberg and Mayer, 2006). Figure 5 illustrates the bioavailable and bioaccessible fractions of a contaminant in soil as defined by its physical location.

The determination of total and non-bioavailable pollutant concentrations may overestimate the magnitude of the environmental and societal problem a contaminated soil represents (Alexander, 2000). Several methods have been proposed to determine bioavailable pollutant concentrations, with earthworms and passive samplers being most common. Earthworms are well suited for assessing bioavailability of contaminants in soil due to the fact that they reside in the soil matrix, have a tolerance for different types of soil, have an epidermal surface and overall ingest more soil compared to other soil organisms (Lanno et al., 2004). Over the past few decades, extensive work has been conducted on the determination of bioavailability using biomimetic methods. Equilibrium passive sampling devices (such as polyethylene, polyoxymethylene or silicone), have been shown useful to determine the bioavailability of hydrophobic organic compounds (Vinturella et al., 2004; Hale et al., 2012; Denyes et al., 2016).

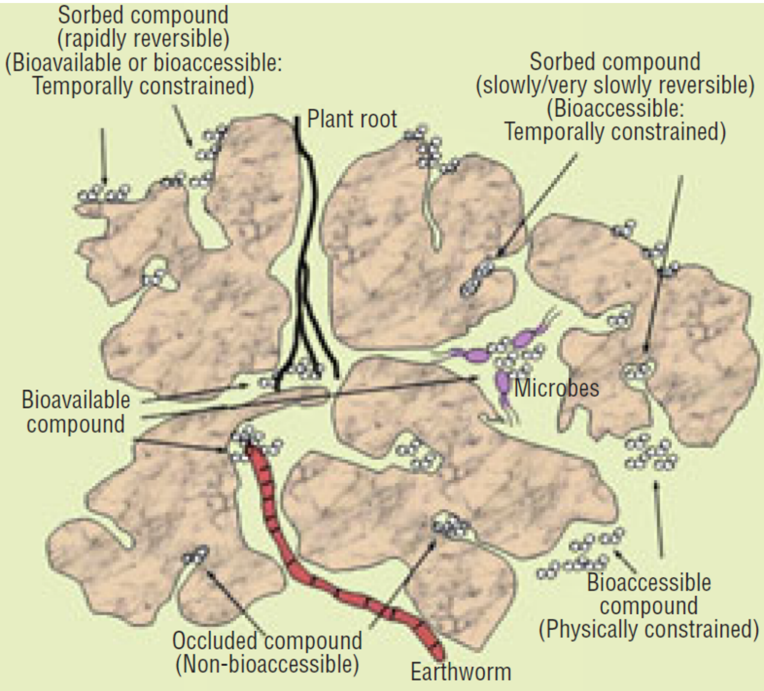


Figure 5 Readily bioavailable and bioaccessible fractions of contaminants (white spherical objects) in soil are those not sorbed or occluded on the soil particles. Earthworms, plant roots and microbes can take up available and bioaccessible fractions of the contaminants (Semple et al., 2004)

The comparison between measuring bioavailability using passive sampler and earthworms is further discussed in section 1.8.1

1.3 Soil remediation techniques

To restore legacy-polluted soils, a range of different remediation techniques can and have been used. Common practice is often excavation followed by transport to a landfill and possible incineration. This generates considerable disturbance to the environment, is not sustainable or economically feasible on a large scale (Paul and Ghosh, 2011; Gomes et al., 2013). Gomes et al. (2013) presented a comprehensive classification of remediation methods for PCB-contaminated soils, dividing the methods in to in situ and ex situ methods, differentiating between biological, physical, chemical and thermal methods, and also included natural attenuation.

Soil washing can be carried out both in situ and ex situ and involves a physical washing of the soil and then chemical treatment of the water used for washing. This process involves high energy contact between the contaminated soil and an aqueous based washing solution (Semer and Reddy, 1996) and is often only economically feasible if the soil washing process is effective and the equipment used is of relatively low cost (Wu and Marshall, 2001).

Focusing on in situ remediation methods, biological microbial degradation with compound specific bacteria can be used (Leigh et al., 2006). Microbial degradation of PCBs requires diverse metabolic activities due to the high number of PCB-congeners (Michel et al., 2001). Phytoremediation is the use of plants to remove contaminants from soil and has been receiving increasing attention (Lunney et al., 2004; Ficko et al., 2010; Huang et al., 2011; Mitton et al., 2012; Wu et al., 2012; Passatore et al., 2014; White, Jason et al., 2015). For successful phytoremediation, the ideal plant has a rapid growth, high biomass, deep roots, is easy to harvest and has a good tolerance (and ability to accumulate) to contaminants (Gomes et al., 2013). Natural attenuation is the biodegradation of contaminants by natural biological processes. This requires monitoring and is a very slow process which works best in well aerated and easily permeable soils (Castelo-Grande et al., 2010).

With regards to physical methods, both capping and sorbent amendment can be used as good alternatives. Capping and isolation with barriers around the polluted area can be used in order to physically separate the contaminated soil from the surrounding clean soil. These methods are designed to reduce the spreading of contaminants to nearby sites, and from a risk management point of view are good methods, however they do not treat the source of the contamination and pollutants remain in the soil, although they are sequestered (Castelo-Grande et al., 2010).

In situ stabilization via sorbent amendment has received increasing attention in recent years, and involves the addition of small amounts of highly sorbing materials, often referred to as carbonaceous geosorbents (CG), to contaminated soils. Activated carbon (AC) has been commonly used and shown to considerably reduce the bioavailable concentrations of organic pollutants (Zimmerman et al., 2004; Brändli et al., 2008). Sorbent amendment added to soils can alter the geochemistry of the soil, increase contaminant binding, reduce contaminant exposure risks to people and the environment as well as limit bioremediation (Cornelissen et al., 2005; Ghosh et al., 2011).

Use of biochar as the sorbent for remediation of polluted soils is rapidly gaining popularity (Denyes et al., 2013). The European Commission's Circular Economy Action Plan includes legislative proposal on waste and provides long term targets to reduce landfilling and increase recycling and reuse (European Commission, 2017). A shift towards a more circular economy will lead to more sustainable solutions to modern environmental problems. With the Europeans Commissions goal of less soil sent to landfills as waste, in situ clean up strategies like sorbent amendment become more relevant.

Life cycle assessment (LCA) is the analysis of the total environmental impact of a product. The analysis views the different stages of the life of a product, from raw material via processing, manufacture, distribution and use to disposal or recycling. Use of biomass-derived activated carbon (where CO_2 was sequestered during the production) as capping material on contaminated sediments, was found to reduce the overall environmental impact compared to that of natural recovery (Sparrevik et al., 2011). Biochar has found to have lower energy demand and global warming potential impact than activated carbon (AC) and if engineered correctly be at least as effective as AC and at a lower cost (Alhashimi and Aktas, 2017). The biochar production technique has to be evaluated from both environmental/climate, health and social perspectives (Sparrevik et al., 2013; Smebye et al., 2017).

1.4 Carbonaceous geosorbents

Carbonaceous geosorbents (CGs) is an umbrella term for carbon rich materials that have the ability to sorb pollutants and can occur naturally and/or be man-made. Examples of natural CGs are char, coke, charcoal, soot and kerogen, while man-made materials include coal hydrochar, biochar and activated carbon (AC) (Luthy et al., 1997; Jonker et al., 2004; Cornelissen et al., 2005; Millward et al., 2005; Pignatello et al., 2006). The term black carbon (BC) is also often used in soil science and environmental literature to refer to pyrogenic carbonaceous materials (PCMs) dispersed in the environment from wildfires and fossil fuel combustion (Pignatello et al., 2006; Lehmann and Joseph, 2015).

Many of these geosorbents can be used in the remediation of contaminated soil. AC is the most commonly used geosorbent, and due to its high surface area and carbon content it has shown excellent sorption properties (Paul and Ghosh, 2011). CGs have been shown to have very high sorption capacities for persistent organic pollutants (Luthy et al., 1997; Jonker et al., 2004; Millward et al., 2005; Pignatello et al., 2006). AC, in common with other CGs, contains condensed, rigid and planar stacks of highly disordered aromatic graphene sheets that have high carbon contents, relatively few polar functional groups, very large microporous networks and very high specific surface areas (Allen-King et al., 2002; Zhu and Pignatello, 2005; Cornelissen et al., 2006a). AC is produced from biomass or anthracite coal that has been exposed to an activation process (Hale et al., 2016), often in the form of high temperature steam (500-1100 °C) or strong dehydrating agents (Brändli et al., 2009). Commercial production of AC is expensive, while biochar has lower production costs and offers a more sustainable production (Denyes et al., 2012). Biochar was therefore chosen as a sorbent of PCBs in this work.

1.4.1 Sorption of PCBs to soils and carbonaceous geosorbents (CGs)

Sorption of contaminants by soils is described as intrinsically heterogeneous, even at a microscopic scale, due to variable composition and structure at both interparticular and intraparticular soil levels (Weber et al., 1992).

Within a dual soil organic matter (SOM) model, SOM is conceptualised as a macromolecule consisting of two organic matter domains; a completely amorphous, young organic matter (AOM) referred to as "soft" or "rubbery" and a condensed, older organic matter referred to as "hard" or "glassy" (Young and Weber, 1995; Xing and Pignatello, 1997). Sorption of hydrophobic organic contaminants (HOCs) like PCBs to soils follows an accepted paradigm of a combination of absorption in amorphous organic matter (AOM) and adsorption to condensed organic matter. The condensed older organic matter that is diagenetically altered is referred to interchangeably as black carbon (BC), carbonaceous geosorbents (CG) and high surface area carbonaceous material (Cornelissen et al., 2005).

Cornelissen et al (2005) state that extensive sorption to CG can have major consequences for overall organic compounds binding to sediments and soils, and thus for the fate of these compounds in the environment. Presence of CG in field contaminated soils may explain elevated sorption of hydrophobic contaminants, multiphasic desorption, reduced uptake by organisms, and limited bioremediation. The movement of PCBs and sorption to soil and carbonaceous geosorbents is shown in Figure 7 section 1.5.1.

1.5 Biochar

Lehmann & Joseph (2009) define biochar as the carbon-rich product obtained when biomass such as wood, manure or leaves, is heated in a closed container with little or no available air. Verheijen et al. (2010) state that biochar is biomass that has been pyrolyzed in a zero or low oxygen environment. Biochar distinguishes itself from charcoal and other carbon based materials in that it is intended for use as a soil ameliorator or for a use in a broader environmental management perspective (Lehmann and Joseph, 2015). Biochar is a newly constructed term (Ahmad et al., 2014), for a concept that is both an extremely ancient and very new to our current thinking. Amazonian Indians added such materials to the Terra Preta soils of the amazon Basin, which, 1000 years after their creation, remain more fertile than surrounding lands (Lehmann and Joseph, 2015).

When added to soil, biochar can sustainably sequester carbon and improve soil function (Kookana et al., 2011). The four major areas where biochar is being used in environmental management include (i) soil improvement, (ii) waste management, (iii) climate change mitigation, and (iv) energy production (Lehmann and Joseph, 2015). Figure 6 shows the way in which biochar can help with climate change as its use results in a carbon neutral or negative process (Lehmann, 2007). Plants actively withdraw carbon dioxide from the atmosphere, via the photosynthesis, and sequester carbon dioxide in their biomass or in soil organic matter (Lackner, 2003). Producing biochar from the plant biomass gives a material which has a twofold higher carbon content than the original biomass, and is more resistant to decomposition. Therefore, biochar directly removes carbon dioxide from the atmosphere by drawing organic carbon from the photosynthesis and decomposition of biomass (Lehmann, 2007).

An increasing number of studies are showing the potential of biochar to serve as an alternative, more cost effective and greener technology than AC (Denyes et al., 2012, 2013).

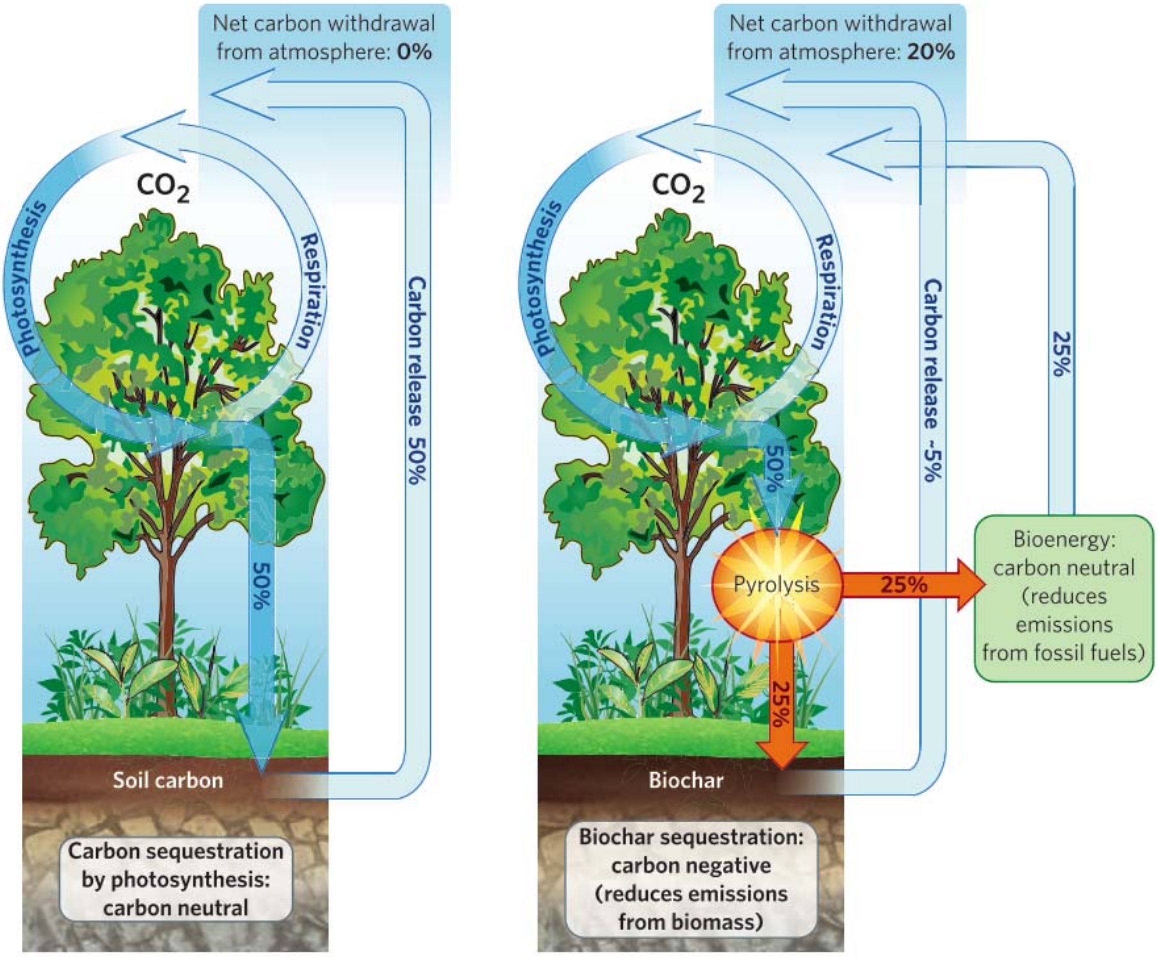


Figure 6 Motivation for applying biochar systems (Lehmann, 2007)

1.5.1 Biochar in soil remediation

Biochar can be added to soil in order to sequester organic (Hale et al., 2016) and inorganic (Beesley et al., 2011) pollutants, or a combination of both (Ahmad et al., 2014). When added to soil a transfer of pollutants from the contaminated soil to the biochar itself will take place (Figure 7). Biochar can sorb organic pollutants strongly and the amendment of biochar to contaminated soils provides a promising method for remediation. The strong affinity of pollutants to biochar can render the pollutants less available to organisms and hinder their transportation into off-site environments (Chen and Chen, 2009; Gomez-Eyles et al., 2011b;

Denyes et al., 2012, 2013; Jakob et al., 2012; Wang et al., 2013b, 2014, 2016; Brennan et al., 2014; Bielska et al., 2017) .

When a strong sorbent like biochar is added to a contaminated soil, the pollutants are transferred from the weaker sorption sites of the contaminated soil (contained in the soil organic matter) to the stronger sorption sites of the biochar. The pollutants first desorbe from the soil matrix, then migrate by sorption-retarded molecular diffusion or by pore water flow through the soil pore space into the vicinity of the nearest biochar particle. The pollutants are then sorbed to the biochar particle (Lehmann and Joseph, 2015). Figure 7 illustrates the movement of pollutants from soil particles to biochar particles. In addition there is some sorption of the pollutants to native carbonaceous geosorbents in the soil, but this is not expected to be as strong as the sorption of the pollutants to the biochar.

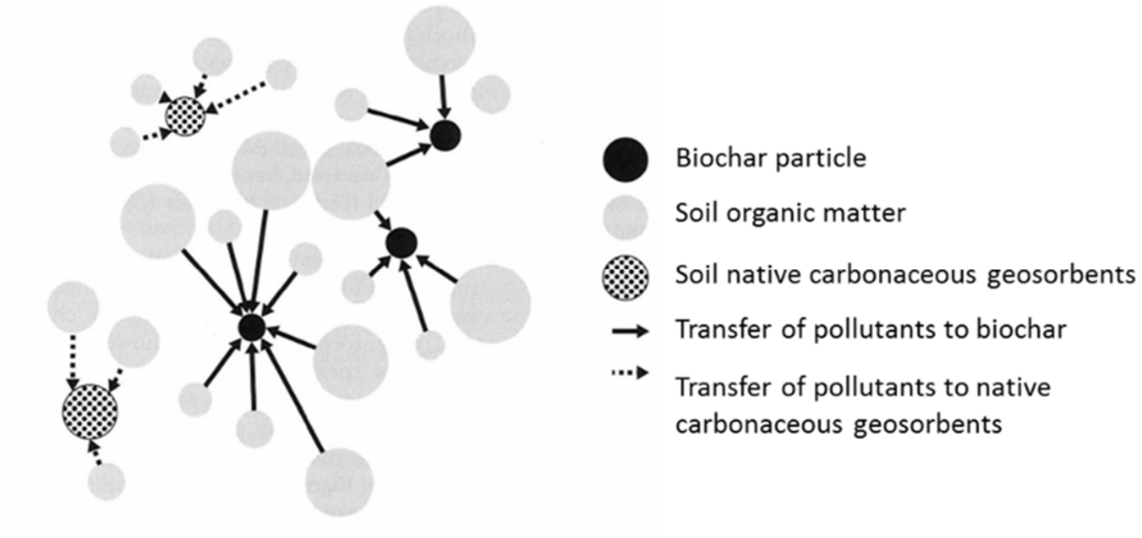


Figure 7 Sketch of the sequestration of organic pollutants in a contaminated soil when biochar is added. Modified from (Lehmann and Joseph, 2015)

1.5.2 Assessing the effects of biochar remediation of contaminated soil

There are several methods that can be used in order to assess whether a biochar amendment to a contaminated soil has been effective. These methods often use the following end points before and after amendment; total pollutant concentrations, uptake in plants and animals and bioavailable concentrations measured using passive samplers (Beesley et al., 2011; Lehmann and Joseph, 2015; Denyes et al., 2016).

1.6 The uptake of PCBs by earthworms

Earthworms are invertebrates, belonging to the Phylum Annelida, order Oligocheta, class clitella (Edwards and Lofty, 1977). There are over 3000 described earthworm species worldwide. Earthworms are found in leaf litter, manure, and some arid areas, bust most species prefer wetter, more heavily vegetated regions. Earthworms can range in size from 2 to 100 cm (Söderhäll, 2010). The species *Eisenia fetida* is associated with environments with a high organic matter content and is known to respond well to adverse environmental conditions (Monroy et al., 2006).

The structure of earthworms varies little between species. They are cylindrical animals that consist essentially of two concentric tubes, the body wall and the digestive system, separated by a fluid-filled cavity, the coelom, divided into segments by septa (Lee, 1985).

The body wall consists of an outer cuticle, the epidermis, a layer of nervous tissue, circular and longitudinal muscle layers, and the peritoneum, which separates the body wall from the coelom (Edwards and Lofty, 1977). The external earthworm anatomy is shown in Figure 8. The earthworm segments vary in width, usually being widest in the anterior (head part) and the clitellum part (blue ring in Figure 8). The pigmentation appear as dark segmental bands separated by lighter intersegmental zones in *Eisenia fetida* (Edwards and Lofty, 1977).

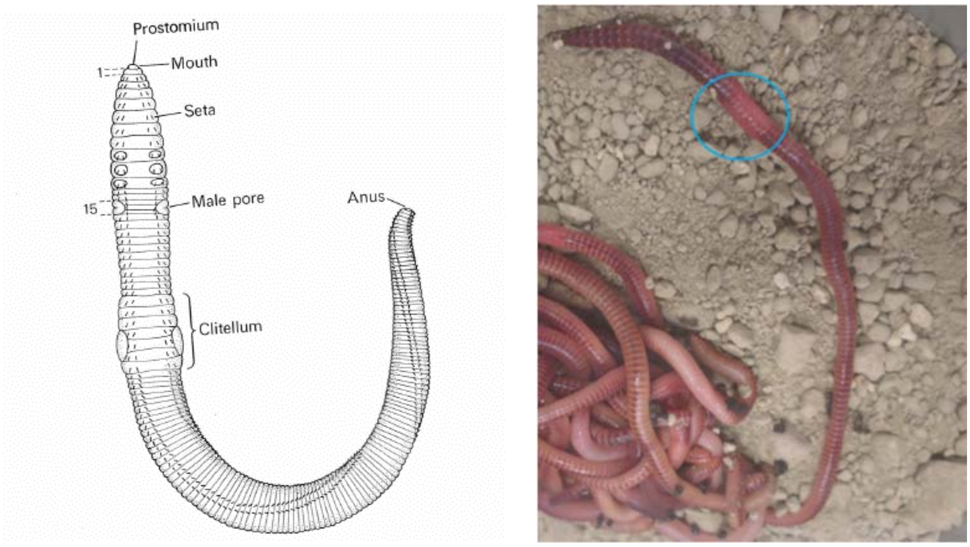


Figure 8 Earthworm anatomy. Left: external morphology showing the prostomium which is before the first body segment, the mouth, seta which are used to anchor the worm during movement,the male pore on body segment number 15 (earthworms are hermaphrodites and have both male and female genitals), the clitellum which is a gland for cocoon production. (Handreck, 1978). *Right: Earthworm with visual segmentation and a distinct clitellum (blue circle)*

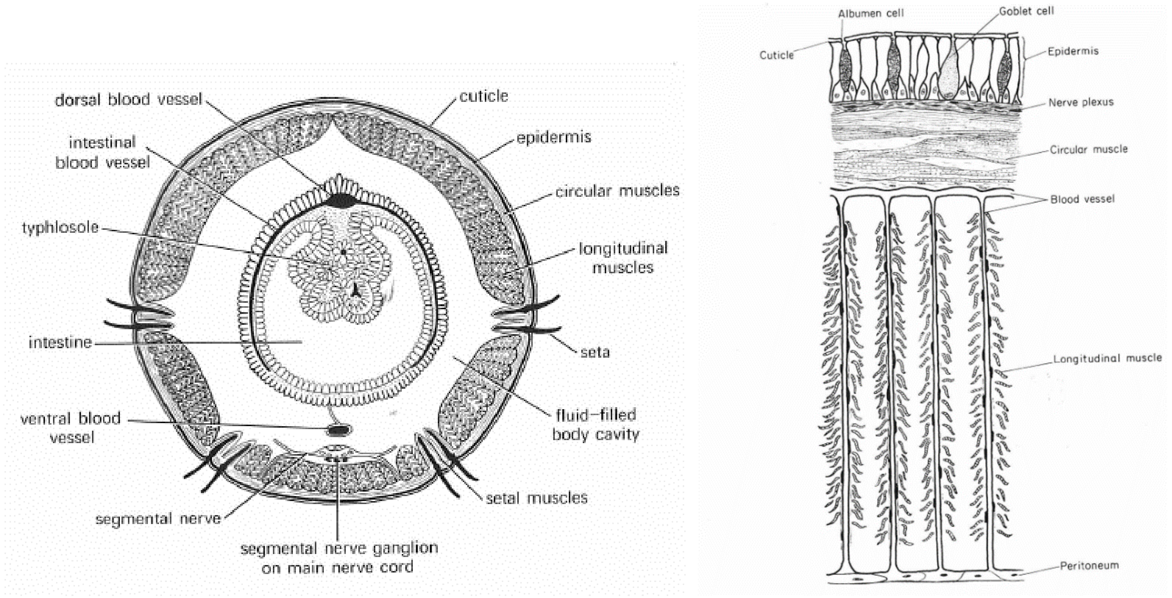


Figure 9 Left: schematic cross section showing (from the surface to the interior of the worm) the cuticle, the epidermis, circular muscles, longitudinal muscles, with seta and setal muscles piercing through, the fluid-filled body cavity (coelom) and the intestine. Nerves and blood vessels are also shown (Handreck, 1978). *Right: Transverse section through the earthworm body wall showing the cuticle with the underlying epidermis of different types of cells, circular and longitudinal muscles* (Grove and Newell, 1962)

In Figure 9 a cross section and transverse section of the earthworms' body wall is shown. The outer layer of the cuticle consist of two or more layers of interlacing collagenous fibers, with several homogenous non-fibrous layers beneath. The cuticle layer is perforated by many small pores. The underlying epidermis consists of a single layer of different types of cells. Glandular mucous cells in the epidermis secret mucus over the surface of the cuticle to prevent dryness and to facilitate movement through soil (Edwards and Lofty, 1977).

The digestive system consists of a buccal chamber, pharynx, esophagus, crop, gizzard and intestine. Earthworms derive their nutrition from organic matter, in the form of plant material, living protozoa, rotifers, nematodes, bacteria, fungi and other micro-organism, and decomposing remains of animals (Edwards and Lofty, 1977). Figure 10 shows the digestive system of an earthworm.

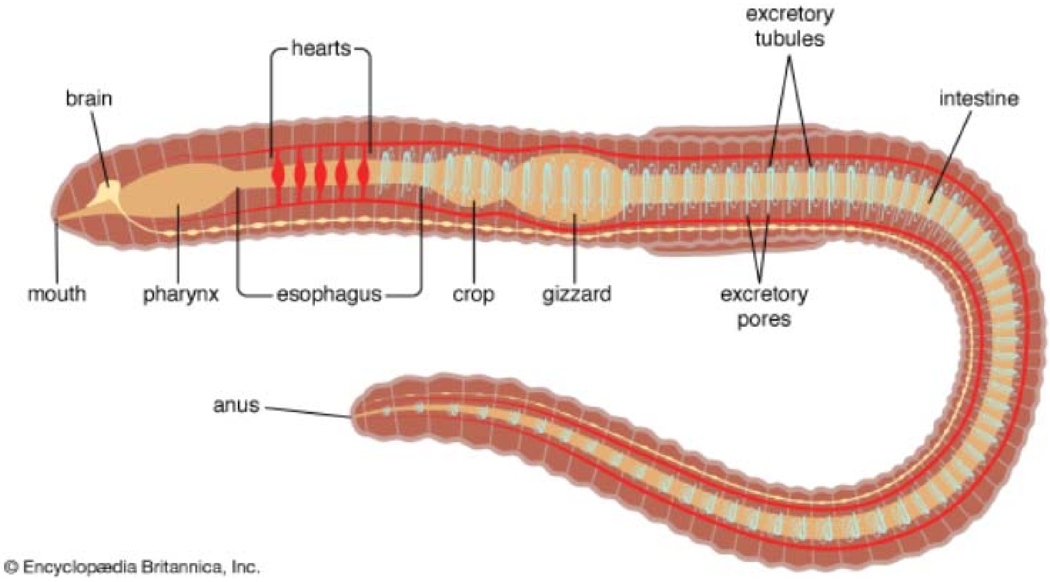


Figure 10 The digestive system of an earthworm (Encyclopaedia Britannica, 2017)

Earthworms play an important role in the dynamic equilibrium of soil and hence play an important role in soil fertility. Soil as a matrix varies continuously in space and time with organic and inorganic materials being added and lost. Earthworms feed on dead plant material and require moisture. They have poorly developed water conservation mechanisms and respiration depends upon diffusion of soil gases through a moist body wall (Lee, 1985).

Hydrophobic organic contaminants like PCBs are taken up by earthworms via passive diffusion from the soil solution through the cuticle or via internal sorption of the compounds from soil passing through the gut and intestine (Lord et al., 1980; Belfroid et al., 1995). The mechanism for uptake from the gut is likely to be the same as for uptake across the skin (passive diffusion)

(Jager et al., 2003). Jager et al. (2003) found that the contribution of gut route increased with increasing hydrophobicity of the compound, and that for PCB-153 the gut route clearly dominated.

Often the chemicals that are able to cause harm to worms are those that are bioavailable (see section 1.2.3). Earthworms live in close contact with the soil, have a thin and permeable cuticle, and also consume large amounts of soil (Jager et al., 2005) which allows them to assimilate bioavailable pollutants. Soil physical and chemical characteristics, in concert with physiology and behavior, determine the bioavailability of chemicals in soil to earthworms (Lanno et al., 2004).

There are several studies in which earthworms in soil have been exposed to organic pollutants like PAHs (Mooibroek et al., 2002; Parrish et al., 2006; Jakob et al., 2012), PCBs (Singer et al., 2001; Langlois et al., 2011; Paul and Ghosh, 2011; Denyes et al., 2012, 2013) and DDTs and their degradation products (Morrison et al., 2000; Denyes et al., 2016; Škulcová et al., 2016)). Denyes et al. (2012) showed that PCBs were bioaccumulated by earthworms (*E. fetida*) when they were exposed to a PCB contaminated soil. These authors reported an 18-fold increase in earthworm tissue PCB-concentration (bioaccumulation factor, BAF of 18.0 ± 2.9) as compared to exposure to non-polluted soil, and thus demonstrated the potential for PCBs to biomagnify within the food chain. In another study looking at PCB availability to earthworms (*Lumbricus terrestris* L.) in a contaminated urban soil, biota to soil accumulation factors (BSAFs) of around 10 were reported for the low chlorinated PCBs (8, 20, 28 and 52). Average BSAFs were 10 to 100 times higher for PCBs (0.71 – 70) than for PAHs (0.13 – 0.41). BSAFs of the PAHs were independent of K_{OW}, while those of the PCBs decreased with increasing K_{OW} (Krauss et al., 2000).

1.7 The uptake of PCBs by plants

Despite plants apparent diversity, all seed plants have the same basic physiology. The vegetative body consists of three organs; leaf, steam and root. The primary function of the leaf is to carry out photosynthesis, that of the stem is to support the leaves and that of the root is to anchor the whole plant and to absorb water and minerals. The stem and leaves are cumulatively referred to as the shoot of the plant (Taiz and Zeiger, 2010). Unlike the growth of animals, vegetative growth is not predetermined, but is variable and has no definite end point. This results in plants developing in a way that is best suited to the local environment (Taiz and Zeiger, 2010).

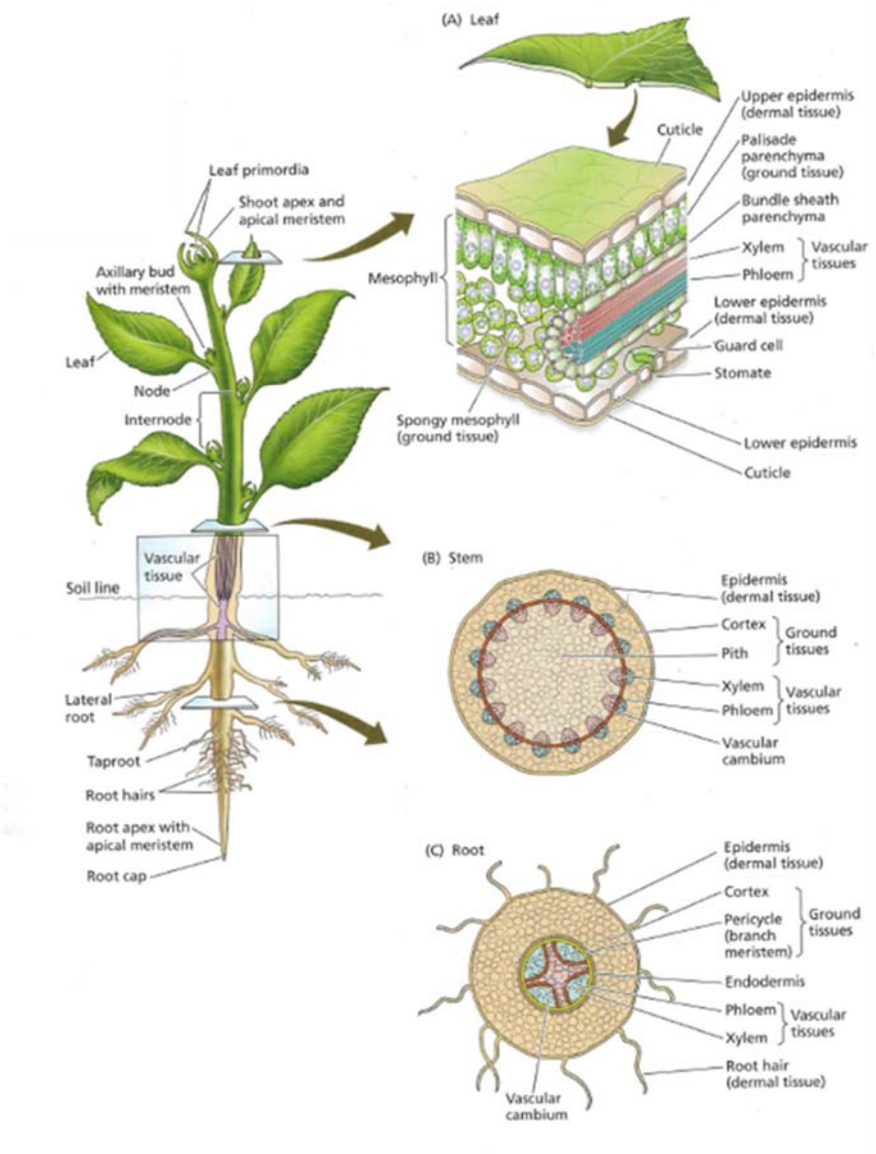


Figure 11 General schematic representation of the body of a plant showing and naming characteristics (Taiz and Zeiger, 2010)

Vascular plants are composed of three tissue systems; dermal, vascular and ground (Figure 11), which are all present in roots, stems and leaves. The functions of the dermal tissue (epidermis and cuticles in the leaves) are mechanical protection and controlling water loss (and aeration via stomata in the leaves). The functions of the ground tissue (cortex, pericycle, pith, palisade parenchyma and spongy mesophyll) are support, metabolic processes (respiration, secretion and photosynthesis), storage and regeneration. The function of the vascular tissues (xylem and phloem) are conduction of water (xylem), nutrients and key substances (phloem) (Raven et al., 2005).

The uptake of water and minerals by the roots is facilitated by root hairs (tubular extensions) which greatly increases the absorptive surface of the root. The cells of the epidermis (root wall) give little resistance to the water and minerals passing into the roots. A substance described as slimy sheath called mucigel lubricates the root during passage through the soil and also gives the roots a closer contact with the soil particles. The layer of soil bound to the root by the by the mucigel and root hairs contains a variety of microorganisms and is called the rhizosphere (Raven et al., 2005).

In addition to bioaccumulation and bioconcentration processes carried out by animals, uptake by plants is another pathway for pollutants contained in contaminated soil to reach the food chain (Sartoros et al., 2005; Collins et al., 2006). As illustrated in Figure 12 plant uptake occurs via the roots of plants alongside water transpiration (Gao and Collins, 2009) in a passive, diffusive process (Trapp and Mc Farlan, 1995).

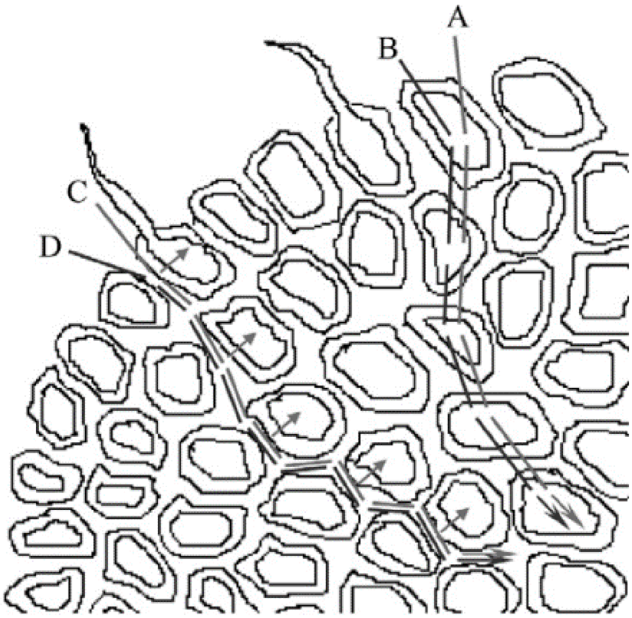


Figure 12 Pathways of water (A and C) and HOCs (B and D) through the plant root epidermis and cortex via symplastic and apoplastic movement (Gao et al., 2011)

Figure 13 shows the processes that occur for the uptake of pollutants to plants from a contaminated soil. The first thing that must happen is desorption of pollutants from the soil to the soil pore water or water. Previous experiments have shown that the uptake occurs following the attainment of equilibrium between pollutant concentrations in the soil pore water or water and the aqueous phase of the plant roots (Collins et al., 2006). In addition to this, sorption of the organic pollutant onto lipophilic root components can occur (Briggs et al., 1982). From the roots, water, solutes and organic pollutants are transported through the xylem to the above ground plant parts. This flux is driven by a water potential gradient, created by transpiration (Collins et al., 2006). From the xylem this transport may allow for lateral diffusion into adjacent tissues. Pollutants can become concentrated in plant shoots due to the equilibrium that exists between chemical concentrations in the aqueous phase of the xylem and the plant shoot, as well as sorption onto lipophilic shoot components (Trapp and Mc Farlan, 1995).

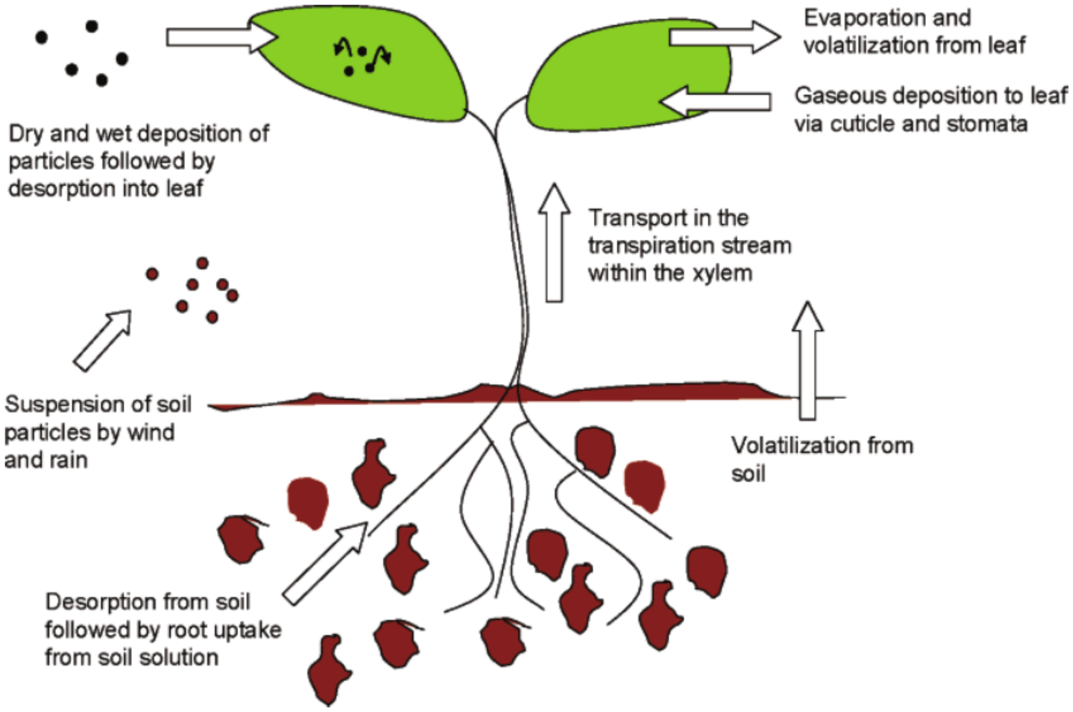


Figure 13 Processes that occur when plants take up pollutants from soil (Collins et al., 2006)

In addition to the uptake of organic pollutants to plants from the soil pore water to the roots, the organic pollutants can be taken up by the above ground parts of plants via depositional processes.

1.7.1 Ryegrass

Ryegrass (*Lolium Perenne*) is from the clade of angiosperms (flowering plants) and is a monocot (giving one seedling from one seed). Monocots comprise about 90 000 species with the grass family (poaceae) being the largest with around 9000 species. The vascular tissues of monocots is scattered throughout the stem giving the stem limited mechanical strength. The first root to emerge from the seed dies off so no strong central root forms (Raven et al., 2005). Monocots like ryegrass sprout roots from shoot tissue near the base (adventitious roots) and the fibrous root system of grasses (Figure 14) shows this rooting pattern (Hannaway et al., 1999).

Figure 14 The shoots and roots of ryegrass. Left: Ryegrass from this work four weeks after germination. Right: sketch of ryegrass modified from Encyclopaedia Britannica, 2016.

Perinneal ryegrass (*Lolium perenne* L.) is native to Europe, temperate Asia, and north-Africa and is widely distributed throughout the world (Hannaway et al., 1999). It is the predominant forage grass in European agriculture, where it provides the major supply of nutrients for grazing sheep and cattle (Lasseur et al., 2011). High palatability and digestibility make this grass species highly valued for livestock (Hannaway et al., 1999).

1.7.2 Turnips

The turnip *(Brassica rapa* ssp*. rapa)* is also from the clade of angiosperms but is a dicot (giving two seedlings from one seed). The Brassicae or mustard family is a clade of about 338 genera and some 3709 species distributed worldwide (Al-Shehbaz et al., 2006). In dicots the radicle (first organ to appear when a seed germinates) grows to become a taproot. The taproot grows downward, and roots grow laterally from it. In turnips the taproot serves as a storage organ and becomes swollen with foodstuff (Encyclopaedia Britannica, 2016). Unlike the monocots, the vascular tissues of the dicots is arranged in rings around the periphery, giving the stems some mechanical strength (Raven et al., 2005). Figure 15 shows to the left three turnip plants from this work four weeks after germination and to the right a mature taproot.

Figure 15 The roots, stem and leaves of turnip. Left: Three turnip plants four weeks after germination. Right: sketch of a mature turnip plant modified from Encyclopaedia Britannica, 2016.

Brassica rapa L. is a plant with various subspecies including the turnip. *Brassica rapa* is a biennial plant than can become 0.5 m tall. The turnip has been used as a vegetable for human consumption in Europe since prehistoric times (Undersander et al., 1991). Figure 16 shows the three diploid taxa; *B. rapa/B. campestris* (turnip, Chinese cabbage), *B. nigra* (black mustard) and *B. Oleracea* (cabbage, kale, broccoli, Brussels sprouts, cauliflower and kohlrabi), which are referred to as the U triangle (U, 1935) and the genomic relationship to their hybrid taxa; *B. napus* (rapeseed, rutabaga), *B. juncea* (Indian mustard) and *B. carinata* (Ethiopian mustard) (Lowe et al., 2002).

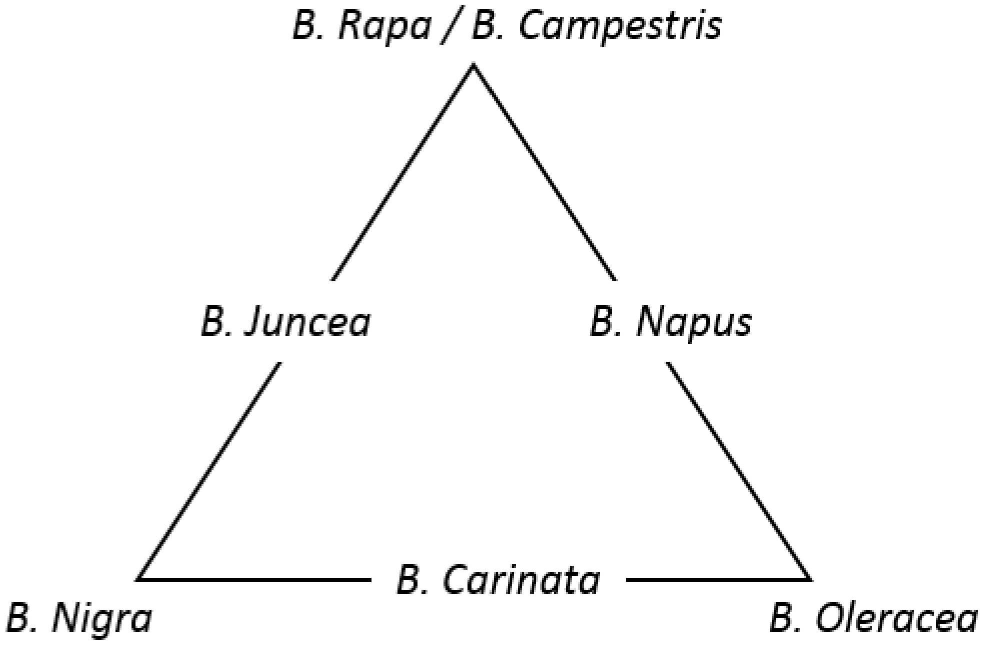


Figure 16 A classical U triangle depicting the familiar relationship between Brassicae species. Figure modified from (Demeke et al., 1992)

1.8 Passive sampling to measure bioavailability

Passive sampling is a method that can be used in order to determine the bioavailable fraction of pollutants. Passive sampling allows the determination of very low (pg level) bioavailable concentrations (Cornelissen et al., 2010). The method involves deploying a polymer to contaminated water, contaminated soil or sediment pore water and allowing a passive accumulation of contaminants to the polymer to occur. This free flow of pollutants comes as a result of a difference in chemical potential. The net flow of analyte molecules from one medium to the other continues until equilibrium is established in the system, or until the sampling period is stopped (Górecki and Namienik, 2002).

One of the first passive samplers to be used was the semipermeable membrane device (SPMDs) (Huckins et al., 1990). The sampling device consists of an outer membrane-tube of low density polyethylene (LDPE) which is filled with a synthetic lipid (Vinturella et al., 2004). The synthetic lipid was assumed to mimic lipids in organisms. The double phase semipermeable membrane device was since simplified by removing the synthetic lipid to leave the sheet of LDPE. The sampling rate of HOCs was shown to be higher after this simplification (Booij et al., 1998). In contrast to double phase membrane devices, single-phase devices are cheaper, easier to deploy, have less complex sorption behavior, can attain HOC-equilibrium faster and will not lose the synthetic lipid-phase if ruptured (Hale et al., 2010). Figure 17 shows PAH-molecules diffusing through pores in the polymer to become sorbed by the polyethylene membrane.

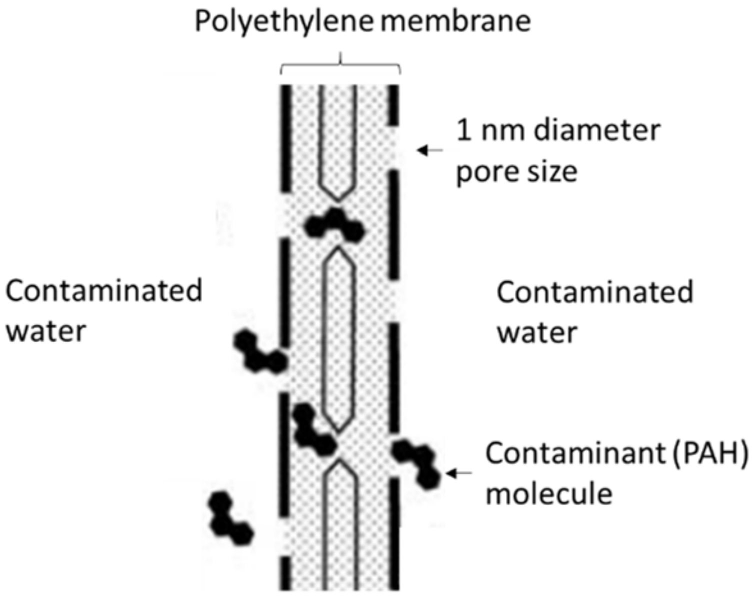


Figure 17 Schematic of an LDPE sampling PAHs, modified from (Williamson et al., 2002)

A number of different materials can be used as single phase equilibrium passive samplers including polydimethylsiloxane (PDMS), polyoxymethylene (POM) and low-density polyethylene (LDPE), often referred to as simply polyethylene (PE) (Cornelissen et al., 2008).

The PE contains transient cavities approximately 1 nm in diameter (Figure 17). Many organic contaminants are of a similar size to this and therefore only dissolved contaminants, and not those that are particle-bound, are assimilated by the LDPE sampler (Huckins et al., 1993).

The bioavailable pollutant concentration (C_W) is determined at equilibrium using the predetermined PE-water partitioning coefficient $K_{PE\text{-}water}$ and the concentration accumulated in the PE (C_{PE}), according to equation 1:

Equation 1 The bioavailable pollutant concentration C_w

$$C_W = \frac{C_{PE}}{K_{PE-water}}$$

Where C_w is the freely dissolved concentration of pollutant in water (µg/mL H_2O), C_{PE} is the concentration of pollutant in PE (µg/g PE) and $K_{PE\text{-}water}$ is the partitioning coefficient between PE and water (g/mL) (Lohmann, 2012).

Passive samplers are often used for in situ environmental monitoring (Huckins et al., 1990; Mayer et al., 2003) and their application in soil and sediment remediation represents a promising method for site monitoring and for determining treatment efficiency of sorbent amendment that result in changes of soil/sediment pore water concentrations (Oen et al., 2011b).

It is generally assumed that equilibrium passive samplers need to be deployed for time periods of 4 weeks or more to attain equilibrium (Gomez-Eyles et al., 2011a) and accurately predict bioavailability. This time period could be shortened if performance reference compound (PRCs) are additionally used as they negate the need for equilibrium. Performance reference compounds (PRCs) are analytically non interfering organic compounds with moderate to high K_{OW} values (Booij et al., 2003), spiked to passive samplers in order to identify compounds that attain sorption equilibrium during sampling period (Booij et al., 2002). However the PRCs used must have the same dissipation rate as uptake rate of the pollutant in order to ensure that non-equilibrium is corrected for (Apell and Gschwend, 2014).

1.8.1 Comparison of uptake of pollutants by passive samplers to uptake by biota

Studies have shown that passive samplers can be used to estimate uptake of HOCs to biota (Vrana et al., 2005). A promising correlation was reported between the uptake of PAHs in polyethylene passive samplers and in the benthic organism *Nereis virems* ($R^2 = 0.67$), with the PEs taking up less pollutants than the organisms (Vinturella et al., 2004).

Exposure of the amphipod *Hyalella azteca* to PAH contaminated sediments demonstrated that PAH pore water concentrations determined using passive samplers were able to predict toxic and non-toxic effects of the sediments (Hawthorne et al., 2007). A linear relationship was demonstrated between lipid normalised PCB congener concentrations in the shallow-water blackworm *Lumbriculus variegatus* and pore water concentrations using passive samplers (Sun and Ghosh, 2007). A close to 1:1 relationship was demonstrated for the partitioning of PCBs from sediment pore water into PE passive samplers and the lipid of benthic organism *Nereis virems* (Friedman et al., 2009).

There are also a few studies that have used a combination of passive sampler, earthworms and plants and compared the uptake of HOCs to these different phases in amendment experiments. Gomez-Eyles et al. (2011a) found that passive samplers predicted PAH-accumulation in earthworms and ryegrass roots from soils. Denyes et al. (2016) studied the uptake of DDTs to passive samplers, earthworms and plants following the amendment of two types of biochar (and an AC) to a soil. The results from the study showed that POM passive samplers predicted DDT accumulation reduction following carbon amendment in the earthworms *(Eisenia fetida*), but not for the squash (*Cucurbita pepo*). Paul et al. (2011) showed that there was a linear relationship between aqueous PCB concentration (measured by POM passive samplers) and earthworm (*Eisenia fetida*) concentrations, which held up even after a reduction in PCB uptake by 2 orders of magnitude due to AC-amendment to the soil.

1.9 Aims and hypothesis of this thesis

In recent years the popularity of biochar as a soil amendment has substantially increased, mostly in response to the realization that it can improve soil quality, from chemical, biological, physical and agricultural perspectives, as well as its ability to sequester carbon. Biochars ability to sorb contaminants and thus remediate soils presents one novel environmental use. The scope of this thesis is therefore to investigate the suitability of biochar as a remediation strategy for a PCB contaminated soil. The work will provide additional knowledge in the area of pollutant immobilization in agricultural soils following biochar amendments.

The aim of this study was **to investigate the remediation effects of biochar on PCBs spiked to an agricultural soil**. Seven PCBs were selected based on variable chemical and physical properties (hydrophobicity, degree of chlorination, octanol-water partition coefficient, Henrys law constant). Two different biochars produced from mixed wood shavings and rice husk, and applied to the soil at 0% dose as well as 1% and 4 % were tested. Two different plants were selected (ryegrass and turnip), that represent different root systems. In addition, one earthworm species and a polyethylene (PE) passive sampler was used. The sub-aims of this work are:

- **To investigate the relationship between the uptake of PCBs by worms, plants and passive samplers.**
- **To compare the sorption capacity of two biochars, one made using a controlled high-technology method, and one made using an uncontrolled low-technology method.**

This work will carry out a pot trial in which all phases will be added at the same time. This is in contrast to several previous studies that have carried out experiments with the different phases (earthworms, plants and/or passive samplers) separately (White et al., 2005; Gomez-Eyles et al., 2011a; Paul and Ghosh, 2011; Jakob et al., 2012; Denyes et al., 2016). The binding and uptake of PCBs to the various phases when in the same pots and over the same time span will be monitored.

The aims will be achieved by measuring the following endpoints:

- Sorption of the PCBs to the biochars.
- Uptake of PCBs to earthworms.
- Uptake of PCBs to plants.
- Uptake of PCBs to PE passive samplers.

The hypotheses for this work are:

1. Soil: The PCBs will sorb strongly to biochar and the sorption will differ based on biochar type and amendment dose.
2. Earthworms: The earthworms will lose mass in all pot treatments, they will take up PCBs from the contaminated soil and there will be a difference in uptake for different PCB-congeners. The presence of biochar in soil will reduce the mass loss of worms as well as the uptake of PCBs by the worms. There will be a dose effect with earthworms losing less mass as well as taking up less PCBs when a higher dose of biochar is amended to the soil. There will be a biochar type effect with variable mass loss as well as sorption for the two biochars. The worms will lose less mass in non-spiked soil than in spiked soil.
3. Plants: All treatments will give plant yield, the plants will take up PCBs and there will be a difference in uptake between different PCB-congeners. The presence of biochar in soil will increase the mass of the plants but reduce the uptake of PCBs. There will be a dose effect with plants giving higher mass yield but less uptake of PCBs when a higher dose of biochar is amended to the soil. There will be a biochar type effect with variable mass yield as well as PCB-sorption for the two biochars. The plant mass will be less for spiked soil than non-spiked soil.
4. Passive samplers: The PE passive samplers will take up PCBs and there will be a difference in uptake between different PCB-congeners. The presence of biochar in soil will reduce the uptake of PCBs to the PE passive samplers. There will be a dose effect; with PE passive samplers taking up less PCBs when a higher dose of biochar is amended to the soil. There will be a biochar type effect with variable sorption.
5. A correlation will exist between the reduction in PCB-uptake by plants and worms and the reduction in PCB-uptake by passive samplers.

2 Materials and methods

2.1 Chemicals

Compounds used for spiking the soil, surrogate standards, internal standard, solvents and other chemicals used in the trial are listed in appendix I. Appendix I also gives concentrations of chemicals, solvent purities (analytical grade or above 96 % for all solvents), other specifications and manufacturers.

2.2 Materials

Within the experiments the following definitions are used:
Phases: earthworms, plants (ryegrass and turnips) and PE passive samplers
Treatment/amendment: amount of biochar added; 0% (control), 1%, or 4% to spiked soil
Function/batch: control pots (0% biochar), amended pots (1 and 4% biochar) and unamended comparison pots (spiked and non-spiked soil with no biochar added, but one of the phases).

Mass when added to the pots and/or mass of extracted sample of soil, biochar, soil-biochar system, earthworms, plants and PE passive samplers used in the pot experiment are listed in appendix II.

2.2.1 Biochar

Two biochars; rice husk and mixed wood shavings, produced from different feedstocks and under different conditions (production technology method, pyrolysis temperature and pyrolysis time) were used. Figure 1 shows the rice husk and mixed wood shavings biochars after sieving to <2 mm grain size.

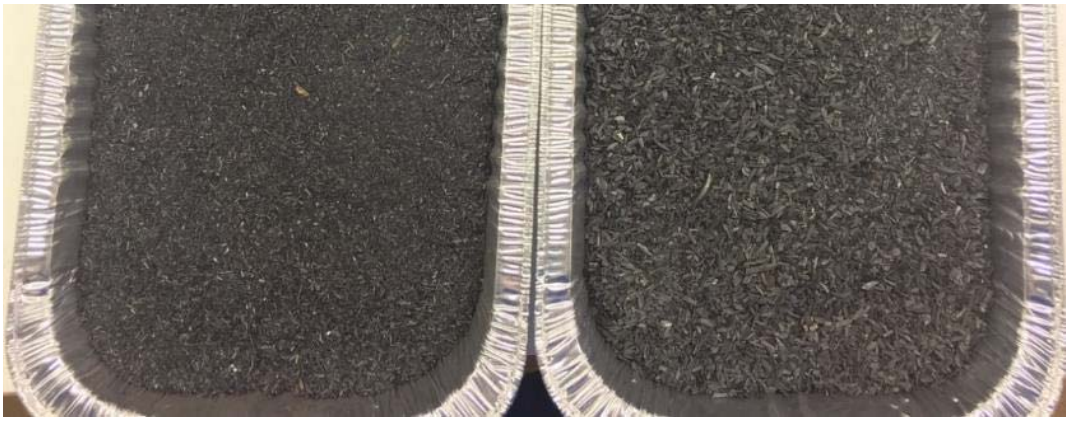


Figure 18 The biochars (sieved to <2mm grain size) used in the experiment. Left: Rice husk biochar and Right: mixed wood sieving biochar

Rice husk biochar

The rice (*Oryza sativa.* L.) husk biochar (Figure 18 left) was produced in a locally constructed pyrolysis unit (kiln) in Lampung, Indonesia at chamber temperature around 300 °C. Optimal pyrolysis time (selected based on amount of carbon recovered) for rice husk was 3.5 hours giving a biochar yield of 30.4% (Martinsen et al., 2015). This biochar represents the biochar produced using an uncontrolled, low technology method.

Mixed wood biochar

The mixed wood biochar was made from mixed wood shavings (Figure 18 right) at 700 °C and a residence time of 20 min with a Pyreg 500W unit at Swiss Biochar, Switzerland (Kupryianchyk et al., 2016). This biochar represents the biochar produced using a controlled, high technology method.

2.2.2 Soil

The soil, classified as a loam (40% sand, 44% silt and 17% clay) was sampled from 20 cm depth from Norderås, an agricultural field near Ås, at the Norwegian University of Life Sciences, Norway (UTM 32-N6617041/E599609) the 13th of November 2014. In total 240 kg of soil was collected and transported back to the laboratory for further use (Figure 19 left). The soil was dried using a combination of air-drying and the application of a heat-fan (3 days in room temperature and 4 x 1 hour with heat fan). Half of the soil was sieved to <2mm and half to <12mm and the two fractions were mixed. Soil was stored at room temperature prior to use.

Figure 19 The soil and perlite used in the experiment. Left: agricultural soil from Ås, Norway and Right: perlite

Half of the collected soil was spiked with seven PCBs (PCB-; 28, 52, 101, 118, 138, 153, 180, obtained as neat solids) and PAHs from the PAH-mix B (500 µg/mL of each of the 16 USEPA PAHs in 1.0 mL of acetone). The pollutants were dissolved in 60 ml of acetone, which itself was dissolved in 6 L of deionized water. The spiking solution contained 825.1 µg/L PCBs and 41.3 µg/L PAH. The co-solvent effect (1%) was therefore not considered to be a problem (Schwarzenbach et al., 2002). Spiking was carried out batch wise and the soil was mixed with the spiking solution using a cement mixer (Atika Betonmischer MIX 130, 600W, 230V). The soil batches were rotated for 30 minutes following the initial addition of the spiking solution and 6 L of water. An additional 6 L of deionized water was mixed into the soil to result in a soil a water content of between 10 and 20% (assuming from tests of the water holding capacity (WHC) of the soil, that the stored soil already contained around 10% water). The spiking gave a theoretical concentration of 0.0833 µg PCB/g soil and 0.0375 µg PAH/g soil. For the remainder of this thesis the PAHs will not be included due to unreliable results from the analysis of the samples. For the remaining 120 kg of soil, the same mixing process was carried out but without the addition of PCBs and PAHs (i.e. by adding water and mixing in the same way), resulting in the non-spiked soil.

The spiked soil was stored for 13 months prior to starting the pot experiment. The soil was mixed regularly by hand in order to homogenize the distribution of the PCBs and in effect resulted in an aged soil. The non-spiked soil was stored in plastic containers for the same period of time and was also mixed.

The water holding capacity (WHC) of the soil was determined by drying a known amount of soil for 24 hours at 110 °C, weighing the same sample fully water saturated, followed by repeating the drying step. The weight difference between the fully water saturated soil sample and the fully dry one gave the WHC of the soil. Based on previous pot trials (Jakob et al., 2012; Hale et al., 2013) and the pre-experimental trials carried out here, an irrigation rate that maintained 60% of the soils WHC was chosen.

Perlite (4.4 wt %), see Figure 19 (right) was added to all pots in order to improve the soil structure and increase aeration. The manufacturers state that perlite is a chemically inert substance. Perlite has been used in previous similar trials, for example a trial testing differences in pesticide bioaccumulation by plants and earthworms from compost and soil, with the same overall aim of improving soil structure (Peters et al., 2007).

2.2.3 Earthworms

The earthworms, *Eisenia fetida* (also called tiger worms and red wigglers) were purchased from Riverside Products, Norway. They were bedded in damp peat and fed on cellulose and sheep manure-pellets during breading. See Figure 20 (left).

Figure 20 The earthworms and plant seeds used in the experiment. Left: earthworms. Right: ryegrass seeds to the left and turnip seeds to the right

2.2.4 Plants

Ryegrass seeds, *Lolium perenne* L. (common name perennial ryegrass) were obtained from the Norwegian University of Life Sciences and turnip seeds (*Brassica rapa* ssp. *rapa*) from Nelson Garden were purchased from Plantasjen (Oslo, Norway). Figure 20 (right) shows the ryegrass seeds to the left and turnip seeds to the right. Seeds were used as received in the pot experiments.

2.2.5 Polyethylene (PE) passive samplers

Polyethylene bags, with thickness 26 µm, were purchased from VWR International (Leicestershire, UK). PE was cut into 150 sheets of approximately 0.1 g with dimensions of 2 x 5 cm (Figure 21 left). The PE passive samplers were precleaned prior to use by rinsing in respectively hexane, methanol and deionized water for 24 hours for each solvent (Hale et al., 2010), and stored in deionized water in a glass beaker prior to the pot experiment (Figure 21 right).

Figure 21 Polyethylene (PE) passive samplers used in the experiment. Left: a sheet of PE passive sampler. Right: 150 sheets of PE passive samplers stored in deionized water

2.3 Pot experiment

The pot experiment was conducted at Fytotronen, Department for Biosciences, University of Oslo, Norway.

2.3.1 Experimental design

A pot experiment was carried out in order to assess the effect of the addition of biochar to spiked soil on the uptake of PCBs to earthworms, plants and PE passive samplers. The soil treatments were as follows; 0% (control), 1% or 4% biochar added (amended) to spiked soil, and in addition unamended comparison pots consisting of either spiked soil or non-spiked soil, without the addition of biochar, but with the addition of just one of the phases described above. There were 5 replicates of the pots amended with biochar (0%, 1% or 4%) and 4 replicates of the pots containing unamended comparisons. The treatments as well as phases added to pots and the function of the replicate treatments tested are given in Table 2.

Table 2 List of treatments and function of the different treatment

Soil treatment	Phases added	Function	Replicate names
Spiked soil, 0% biochar	Ryegrass and PE passive sampler	Control (ryegrass)	0R1 - 0R5
Spiked soil, 1% rice husk biochar	Ryegrass and PE passive sampler	Amended (ryegrass)	1RR1 - 1RR5
Spiked soil, 4% rice husk biochar			4RR1 - 4RR5
Spiked soil, 1% mixed wood biochar			1MR1 - 1MR5
Spiked soil, 4% mixed wood biochar			4MR1 - 4MR5
Spiked soil, 0% biochar	Turnip, earthworm and PE-passive sampler	Control (turnip)	0T1 - 0T5
Spiked soil, 1% rice husk biochar	Turnip, earthworm and PE-passive sampler	Amended (turnip)	1RT1 - 1RT5
Spiked soil, 4% rice husk biochar			4RT1 - 4RT5
Spiked soil, 1% mixed wood biochar			1MT1 - 1MT5
Spiked soil, 4% mixed wood biochar			4MT1 - 4MT5
Spiked soil	Earthworm	Unamended comparisson	SSEW1 - SSEW4
	Ryegrass		SSR1 - SSR4
	Turnip		SST1 - SST4
	PE passive sampler		SSPE1 - SSPE4
Non-spiked soil	Earthworm		NSEW1 - NSEW4
	Ryegrass		NSR1 - NSR4
	Turnip		NST1 - NST4
	PE passive sampler		NSPE1 - NSPE4

Abbreviations in the replicate names column; R: ryegrass, RR: rice husk biochar and ryegrass, MR: mixed wood biochar and ryegrass, T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip, SS: spiked soil, EW: earthworm, PE: polyethylene passive sampler, NS: non-spiked soil

Every pot received soil and perlite and in addition one or more of the following phases; biochar, ryegrass seeds, turnip-seeds, earthworms and one PE passive sampler. Worms were only added to the pots with the turnip seeds. This was based on initial tests showing that the root system of the ryegrass took up so much room that the worms did not have enough space and did not thrive. The pot experiment was carried out in a growth room (further details are given below). The pot replicate names can be found in Table 2 and Figure 22.

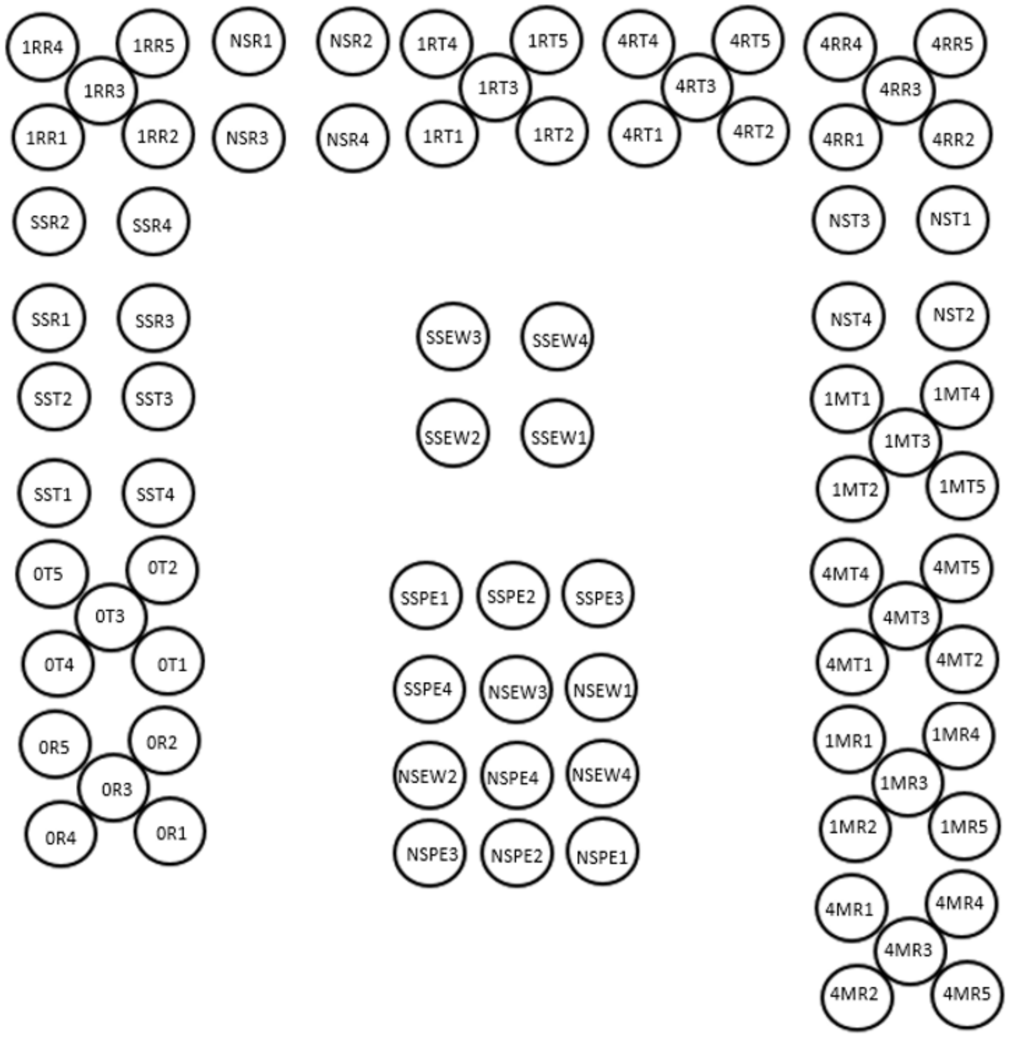


Figure 22 Set up of pots in Fytotronen growth room

Control and amended pots were prepared 8 weeks prior to the start of the pot experiment (see Figure 23 left). Control pots (see Table 2) were prepared by adding 1000 ± 0.05 g of spiked soil and 4.4 w% (44 g) of perlite. Amended pots (see Table 2) were prepared by adding 1000 ± 0.05 g of spiked soil, 4.4 w% (44 g) of perlite and biochar at one of two different doses; 1% (10.01 ± 0.03 g) and 4% (40.01 ± 0.03 g). The pots were mixed thoroughly by hand (2 times over the 8 weeks) to ensure a homogeneous distribution of biochar. After the 8 week period the unamended comparison pots (Table 2) were prepared in the same way as the control- and amended pots. All pots were prepared for the experiment by adding PE passive samplers, plant seeds and earthworms according to the treatments listed in Table 2.

Figure 23 Left: biochar has been added to the pot, but not yet mixed in with the soil. Right: a PE passive sampler is placed on top of the soil before being pushed 4 cm below the soil surface

The pots used were galvanized zinc-coated steel pots with dimensions of 18 cm inner diameter and height 17 cm (product name Socker, IKEA Alnabru, Norway). Eight holes with 1mm diameter were made in the bottom of each pot using a spike-board, to ensure drainage of water.

PE passive samplers were dried with a paper tissue, weighed individually (0.112 ± 0.009 g) and one PE passive sampler sheet was added 4 cm deep in the soil per pot (see Figure 23 right). The earthworms were rinsed with spring water and depurated on filter paper in large plastic containers for 24 hours prior to potting. The depurating procedure was carried out in a similar way, but on a much larger scale, than in previous studies (Hale et al., 2013). After depuration the worms were weighed in batches of 25 worms (30.2 ± 2.4 g). OECD procedure 222 was followed with respect to the ratio of worm to soil (OECD/OCDE, 2004). For the pots receiving ryegrass, 0.1 g of seeds were sprinkled on the top soil in the pot and the top 2 cm of surface soil was mixed. For the turnip-pots, three turnip seeds were pushed down 2 cm in to the soil with a minimum distance of 6.5 cm between each seed.

Figure 24 Left: a batch of 25 earthworms before washing, weighing and added to a pot. Right: Turnip seeds before being added to the pots

Following the addition of all individual phases to the pots, a fabric mesh was secured with a rubber band over the top of the pots to hinder the earthworms from escaping. The water content was maintained at 60% of soil water holding capacity (WHC) throughout the experiment (section 2.2.2). Some of the pots from the experiment are shown in Figure 25. The setup of pots in the growth room is shown in Figure 22 and Figure 26. An explanation of the names of the replicate of the samples in Figure 22 is given in Table 2.

Figure 25 Left: unamended comparison pots with non-spiked soil and ryegrass in 4 replicates. Right: amended pots with 4% mixed wood biochar, turnip, PE-passive sampler and earthworms in 5 replicates

Figure 26 Photograph taken from the growth room at Fytotronen

2.3.2 Conditions in the growth room

The conditions in the growth room were designed to simulate a Norwegian summer day and night cycle, with 14 hours of light and 10 hours of dark. The temperature was set at 20°C during the day hours and 15°C during the night hours. Day light simulation was carried out using metal (Na) halide bulbs set to around 200 PPF (µmol/m^2/s), which is equivalent to 14 200 lux. The air humidity was kept constant at 80% throughout the experimental period. The test conditions were adjusted to fit recommendations for testing of chemicals in earthworms (OECD/OCDE, 2004). Weeds that grew in the pots during the trial was not removed, this to minimize "interference" with the system in the pot thus making sure the plant roots were not damaged.

2.3.3 Collection of samples

After between 24 and 30 days, earthworms, plants and PE passive samplers were removed from pots. The ryegrass shoots were cut at the base just above the soil surface (Figure 27 left). The ryegrass roots were not sampled, due to the intricate network of ryegrass roots in the soil. The large quantity of very small roots resulted in soil particles adhering to them and the particles could not be rinsed off easily. A previous study focusing on the bioaccumulation of PAHs from activated carbon amended sediment to snails, questioned whether results obtained were reliable due to soil particles adhered to folding in the snails body surface and then been analysed along with the snails (Cornelissen et al., 2006b). A similar phenomenon was considered possible here and for that reason the ryegrass roots were not sampled. Turnips were sampled by gently removing them from the soil, dusting soil off the roots and washing with deionized water. The smallest parts of the turnip roots (<1mm in diameter) were removed due to soil particles that could not be removed (Figure 27 right). The ryegrass and turnip samples were wrapped in aluminium foil and stored in separate air diffusion minimizing bags prior to extraction.

Figure 27 Left: sampled ryegrass shoots. Right: sampled turnip shoots and roots

PE passive samplers were removed from pots (Figure 28 left), gently rinsed with deionized water, dried with a paper tissue before being stored in glass vials. Earthworms were removed from pots (Figure 28 right) and the rinsing and depuration process described in section 2.3.1 was repeated but in separate glass jars and without the use of the filter paper in order to avoid losses of PCBs to that media. The soil remaining in the pots was then mixed using a spoon and a subsample of soil was transferred to glass vials.

Figure 28 Left a sampled PE passive sampler before rinsing with deionized water. Right: Sampled earthworms prior to rinsing and depuration for 24 hours

Due to experimental challenges related to the amount of worms received, the pots with 1% (1RT1-1RT5) and 4% (4RT1-4RT5) rice husk biochar, as well as pot SSEW4 had a 6 day shorter trial period than the other pots.

Earthworms, plants, PE passive samplers and sub samples of the soil (spiked with and without biochar and non-spiked soil) were stored at -18 °C prior to sample processing and analysis.

2.4 Sample processing

Sample preparation, extraction and clean up, prior to analysis was carried out at RECETOX Research Centre for Toxic Compounds in the Environment, Brno, Czech Republic.

2.5 Sample preparation

All samples were freeze dried for 24 hours in a Christ Gamma 1-16 LSC freeze dryer (Figure 29) using vacuum (0.120 mbar) set at -50°C.

Figure 29 The Christ Gamma 1-16 LSC freeze dryer used to freeze dry the samples

2.5.1 Soil and soil-biochar system

For the remainder of this experimental section and when considering results, all samples referred to as soil and soil-biochar samples also contain 4.4 wt % perlite. The soil samples (controls as well as the unamended comparison pots) and the soil-biochar samples (amended pots) were extracted. A spatula was used to homogenize the soil and soil-biochar samples before transfer to extraction thimbles. Mass of the samples was 10.07 ± 0.06 g dry weight.

2.5.2 Earthworms

The mass of the earthworm samples (Figure 30 left) was 3.77 ± 1.06 g dry weight. Anhydrous sodium sulfate (5 g) of was added to each earthworm sample before grinding with a mortar and pestle into fine powder (Figure 30 right) and then transferred to extraction thimbles. The sodium sulfate was added to the worm samples to bind traces of excess water in the sample.

Figure 30 Left: freeze dried earthworms after weighing. Right: mixing of earthworms and sodium sulfate powder

2.5.3 Plants

Ryegrass and turnip samples (Figure 31) were transferred to extraction thimbles, where they were weighed and cut into smaller pieces with scissors. All recovered mass was extracted which was approximately 0.5 g for ryegrass and 0.3 g for turnips.

Figure 31 Left: a sample of freeze dried ryegrass. Right: a sample of freeze dried turnips

2.5.4 PE passive samplers

PE passive samplers were transferred from the glass vials used for storage to glass vials suited for solvent extraction.

2.6 Sample extraction

2.6.1 Soil, soil-biochar system, earthworms and plants

Samples (soil, soil-biochar, earthworm and plant) were extracted with 80 mL of an acetone:hexane mixture (1:1) (Škulcová et al., 2016), to which 3-5 boiling stones and 25 µL of surrogate standard was added.

A surrogate standard was used in order to follow method recovery. PCB 81 (0.1 mg/mL in isooctane) and PCB 126 (0.1 mg/mL in isooctane) were used to make up the surrogate standard. The compounds were purchased from Chiron AS (Trondheim, Norway). The standard concentration was 20 µg/mL in hexane.

The extraction was carried out using the Randall method (Eljarrat et al., 2000) with a VELP (SER 148/6) Randall-Soxhlet instrument (VELP Scientifica, Usmate Velate MB, Italy). The extraction consisted of 1 hour immersion, 1 hour dripping and 30 minutes of evaporation. Figure 32 (left) shows the VELP (SER 148/6) Randall-Soxhlet instrument and thimbles with green plant samples immersed in solvent vials. Figure 32 (right) shows solvent vials with earthworm and soil samples after the extraction process.

Figure 32 Left: VELP (SER 148/6) Randall Soxhleth instrument showing thimbles with plant samples immersed in solvent vials. Right: Samples of earthworms (brown colour) and soil after the extraction process

After extraction, soil, soil-biochar and plant sample extracts (between 1 and 5 mL) were collected, transferred to 20 mL glass vials and diluted in to 10 mL with the addition of acetone:hexane (1:1). The 1:1 mixture of acetone and hexane was chosen based on the fact that different solvents have different capacities to extract organic compounds, and that the mixture of acetone and hexane previously has shown to give good extraction efficiency (Gomez-Eyles et al., 2010; Škulcová et al., 2016). Earthworm extract samples were collected and diluted to 5 mL with chloroform prior to clean-up. The dilution of samples after extraction was carried out in order to obtain samples of known volume with concentrations that were suspected to be within the operating window of the analytical follow up.

2.6.2 PE passive samplers

PE passive samplers were extracted for 2 days in 20 mL of acetone:hexane (1:1) on a table top shaker at 10 rpm (Hale et al., 2010). The surrogate standard was added to the solvent prior to extraction.

2.7 Sample clean-up and preparation for analysis

2.7.1 Gel permeation chromatography (GPC)

Gel permeation chromatography, a type of size exclusion chromatography (SEC) that separates analytes based on size of molecules, was used, for clean-up of the earthworm samples. The method is used to remove large molecules like proteins, lipids and dyes (Mooibroek et al., 2002; Hubert et al., 2003). 1.5 mL of the earthworm extract sample in 5mL of chloroform (Cejpek et al., 1995) was filtrated through a 0.45 µm filter (Figure 33 left) and added to the top of the GPC-column (Figure 33 right) after rinsing the column with 2 mL of pure chloroform. A DeltaChrom KVAR 400 parallel GPC Clean-up System (Watrex Praha, Czech republic) was used.

Figure 33 Left: filtering of an earthworm sample. Right: adding the filtered sample to the DeltaChrom KVAR 400 GPC-column

2.7.2 Silica gel column chromatography

The soil, soil-biochar, earthworm, plant and PE passive sampler samples were all cleaned up using a silica gel column clean-up. The silica gel was activated (155 °C for 12 hours) prior to use in a Martinek Laboratorni Pece oven. Columns were constructed using 5 g silica gel for clean-up of earthworm-, PE- and soil samples, and 15 g silica gel for clean-up of plant samples. The volumes of solvent used for the extraction steps and clean-up for all samples is shown in Table 3.

Table 3 Volume of solvent used for extractions and clean-up for plant-, earthworm-, PE passive sampler and soil samples.

Parameter		Eartworms	Plants	PE passive samplers	Soil
Extraction	Input solvent (ml)	80	80	80	80
	Solvent	50:50 hexane: aceton	50:50 hexane: aceton	50:50 hexane: aceton	50:50 hexane: aceton
	Output sample (ml)	10	10	10	10
	Solvent	Chloroform	50:50 hexane: aceton	50:50 hexane: aceton	50:50 hexane: aceton
GPC	Input sample (ml)	1	-	-	-
	Solvent	Chloroform	-	-	-
	Output sample (ml)	5	-	-	-
	Solvent	Chloroform			
Clean up	Input sample (ml)	5	5	2	1
	Input solvent (ml)	30	60	20	30
	Solvent	Hexane	Hexane	Hexane	Hexane

2.7.3 Blow down of samples and spiking with internal standard

After the clean-up, the volume of the samples was blown down, using purified nitrogen gas, to about 0.5 mL and transferred to tapered GC-vials. The samples were then spiked with a standard PCB-77 solution (20 µg/mL in hexane) to a concentration of 1 µg/mL. All samples were stored at -18 °C prior to the analysis.

2.7.4 Determination of earthworm lipid content

About 8 mL (80%) of the remaining earthworm sample after the extraction was blown down (using purified nitrogen gas) to remove all water. The mass remaining was considered to be the lipid content.

2.8 Gas chromatography – mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out in the lab of the Department for Environmental Engineering at the Norwegian Geotechnical Institute (NGI) in Oslo, Norway.

The results from the GC-MS analysis (µg/g) can be found listed in appendix III. Replicates that are not listed in appendix III were lost during the experiment or excluded because the data was not deemed reliable based on the recovery of the surrogate standards.

Analysis was carried out using Agilent Technologies 6850 Network GC system with a 5973 mass selective detector (Agilent Technologies, USA). A fused silica capillary column type HP-5MS (5% phenylmethylsiloxane) with dimensions 30 m x 250 µm (inner diameter) x 0.25 µm film thickness was used. Helium was used as the carrier gas and maintained at a constant flow of 1 mL/minute. A splitless injection of 1 µL with an injector temperature of 280 °C was performed. The temperature program was as follows: 80 °C for 1 minute, increasing at 20 °C/minute to 180 °C, increasing at 4 °C/minute to 200 °C, increasing at 5 °C/minute to 280 °C, increasing at 40 °C/minute to 310 °C and a final hold of 5 minutes. The GC-MS ionization potential was 70 eV, the ion source and the transfer line temperature were 230 °C and 310 °C respectively. The MS was operated in selected ion monitoring (SIM) mode for quantitation of target compounds. The compounds were identified based on their mass spectra using the base peak and two qualifier ions for each compound and quantified using the internal standard. All values were reported as µg/g dry weight. The GCMS was calibrated with a 5 point PCB-calibration curve, ranged from 0.005 to 0.5 mg/L. Detection limits were 0.01 µg/g.

2.9 Quality assurance/ Quality control (QA/QC)

2.9.1 Pre-experimental trials

Small scale pot trials were conducted in the laboratory at NGI and a bigger pot trial at the growth room at Fytotronen prior to the main pot experiment. The aim of these trials was to become familiar with the best methods to use when carrying out a large scale pot trial. The trials investigated soil properties such as aeration and water holding capacity and how these parameters were affected by biochar amendment. The trials also allowed pre-testing with different biochar doses in order to select the most appropriate doses. The growth of different plants (tomato, radish, squash, ryegrass turnip and beetroot) was tested with and without biochar amendment. Earthworm behaviour was studied by varying the amount of worms in a pot and testing how the worms behaved in pots with and without biochar and plants. The pre-experimental trials are further discussed in chapter 3.

2.9.2 Blanks and replicates

Blank samples were prepared and analysed. Blank samples of the non-spiked soil, spiked soil, perlite, earthworms and PE passive samplers that had not undergone the pot trial were prepared and extracted in the same way as the real samples. Blank samples were always run in triplicate. All blank samples were spiked with surrogate standards prior to extraction. Blank samples are further discussed in section 4.1.

2.9.3 Unamended comparisons and replicates

Comparison samples were samples of spiked and non-spiked soil with only one other phase added to the pots (that is either ryegrass, turnip, earthworms or PE passive sampler). All comparison samples were used in the pot trial (in comparison to the blank samples that did not undergo the pot trial) and were prepared in 4 replicates. They were processed, spiked with surrogate standards and analysed in the same way as all other samples in the pot trial. Unamended comparison samples are further discussed in section 4.2.

2.9.4 Surrogate standards

Surrogate standards were spiked to all samples prior to extraction in order to follow method recovery. The surrogate standards are further discussed in section 4.3.

2.9.5 Treatment equality

The control pots (0% biochar) were treated with the same mixing regime as pots amended with 1% and 4% of biochar. Non-spiked soil was treated in the same way as spiked soil with respect to mixing of the soil regularly.

2.10 Statistics

Data points that were suspected to be outliers were tested using Dixons Q-test and removed accordingly. All graphical material (plots) and statistical analysis was carried out using the GraphPad Prism 7 scientific software. To compare the difference between pair of means in the control and amended samples Tukey multiple comparison test was used following a one-way ANOVA test. In the following discussion of the results, all concentration data given are treated with Tukey multiple comparison test following a one-way ANOVA test, using a 95% confidence interval of difference. All statistical analysis are listed in appendix IV.

2.11 Data interpretation

In this study the concentration of pollutants in different phases (earthworms, plants, PE passive samplers as well as the soil and soil-biochar system) was used as the end points of the pot experiment.

2.11.1 Mass balance

A mass balance was set up, finding the total recovery of all PCB congeners from all phases. Mass balances were calculated for all PCB congeners for all treatments using Equation 2.

Equation 2 The recovery of the PCB congeners from all phases following the pot trial

$$M_{total} = (m_{earthworms} \times c_{earthworms}) + (m_{plant} \times c_{plant}) + (m_{PE\ passive\ sampler} \times c_{PE\ passive\ sampler}) + (m_{soil\ or\ soil-biocar\ system} \times c_{soil\ or\ soil-biochar\ system})$$

Where M_{total} is the total mass (µg) of the PCBs in all phases (worms, plant, PE passive sampler and soil/soil-biochar system), m is mass (g) of sample and c is concentration (µg/g) of PCBs detected in the sample. The mass balance is further discussed in section 4.4.

2.11.2 Phase to soil accumulation factors (PSAF)

The PCB concentration accumulated by the earthworms, plants and PE passive samplers were assessed as phase (earthworm/plant/PE) to soil - accumulation factors (PSAF).

The PSAFs were calculated using Equation 3.

Equation 3 The phase (earthworms, plant or PE) to soil accumulation factor (PSAF)

$$\text{PSAF} = \frac{c_{PCB\ (earthworm\ (lw),\ or\ plant\ (dw) or\ PE\ passive\ sampler)}}{c_{PCB\ (soil\ or\ soil-biochar\ system\ (dw))}}$$

Where $c_{\text{PCB(earthworm (lw) or plant (dw) or PE passive sampler)}}$ is the detected concentration (µg/g) of PCB in the different phases; earthworm based on lipid weight (lw), plant based on dry weight (dw) and PE passive sampler. $c_{\text{PCB(soil or soil-biochar system (dw))}}$ is the concentration (µg/g) of PCB in the soil based on dry weight (dw) from the corresponding pot. The PSAFs are discussed in chapter 9

3 Pre-experimental trials

The information gained in the pre experimental trials was invaluable for the main trial. The doses of biochar tested were 0%, 3%, 5% and 10 %. Figure 34 (left) shows the soil amended with 0% and 5% mixed wood biochar. The 10% addition resulted in a visual change where the colour went from soil brown to a biochar black. In addition, a 10 % dose is high in comparison to what has previously been used in field studies, where doses are often between 0.4 and 5% (Asai et al., 2009; Cornelissen et al., 2013; Obia et al., 2016; Pandit et al., 2017). Field trials using AC often amend 2% AC to contaminated soils (Brändli et al., 2008, 2009; Jakob et al., 2012).

Figure 34 Left: A table top trial after three weeks. The soil in the pot with 0% biochar was dryer and more compact than the pot with 5% biochar. All 5 earthworms added to the 0% biochar pots had escaped, while only 2 out of 5 worms had escaped from the 5% biochar pot. Right: A table top trial, 11 days into the experiment. Turnip seedlings in pots in the top row, little response from radish seeds in the middle row and little response from tomato seeds in the bottom row. From left to right the pots contained non-spiked soil + 0% biochar, non-spiked soil + 5% biochar, spiked soil + 0% biochar and spiked soil + 5% biochar

The production of biochar in high enough quantities to satisfy a 10 % amendment may also be challenging in relation to the quantity of feedstock available. In farmer-led field trials carried out in Zambia, the addition of low-dosage biochar (1% in planting basins) was tested as a realistic way to increase maize yields (Cornelissen et al., 2013), in contrast to the alternative previously suggested unrealistically high application rates of 39% needed to obtain optimal crop yield (Jeffery et al., 2011). In addition, the use of such a high biochar dose may result in negative environmental consequences. Bielska et al. (2017) reported that *Folsomia candida* benefited from the reduction of *p,p'*-DDE bioavailability following a 1% and 5% biochar addition to contaminated soils, while a 10% amendment led to a nullification of the positive effects of biochar amendment caused by biochar-induced toxicity. Based on this information, two does were chosen for the main trial; 1% and 4%.

It was crucial that the plants chosen for the main experiment would produce a large enough yield for further testing, over the four week experiment. Given the soil used in the experiment (loam from an agricultural field as described in section 2.2.2), the plants chosen for the pre-trial testing were; tomato, *Solanum lycopersicum*, (Gartler et al., 2013), radish, *Raphanus sativus*, (van Zwieten et al., 2010; Gartler et al., 2013), squash/zucchini, *Cucurbita pepo,* (Jakob et al., 2012; Gartler et al., 2013), ryegrass, *Lolium perenne* L., (Jakob et al., 2012), turnip, *Brassica rapa* L., (Khan et al., 2015) and beetroots, *Beta vulgaris*, (Gartler et al., 2013). This decision was based on an initial literature study which showed that these species had been tested before and were therefore expected to produce enough biomass. Figure 34 (right) shows one of the pre-trials with the seeds of turnips, radish and tomato being tested.

Ryegrass and turnips were chosen for the main trial based on the fact that they showed the most consistency (giving seedlings from the seeds) and the best plant yield, as well as thriving in both the soil and soil-biochar systems. Ryegrass and turnip cover a broad range of plant physiological and morphological characteristics, including luxuriant root system for the ryegrass and abundant root-derived lipids (linoleic acid) for turnip. There are numerous previous studies on ryegrass (Rezek et al., 2008; Alam et al., 2010; Janus et al., 2015) and some that are focused on turnips (Tammeorg et al., 2014; Khan et al., 2015).

Initially three soil:water ratios were tested (10 wt%, 20 wt% and 30 wt% of water added), with 30 wt% water resulting in a supersaturated fluid soil. The amount and frequency of irrigation was decided following the determination of the WHC of the soil (18%) (described in section 2.2.2), as well as visual observations for the soil-biochar system, the plant growth and the behaviour of earthworms during the pre-experimental trials (Figure 34). 60% of the WHC gave optimal irrigation of the soil, as has been suggested in previous trials (Jakob et al., 2012; Hale et al., 2013; Brennan et al., 2014), and was therefore maintained throughout the trial.

The pre-trial gave very important information about using earthworms. It was important to ensure that the worms remained in the pots, had enough space to thrive and actually survived the pot trial. In order to keep the earthworms in the pots, two measures were taken as a result of the pre-trial. The first was to make the size of the holes in the pots smaller in the main trial compared to the pre-trials (from 2 mm in diameter to 1 mm) and the second was to add a mesh on top of the pots (Figure 35 left). There was little problem with worm survival in the pre-trials, and most earthworms (that remained in the pots during the pre-trial) survived. The root system of the ryegrass was found to be too extensive for the earthworms to thrive in (Figure 35 right) and this led to the decision to not include earthworms in pots with ryegrass for the main trial.

Figure 35 Left: earthworm escaping through hole in bottom of the pot. Right: the root system in a pot with 5 week old ryegrass

Perlite was tested to see if it could improve the soil aeration. Perlite appeared to make conditions more conducive for both plant growth and earthworm survival (Figure 36). Perlite has been used in previous pot trials (Peters et al., 2007; Whitfield Åslund et al., 2007; Lunney et al., 2010; Jeffery et al., 2015) and was used in the main trial.

Figure 36 Soil from pots in the pre-trial in Fytotronen. Left: No perlite added. Right: 10 g of perlite added

4 Quality Control

4.1 Blanks

Blank samples were non-spiked soil, perlite, earthworms and PE passive samplers that had not been used in the main pot experiment.

None of the PE passive sampler blanks contained PCBs at concentrations above the analytical limit of detection (LOD) (0.01 µg/g) and none of the perlite or earthworm blank samples contained PCB-congeners 101, 118, 153 and 180 at concentrations above the LOD.

The smaller PCBs (28 and 52) were detected in the blank samples. However, average detected concentrations of PCB-28 and PCB-52 in blank earthworm samples were far below those for the spiked soil and biochar amended systems. However, for the blank non-spiked soil and the blank perlite samples, concentrations were higher than for some of the amended treatments. We are unsure of the reason for this, a reason could be due to a contamination during laboratory work.

4.2 Unamended comparisons

Unamended comparison samples were spiked and non-spiked soil without the amendment of biochar, but with only one other phase added to the pots (that is either ryegrass or turnip or earthworms or PE). All comparison samples we used in the pot trial.

For the non-spiked soil unamended comparison pots, the concentrations of all PCB congeners (except for PCB-180) were detected above the LOD (0.01µg/g) in earthworm comparison samples. However, detected concentrations were far below those for the spiked soil and biochar amended systems for all of the treatments.

The detected concentrations of PCBs in the non-spiked soil unamended comparison pots for the plant samples were in general lower than in the control pots (0% biochar). Concentrations of PCBs in plants in biochar amended pot were both higher and lower than the plants from the corresponding non-spiked soil pots. It appears that the plant system in the presence of biochar complicated the picture.

None of the PE passive samplers from the non-spiked soil comparison pots contained PCB-congeners at concentrations above LOD and none of the soil samples from the non-spiked soil comparison contained PCB-congeners 101, 118, 153 and 180 at concentrations above the LOD.

Average detected concentrations of PCB-28 and PCB-52 in non-spiked soil samples exceeded the average concentrations for treatments with 4% rice husk and 4% mixed wood biochar in ryegrass pots as well as in 1% rice husk in turnip pots. Again we are unsure of the reason for this, but suggest a contamination from the laboratory. Concentrations were below those for the spikes of soil and biochar amended systems for the other treatments.

4.3 Surrogate standards and data exclusion

PCB 81 (0.1 mg/mL in isooctane) and PCB 126 (0.1 mg/mL in isooctane) were used as surrogate standards. Concentrations were corrected for the recovery of the average of the surrogate standards. PCBs 28, 52 and 101 were corrected for using PCB 81 and PCBs 118, 138, 153 and 180 were corrected for using PCB 126. Following the data analysis, the samples that were excluded from further work were; 7% of soil samples, 34% of plant samples and 48% of PE passive sampler samples. Due to no recovery of the surrogate standard in the sample.

4.4 Mass balance

A 100 % mass balance is one for which all spiked PCBs are recovered in the different phases added to the pots. Using the following equation;

$$M_{total} = (m_{earthworms} \times c_{earthworms}) + \left(m_{plant} \times c_{plant}\right) + \left(m_{PE\ passive\ samplers} \times c_{PE\ passive\ samplers}\right) + (m_{soil\ or\ soil\ biochar\ system} \times c_{soil\ or\ soil\ biochar\ system})$$

a mass balance was constructed for all PCB congeners and all treatments. In the experiments all phases were extracted and thus a mass balance could be constructed on this basis. A comparison of the result of the mass balance with the mass of PCBs initially spiked to the soil was made to determine the percentage recovery of the PCBs (Table 4) and appendix V.

Mass balances between 50 and 150% were deemed acceptable based on the fact that complex environmental matrices were used in the experiments. The mass balance for the 0% biochar (control) turnip pots ranged from 58% to 129% for all PCB congeners. Mass balances for 0% biochar (control) ryegrass pots also had good mass balances (up to 65%) for most PCB congeners, however somewhat lower values (37 - 49%) for PCBs 118, 138 and 180 were recorded in some cases. Mass balances for unamended comparison pots (range 26 – 151%) as well as the blanks (range 34 – 105%) were acceptable for most PCB congeners (appendix V).

Table 4 Mass balances expressed as total recovery (%) for treatments

Soil treatment	Phases added	Batch	Total recovery (%)							
			PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	∑ PCB-7
Spiked soil, 0% biochar	Ryegrass and PE passive sampler	Control (ryegrass)	54	63	51	37	65	46	49	52 ± 10
Spiked soil, 1% rice husk biochar	Ryegrass and PE passive sampler	Amended (ryegrass)	64	76	82	56	106	84	93	80 ± 17
Spiked soil, 4% rice husk biochar			0,04	6	0,05	0,03	0,07	13	28	7 ± 10
Spiked soil, 1% mixed wood biochar			42	50	60	49	76	93	99	67 ± 22
Spiked soil, 4% mixed wood biochar			0,07	3	3	6	3	51	70	20 ± 28
Spiked soil, 0% biochar	Turnip, PE-passive sampler and earthworm	Control (turnip)	95	129	118	76	124	96	58	100 ± 26
Spiked soil, 1% rice husk biochar	Turnip, PE-passive sampler and earthworm	Amended (turnip)	0,5	8	2	1	8	33	50	14 ± 19
Spiked soil, 4% rice husk biochar			90	114	99	77	129	91	94	99 ± 17
Spiked soil, 1% mixed wood biochar			64	79	93	65	116	87	83	84 ± 18
Spiked soil, 4% mixed wood biochar			95	108	93	69	127	118	125	105 ± 21
Spiked soil	Earthworm	Unamended comparisson	27	29	112	83	151	109	111	89 ± 46
	Ryegrass		44	54	56	47	64	105	115	69 ± 29
	Turnip		56	62	70	51	95	70	76	69 ± 14
	PE passive sampler		48	57	51	37	73	52	64	55 ± 12
Spiked soil not undergone pot trial	None	Blank	34	40	70	59	84	96	105	70 ± 27

Mass balance ranged from below 1 to 95% for all amended treatments (1% and 4%, both biochars) for PCB-28. Mass balances ranged from 3 to 114% for PCB-52, below 1 to 99% for PCB-101, below 1 to 77% for PCB-118, below 1 to 130% for PCB-138, 13 to 118% for PCB-153 and from 28 to 125% for PCB-180. In general, mass balances were higher for larger PCBs, perhaps due to the lower volatility of these congeners. Individual data points with mass balances outside the range defined above as acceptable, we excluded from further data analysis. The low recovery for some PCB congeners for some of the biochar amended treatments (below 1 to 49%) is thought to be due to the extraction method used (1:1 acetone:hexane). This solvent combination may not have been optimal for extracting PCBs from biochar due to their strong binding to the biochar (Wang et al., 2013a) and a different solvent may have resulted in a better PCB extraction efficiency for the biochar-sorbed PCBs. A previous study quantifying native PAHs in biochars concluded that use of toluene as extraction solvent was optimal as it gave the highest extracted PAH concentration and best surrogate standard recovery (Hale et al., 2012).

5 Results earthworms

5.1 Visual observations

In 64 % of pots, all earthworms that were added at the start of the pre-trial were recovered (Figure 37). For the reminder of the pots between 1 and 4 earthworms were found dead in the soil at the end of the trial, and for the replicate pot 1 with 4% rice husk biochar and turnips (4RT1) all the earthworms had collected in a pile and were dead on the soil surface. The reason for this is unknown.

The colour and tissue quality of the sampled earthworms did not appear to be different compared to when the earthworms were potted. Some earthworms appeared deformed and desegmented at the end of the trial (Figure 38). The earthworms from the 0% biochar and 1% and 4% mixed wood biochar pots appeared to have higher degree of deformities than worms from the other pots.

Figure 37 Earthworms from pot with 4% mixed wood biochar, replicate number 4 (4MT4), after depuration for 24 hours. Left: worms and gut content. Right: earthworms after rinsing

Reasons for the deformities and desegmentation of the worms is unknown. It is possible that the earthworms attempted to remove accumulated PCBs by releasing segments of their tails in order to reduce the overall contaminant burden. The soil itself could also have played a role, where compact and aggregated parts of the soil could have resulted in the worms becoming less mobile and forming the "lumps" due to pressure build up (personal communication with Lucie Bielska).

Figure 38 Examples of observed features on some of the sampled earthworms. Left: "lumps" on a worm from replicate pot 2 with 0% biochar (0T2). Right: Narrowing on earthworms from pot with 1% mixed wood biochar, replicate number 5 (1MT5)

5.2 Percentage mass loss of earthworms during the trial

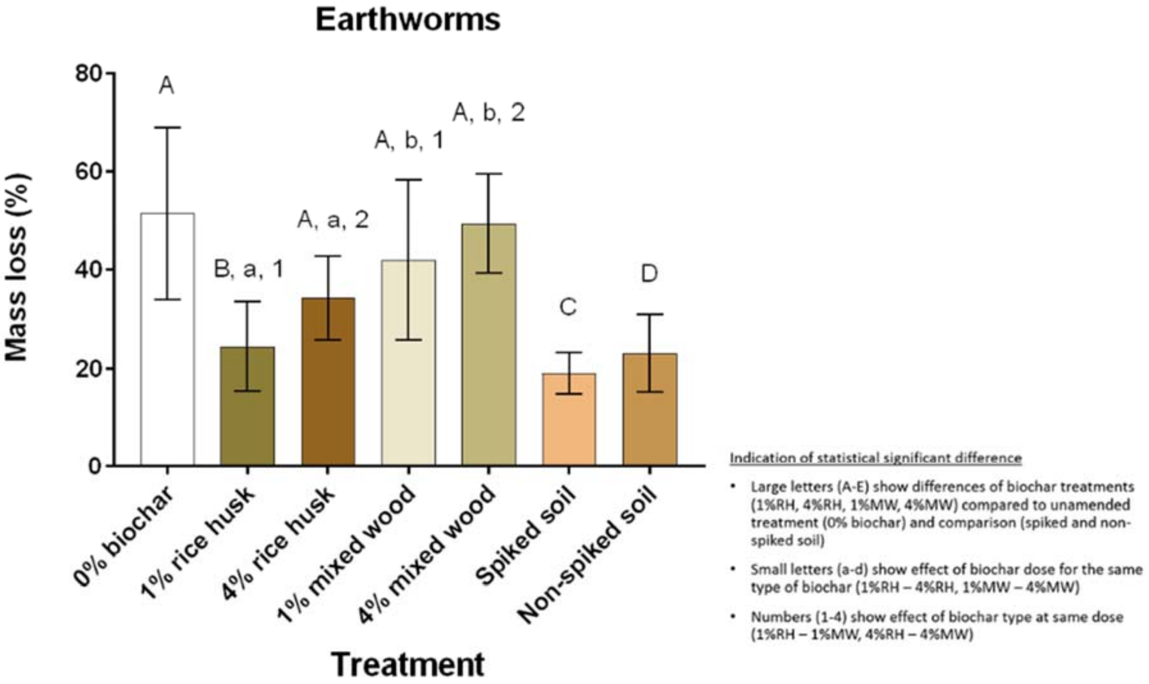
Table 5 and Figure 39 show the loss of mass for the earthworms in amended and control pots as well as unamended comparison pots. The mass loss was determined by comparing the initial potting mass with that after the trial for each pot (not for each worm). The batches of earthworms lost between 19% and 51% of their starting mass during the period of the trial.

Table 5 Loss of mass in earthworm batches for pots treated with 0% biochar (control), 1% rice husk biochar and 4% biochar

Treatment	Batch	Loss of mass (%)
0% biochar	Control	51 ± 18
1% rice husk	Amended	24 ± 9
4% rice husk		34 ± 8
1% mixed wood		42 ± 17
4% mixed wood		50 ± 10
Spiked soil	Unamended comparison	19 ± 4
Non-spiked soil		23 ± 8

5.2.1 Amended and control pots

For the amended and control pots, the largest loss of mass occurred in the control pot series with 0% biochar (51 ± 18% loss) and 4% mixed wood biochar (50 ± 10 % loss), although there was no statistically significant difference from the control. The smallest weight loss was in the pot series with 1% rice husk biochar with a 24 ± 9 % loss (statistical significant difference from the control, p-value 0.0235). This suggests that the rice husk biochar has a conducive effect on the earthworm growth. For the other treatments (4% rice husk biochar and 1% mixed wood biochar) there were no statistically significant effects (p-values ranging from 0.2256 to >0.9999) but it appears that earthworms in pots with rice husk biochar lost less weight than those in pots with 0% biochar and mixed wood biochar. This may have been due to the shorter trial period for these pots.



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar) and comparison (spiked and non-spiked soil)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)

Figure 39 Mass loss (%) of earthworms from different biochar treatments. The mass is based on fresh weight and the loss of mass is calculated as an average for all replicates in the treatment

5.2.2 Unamended comparison pots

For the unamended comparison pots the loss of mass in the batches of earthworms were 19 ± 4 % and 23 ± 8 % for the pots with spiked soil and non-spiked soil, respectively. This difference was not statistically significant (p-value 0.9987) and possibly in contrast to what would have been expected where a negative toxic effect of the PCBs themselves on the earthworms may have been expected to result in a greater loss of mass for the earthworms in the spiked soil than the non-spiked soil. Several previous studies have reported variable observations with respect to weight loss and presence of pollutants.

A glass beaker trial showed that during a 28 day trial period there was a 6.7% loss of earthworm mass in a freshly spiked unamended soil compared to a 9.8% mass loss in non-spiked control soil and a 21.6% mass loss in 19 month aged spiked unamended soil compared to an 18.5% mass loss in control non-spiked soil. The mass loss for worms in 2% AC amended freshly spiked soil and aged soil were 15% and 32% respectively (Paul and Ghosh, 2011).

No food was provided for the earthworms in the duration of the pot experiment here as for other previous experiments (Paul and Ghosh, 2011; Hale et al., 2013). The reason for this was to avoid adding organic substances that could possibly compete with PCB sorption sites on the biochar. The loss of earthworm mass might therefore be explained by starvation during the pot experiment (Gomez-Eyles et al., 2011b).

5.2.3 Hypotheses

Prior to the pot experiment it was hypothesized that; 1) the earthworms in all treatments would lose mass during the trial 2) the presence of biochar in soil would reduce the mass loss of the earthworms, 3) there would be a dose effect; with earthworms loosing less mass when a higher dose of biochar was amended to the soil, 4) there would be a difference between the two biochar's with regard to earthworm mass loss and 5) in the unamended comparison pots the mass loss in the worms would be less for non-spiked soil than spiked soil.

The results from the trial generally supported the first and second hypotheses as there was a mass loss for earthworms in all treatments and a difference in the mass loss between the control and amended pots, with earthworms losing less mass in biochar amended pots. The third hypothesis was falsified as there seemed to be dose effect, however earthworms lost less mass when a lower biochar dose was amended to the soil (possibly contrary to expectations). The fourth hypothesis was confirmed as earthworms from pots with rice husk biochar lost less mass than the worms in the pots with mixed wood biochar. The fifth hypothesis was falsified as there was no statistically significant difference in worm mass loss between non-spiked and spiked soil.

5.3 Uptake of pollutants by earthworms in pots containing turnips

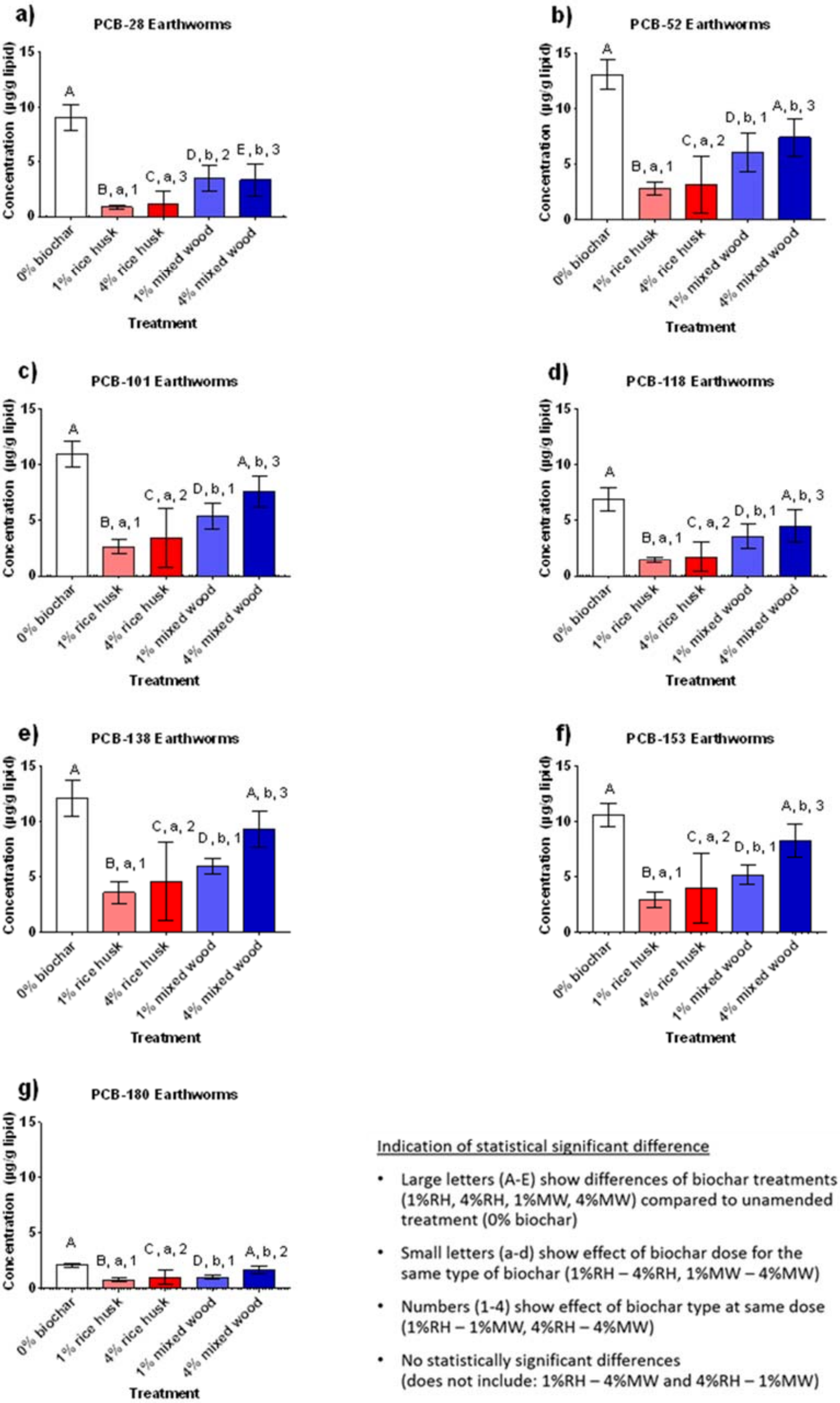
5.3.1 Absolute concentrations

Table 6 shows concentrations (µg/g lipid mass) of PCBs in earthworms in pot series containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar and concentrations of PCBs in the unamended comparison pots.

Table 6 Earthworm concentrations of 7 PCB-congeners (µg/g lipid mass) for pots with 0% biochar (control), 1% and 4% of either mixed wood or rice husk biochar as well as unamended comparison pots with spiked and non-spiked soil

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	7-PCBs
0% biochar	Control	9,1 ± 1,1	13,0 ± 1,3	11,0 ± 1,1	6,9 ± 1,0	12,1 ± 1,7	10,6 ± 1,0	2,1 ± 0,2	9,3 ± 3,7
1% rice husk	Amended	0,9 ± 0,2	2,8 ± 0,6	2,7 ± 0,7	1,5 ± 0,2	3,6 ± 1,0	2,9 ± 0,7	0,8 ± 0,2	2,2 ± 1,1
4% rice husk		1,2 ± 1,2	3,2 ± 2,5	3,4 ± 2,6	1,8 ± 1,3	4,6 ± 3,5	4,0 ± 3,1	1,0 ± 0,7	2,7 ± 1,4
1% mixed wood		3,5 ± 1,2	6,0 ± 1,7	5,4 ± 1,1	3,6 ± 1,1	5,9 ± 0,7	5,2 ± 0,8	1,0 ± 0,2	4,4 ± 1,8
4% mixed wood		3,3 ± 1,5	7,4 ± 1,6	7,6 ± 1,4	4,5 ± 1,4	9,3 ± 1,7	8,3 ± 1,5	1,7 ± 0,4	6,0 ± 2,8
Spiked soil	Unamended comparison	7,9 ± 1,7	12,9 ± 2,3	11,0 ± 0,9	8,8 ± 3,5	12,2 ± 1,5	10,2 ± 1,1	1,8 ± 1,2	9,3 ± 3,7
Non-spiked soil		0,4 ± 0,3	0,5 ± 0,5	0,2 ± 0,4	0,1 ± 0,2	0,1 ± 0,2	0,1 ± 0,2	0 ± 0	0,2 ± 0,2

Figure 40 shows concentrations in µg PCB/g of lipid earthworm mass for the different treatments for each of the 7 PCB congeners. In the different bar plots the white bar represents the PCB concentrations in 0% biochar (control) treatments, while the PCB concentrations in the 1 and 4% rice husk biochar pots are represented with two shades of red and the PCB concentrations in the 1 and 4% mixed wood biochar pots are represented with two shades of blue. To indicate statistical significant differences large letters (A-E) are used above the bars, where the 0% biochar (control treatment) is labelled A. The biochar treatments labelled A as well are not statistically significantly different from the 0% biochar treatment, while letters B-E indicate a statistical significant difference. Small letters (a-d) indicate statistical significant differences between biochar dose for the same type of biochar, with a and b indicating statistical difference between 1% and 4% doses of rice husk biochar and c and d indicating statistical difference between 1% and 4% doses of mixed wood biochar. Numbers (1-4) indicate statistical significant differences between biochar types at the same dose. 1 and 2 indicate statistical difference between 1% rice husk biochar and 1% mixed wood biochar and 3 and 4 indicating statistical difference between 4% rice husk biochar and 4% mixed wood biochar.



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 40 Earthworm concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

For all PCBs, the average detected concentrations in earthworms in the 0% biochar pots were significantly higher than in pots with 1% (p-values between < 0.0001 and 0.001) and 4% (p-values between < 0.0001 and 0.01) rice husk biochar and 1% (p-values between < 0.0001 and 0.01) mixed wood biochar. For the 4% mixed wood treatments the effects were not significant (p-values between 0.0643 and 0.6339), except for PCB-28 (p-value < 0.0001) and PCB-52 (p-value 0.0034). These findings illustrate that the amendment of biochar to soil was efficient in reducing the uptake of PCBs by earthworms.

The largest uptake of PCBs to earthworms was seen for PCB-138 for most treatments and the smallest uptake was seen for PCB-180. The following generalisation can be made related to PCB uptake for largest to smallest: PCB-138 > PCB-153 > PCB-52 > PCB-101 > PCB-118 >PCB-28 > PCB-180. There does not appear to be a trend based on the molecular size of the PCB, although PCB-180 was taken up the least and is the largest of the PCBs tested. The planar PCBs (28 and 118) are taken up to a smaller extent than the non-planar PCBs suggesting differences in uptake due to stereochemistry.

5.3.2 Unamended comparison pots

For the spiked soil unamended comparison pots (Table 6) the highest uptake of PCB to the earthworms was for PCB-52 (12.9 ± 2.3 µg/g) followed by PCB-138 (12.2 ± 1.5 µg/g) and the lowest was for PCB-180 (1.8 ± 1.2 µg/g). For the non-spiked soil unamended comparison pots the sum PCB-7 concentration was 0.2 ± 0.2 µg/g. Since the soil in these pots was not spiked with PCBs this result must be due to a diffuse contamination during the trial or volatilization of the lower chlorinated PCBs from pots with spiked soil. The average uptake of PCBs by earthworms was in very good agreement (both were 9.3 ± 3.7 µg/g) for the 0% biochar treatment and the unamended comparison spiked soil pot.

5.3.3 Percentage reduction in PCB uptake by earthworms

Table 7 shows the reduced uptake of PCB congeners to earthworms following the amendment of biochar. The highest reduction for a single PCB in a treatment was 90% for PCB-28 in the 1% rice husk treatment. Average reductions for sum PCB-7 congeners were; 76%, 69%, 53% and 34% for 1% rice husk, 4% rice husk, 1% mixed wood and 4% mixed wood treatments respectively. In most cases there was a significant effect of the biochar type, with rice husk biochar resulting in the greatest reduction in uptake of PCBs by earthworms. There was no significant effect of biochar dose for either biochar, suggesting a 1 % addition of these biochars to a PCB contaminated soil may be enough to observe a good remediation efficiency.

Table 7 Reductions (%) in earthworm PCB-concentrations for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	7-PCBs
1% rice husk	Amended	90	79	76	79	70	72	64	53
4% rice husk		87	76	69	75	62	62	51	34
1% mixed wood		62	54	51	48	51	51	51	76
4% mixed wood		63	43	31	35	23	22	19	69

5.3.4 Comparison with previous literature studies

A greenhouse trial on the use of biochar to reduce soil PCB bioavailability to earthworms (*Eisenia fetida)* used a biochar made from wood waste biomass produced under similar condition as the mixed wood biochar used in this experiment. The authors reported a reduction in bioaccumulation of PCBs into the worm tissue of 53% and 88% for treatments with 2.8% and 11.1% biochar, respectively (Denyes et al., 2012). In another experiment, the reduction in bioaccumulation of PCBs to *E. fetida* after 2% AC amendment was found to be inversely related to the chlorination level of the PCBs (Paul and Ghosh, 2011). The observation was explained by the fact that the lower chlorinated PCBs have a higher aqueous solubility and faster rate of mass transfer from the pore water to the AC particles than the larger PCBs. Similar trends were found for marine organisms in PCB-polluted sediment amended with AC (Millward et al., 2005; Sun and Ghosh, 2007). Jakob et al. (2012) also showed that the amendment of AC to a PAH contaminated soil reduced the uptake of PAH to earthworms. This is further discussed in section 9.4. Amendment with powdered AC (PAC) resulted in significant earthworm mass loss and the authors speculated that the PAC could have caused toxic effects (rather than the PAHs themselves). This observation was confirmed by a further study where PAC, biochar and ferric oxyhydroxide were amended to a soil and the effect on organisms was tested. *E. fetida* was observed to prefer biochar over the other amendments as well as the unamended non-polluted agricultural soil (Hale et al., 2013). PAC resulted in negative effects to the organisms. Denyes et al. (2016) assessed DDT bioavailability following biochar and AC amendment to a contaminated soil. The addition of 2.8% of Burt's biochar and blue leaf biochar showed an up to 49 % and significant reduction in DDT accumulation by *E. fetida* as well as there not being any harmful effects on the earthworms. The GAC, in contrast, resulted in the earthworms showing avoidance behaviour and mass loss, as well as no significant reduction in DDT accumulation.

5.3.5 Hypotheses

It was hypothesized prior to the pot trial that: 1) the earthworms would take up PCBs and there would be a difference in uptake between different PCB-congeners, 2) the presence of biochar in soil would reduce the uptake of PCBs in the earthworms, 3) there would be a dose effect; with earthworms taking up less PCBs when a higher dose of biochar is amended to the soil and 4) there would be a difference between the two biochars with regard to earthworm uptake of PCBs. The results from the trial generally supported the first and second hypothesis as there was a difference in the uptake between different PCBs and biochar reduced the uptake of PCBs by earthworms. The third hypothesis was falsified as there was no statistically significant effect of biochar dose. The fourth hypothesis was confirmed as there was a statistically significant effect of biochar type, with rice husk biochar giving a higher reduction of PCB concentrations in the worms.

6 Results plants

6.1 Visual observations

6.1.1 Ryegrass mass yield

Ryegrass seeds grew in all pots. The size of the individual blades of grass and total amount of grass was very variable. Above ground ryegrass samples (shoots and not roots) were weighed per pot and further processed for each pot. Figure 41 shows the smallest ryegrass yield to the left (0.10 g dry weight) in unamended comparison replicate pot 3 with spiked soil (SSR3) and the largest ryegrass yield to the right (1.0 g dry weight) in replicate pot 4 amended with 1% rice husk biochar (1RR4).

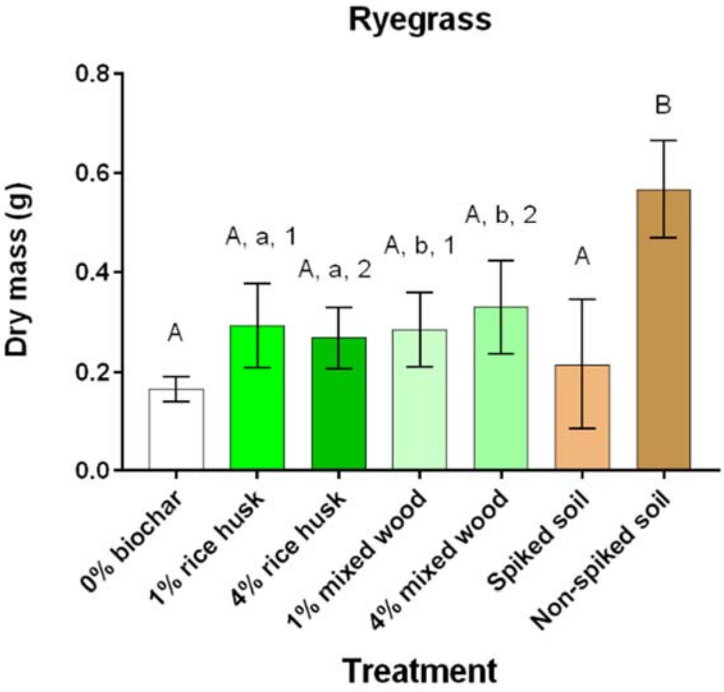
Figure 41 Ryegrass yield in two pots. Left: ryegrass in the unamended comparison pot with spiked soil and ryegrass, replicate number 3 (pot SSR3). Right: ryegrass in pot amended with 1% rice husk biochar, replicate number 4 (1RR4)

Table 8 and Figure 42 shows average dry mass of the above ground ryegrass for each treatment as well as the unamended comparisons. The largest masses were for the pot series with 4% mixed wood biochar (up to 0.33 ± 0.09 g). The smallest masses were recorded for the pot series with 0% biochar (0.17 ± 0.03 g). Although no statistically significant differences were observed for either biochar type or dose (p-values ranging from 0.0867 to >0.9999) it appears that ryegrass grows better in the biochar amended (both biochars and both doses) pots compared to 0% biochar pots. Previous studies have indicated that plant growth can be improved following the amendment of biochar to soils and this is discussed at the end of this section.

Table 8 The above ground mass (g) of the ryegrass shoots. The mass is the mean of the 5 replicates for the treatments (4 replicates for the unamended comparison) and the standard deviation

Treatment	Batch	Above ground mass ryegrass (g)
0% biochar	Control	0.17 ± 0.03
1% rice husk	Amended	0.29 ± 0.08
4% rice husk		0.27 ± 0.32
1% mixed wood		0.28 ± 0.07
4% mixed wood		0.33 ± 0.09
Spiked soil	Unamended comparison	0.22 ± 0.13
Non-spiked soil		0.57 ± 0.10

The mass of ryegrass shoots from the spiked soil unamended comparison pots was 62% lower than the mass of the shoots for the non-spiked soil (statistically significantly different with p-value <0.0001). This observation likely reflects the negative effect the PCBs themselves have on the ryegrass and is supported by previous work. For example, a decrease of 23% biomass yield has been reported for ryegrass shoots in soil spiked with PAHs compared to non-spiked soil (Alam et al., 2010).



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar) and comparison (spiked and non-spiked soil)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)

Figure 42 Above ground mass (g) of ryegrass from different biochar treatments. The mass is based on dry weight of the ryegrass and is calculated as an average for all replicates in the treatment

6.1.2 Turnip mass

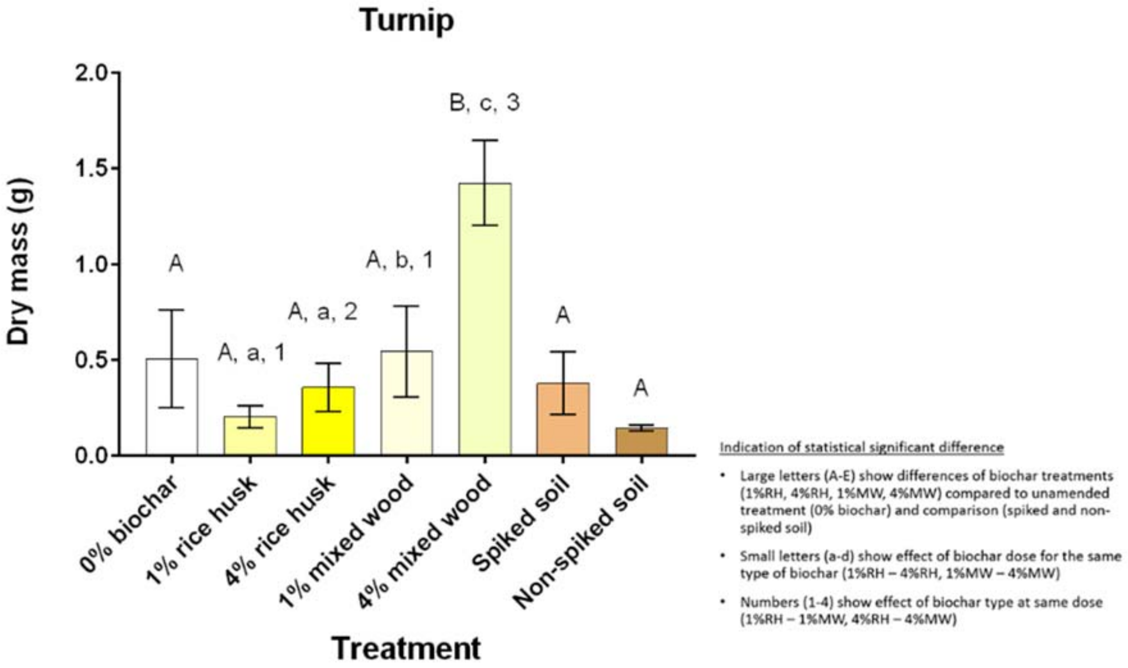
All three turnip seeds grew in 58% of the pots, while two of the seeds gave plants in 30% of the pots, two of the pots gave four turnips, and the final two pots grew either one or no turnips. All of the plant mass was used in further work and was considered on a per pot basis, not a per plant basis. Both the above and below ground mass was considered for turnips (except for the smaller roots with <1 mm in diameter), however the root system for the turnips was not extensive. The size of the individual turnips showed great variance. Figure 43 shows the smallest turnip sample yield to the left (0.05 g dry weight) in unamended comparison replicate pot 4 with non-spiked soil (NST4) and the largest plant yield to the right (1.69 g dry weight) from replicate pot number 1 amended with 4% mixed wood biochar (4MT1).

Figure 43 Turnip yield from two pots. Left: a single small turnip sampled from the unamended comparison pot with non-spiked soil and turnip, replicate number 4 (NST4). Right: three larger turnips sampled from pot amended with 4% mixed wood biochar replicate number 1 (4MT1)

Table 9 and Figure 44 show measured average above ground dry mass for each treatment as well as the unamended comparisons. The largest mass was recorded for the pot series with 4% mixed wood biochar (1.2 ± 0.6 g) and the smallest mass was for the pot series with 1% rice husk biochar (0.16 ± 0.05 g). Turnips in pots with mixed wood biochar had a higher yield compared to those from 0% biochar pots (statistically significant for 4% mixed wood biochar with p-value <0.0001 but not significant for 1% with p-value 0.9999) and compared to pots amended with rice husk biochar (statistically significant comparing the two 4% biochar treatments with p-value <0.0001 but not significant for the two 1% biochar treatments with p-value 0.2182). Brennan et al. (2014) reported that mass of maize root (dry weight) was unaffected by amendment with two different biochars and AC, but that maize shoot mass significantly increased for maize biochar and AC compared to controls.

Table 9 The mass (g) of the turnip shoots and roots. The mass is the mean of the 5 replicates for the treatments (4 replicates for the unamended comparison) and the standard deviation

Treatment	Batch	Mass turnip (g)
0% biochar	Control	0.50 ± 0.26
1% rice husk	Amended	0.16 ± 0.05
4% rice husk		0.29 ± 0.24
1% mixed wood		0.54 ± 0.24
4% mixed wood		1.2 ± 0.6
Spiked soil	Unamended comparison	0.38 ± 0.16
Non-spiked soil		0.12 ± 0.05



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar) and comparison (spiked and non-spiked soil)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)

Figure 44 Mass (g) of turnip from different biochar treatments. The mass is based on dry weight of the turnips and is calculated as an average for all replicates in the treatment

The mass of turnips from the unamended comparison pots was 32% higher for the spiked soil than for the non-spiked soil, (although not statistically significantly different with p-value 0.6675) in contrast to the findings for ryegrass. It appears that turnips are a species that are less sensitive to the presence of pollutants in soil. In chronic plant tests to look at how the presence of pyrene in soil affected turnip (*B. rapa*) biomass, turnips were grown in soils with 10000 mg/kg pyrene and in a non-polluted control. The yield of turnip was the same in both soils and the authors concluded that turnips were unaffected by high concentrations of pyrene (Kalsch et al., 2006). In another pot trial, the effects of hexachlorocyclohexane (HCH) on germination and growth of turnips was tested. Results showed that the turnip yield was not greatly affected by the presence of HCHs and the authors even suggested that the turnips were capable of mitigating the negative effects of HCH, displaying a certain degree of resistance, and making them ideal for phytoremediation (Pereira et al., 2010).

6.1.3 Hypotheses

Prior to the pot experiment it was hypothesized that; 1) all treatments would give plant growth, 2) the presence of biochar in soil would increase the mass of the plants, 3) there would be a dose effect; with higher plant mass when a higher dose of biochar was amended to the soil, 4) there would be a difference between the two biochar's with regard to plant mass and 5) in the unamended comparison pots the plant mass would be less for spiked soil than non-spiked soil.

The results from the trial supported the first hypothesis, and the second hypothesis was supported for ryegrass but not for turnips. The third hypothesis was falsified for the ryegrass but supported for the turnips as the 4% treatments seemed to give better plant yield than the 1% treatments. The fourth hypothesis was falsified for the ryegrass but supported for the turnips as mixed wood biochar seemed to give higher plant mass yield than the rice husk biochar (the six days shorter trial time for the turnips in the rice husk pots could be a contributing factor here). The fifth hypothesis was supported for the ryegrass as the mass of the ryegrass from non-spiked soil was significantly statistically higher than the plants mass from the spiked soil, but falsified for the turnips as plant mass from spiked soil was higher than the turnip plant mass from the non-spiked soil although not statistically significant.

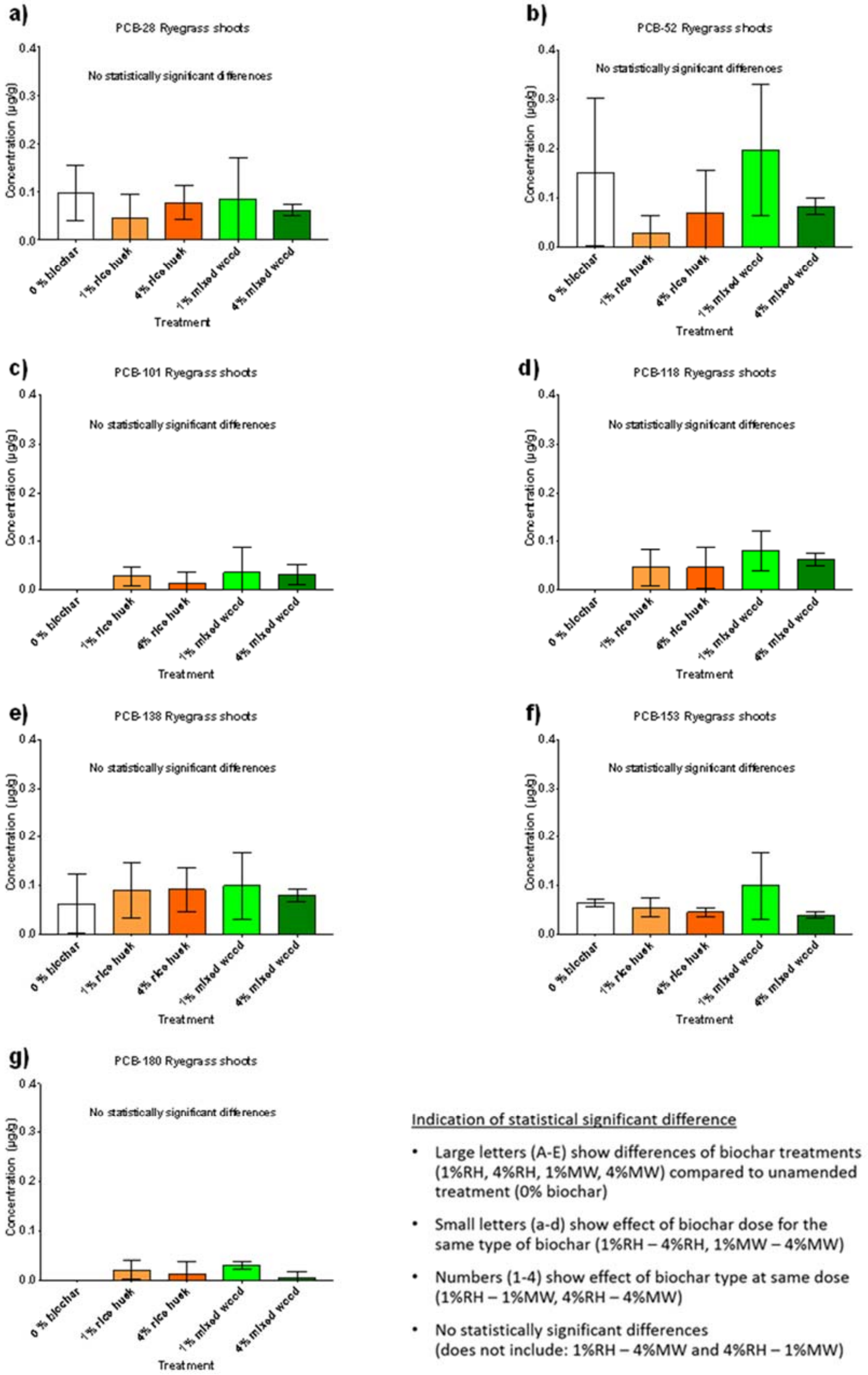
6.2 Uptake of pollutants by ryegrass

6.2.1 Absolute concentrations

Table 10 and Figure 45 show concentrations (µg/g) of PCBs in ryegrass in pots containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar. Table 10 also shows concentrations of PCBs in unamended comparison pots.

Table 10 Ryegrass shoots concentrations of PCB-congeners (28, 52, 101, 118, 138, 153, 180 and average for 7-PCBs) (µg/g) for pots with 0% biochar (control), 1% and 4% of either mixed wood or rice husk biochar as well as amended and unamended comparison pots with spiked and non-spiked soil

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	7-PCBs
0% biochar	Control	0.097 ± 0.057	0.151 ± 0.150	0 ± 0	0 ± 0	0.063 ± 0.060	0.043 ± 0.038	0 ± 0	0.051 ± 0.058
1% rice husk	Amended	0.045 ± 0.050	0.027 ± 0.037	0.029 ± 0.019	0.047 ± 0.037	0.091 ± 0.057	0.055 ± 0.019	0.021 ± 0.019	0.045 ± 0.024
4% rice husk		0.078 ± 0.035	0.069 ± 0.087	0.014 ± 0.023	0.046 ± 0.042	0.092 ± 0.045	0.046 ± 0.009	0.014 ± 0.024	0.051 ± 0.030
1% mixed wood		0.086 ± 0.085	0.197 ± 0.133	0.036 ± 0.051	0.081 ± 0.041	0.099 ± 0.067	0.099 ± 0.067	0.031 ± 0.008	0.090 ± 0.055
4% mixed wood		0.062 ± 0.012	0.082 ± 0.016	0.031 ± 0.021	0.063 ± 0.012	0.080 ± 0.013	0.040 ± 0.006	0.006 ± 0.012	0.052 ± 0.028
Spiked soil	Unamended comparison	0.144 ± 0.057	0.257 ± 0.224	0.018 ± 0.030	0.112 ± 0.053	0.142 ± 0.023	0.066 ± 0.012	0.009 ± 0.015	0.107 ± 0.086
Non-spiked soil		0.040 ± 0.019	0.035 ± 0.002	0.022 ± 0.019	0.057 ± 0.018	0.069 ± 0.028	0.041 ± 0.011	0 ± 0	0.038 ± 0.022



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 45 Ryegrass shoots concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

Concentrations of PCB congeners in ryegrass from pots containing 1 and 4% of either rice husk or mixed wood biochar showed no statistically significant difference (p-values ranging from 0.0766 to >0.9999) when compared to the pot series with 0% biochar. The average concentration for the sum PCB-7 was 0.051 ± 0.058 µg/g for the 0% biochar treatment, while concentrations ranged from 0.045 ± 0.024 µg/g to 0.090 ± 0.055 µg/g for the biochar treatments. One of the reasons why there were no statistically significant differences observed in this work could have been related to the fact that only the concentration of PCBs in the shoots of the ryegrass were quantified. Accumulation of PCBs occurs first in the ryegrass root system, before the PCBs move to the shoots. This represents an area for further investigation.

Although not significantly different, trends comparing the PCB congeners can be summarized as follows; concentrations of PCB 28, 138 and 153 in ryegrass for both biochar types and doses were similar to the 0% biochar treatment. For PCB-52, the concentrations in 0% biochar and 1% mixed wood biochar pots were similar, while for both doses of rice husk and 4% mixed wood biochar, concentrations were lower compared to 0% biochar treatment. For the 0% biochar treatment, PCB concentrations for congeners 101, 118 and 180, were below the analytical LOD (0.01 µg/g). Concentrations can be seen in appendix III. These results show that there was no overall difference in PCB uptake for the different biochar doses or types, except for PCB-52 where amendment with 4% biochar gave a lower concentration when compared to the 0% biochar pots.

6.2.2 Unamended comparison pots

For the spiked soil unamended comparison pots (Table 10), the highest uptake of PCB to the ryegrass was for PCB-52 (0.26 ± 0.22 µg/g) followed by PCB-28 (0.14 ± 0.06 µg/g) and the lowest was for PCB-180 (0.009 ± 0.015 µg/g). The following generalisation can be made for PCB uptake to ryegrass from spiked soil in the unamended comparison pots from highest to lowest uptake: PCB-52 > PCB-28 > PCB-138 > PCB-118 >PCB-153 > PCB-101 > PCB-180. There does not appear to be a trend based on the molecular size of the PCB, although PCB-180 was taken up the least and is the largest of the PCBs tested. There does not appear to be differences in uptake based on stereochemistry when comparing the planar PCBs (28 and 118) to the non-planar ones.

For the non-spiked soil unamended comparison pots, the sum PCB-7 concentration was 0.04 ± 0.02 µg/g. This slight contamination (as these pots were not spiked) most likely comes from a diffuse source, possibly as a result of volatilization of the PCBs from pots with spiked soil to the ryegrass shoots in the non-spiked pots. This observation has been reported before, where PCBs were detected in control plants grown in the vicinity of PCB contaminated plants. Both plants had the same chromatographic profile leading the authors to conclude that there was a vapour or particulate mediated transfer of PCBs (Ye et al., 1992).

6.2.3 Percentage reductions in PCB uptake to ryegrass shoots

Table 11 shows the reduction in uptake of PCBs by ryegrass following the amendment of biochar. In many cases, negative reductions (i.e. increases in uptake as the concentrations in the biochar amended pots were higher than in the pots with 0% biochar) were seen. This is most likely explained by limitations in the analytical method for the low concentrations of highly chlorinated PCBs which resulting in working very close to the LOD.

Table 11 Reductions (%) in ryegrass shoot PCB-concentrations for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
1% rice husk	Amended	53	82	-	-	-44	-28	-
4% rice husk		20	54	-	-	-46	-6	-
1% mixed wood		12	-30	-	-	-58	-129	-
4% mixed wood		36	46	-	-	-26	8	-

-indicates concentrations below the limit of detection for the analytical method for the 0% biochar treatment

However, there were some cases where the addition of biochar (both mixed wood and rice husk) reduced the uptake of PCBs by ryegrass. These were for PCB-28 for all treatments, for PCB-52 for all treatments except 1% mixed wood biochar and for PCB-153 for the 4 % mixed wood biochar. The highest reduction was 82% for PCB-52 in the 1% rice husk treatment.

6.3 Uptake of pollutants by turnips

6.3.1 Absolute concentrations

Table 12 and Figure 46 show concentrations (µg/g) of PCBs in turnips in the pots containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar. Table 12 also shows concentrations of PCBs in unamended comparison pots.

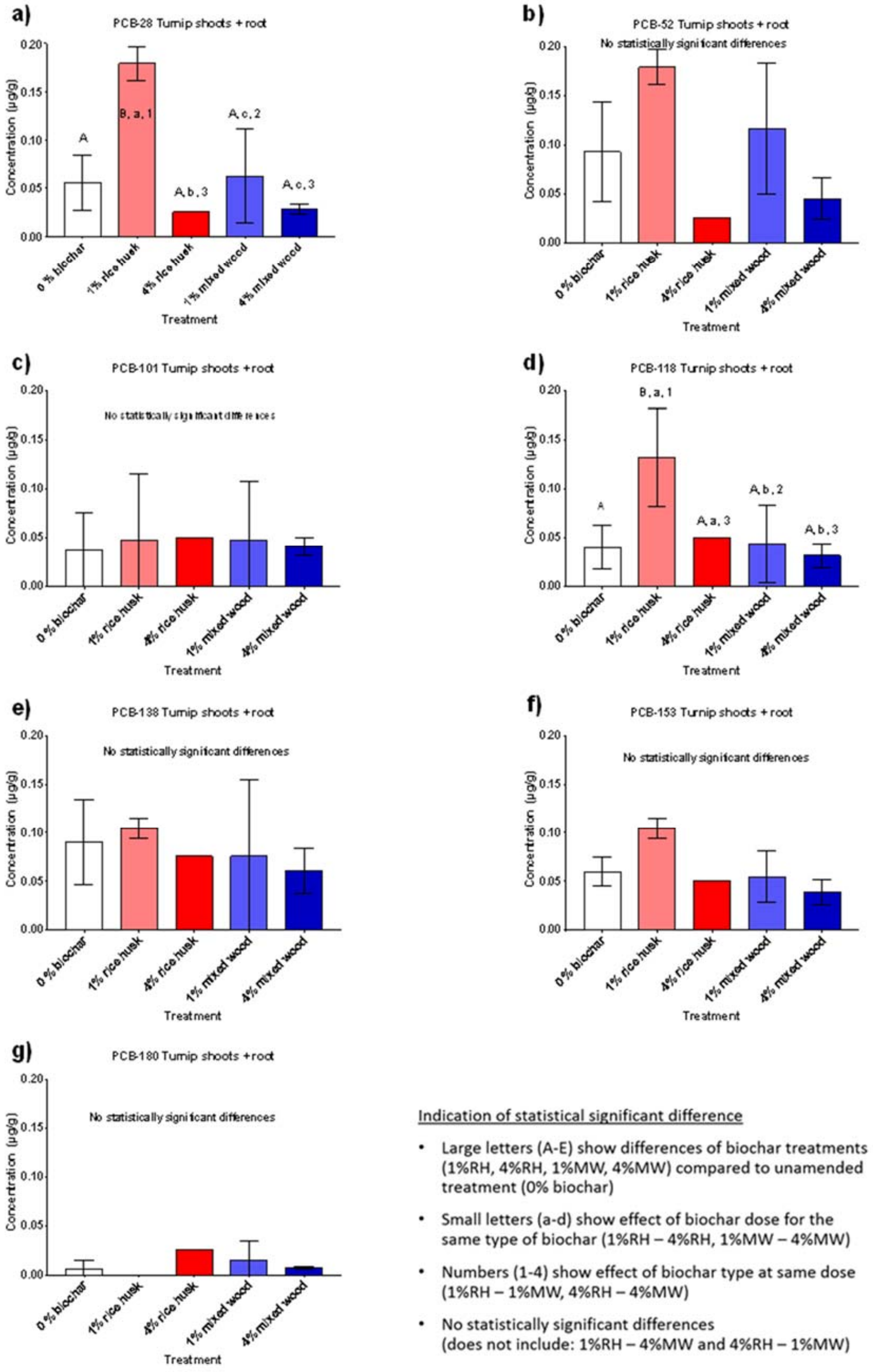
Concentrations of PCB congeners in turnips from pots containing 1 and 4% of both rice husk and mixed wood biochar did not show statistically significant differences for PCB-congeners 52, 101, 138, 153 and 180 (p-values ranging from 0.0901 to >0.9999) when compared to the pot series with 0% biochar. For PCB congeners 28 and 118 the 1% rice husk treatment gave significantly higher concentrations (p-values 0.0078 and 0.0343 respectively) than the 0% biochar treatment.

Table 12 Turnip shoots and root concentrations of 7 PCBs (µg/g) for pots with 0% biochar (control), 1% and 4% of either mixed wood or rice husk biochar as well as amended and unamended comparison pots with spiked and non-spiked soil

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	7-PCBs
0% biochar	Control	0.056 ± 0.029	0.093 ± 0.051	0.038 ± 0.039	0.041 ± 0.023	0.090 ± 0.044	0.060 ± 0.015	0.006 ± 0.009	0.055 ± 0.031
1% rice husk	Amended	0.179 ± 0.018	0.179 ± 0.018	0.048 ± 0.068	0.133 ± 0.050	0.105 ± 0.011	0.105 ± 0.011	0 ± 0	0.107 ± 0.066
4% rice husk*		0.025	0.025	0.050	0.051	0.076	0.051	0.025	0.043 ± 0.019
1% mixed wood		0.063 ± 0.049	0.116 ± 0.067	0.048 ± 0.059	0.043 ± 0.040	0.076 ± 0.079	0.055 ± 0.026	0.015 ± 0.020	0.060 ± 0.031
4% mixed wood		0.028 ± 0.005	0.044 ± 0.021	0.041 ± 0.009	0.032 ± 0.012	0.061 ± 0.023	0.039 ± 0.012	0.007 ± 0.001	0.036 ± 0.016
Spiked soil	Unamended comparison	0.069 ± 0.009	0.069 ± 0.009	0.031 ± 0.044	0.057 ± 0.008	0.113 ± 0.017	0.057 ± 0.008	0.031 ± 0.044	0.061 ± 0.028
Non-spiked soil		0.075	0.075	0 ± 0	0.038 ± 0.054	0.038 ± 0.054	0.038 ± 0.054	0 ± 0	0.038 ± 0.031

*The concentration is based on the result from only one sample

The addition of biochar (mixed wood and rice husk) did not reduce the uptake of PCBs to turnips, and actually appeared to promote it in some cases as concentrations were higher for the biochar amended pots. The average concentration for the sum PCB-7 was 0.055 ± 0.031 µg/g for the 0% biochar treatment, while average concentrations ranged from 0.036 ± 0.016 µg/g to 0.107 ±0.066 µg/g for the biochar treatment pots. To the best of our knowledge, this is the first time a turnip-biochar trial has reported an increase in pollutant uptake following biochar amendment. This suggests the need for caution if turnips are to be grown with the intention of eating in a PCB polluted soil that has been amended with biochar. Khan et al. (2015) reported that the addition of either 2% or 5% of four different biochar all significantly reduced PAH concentrations in turnip roots compared to 0% biochar, with the 5% amendment being the most effective. In this masters thesis, turnip plants were allowed to grow for just 30 days, and as can be seen from Figure 43 this was not sufficient for a mature turnip root to develop. In contrast, Khan et al. (2015) let the turnips reach maturity (8 weeks) and this could be a reason for the difference in their results compared to those obtained here. In a further study with turnips grown in a PAH polluted soil, PAHs were detected in the roots of turnips. In contrast to this study, the shoots were not analyzed (Ashraf and Salam, 2012). It seems to be apparent that the roots of the turnips also play a part in taking up pollutants.



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 46 Turnip shoots and root concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

6.3.2 Unamended comparison pots

For the spiked soil unamended comparison pots (Table 12) the highest uptake of PCB to the turnips was for PCB-138 (0.11 ± 0.02 µg/g) and the lowest was for PCB-101 and PCB-180 (both concentrations 0.03 ± 0.04 µg/g). For the non-spiked soil unamended comparison pots the sum PCB-7 concentration was 0.04 ± 0.03 µg/g. PCB-uptake by plants has been theoretically estimated, based on information on chemical properties (Log K_{OW}) and plant physiology (Ryan et al., 1988). These authors demonstrated that PCBs would not be expected to be present in significant amounts in above-ground plant tissue, unless they are deposited from the air onto the plant surface. Indeed they suggested that only low chlorinated compounds would be taken up inside plant tissues, as PCB water solubility decreases with the degree of chlorination (Ryan et al., 1988).

The following generalisation can be made for PCB uptake to turnips from spiked soil in the unamended comparison pots from highest to lowest uptake: PCB-138 > PCB-52 = PCB-28 > PCB-118 = PCB-153 > PCB-101 = PCB-180. There does not appear to be a trend based on the molecular size of the PCB, although PCB-180 was taken up the least and is the largest of the PCBs tested. There does not appear to be differences in uptake based on stereochemistry when comparing the planar PCBs (28 and 118) to the non-planar ones.

6.3.3 Percentage reduction in PCB uptake by turnips

Table 13 shows the reduction of PCB uptake following the addition of biochar (mixed wood and rice husk). Results are variable, and in over half of the treatment variables, negative reductions (i.e. increases) are seen. As for ryegrass, working close to the LOD may be the explanation for this. There are very few consistent trends in the data, however for PCBs 138 and 153 all treatments except the 1% rice husk biochar reduced PCB uptake compared to the 0% biochar treatment. The highest reduction was 73% for PCB-52 in the 4% rice husk treatment.

Table 13 Reductions (%) in turnip shoots and root PCB-concentrations for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
1% rice husk	Amended	-222	-93	-27	-227	-16	-75	*
4% rice husk		55	73	-33	-25	16	15	-320
1% mixed wood		-13	-26	-27	-7	16	8	-150
4% mixed wood		49	52	-8	21	33	34	-21

*no detection of the compound over the LOD for the analytical method

6.4 Comparison with previous literature studies of plants

There are variable effects of the presence of pollutants on the behaviour of brassica. Rape plant (*Brassica napus* L.) roots contain lipids in their tissues and these lipids have been reported to enhance the uptake potential of chlorinated compounds (Kipopoulou et al., 1999). In a study looking at the degradation of PCBs in the rhizosphere of rape (*Brassica napus* L.), neither the rape root or shoot mass was affected by the presence of PCBs, and the plants did not show stress-related symptoms caused by the presence of pollutants (Javorská et al., 2009). Analysis of rape (*Brassica napus* L.) that was grown at a PCB-polluted site showed no transfer of pollutants to the biomass (Smith et al., 2013). However, other studies have reported that the presence of PCBs in sludge and soil can give uptake and translocation to cabbage (*Brassica oleracea* L.) (Webber et al., 1994). Ye et al. (1992) reported measurable concentrations of PCBs in all parts of tomato plants grown in contaminated soil. Concentrations were found to be highest in the leaves and lowest in the fruits. The prominent PCB-congeners found in plant tissue were 31, 52, 103, 101, 87, 138 and 153. Similar results were found for barley plants, where concentrations in stems were found to be one order of magnitude lower than in the leaves. Studies on the effect of PCB on turnips (*Brassica rapa*) were not found.

The increase in plant yield following the amendment of biochar has been observed in previous pot and field experiments (especially in degraded tropical soils), supporting the observations here. When biochar is added to soil it can improve soil properties and thus result in an increase in crop yield (Jeffery et al., 2011; Carter et al., 2013; Cornelissen et al., 2013; Khan et al., 2015). The effect of rice husk biochar (25, 50 and 150 g/kg) was tested in a pot experiment using a Cambodian acrisol and the effect on lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*) growth was determined. Biochar treatments were found to increase biomass in for both plants (Carter et al., 2013). Cornelissen et al. (2013) found that amending sandy, acidic soils with biochar improved physical and chemical soil characteristics (pH, nutrients and CEC increased), which contributed to an increased crop yield for maize. Jeffery et al. (2011) report in a meta-analysis that biochar can improve crop yield and suggested that the main mechanisms that explain this are the liming effects of biochar, improved water holding capacity (WHC) of the soil following amendment and improved crop nutrient availability following amendment. Biochar itself is alkaline (the pH of the biochars used here were 7.3 for the rice husk biochar and 8.3 for the mixed wood) and when added to acidic soil it can neutralize the acidity. Although this soil was not acidic, a liming effect may still have occurred which in turn could have made conditions more conducive for plant growth. In a pot trial ryegrass (*Lolium perenne* L.) showed no significant increase in biomass following the amendment of 10% or 30% wheat straw biochar (produced in a kiln) to a sandy loam soil. However, the biochar did significantly increase soil water content (O'Toole et al., 2013).

A three year field experiment investigating the effects of biochar amendment (debarked spruce chips and pine biochar, with 0, 5 and 10 t/ha amendment applications) to a fertile sandy clay loam (50% sand, 26% silt and 24% clay) in boreal conditions in Finland showed no significant difference in biomass yield for turnips (*Brassica rapa*) when compared to unamended control. However, the number of seeds per plant increased during a dry season, possibly due to a positive effect of biochar on the otherwise water deficient soil (Tammeorg et al., 2014). Jakob et al. (2012) reported a negative effect on plant growth for ryegrass (*Lolium perenne*), carrots (*Daucus carota*) and squash (*Cucurbita pepo* ssp. *pepo* convar. *giromontiina*) following 2% PAC amendment, while 2% GAC increased the rate of growth for the plants.

Brennan et al. (2014) reported that the amendment of two different biochars and one AC did not affect the uptake of PAHs by maize roots as compared to controls. However, shoot uptake of PAHs was significantly reduced by the amendment of maize biochar and AC for all PAHs, but not by pine wood biochar. The authors attribute this difference to the differences in the biochars which affect soil properties (electrical conductivity, cation exchange capacity, soluble nitrogen, phosphorous and potassium (NPK), bulk densities, particle size distributions and oxygen-content) and that this in turn could have affected PAH uptake.

In a greenhouse trial Langlois et al. (2011) showed reductions in PCB concentrations in pumpkin shoots and roots (*Cucurbita pepo ssp. Pepo*) following the amendment of 0.2, 0.8, 3.1, and 12.5% AC amendment to an industrially contaminated soil. In general, a larger AC dose resulted in lower PCB concentrations in the plant tissues. Reported reductions were between 89 and 97% for the roots and between 17 and 63% in the shoots of the plants. Denyes et al. (2012) showed in a 50 day greenhouse trial using the pumpkin (*Cucurbita pepo ssp. Pepo*) that the addition of 2.8 wt % wood waste biochar reduced PCB root concentrations between 58 and 77 % and the addition of 11.1% of biochar reduced PCB concentrations between 83 and 89 %. In a similar experiment, but carried out in the field, Denyes et al. (2013) reported 74%, 72% and 64% reductions in PCB root concentrations in pumpkin (*Cucurbita pepo ssp. Pepo*) following the amendment of 2.8% granulated activated carbon (GAC), Burt's biochar and blue leaf biochar respectively. In a greenhouse experiment the plant species pakchoi (*Brassica chinensis* L.) and carrots (*Daucus carota*) showed reduced uptake of PCBs following the amendment of 2% wheat straw and pine needle biochars. Root concentrations were reduced by 61 to 94 % and 18 to 62 % for pakchoi and carrots respectively (Wang et al., 2013b).

A pot experiment was conducted to evaluate the effect of biochars (2% of bamboo and rice straw biochars) on the bioavailability of the HOC di-(2-ethylhexyl) phthalate (DEHP) on pakchoi (*Brassica chinensis* L.). Two soils were tested with variable organic carbon contents (OC, 2.2 and 0.35 %). DEHP concentrations in plant shoots grown in the soil with high OC were lower than those grown in low OC soil. Compared to the control, the biochar amendment decreased the DEHP concentrations in shoots grown in the low OC soil, whereas there were no significant difference in the high OC soil. This confirms the importance of measuring and understanding the soil properties before amendments are carried out (He et al., 2016).

6.5 Hypotheses for ryegrass and turnips

Prior to the pot trial it was hypothesized that 1) the plants would take up PCBs and that there would be a difference in uptake between different PCB-congeners, 2) the presence of biochar in soil would reduce the uptake of PCBs in the plants, 3) there would be a dose effect; with plants taking up less PCBs when a higher dose of biochar is amended to the soil, 4) there would be a difference between the two biochars in regard to plant uptake of PCBs.

The results from the trial generally supported the first hypothesis as PCBs, although low concentrations, were detected in the plants and there were differences for different PCBs. The second hypotheses was not supported, but the presence of biochar seemed to reduce the uptake of some PCB congeners for ryegrass. The third hypothesis was falsified as there was no statistically significant effect of biochar dose. The fourth hypothesis was falsified with biochar type generally not having an effect, except for rice husk biochar being more effective (lower PCB concentrations) for some PCB congeners in the ryegrass samples.

7 Results PE passive samplers

7.1 Visual observations

When the PE passive samplers were removed from the pots they were observed to have one or more tiny holes or tares, through which the roots of the ryegrass were growing (Figure 47). In 14 of the 58 pots with a PE passive sampler, a small fraction (up to around 10 %) of the total surface area of the PE passive sampler was above the soil surface at the end of the pot trial. This was likely due to the fact that the soil surface sank as the pots were repeatedly irrigated. This may have introduced some bias to the results (Figure 48).

Figure 47 Left: Tiny holes on a PE passive sampler from the unamended comparison pot with spiked soil and PE passive sampler, replicate number 1 (pot SSPE1). Right: Ryegrass roots piercing through a PE passive sampler in the pot with ryegrass amended with 4% rice husk biochar, replicate number 1 (pot 4RR1)

Figure 48 Left: PE passive sampler above the soil surface in the unamended comparison pot with spiked soil and PE passive sampler, replicate number 3 (pot SSPE3). Right: PE passive sampler from the pot with turnip, amended with 4% mixed wood biochar, replicate number 5 (pot 4MT5)

7.2 Uptake of pollutants by PE in pots containing ryegrass

7.2.1 Absolute concentrations

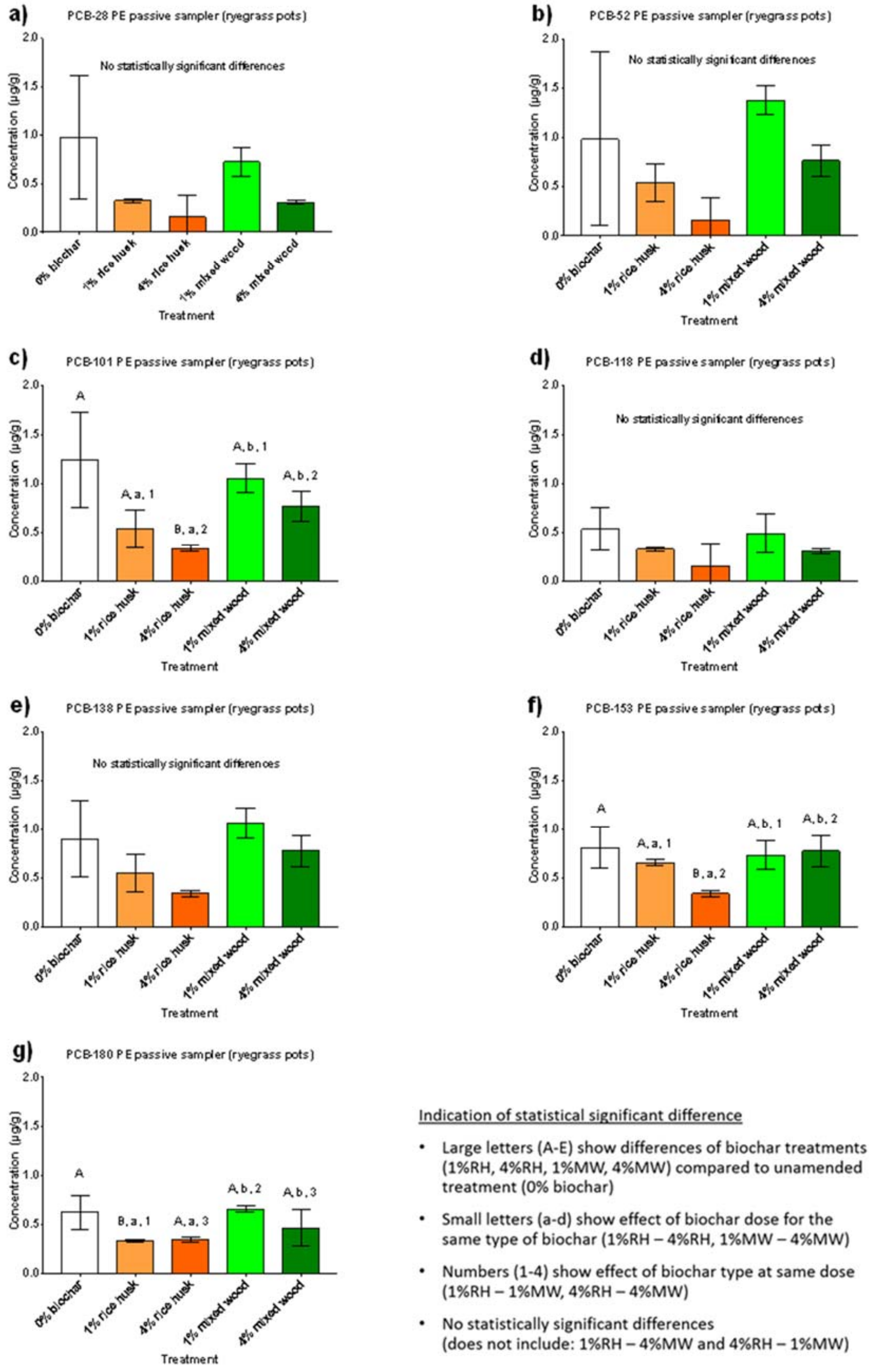
Table 14 and Figure 49 show concentrations (µg/g PE) of PCBs in the PE passive samplers from ryegrass pots containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar. Table 14 also shows concentrations of PCBs in unamended comparison pots.

Table 14 PE passive sampler concentrations of PCB-congeners (28, 52, 101, 118, 138, 153, 180 and average for 7-PCBs) (µg/g) for pots with 0% biochar (control), 1% and 4% of either mixed wood or rice husk biochar as well as amended and unamended comparison pots with spiked and non-spiked soil

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	7-PCBs
0% biochar	Control (ryegrass pots)	0.98 ± 0.64	0.99 ± 0.88	1.24 ± 0.48	0.54 ± 0.21	0.90 ± 0.39	0.81 ± 0.21	0.63 ± 0.17	0.87 ± 0.24
1% rice husk	Amended (ryegrass pots)	0.33 ± 0.02	0.54 ± 1.19	0.54 ± 0.19	0.33 ± 0.02	0.55 ± 0.19	0.66 ± 0.03	0.33 ± 0.02	0.47 ± 0.14
4% rice husk		0.16 ± 0.23	0.16 ± 0.23	0.34 ± 0.03	0.16 ± 0.23	0.35 ± 0.03	0.35 ± 0.03	0.35 ± 0.03	0.27 ± 0.10
1% mixed wood		0.73 ± 0.15	0.38 ± 0.15	1.05 ± 0.15	0.50 ± 0.20	1.07 ± 0.15	0.74 ± 0.15	0.66 ± 0.03	0.87 ± 0.30
4% mixed wood		0.31 ± 0.02	0.77 ± 0.16	0.77 ± 0.16	0.32 ± 0.02	0.78 ± 0.16	0.78 ± 0.16	0.46 ± 0.19	0.60 ± 0.23
0% biochar*	Control (turnip pots)	1.25	1.56	1.25	0.63	0.95	0.63	0.32	0.94 ± 0.44
1% rice husk	Amended (turnip pots)	0.22 ± 0.19	0.22 ± 0.39	0.54 ± 0.22	0.32 ± 0.03	0.55 ± 0.22	0.44 ± 0.21	0.32 ± 0.03	0.37 ± 0.14
4% rice husk		0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.26 ± 0.17	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.03
1% mixed wood*		1.02	1.36	1.36	0.69	1.04	0.69	0.35	0.93 ± 0.38
4% mixed wood		0.33 ± 0.31	0.56 ± 0.49	0.88 ± 0.51	0.33 ± 0.03	0.66 ± 0.33	0.77 ± 0.17	0.44 ± 0.17	0.57 ± 0.22
Spiked soil	Unamended comparison	0.58 ± 0.67	0.72 ± 0.84	0.85 ± 0.58	0.43 ± 0.29	0.72 ± 0.44	0.86 ± 0.49	0.57 ± 0.33	0.68 ± 0.16
Non-spiked soil		0	0	0	0	0	0	0	0 ± 0

*The concentration is based on the result from only one sample

For PCB congeners 101 and 153 the amendment of 4% rice husk biochar significantly reduced concentrations (p-values 0.0333 and 0.0331 respectively) in the PE compared to the 0% pots, and for PCB-180 the 1% rice husk treatment showed significantly lower concentrations (p-values 0.0407) than the 0 % biochar pots.



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 49 PE passive sampler concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in ryegrass-pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

The concentrations of PCBs in the PE from the amended pots showed no statistically significant difference for PCBs 28, 52, 118 and 138 (p-values ranging from 0.1513 to 0.9961) when compared to the unamended pots. However, in general the concentrations for these PCBs were lower for pots with 1% and 4% rice husk biochar, than the control and mixed wood biochar pots.

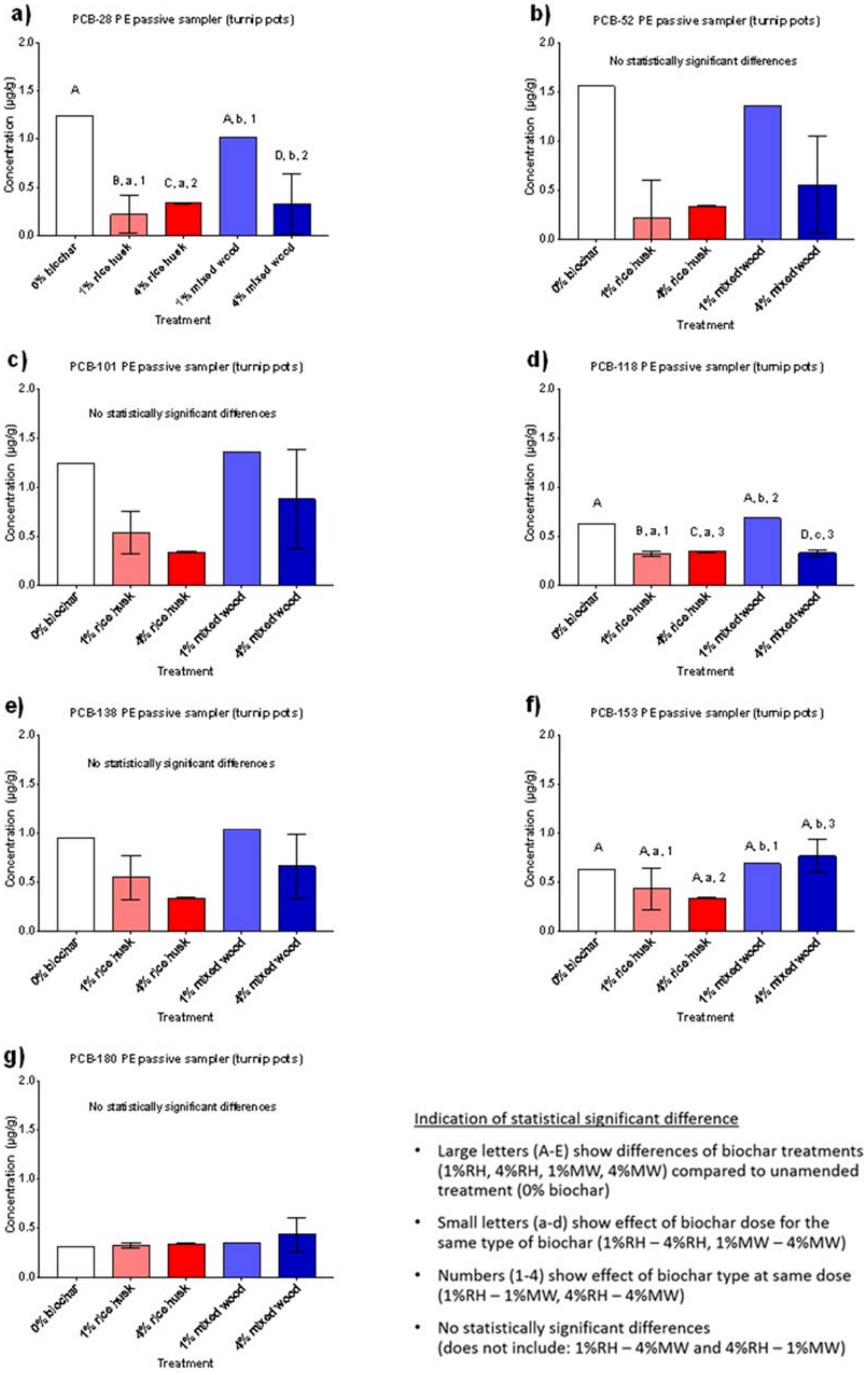
The average concentration for the sum PCB-7 was 0.87 ± 0.24 µg/g for the 0% biochar treatment while concentrations for the 1% and 4% rice husk treatments were 0.47 ± 0.14 µg/g and 0.27 ±0.10 µg/g respectively. For mixed wood biochar, the concentrations were similar to the 0% biochar treatment with sum PCB-7 concentrations 0.87 ± 0.30 µg/g and 0.60 ±0.23 µg/g for 1% and 4% mixed wood biochar respectively.

7.3 Uptake of pollutants by PE in pots containing turnips

7.3.1 Absolute concentrations

Table 14 and Figure 50 show concentrations (µg/g) of PCBs in PE passive samplers from turnip-pot series containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar. Table 14 also shows concentrations of PCBs in unamended comparison pots.

There was no statistically significant difference in the absolute PCB concentrations taken up by PE passive samplers from pots containing 1 and 4% of either rice husk or mixed wood biochar for PCB-congeners 52, 101, 138, 153 and 180 (p-values ranging from 0.074 to >0.9999) when compared to the pot series with 0% biochar. For PCB congeners 28 and 118, both the 1% and 4% rice husk as well as 4% mixed wood treatment showed significantly lower concentrations (p-values ranging from 0.0003 and 0.0286) than the 0% biochar treatment. The average concentration in the PE passive samplers for the sum PCB-7 was 0.94 ± 0.44 µg/g for the 0% biochar treatment while concentrations in the 1% and 4% rice husk treatments were 0.37 ± 0.14 µg/g and 0.33 ±0.03 µg/g respectively. For the 1% and 4% mixed wood biochar treatments, concentrations were 0.93 ± 0.38 µg/g and 0.57 ±0.22 µg/g.



Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 50 PE passive sampler concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in turnip-pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

7.4 Unamended comparison pots

The PE passive samplers from the spiked soil unamended comparison pots (Table 14) showed the largest concentration for a single PCB congener for PCB-153 (0.86 ± 0.5 µg/g) followed by PCB-101 (0.85 ± 0.6 µg/g) and the lowest was for PCB-118 (0.43 ± 0.3 µg/g). The following generalisation can be made for highest to lowest concentrations: PCB-153> PCB-101 > PCB-52 = PCB-138 > PCB-28 > PCB-180 > PCB-118. There does not appear to be a consistent trend based on the molecular size of the PCBs, although PCB 180 was taken up to a lower degree and is the largest of the congeners tested here. However there appears to be a trend related to planarity of the PCBs, where the planar PCBs (28 and 118) are taken up to a smaller extent than the non-planar PCBs. For the non-spiked soil unamended comparison pots none of the PCBs congeners were detected above the analytical LOD.

7.5 Percentage reduction in PCB uptake by PE passive samplers

Table 15 shows the percentage reduction in PCB uptake by PE passive samplers for all pots. The addition of rice husk biochar reduced the uptake of all PCB congeners to the PE passive samplers for both plant pots with the exception of PCB-180 in the turnip pots. The highest reduction was 86% for PCB-52 for the 1% rice husk treatment in the turnip pots. The addition of mixed wood biochar showed more variable responses, with some decreases in uptake of PCBs, and some increases of uptake of PCBs (indicated by the negative reductions). A similar explanation as for the plants can be used here for the increase in uptake. In general the decrease in uptake is greatest for the smaller PCBs concurrent with their lower water solubility.

Table 15 Reductions (%) in PE passive sampler PCB-concentrations for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
1% rice husk	Amended (ryegrass pots)	67	45	56	39	39	19	47
4% rice husk		84	84	73	70	62	57	45
1% mixed wood		26	-40	15	8	-18	9	-5
4% mixed wood		68	22	38	42	14	4	26
1% rice husk	Amended (turnip pots)	82	86	56	49	42	31	-3
4% rice husk		73	78	73	59	64	46	-8
1% mixed wood		18	12	-9	-9	-9	-9	-9
4% mixed wood		74	64	29	48	30	-22	-38

7.6 Comparison with previous literature studies

There are several previous studies that have used passive samplers to look at pollutant uptake following the amendment of biochar (and AC) to soils (Gomez-Eyles et al., 2011b; Oen et al., 2011a; Paul and Ghosh, 2011; Brennan et al., 2014; Denyes et al., 2016; Bielska et al., 2017). Oen et al. (2011) showed that AC amendment after 12-36 months of field aging reduced HOC pore water concentrations which were measured using POM passive samplers compared to unamended soil. In a study conducted by Bielska et al. (2017), the bioavailability of *p*-*p'*-DDE measured using PE passive samplers decreased up to 82% with increased biochar dose (0%, 1%, 5% and 10%).

However, unlike in this work, where all phases were added to the pots at the same time, previous studies have investigated the uptake of various HOCs to passive samplers, plants and/or earthworms in separate systems. Denyes et al. (2016) studied the effects of two types of biochar (and AC) amendment on DDT bioavailability in soil using POM passive samplers, with earthworms (*Eisenia fetida*) and squash (*Cucurbita pepo*) as endpoint measures. The results showed that POM passive samplers predicted the reduction in DDT accumulation following amendment for the earthworms, but not for the plants. Paul & Ghosh (2011) found that application of AC to PCB-polluted soil in pots greatly reduced PCB uptake to earthworms and PCB concentrations in POM passive samplers. Gomez-Eyles (2011a) assessed PAH bioavailability in field contaminated soils using POM passive samplers and then compared the results to actual PAH bioaccumulation in earthworms (*Eisenia fetida*) (14 days of pot trial) and ryegrass (*Lolium Multiflorum*) roots (4 weeks in a greenhouse). There was a good correlation between passive samplers and earthworms showing the potential of passive samplers to predict PAH bioaccumulation in earthworms and plants.

7.7 Hypotheses

Prior to the pot trial, it was hypothesized that 1) the PE passive samplers would take up PCBs and that there would be a difference in uptake between different PCB-congeners, 2) the presence of biochar in soil would reduce the uptake of PCBs in the PE passive samplers, 3) there would be a dose effect; with PE passive samplers taking up less PCBs when a higher dose of biochar is amended to the soil and 4) there would be a difference between the two biochars with regard to PE passive sampler uptake of PCBs. The results from the trial generally supported the first hypothesis as the PE passive samplers took up the PCBs and there was a difference between different PCB congeners. The second hypothesis was confirmed as the presence of biochar seemed to reduce the uptake of PCBs to PE passive samplers, although only statistically significant for rice husk biochar for some PCB-congeners. The third hypothesis was falsified as there was no statistically significant effect of biochar dose. The fourth hypothesis was to certain degree confirmed with rice husk biochar having a better amendment effect, although it was not statistically significant.

8 Results soil-biochar system

8.1 Visual observations

There was a clear difference in colour between the 0%, 1% and 4% biochar treatment, with the colour becoming progressively darker as more biochar was added. The colour differences were clearer at potting than as the trial progressed and as the trial progresses, biochar particles appeared to be more visible on the soil surface (Figure 51, Figure 52, Figure 53, Figure 54 and Figure 55). All pots were irrigated in the same way and the pots without biochar did not seem to hold water as well and the soil seemed more densely packed and cracked at the soil surface as compared to the biochar amended pots. During soil sampling, the soil without biochar seemed wetter and more aggregated than the soil that was amended with biochar (Figure 56). Studies suggest that biochar amended to sandy loam soils increases the soils WHC (Case et al., 2012; Obia et al., 2016) and this could have been the case for this agricultural loamy soil.

Figure 51 Pot with 0% biochar and ryegrass. Left: at start of trial. Right: three weeks into the trial, before irrigation

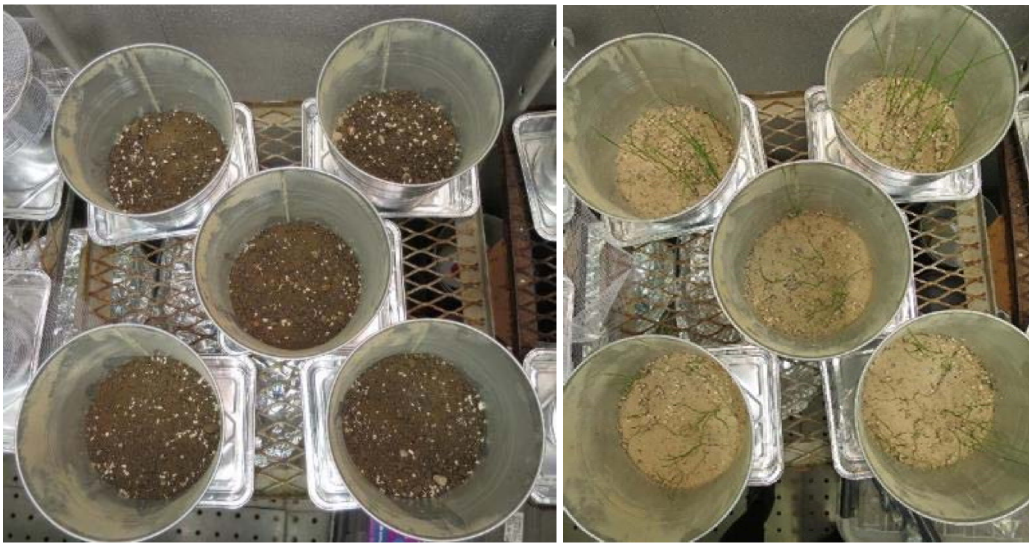


Figure 52 Pots with 1% mixed wood biochar. Left: at start of trial. Right: three weeks into the trial before irrigation

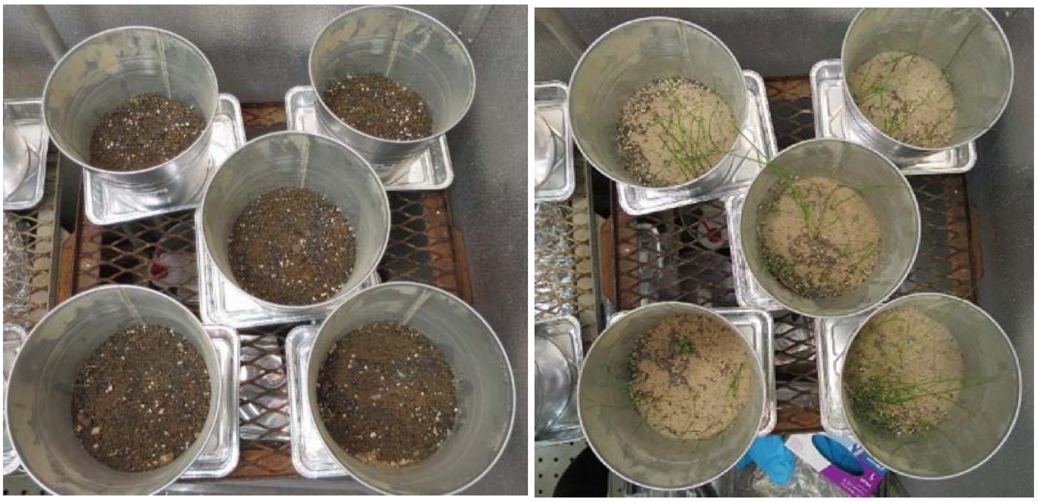


Figure 53 Pots with 4% mixed wood biochar. Left: at start of trial. Right: three weeks into the trial before irrigation

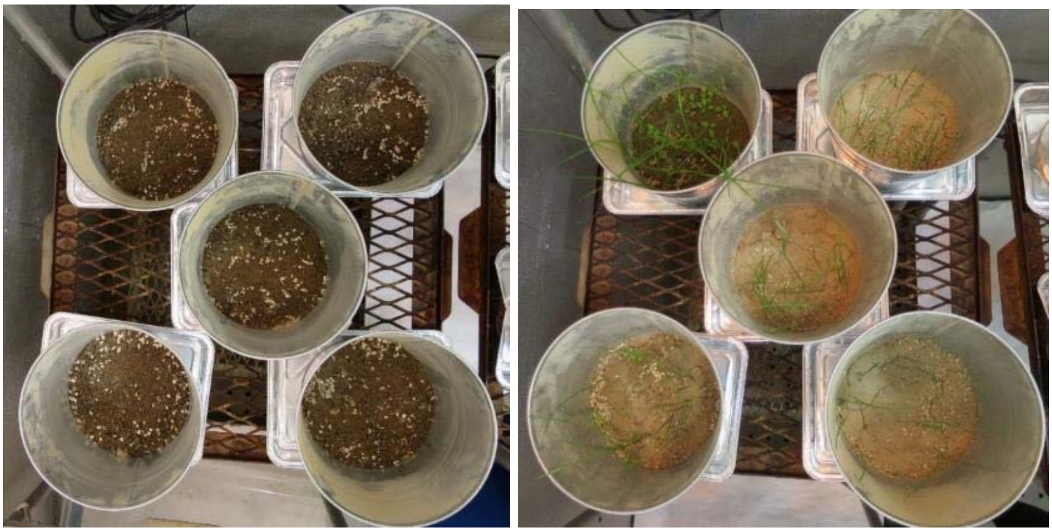


Figure 54 Pots with 1% rice husk biochar. Left: at start of trial. Right: three weeks into the trial before irrigation

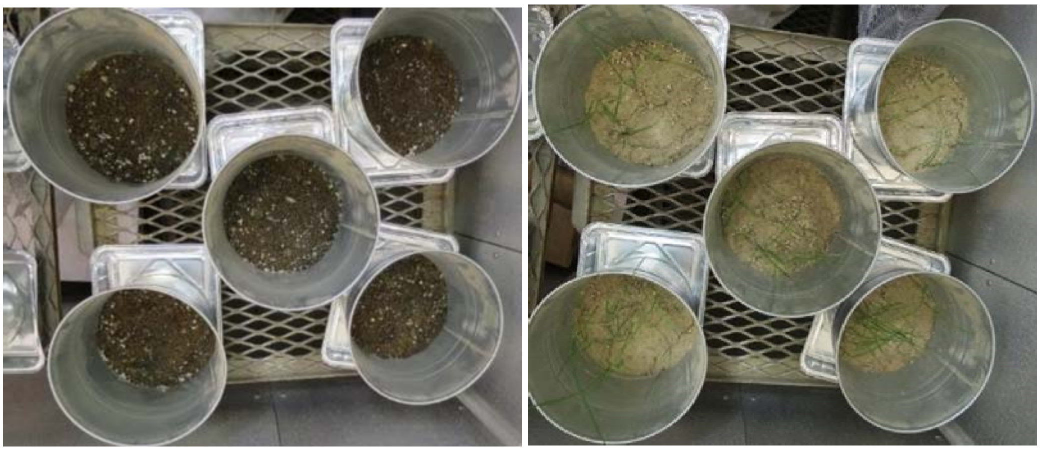


Figure 55 Pots with 4% rice husk biochar. Left: at start of trial. Right: three weeks into the trial before irrigation

Figure 56 Soil when sampled. Left: Soil from pot with 0% biochar. Right: soil from pot with 4% rice husk biochar

8.2 Soil physiochemical properties

The soil used in the experiment is classified as a loam, with 40% wt% sand, 44 wt% silt and 17 wt% clay. The measured water holding capacity (WHC) was 18 %. The soil total organic carbon (TOC) content was 1.4 % wt, and the pH 6.5 (Hale et al., 2013).

8.3 Biochar physiochemical properties

The pyrolysis time for rice husk biochar was 3.5 hours at 300 °C, measured total carbon content, C was 41%, total nitrogen content, N was 1%, surface area, SA (measured using N_2) was 51 m^2/g and the pH determined in water was 7.3 (Martinsen et al., 2015). For the mixed wood biochar, the pyrolysis time was 20 minutes at 700 °C, measured total carbon content, C was 53%, total nitrogen content, N was 0.27%, the surface area, SA (N_2) was 404 m^2/g and pH was 8.3 (Kupryianchyk et al., 2016). Although both biochars were sieved to 2 mm grain size, visually the rice husk biochar had a larger portion of "dusty" smaller particles than the mixed wood biochar.

8.4 Concentration of pollutants in soil-biochar system

8.4.1 Absolute concentrations

Table 16 shows concentrations (µg/g) of PCBs in soil/soil-biochar systems from ryegrass and turnip pot series containing 0% biochar as well as 1 and 4% of both rice husk and mixed wood biochar. The table also shows concentrations of PCBs in the unamended comparison pots.

Table 16 Soil concentrations of 7 PCB-congeners (µg/g) for pots with 0% biochar (control), 1% and 4% of either mixed wood or rice husk biochar as well as amended and unamended comparison pots with spiked and non-spiked soil

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	∑ 7-PCBs
0% biochar	Control (ryegrass + turnip pots)	0.064 ± 0.058	0.083 ± 0.081	0.074 ± 0.060	0.049 ± 0.033	0.082 ± 0.059	0.061 ± 0.045	0.045 ± 0.028	0.065 ± 0.015
1% rice husk	Amended (ryegrass pots)	0.053 ± 0.074	0.063 ± 0.074	0.068 ± 0.096	0.046 ± 0.065	0.087 ± 0.116	0.069 ± 0.069	0.077 ± 0.065	0.066 ± 0.014
4% rice husk		0 ± 0	0.005 ± 0.005	0 ± 0	0 ± 0	0 ± 0	0.010 ± 0.010	0.022 ± 0.013	0.005 ± 0.008
1% mixed wood		0.035 ± 0.056	0.041 ± 0.063	0.050 ± 0.040	0.041 ± 0.023	0.063 ± 0.057	0.076 ± 0.013	0.081 ± 0.004	0.055 ± 0.019
4% mixed wood		0 ± 0	0.003 ± 0.004	0.002 ± 0.003	0.005 ± 0.007	0.003 ± 0.004	0.041 ± 0.028	0.056 ± 0.021	0.016 ± 0.023
1% rice husk	Amended (turnip pots)	0	0.005	0	0	0.005	0.025	0.041	0.011 ± 0.016
4% rice husk		0.072 ± 0.003	0.090 ± 0.005	0.078 ± 0.006	0.061 ± 0.010	0.102 ± 0.005	0.071 ± 0.005	0.075 ± 0.003	0.078 ± 0.014
1% mixed wood		0.051 ± 0.068	0.063 ± 0.070	0.075 ± 0.051	0.053 ± 0.038	0.093 ± 0.069	0.070 ± 0.045	0.068 ± 0.052	0.067 ± 0.014
4% mixed wood		0.075 ± 0.106	0.085 ± 0.113	0.072 ± 0.088	0.054 ± 0.061	0.100 ± 0.133	0.092 ± 0.050	0.100 ± 0.054	0.082 ± 0.017
Spiked soil + ryegrass	Unamended comparison spiked soil	0.037 ± 0.064	0.045 ± 0.074	0.047 ± 0.060	0.039 ± 0.033	0.053 ± 0.070	0.087 ± 0.014	0.096 ± 0.008	0.058 ± 0.024
Spiked soil + turnips		0.047 ± 0.051	0.052 ± 0.054	0.059 ± 0.051	0.043 ± 0.037	0.079 ± 0.068	0.058 ± 0.014	0.063 ± 0.047	0.057 ± 0.012
Spiked soil + earthworms		0.020	0.020	0.090	0.067	0.123	0.087	0.092	0.071 ± 0.039
Spiked soil + PE passive sampler		0.040 ± 0.056	0.047 ± 0.060	0.042 ± 0.060	0.031 ± 0.043	0.061 ± 0.086	0.043 ± 0.061	0.053 ± 0.054	0.045 ± 0.010
Non-spiked soil + ryegrass	Unamended comparison non-spiked soil	0.002 ± 0.003	0.005 ± 0.000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.001 ± 0.002
Non-spiked soil + turnips		0.005 ± 0.000	0.008 ± 0.011	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.002 ± 0.003
Non-spiked soil + earthworms		0.003 ± 0.004	0.007 ± 0.003	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.001 ± 0.003
Non-spiked soil + PE passive sampler		0.005 ± 0.000	0.010 ± 0.007	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.002 ± 0.004

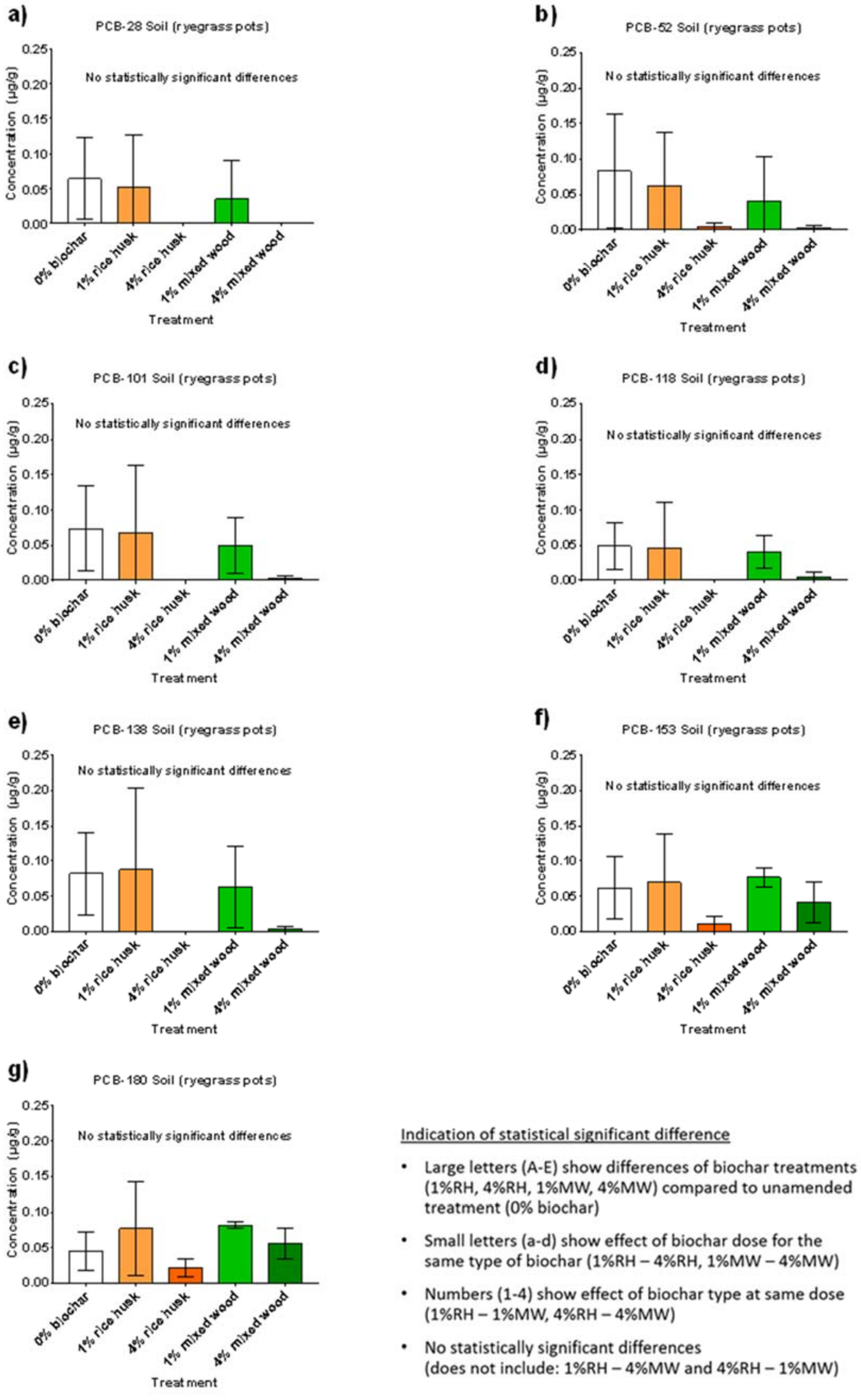
*The concentration is based on the result from only one sample

Figure 57 and Figure 58 show the concentrations (µg/g) of PCBs in soil for the ryegrass pots and for the turnip pots respectively, with 0% biochar, 1 and 4% of either rice husk or mixed wood biochar. In both figures the concentration for the 0% biochar treatment is an average of both the turnip and ryegrass soil samples due to the fact that only two ryegrass samples were obtained.

Concentrations of PCB congeners in soil from ryegrass pots (Figure 57) following amendment of both biochars at both doses showed no statistically significant difference (p-values ranging from 0.2731 to >0.9999) when compared to the pots without biochar. The average sum PCB-7 concentrations in the soil in 0% biochar pots (0.065 ± 0.015 µg/g) were similar to those in pots with 1% rice husk biochar (0.066 ± 0.014 µg/g) and 1% mixed wood biochar (0.055 ± 0.019 µg/g) but concentrations were lower for the 4 % rice husk and mixed wood biochars (0.005 ± 0.008 µg/g and 0.016 ± 0.023 µg/g, respectively). The addition of a higher dose of biochar resulted in lower absolute concentrations extracted from the soil-biochar system.

Concentrations of PCB congeners in soil from turnip pots (Figure 58) following the amendment of both biochars at both doses showed no statistically significant difference (p-values ranging from 0.4112 to >0.9999) when compared to the pot series with 0% biochar. The average sum PCB-7 concentrations in the soil in 0% biochar pots (0.065 ± 0.015 µg/g) were similar to those in pots with 1% (0.067 ± 0.014 µg/g) and 4% (0.082 ± 0.017 µg/g) of mixed wood biochar as well as 4 % rice husk (0.078 ± 0.014 µg/g), while the average sum PCB-7 concentration in the 1 % rice husk pots were lower (0.011 ± 0.016 µg/g).

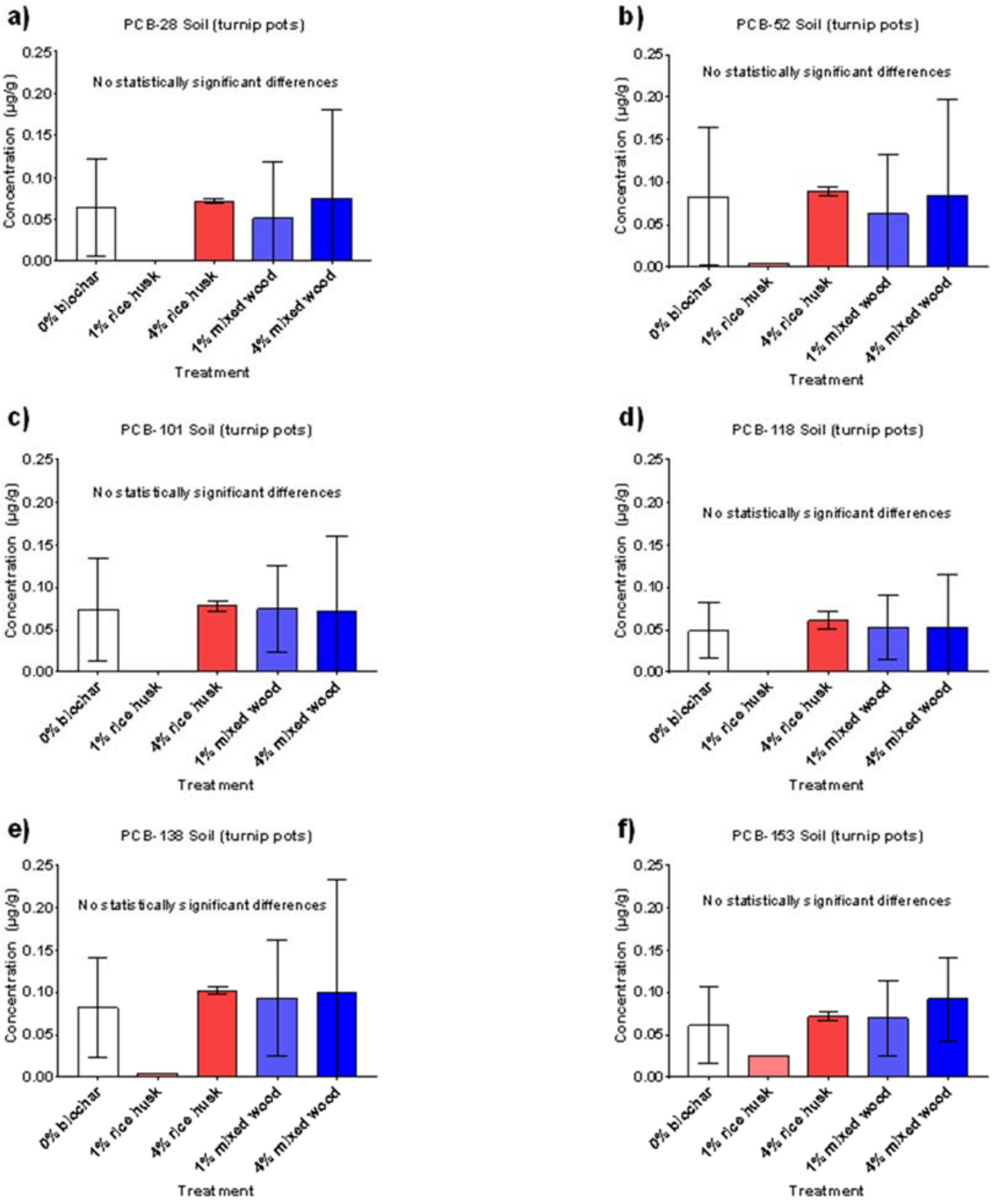
Concentrations of PCBs for the soil-biochar treatments are low and as discussed in the mass balance section (these systems had mass balances between 7 and 20%), this is likely due to the very strong sorption of PCBs to biochar and the subsequent incomplete extraction during the quantification. Biochar has a high adsorption affinity for PCBs (Wang et al., 2013a, 2016) and it has been reported that the higher the black carbon content of carbonaceous sorbents (of which biochar is included), the lower the efficiency of an extraction using hexane-acetone (Beesley et al., 2010; Hale et al., 2012).

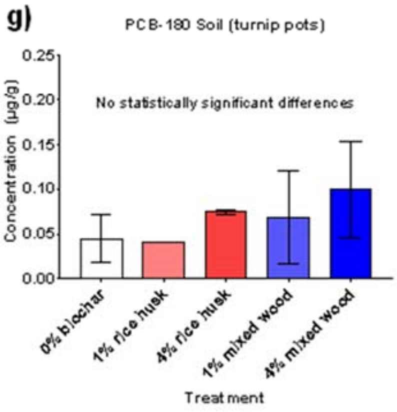


Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 57 Soil concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in soil samples in ryegrass-pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar





Indication of statistical significant difference

- Large letters (A-E) show differences of biochar treatments (1%RH, 4%RH, 1%MW, 4%MW) compared to unamended treatment (0% biochar)
- Small letters (a-d) show effect of biochar dose for the same type of biochar (1%RH – 4%RH, 1%MW – 4%MW)
- Numbers (1-4) show effect of biochar type at same dose (1%RH – 1%MW, 4%RH – 4%MW)
- No statistically significant differences (does not include: 1%RH – 4%MW and 4%RH – 1%MW)

Figure 58 Soil concentrations of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in soil samples in turnip-pots containing 0% biochar or 1 or 4% of either mixed wood or rice husk biochar

The biochars used in this thesis have a high proportion of carbon (41% for the rice husk biochar and 53% for the mixed wood biochar) and this could have affected the extraction efficiency, and hence the PCB concentrations in the biochar-amended treatments. Previously, biochar sorption coefficients for PCBs have been shown to increase with biochar pyrolysis temperature, surface area and pore volume, C-content, aromaticity and thermal stability (Kupryianchyk et al., 2016). The mixed wood biochar used in this work was produced under the highest pyrolysis temperature (700 °C compared to 300 °C for the rice husk biochar), had the highest SA (404 m^2/g compared to 51 m^2/g) and C-content (53.2% compared to 41.2%) and may have been expected to sorb PCBs more strongly and perform better than rice husk biochar as an amendment material. However this was not the case given that rice husk biochar resulted in greater reductions of PCB-concentrations in earthworms and PE passive samplers. The reasons for this are unclear, but may have been due to the larger proportion of fine particles for the rice husk biochar, or may have been due to a biochar property that was not measured here.

8.4.2 Unamended comparison pots

For the spiked soil unamended comparison pots (Table 16) the highest detected concentration of PCB in the soil system was for PCB-138 (0.123 µg/g) in the earthworm pots, and the lowest detected concentration was for PCB-28 and PCB-52 (both 0.02 µg/g), also in the earthworm pots. Looking at all pots (soil from pots with ryegrass, turnips, earthworms and PE passive sampler) it was PCB-138 and PCB-180 that were often detected in the highest concentrations and PCB-118 and PCB-28 detected in the lowest concentrations. This result again highlights that there was no real consistent trend with PCB molecular size, but an effect of PCB planarity where the planar PCBs (28 and 118) are detected in the soil to a smaller extent than the non-planar PCBs. For the non-spiked soil from unamended comparison, only PCB-28 and PCB-52 were detected with concentrations between 0.002 and 0.010 µg/g reflecting the small diffuse contamination.

8.4.3 Percentage reduction in absolute PCB concentrations in the soil

Table 17 shows the percentage reduction in the concentration of PCBs in the soil/soil-biochar systems. Overall, results were variable as expected based on the results of all other phases and the possible incomplete extraction. The most consistent trends were seen for the soil-biochar systems from the turnip pots which most showed reductions following biochar amendment. The highest reduction was 95% for PCB-52 and PCB-138 in the 4% rice husk treatment. For the ryegrass pots, both reductions and increases were observed following amendment.

Table 17 Reductions (%) in soil PCB-concentrations for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment	Batch	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
1% rice husk	Amended (ryegrass pots)	-17	-19	-59	-50	-62	-80	-87
4% rice husk		*	*	*	*	*	73	46
1% mixed wood		23	22	-17	-32	-16	-99	-99
4% mixed wood		*	95	94	84	95	-6	-36
1% rice husk	Amended (turnip pots)	*	95	*	*	95	67	15
4% rice husk		7	13	18	0	-2	7	-58
1% mixed wood		33	39	21	14	7	9	-43
4% mixed wood		2	18	24	13	1	-20	-110

*no detection of the compound over the LOD for the analytical method

8.5 Hypotheses

Prior to the pot trial it was hypothesized that; 1) the PCBs would be sorbed strongly to biochar, 2) sorption to biochar would differ with dose, 3) there would be a difference between the two biochars with regard to soil sorption of PCBs. The results from the trial supported the first hypothesis. The second and third hypotheses were falsified.

9 Phase to soil accumulation factors (PSAFs)

Accumulation of PCBs in earthworms, plants and passive samplers was assessed using phase (earthworms, plants or PE passive samplers) to soil accumulation factors (PSAFs). These PSAFs were calculated by dividing the PCB concentration detected in the different phases by the PCB concentration detected in the soil according to equation 3 below;

$$\mathrm{PSAF} = \frac{{}^{c}\,{}_{PCB\,(worm\,(lw)/\,plant\,(dw)/\,PE)}}{c_{PCB\,(soil\,(dw))}}$$

The PSAF was calculated for all PCB congeners and all treatments. Table 18 shows the calculated PSAFs for the earthworms, plants and PE passive samplers for the individual PCB-congeners as well as the average PSAFs for the sum PCB-7.

The PCB concentrations used to calculate the PSAFs are given in Table 6 (earthworm), Table 10 (ryegrass), Table 12 (turnips), Table 14 (PE passive samplers) and Table 16 (soil) as well as in appendix III. The calculated PSAFs were plotted for each phase (earthworm, plant or PE passive sampler) for each of the two biochar stacked with biochar doses 0%, 1% and 4% in bar plots.

Figure 59 shows the PSAFs stacked bar plots for all three phases in the turnip pots (turnip, earthworm and PE passive sampler) amended with rice husk and mixed wood biochar. Figure 60 shows the PSAFs stacked bar plots in the two phases in the ryegrass pots (ryegrass and PE passive sampler) amended with rice husk and mixed wood biochar. For some stacked bars one biochar treatment is missing (0%, 1% or 4%) due to concentrations of PCBs in either the phase (earthworm, plant or PE) or the soil being below the analytical LOD (appendix III).

Table 18 Phase to soil accumulation factors of 7 PCBs (-) for pots with 0% biochar, 1% and 4% of either mixed wood or rice husk biochar

Treatment		Ryegrass pots						Turnip pots					
		0% biochar	1% rice husk	4% rice husk	1% mixed wood	4% mixed wood	∑ dose (0%, 1% and 4%)	0% biochar	1% rice husk	4% rice husk	1% mixed wood	4% mixed wood	∑ dose (0%, 1% and 4%)
Batch		Control	Amended				∑	Control	Amended				∑
PSAF earth-worm (-)	PCB-28							141	-	16	68	45	270
	PCB-52							157	562	35	96	87	938
	PCB-101							149	-	44	73	105	371
	PCB-118							141	-	29	68	84	322
	PCB-138							148	707	45	64	94	1057
	PCB-153							173	116	56	74	90	509
	PCB-180							47	19	14	15	17	113
	∑ PCB-7							137 ± 41	351 ± 335	34 ± 15	65 ± 24	75 ± 32	
PSAF ryegrass/ turnip (-)	PCB-28	1,5	0,9	-	2,5	-	4,9	0,9	-	0,4	1,2	0,4	2,8
	PCB-52	1,8	0,4	13,8	4,8	32,6	53	1,1	36,2	0,3	1,9	0,5	40
	PCB-101	-	0,4	-	0,7	12,5	14	0,5	-	0,6	0,6	0,6	2,4
	PCB-118	-	1,0	-	2,0	12,4	15	0,8	-	0,8	0,8	0,6	3,1
	PCB-138	0,8	1,0	-	1,6	31,5	35	1,1	20,6	0,7	0,8	0,6	24
	PCB-153	0,7	0,8	4,5	1,3	1,0	8,3	1,0	4,1	0,7	0,8	0,4	7,0
	PCB-180	-	0,3	0,6	0,4	0,1	1,4	0,1	-	0,3	0,2	0,1	0,8
	∑ PCB-7	1,2 ± 0,6	0,7 ± 0,3	6,3 ± 6,8	1,9 ± 1,5	15 ± 14		0,8 ± 0,4	20 ± 16	0,6 ± 0,2	0,9 ± 0,5	0,5 ± 0,2	
PSAF PE passive sampler (-)	PCB-28	15	6	-	21	-	43	19	-	5	20	4	49
	PCB-52	12	9	32	33	307	393	19	45	4	22	7	96
	PCB-101	17	8	-	21	311	357	17	-	4	18	12	52
	PCB-118	11	7	-	12	62	93	13	-	6	13	6	38
	PCB-138	11	6	-	17	308	342	12	109	3	11	7	141
	PCB-153	13	10	34	10	19	85	10	17	5	10	8	51
	PCB-180	14	4	16	8	8	50	7	8	5	5	4	29
	∑ PCB-7	13 ± 2,2	7,2 ± 1,8	27 ± 10	18 ± 8,8	169 ± 154		14 ± 4,7	45 ± 45	4,5 ± 0,7	14 ± 6	7,0 ± 2,7	

- Either phase-sample (plant/worm) or soil sample with concentrations below the analytical limit of detection

No earthworms in pots with ryegrass

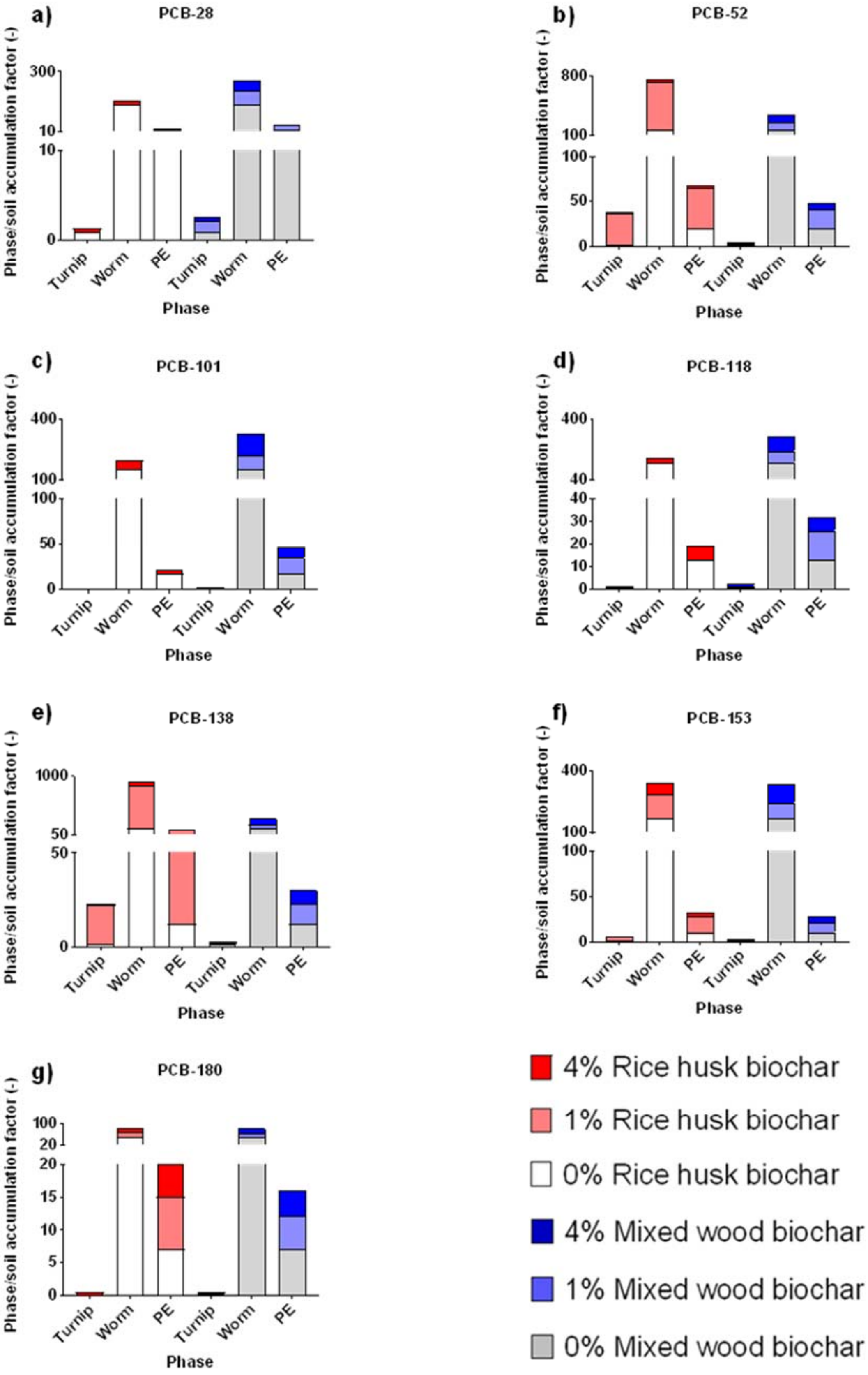


Figure 59 Phase to soil accumulation factors of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in turnip-pots containing 0%, 1% or 4% of rice husk biochar or 0%, 1% or 4% of mixed wood biochar

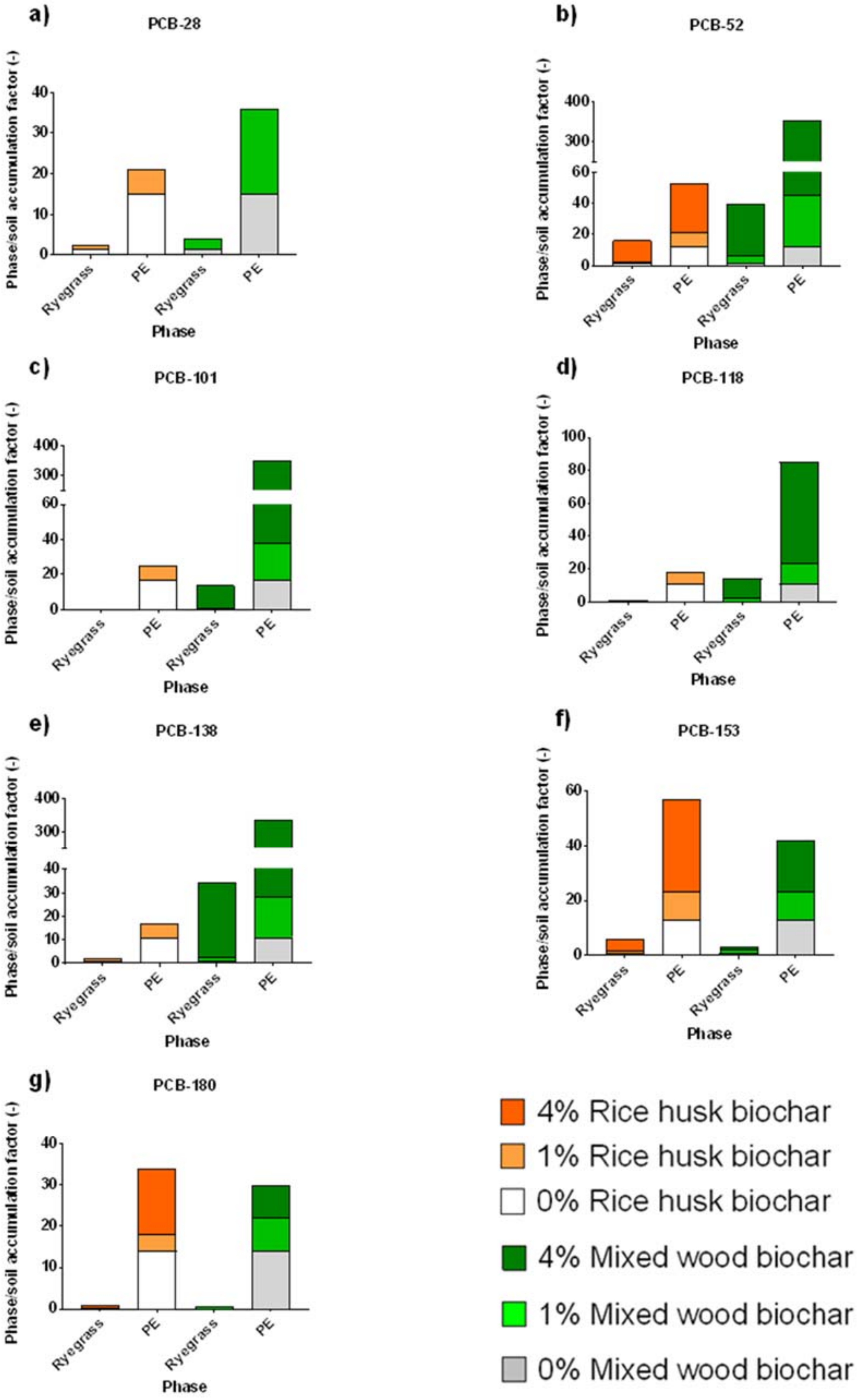


Figure 60 Phase to soil accumulation factors of PCBs (a) PCB-28, b) PCB-52, c) PCB-101, d) PCB-118, e) PCB-138, f) PCB-153 and g) PCB-180) in ryegrass-pots containing 0%, 1% or 4% of rice husk biochar or 0%, 1% or 4% of mixed wood biochar

9.1 PSAF: earthworm vs. plant vs. PE

The PSAF plots show a general trend with regards to the size of the PSAFs, where they can be arranged from highest to lowest by phases as follows; $PSAF_{earthworms} > PSAF_{PE} > PSAF_{turnip}$ and $PSAF_{PE} > PSAF_{ryegrass}$. The results show that the earthworms take up the PCBs to a greater extent than the plants. The uptake routes of PCBs for the earthworms are through dermal uptake and ingestion. Worms are inherently mobile in soil and this factor may also explain why there is a greater uptake of PCBs to earthworms. A similar trend was observed by Denyes et al. (2016), who carried out pot trials with earthworms (*Eisenia fetida*), PE passive samplers and plants (*Cucurbita pepo*) in pots amended with biochar (Burts and blue leaf biochars). The results from their study showed that DDT had a higher sorption affinity for earthworm lipids and PE passive samplers than plants based on the magnitude of the accumulation factors.

There were no clear correlations with PCB congener, apart from the fact that PCB 180 had the lowest PSAFs in both turnip and ryegrass pots, however the plants (especially ryegrass) tended to have the highest accumulation of PCB-52. Ryegrass generally also seem to have higher PSAFs for all PCB-congeners when compared to turnip PSAFs. The exception was the 1% rice husk treatment for the turnips. The measured concentrations of PCBs in the soil in this pot (base on only one soil sample) were all low (one order of magnitude) when compared to soil PCB-concentrations measured in other pots (see appendix III), this was also the case for the soil in the pots with ryegrass and 4% mixed wood biochar, giving high PSAFs. The plant-biochar chemistry will affect the accumulation factors and the reason could be different root system and therefore uptake by plants.

9.2 The effect of biochar type and dose on PSAF

The PSAFs were generally lower for the rice husk biochar than the mixed wood biochar. In the ryegrass pots PSAFs from 1% biochar dose amendment was generally one order of magnitude lower than the 4% biochar PSAFs for both plant and PE passive sampler. In the turnip pots the 4% amended dose tended to give lower PSAFs than the 1% doses for most phases. Brennan et al. (2014) carried out a 21 day pot trial to test the effects of the amendment of 3% wt pine wood and maize stubble biochar (as well as an AC) on the availability of PAHs to maize plants. The results showed non-significant reductions in PAH biota to soil accumulation factor (BSAFs) for pine wood biochar and significant reductions for maize biochar (as well as AC), compared to unamended controls. The maize biochar reduced the BSAFs by 58%, 57% and 65% for 3 ring, 4 ring and 5 ring PAHs respectively. The pine wood biochar reduced the BSAF by 33%, 25% and 27% for 3 ring, 4 ring and 5 ring PAHs respectively. This study highlights the fact that biochar feedstock can affect the remediation performance, in concurrence with what was seen here.

Table 19 shows the reduction or increase (expressed as percent) in PCB PSAFs, comparing biochar amendment treatments with the control treatments (0% biochar). There are no clear trends for the two different types of biochar with respect to PSAF reduction following biochar amendment. Based on the previous discussion this is expected, as is the fact that in some cases the PSAF increases following amendment. A trend emerged for the turnip pots amended with 4% biochar (both rice husk and mixed wood) where PSAFs for earthworms (68 to 89% and 29 to 68% reductions for rice husk and mixed wood biochars, respectively) and PE passive samplers (35 to 80% and 19 to 77% reductions for rice husk and mixed wood biochars, respectively) were both reduced following amendment.

Table 19 Reductions (%) in PCB phase to soil accumulation factors (PSAFs) for biochar treatments (1% and 4% of either mixed wood or rice husk biochar) compared to 0% biochar (control) treatment

Treatment		Ryegrass pots				Turnip pots			
		1% rice husk	4% rice husk	1% mixed wood	4% mixed wood	1% rice husk	4% rice husk	1% mixed wood	4% mixed wood
Batch		Amended				Amended			
PSAF earthworm (-)	PCB-28						89	52	68
	PCB-52					-258	78	39	44
	PCB-101						71	51	29
	PCB-118						80	52	40
	PCB-138					-378	70	57	37
	PCB-153					33	68	57	48
	PCB-180					60	71	68	63
PSAF ryegrass/ turnip (-)	PCB-28	43		-63			60	-40	56
	PCB-52	76	-658	-163	-1689	-3133	75	-66	53
	PCB-101						-26	-26	-11
	PCB-118						0	0	28
	PCB-138	-36		-106	-3983	-1768	32	26	45
	PCB-153	-13	-537	-84	-39	-323	27	19	56
	PCB-180						-152	-65	45
PSAF PE passive sampler (-)	PCB-28	59		-38			76	-2	77
	PCB-52	27	-169	-181	-2479	-140	80	-16	65
	PCB-101	52		-26	-1754		74	-9	28
	PCB-118	35		-11	-467		56	-2	52
	PCB-138	43		-54	-2687	-838	71	4	42
	PCB-153	28	-156	27	-45	-68	53	3	19
	PCB-180	69	-12	42	40	-14	35	28	38

Negative reductions indicate and increase following amendment. See earlier discussion

Wang et al. (2014) calculated the BSAF (biota-soil accumulation factor) of the herbicide atrazine was for two worm species that were exposed to biochar amended and unamended soil. This study found clear differences between earthworm species and biochar dose. The BSAF was 5 times higher (0.42 versus 0.079) for the anecic earthworm *Metaphire Guillelmi* than for *Eisenia fetida* in the unamended pots. Pine wood biochar produced at 400°C and added to the soil at doses of 0.5% and 2%, resulted in reductions of the BSAF for both earthworm species, but there was a much greater reduction of BSAF for *M. guillelmi* than for *E. fetida.* BSAFs were found to be 0.12 and 0.035 for 0.5% and 2% biochar respectively for *M. guillelmi* and 0.049 and 0.040 for 0.5% and 2% biochar respectively for *E. fetida* (Wang et al., 2014). Comparing the PSAFs calculated here with those reported in the literature should be done with caution due to differences in experimental set ups, differences in the plant and earthworm species and passive samplers used. Variability of PCB BSAFs have been reported as large between different soils in the same trial (Krauss et al., 2000)

9.3 PSAF: unamended comparison pots

Table 20 shows the PSAFs from the unamended comparison pots with spiked soil. The general trend with regards to the size of the PSAFs from highest to lowest for the phases was as follows; $\mathrm{PSAF}_{\mathrm{earthworm}} > \mathrm{PSAF}_{\mathrm{PE}} > \mathrm{PSAF}_{\mathrm{ryegrass}} > \mathrm{PSAF}_{\mathrm{turnip}}$.

Table 20 PSAF from unamended comparison pots spiked soil

Phase/PCB-congener	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
Earthworm	180	257	180	188	152	134	28
Ryegrass	3.3	5.1	0.3	2.4	1.8	0.9	0.1
Turnip	1.6	1.4	0.5	1.2	1.4	0.7	0.4
PE passive sampler	13	14	14	9.2	9.0	11	6.9

PFAS values from the spiked soil unameded comparison pots are in most cases comparable with PSAFs in 0% biochar treatment (the exception is the ryegrass PSAFs). They are also higher (or similar in a few cases) than PSAFs for all phases from the turnip pots amended with 4% rice husk, 1% mixed wood and 4% mixed wood treatments. In the ryegrass pots the same was the case for only 1% rice husk biochar amendment. The PCB-congener trends for the plants are similar to those for the amended pots with PSAFs being highest for PCB-52 and PCB-28 and lowest for PCB-180. There are no clear trend for PSAFs for earthworms or PE passive samplers with respect to the different PCB-congeners.

9.4 The wider context of PSAFs

The PSAF is a useful measure of how effective a biochar amendment to contaminated soil has been for different environmental phases. The closer the PSAF is to zero, the lower the amount of PCBs taken up by the plant, earthworm or PE passive sampler and hence a greater remediation efficiency. Measuring the uptake and accumulation of pollutants to different plants before and after amendment helps land planners to assess risks associated with contaminated land. By reducing the PSAF by amending a contaminated soil with biochar, the uptake of pollutants and transfer to the food chain can be reduced. On the opposite side, phytoremediation has the aim of using plants to take up pollutants making it is desirable that the PSAF for the plant is as high as possible.

Previous studies that have looked at PCB polluted soil and different plants have confirmed the low uptake capability of PCBs to plants, where very low bioaccumulation factors (BAF) in shoots have been reported. A PCB BAF in cabbage (*Brassica oleracea*) of 0.0042 was reported in a field study (Webber et al., 1994). Passatore reviewed phytoremediation and bioremediation of PCBs and reported following PCB shoot BAFs from various studies; 0.45 for *Carex aquatilis*, 0.29 for *Carex normalis*, 0.14 – 0.20 for *Cucurbita pepo*, 0.29 for *Cucurbita moschata* (BAF was 2 for part of the shoot), 0.28 for *glycine max*, 0.19 for *Zea mais* and 1.1 for *Vicia cracca.* Further experiments have shown that the uptake of PCB congeners to soybean sprouts was primarily dependent on the water solubility of the PCB, and not on the absorption by sprouts (Suzuki et al., 1977). Active transport of PCBs through the plant xylem system was shown to be lacking for tomatoe plants (Ye et al., 1992). However, other previous studies have demonstrated BAF>1 for 26 different weed species and for pumpkin (*Cucurbita pepo*) (Whitfield Åslund et al., 2007, 2008; Ficko et al., 2010). Langlois et al. (2011) reported after a 60 day greenhouse trial pumpkin *(Cucurbita pepo ssp. pepo*) shoot BAFs between 0.04 and 0.19 and root BAFs between 3.8 and 11.5 as well as earthworm (*E. fetida*) BAFs of between 33 and 88. The authors reported that amendment with AC diminuated the BAFs. Denyes et al.

(2012) reported a PCB BAF for *Cucurbita pepo ssp. Pepo* tissue of 0.11 in a 50 day greenhouse experiment.

Several studies have shown that earthworms take up PCBs with reported BAFs for the earthworms *E. fetida* and *Lumbricus terrestris* between 1 and 88 (Krauss et al., 2000; Langlois et al., 2011; Paul and Ghosh, 2011; Denyes et al., 2012). Krauss et al. (2000) reported BSAFs ≥ 10 for low chlorinated PCBs (8, 20, 28 and 52) and BSAFs around 1 for high-chlorinated PCBs (101, 118, 138, 153, 180, 199, 206 and 209) for the earthworm species *Lumbricus terrestris.* Langlois et al. (2011) reported BAFs between 33 and 87.7 for the earthworm *E. fetida* and PCBs after a 60 day greenhouse trial. Paul and Ghosh (2011) showed *E. fetida* BSAFs ranging from 2 to 7 for di- to penta-homologues of PCBs following a 28 day glass beaker trial, and Denyes et al. (2012) reported BAFs of 18 for PCBs in *E. fetida* PCB for a 50 day greenhouse experiment.

Jakob et al. (2012) conducted a field trial using PAH-contaminated soil in the period 2008-2010 using the plants ryegrass (*Lolium perenne* L.), carrots (*Daucus carota*) and squash (*Cucurbita pepo ssp. pepo* var. *giromontiina*) and earthworms (*E. fetida)*. The authors reported reduced BSAFs for the plants and earthworms for PAHs following the amendment of 2% granular activated carbon (GAC) and powder activated carbon (PAC). GAC and PAC reduced earthworm BSAFs by 47% and 72% respectively and an average BSAF reduction of 46% and 53% was reported for the plants following amendment with 2% GAC and PAC (Jakob et al., 2012).

10 Correlations between phases

In order to investigate whether there were correlations between the PSAFs for the different phases (Table 18) they were plotted against each other. Figure 61 shows the correlations, where each PCB congener was treated as a separate point (using the average of measurements), for a) earthworms vs. PE passive samplers b) earthworms vs. turnips c) turnips vs. PE passive samplers and d) ryegrass vs. PE passive samplers.

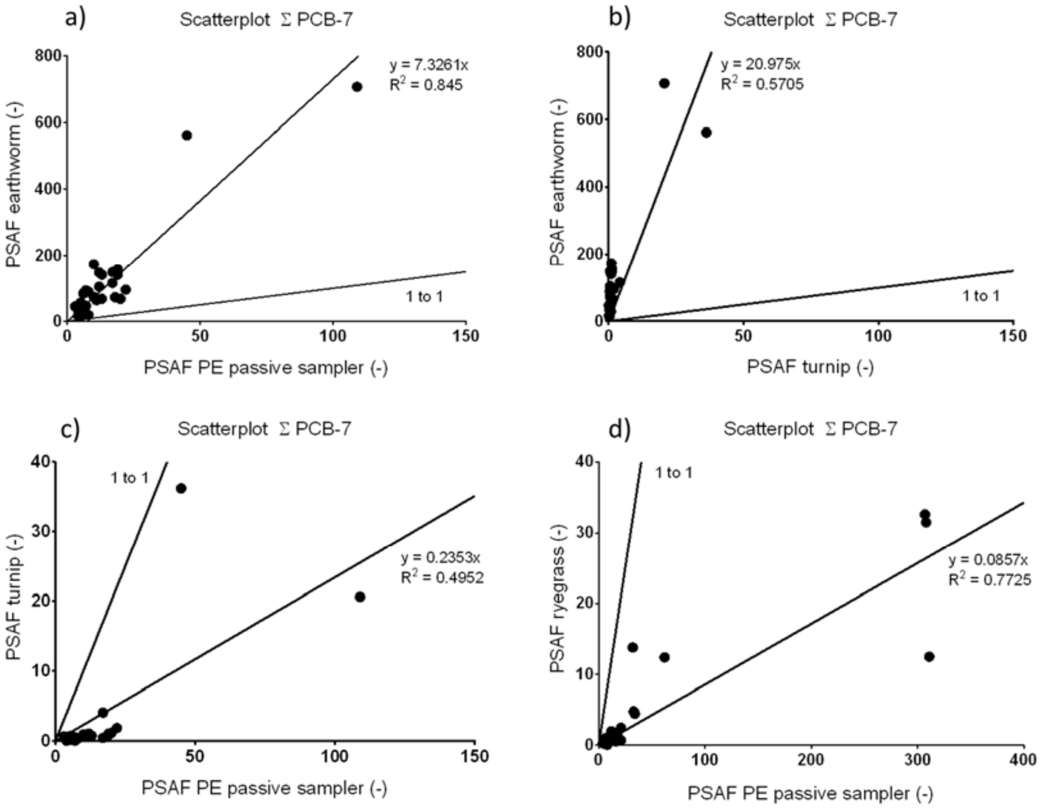


Figure 61 Correlation plots showing phase (earthworm, plant and PE) to soil accumulation factor (PSAFs) for all PCBs (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180) for a) earthworms vs. PE passive samplers, b) earthworms vs. turnips, c) turnips vs. PE passive samplers and d) ryegrass vs. PE passive samplers

A regression analysis was carried out and results showed that the PSAFs exhibiting the most correlation with each other were earthworms and PE passive samplers (coefficient of determination, r^2 = 0.85). The correlations between the other phases (Figures 30b-d) do not show such good correlations (r^2 = 0.57 for the turnip and earthworms, r^2 = 0.50 for the turnip and PE, r^2 = 0.77 for the ryegrass and PE). This indicates that using one of these phases in order to predict the uptake in another should not be done.

The regression for the earthworm versus PE PSAFs is $PSAF_{earthworm} = 7.33 \times PSAF_{PE\ passive\ sampler}$ and it is therefore evident that the worms and PE passive sampler phases behave differently as the earthworms take up 7 times more PCBs than the PE passive samplers (evident form the gradient of 7.33). However, the results show that the accumulation of PCBs in PE passive samplers can be a good proxy for the accumulation of PCBs in earthworms, if this fact is taken in to consideration. Indeed one of the main benefits of passive samplers is that they can be used to approximate uptake by biota without suffering some of the disadvantages of using biota (such as death, population changes and transformation of chemicals) and the correlation here supports this. Gomez-Eyles et al. (2011a) reported that POM passive samplers were used to assess PAH bioavailability and that the results could be compared to actual bioaccumulation of PAHs to earthworms (*Eisenia fetida*) and ryegrass (*Lolium multiflorum*) roots.

There are several other previous studies that have also reported that passive samplers can be used as proxies to predict the uptake of organic compounds by various organisms. In one of these studies the authors investigated the use of PE passive samplers to mimic the uptake of PAHs from sediments by benthic polychaetes (a species of marine worm) and concluded that there was a significant relationship between PAH-concentrations in the worms and the PE. They also reported that the polyethylene took up less PAHs than the worms (Vinturella et al., 2004). In another study, the bioavailable DDT concentration, determined using POM passive samplers, correlated well (<50% variability) with measured invertebrate uptake (Denyes et al., 2016). Paul & Ghosh et al. (2011) showed that there was a linear relationship between aqueous PCB concentration (measured by POM passive samplers) and earthworm (*Eisenia fetida*) concentrations, which held up even after a reduction in PCB uptake by 2 orders of magnitude due to AC-amendment to the soil.

In Figure 61 there are a few points where the PSAFs are orders of magnitude higher than the majority of the other points. As described earlier this is related to the fact that the measured concentrations of PCBs in the soil (turnip pots) were low, compared with the fact that measured concentrations were high for the separate phases (both earthworm, plant and PE passive samplers), resulting in high PSAFs for PCBs 52 and 138. However the data quality was high and there was no reason do discard them as outliers.

In Figure 62 a "zoom in" PCB-7 correlation plot for PSAFs of earthworms vs. PE passive samplers (shown in Figure 61 a) was plotted for all PSAF data points except the ones for PCB-52 and PCB-138 for the 1% rice husk biochar treatment. Individual PCB congeners for all treatments are shown. There was no trend with regard to PCB molecular size or planarity and this may suggest that amendment with different types and doses of biochar does not result in a common trend for each of the PCB-congeners. When the highest points were removed, there was no correlation between the PSAFs for the earthworms and the passive samplers.

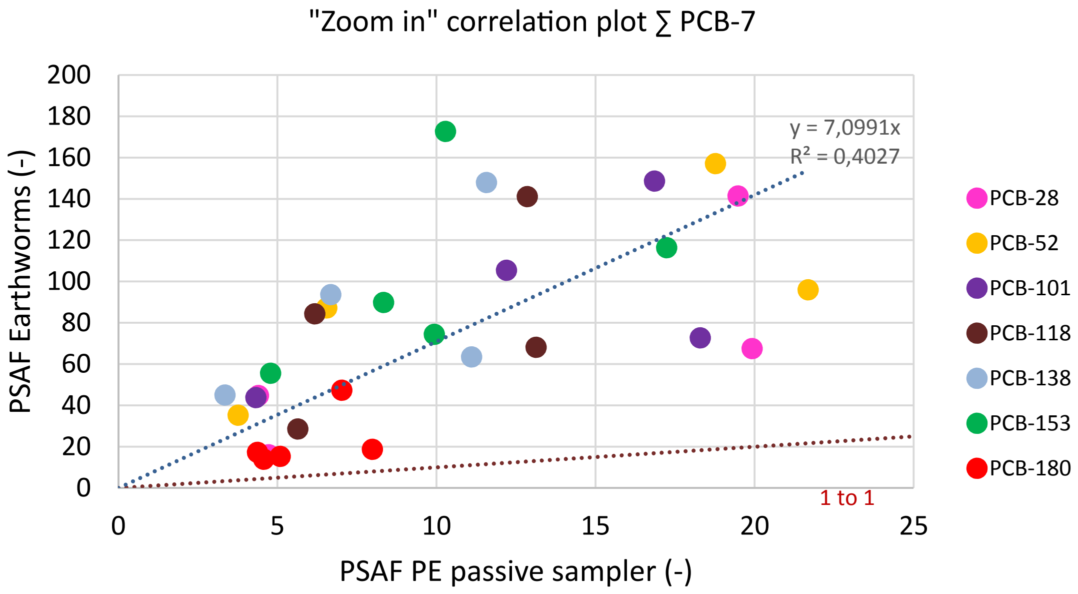


Figure 62 "Zoom in" correlation plot showing phase to soil accumulation factor (PSAFs) for all 7 PCBs for earthworms vs. PE passive samplers. Data is shown for all treatments (0% biochar, 1 % mixed wood, 4 % mixed wood, 1 % rice husk and 4 % rice husk biochar) thus giving 5 points per PCB. The plot visually excludes the high PSAFs attained for PCB-52 and PCB-138 for the 1% rice husk biochar treatment in turnip pots.

10.1 Hypothesis

Prior to beginning the experiment, it was hypothesized that a correlation between the uptake by plants, earthworms and passive samplers would exist. The results supported the hypothesis for the correlation between earthworms and PE passive samplers, but not for the correlation between the other phases.

11 Conclusion

In this thesis a pot experiment was conducted using spiked PCB polluted soil that was amended with biochar (two different types, two different doses). The uptake of PCBs to plants (two types), earthworms and PE passive samplers was assessed with and without amendment.

The main findings from the work can be summarized as follows:

Earthworms:

- The earthworms lost mass in all treatments, however they lost less in the biochar amended pots. A lower dose and the presence of rice husk biochar led to a smaller mass loss than the other treatments. There was no statistically significant difference in worm mass loss between non-spiked and spiked soil. The earthworms therefore showed a preference for the presence of biochar and mass loss did not seem to be affected by the presence of PCBs.
- The earthworms took up PCBs. Uptake was both dependant on PCB congener and biochar type, with rice husk biochar giving highest reduction in PCB-concentrations (up to 90%). There was no effect of biochar dose suggesting that the remediation of PCB polluted soil with biochar could be effectively achieved with a small biochar addition.

Plants:

- There were different observations for the two plants in relation to mass yield in the presence of biochar. Ryegrass yield increased with the presence of both biochars. The turnip yield was reduced with the addition of rice husk biochar but increased with 4% amendment of mixed wood biochar. The ryegrass yield was lower in the presence of PCBs, but the same trend was not seen for the turnips. This suggests that the PCBs have a negative effect on ryegrass, but that turnips could possibly be used in phytoremediation. This finding also illustrates the differences in behaviour of these two plants both with regards to their interactions with biochar and PCBs.

- Low concentrations of PCBs were detected in both plants with some difference between the PCB congeners. Plant uptake was generally not affected by either type or dose of biochar. In ryegrass a trend of reduced PCB concentrations for low chlorinated PCB congeners (28 and 52) was seen for both doses of rice husk biochar. In the turnips statistically significantly higher PCB concentrations of planar PCB congeners (28 and 118) were found in the 1% rice husk biochar treatment.

PE passive samplers

- PE passive samplers sorbed PCBs and the uptake was PCB congener specific. Biochar reduced the uptake of PCBs to PE passive samplers and there were no real effects of biochar type or dose, however rice husk biochar seemed to perform better with respect to reduced PCB concentrations (up to 86%) than mixed wood biochar.
- There was a correlation between the uptake of PCBs by PE passive samplers and by earthworms. However there was no correlation between the uptake of PCBs by PE passive samplers and by plants. This finding illustrates that PE passive samplers can be used as biomimetic models of worms with regards to pollutant uptake.

The overall aim of this thesis was to investigate the remediation effects of biochar on PCBs spiked to an agricultural soil. The sub aims investigating the relationship between uptake of PCBs by earthworms, plants and passive samplers as well as comparing sorption capacity of two biochars, one made using a controlled high-technology method, and one made using an uncontrolled low-technology method. These aims were achieved through the pot experiment and knowledge related to the use of biochar to amend PCB polluted soil has been gained.

The results of this thesis show a relationship in uptake of PCBs between earthworms and PE passive samplers suggesting that accumulation of PCBs in PE passive samplers is a good proxy for the accumulation of PCBs in earthworms. No relationships were found for PE passive samplers and ryegrass shoots or 3-4 week mature turnips. The results further indicated that the sorption capacity of the rice husk biochar made using an uncontrolled low-technology method performed better than the mixed wood biochar. The reason could be due to the visual larger portion of dusty smaller particles of the biochar and/or other physical-chemical properties not dealt with here.

The current move towards a more circular economy seeks to treat contaminated soil as a resource rather than as a waste. Circular economy is a system in which resource input and waste, emissions, and energy loss are reduced by closing and/or narrowing material and energy loops. In order to achieve this a difference from the current model "take, make and dispose" must be adopted (European Commission, 2017). In this regard, remediating contaminated soil and using this resource in a more sustainable way will reduce the amount of soil that is sent to landfill and close this material loop. In recent years the popularity of biochar as a soil amendment has substantially increased. In addition to biochar being able to bind organic pollutants, producing biochar from biomass waste can aid waste handling issues, the amendment of biochar to soil improves soil quality and biochar is able to sequester carbon, thus having a positive impact on climate change. It is however important to assess from a complete life cycle perspective the possible benefits of the amendment of soil with biochar in order that the most sustainable remediation strategy is chosen.

This thesis provides additional knowledge in the area of pollutant immobilization in agricultural soils following biochar amendments.

12 Future outlook

During the experimental work several areas were identified as topics for future work. The first is air sampling where passive air samplers could be placed just above the soil surface at the same height as the shoots of the plants in order to identify PCBs that are lost from the pots via this pathway.

Another area for future work is the analysis of the roots of the ryegrass in order to quantify the amount of pollutant that is accumulated there. Scaling the experiment up would also provide very useful information and may allow earthworms and ryegrass to be studies in the same system. The experiment would have benefitted from a better extraction of the soil-biochar systems in order to ascertain more information about the sorption of PCBs to the biochar. In addition, analysing the excrements from the earthworms would provide more answers related to whether pollutants are taken up by the gut or through the skin. As these pathways were not explicitly considered separately in this work it is difficult to assess which pathway was operating and to what extent. In future work the use of performance reference compounds (PRCs) could be considered in order to allow a quantification of how close the experimental system was to equilibrium.

A longer trial would also be beneficial as it would allow the turnip roots to reach maturity and would allow the longevity of biochar to be assessed. Once biochar is added to soil it is important to know how long it remains in the soil and whether the pollutants that have been sequestered stay that way could. It would also be interesting to test different biochar types with different properties including; feedstock, pyrolysis time, pyrolysis temperature and pyrolysis method, as these affect biochar physico-chemical properties. If a field trial were to be carried out then a soil with a range of native pollutants could be investigate (organics and metals) in order to understand the cocktail effect (additive effect).

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Appendix

Appendix I - List of Chemicals

Appendix Ia: Compounds used for spiking and standards

Usage	Name	Concentration and solvent	Manufacturer
Soil spike	EPA 525 PAH Mix-B	500 µg/mL each in acetone	Sigma-Aldrich, Scnelldorf Germany
	PCBs (28, 52, 101, 118, 138, 153 and 180)	10 mg of powder in each vial	CHIRON AS, Trondheim Norway
Surrogate standard	PCB-81	0.1 mg/mL in 1,1 mL of isooctane	CHIRON AS, Trondheim Norway
	PCB-126	0.1 mg/mL in 1,1 mL of isooctane	CHIRON AS, Trondheim Norway
Internal standard	PCB-77	10 mg of powder	CHIRON AS, Trondheim Norway

Appendix Ib: Solvents

Usage	Name	Purity	Manufacturer
PE passive sampler rinsing	Methanol	min. 99.8%	Merck KGaA, Darmstadt Germany
Soxtech	Hexane	min. 97.0%	Sigma-Aldrich, Scnelldorf Germany
Soxtech	Acetone	min. 99.8%	Sigma-Aldrich, Scnelldorf Germany
GPC	Chloroform	min. 99.5%	Lach-ner, Neratovice Tsjekkia

Appendix Ic: Other chemicals

Usage	Name	Specifications	Manufacturer
Pots	Perlite	No.2 extra pull (0.6-3.0 mm)	Horticoop
Worm sample preparation	Sodium sulfate, Na2SO4	min. 99% purity	Penta
Clean up	Activated silica gel	0.063-0.100 mm	Merck KGaA, Darmstadt Germany
Blow down	N2-gas		
General	Deionized water		

Appendix II – Potting and sample data

Appendix IIa: Mass of phases added to control and amended ryegrass replicate pots and mass of samples extracted

Treatment	Phases added	Function	Replicate pot	Mass soil potting (g)	Mass biochar potting (g)	Mass PE passive sampler potting (g)	Mass soil or soil-biochar system sample (g)	Dry mass ryegrass shoots/turnip sample (g)
0% biochar	Ryegrass, PE passive sampler	Control	0R1	1000.01	0	0.104		0.13
			0R2	1000.05	0	0.0988		0.18
			0R3	1000.05	0	0.105	10.03	0.19
			0R4	1000.06	0	0.107	10.01	0.15
			0R5	1000.04	0	0.098	10.01	0.18
1% rice husk		Amended	1RR1	999.96	10.01	0.11	10.03	0.39
			1RR2	1000.03	10.07	0.106		0.19
			1RR3	999.96	9.99	0.1	10.13	0.32
			1RR4	1000.01	9.97	0.122		1
			1RR5	1000	10.01	0.117		0.27
4% rice husk			4RR1	1000.04	40.05	0.1	10.01	0.26
			4RR2	1000	40.01	0.1	10.1	0.36
			4RR3	1000.01	39.99	0.102	10.11	0.26
			4RR4	999.99	40.01	0.113		0.19
			4RR5	1000.04	40.02	0.112		0.27
1% mixed wood			1MR1	1000.05	9.99	0.114	9.99	0.23
			1MR2	1000.04	10.02	0.106	10.12	0.29
			1MR3	1000	10.05	0.117		0.25
			1MR4	1000.02	10.05	0.109	10.18	0.24
			1MR5	999.98	9.99	0.106		0.41
4% mixed wood			4MR1	1000.02	40.03	0.093		0.24
			4MR2	1000.02	39.98	0.123	10.16	0.46
			4MR3	999.99	40.04	0.11	10.01	0.31
			4MR4	999.98	40.02	0.113		
			4MR5	1000.02	39.98	0.098		0.31

Abbreviations in the replicate names column; R: ryegrass, RR: rice husk biochar and ryegrass, MR: mixed wood biochar and ryegrass

Appendix IIb: Mass of phases added to control and amended turnip replicate pots and mass of samples extracted

Treatment	Phases added	Function	Replicate pot	Mass soil potting (g)	Mass biochar potting (g)	Mass PE passive sampler potting (g)	Mass earthworms potting (g)	Mass soil or soil-biochar system sample (g)	Dry mass ryegrass shoots/turnip sample (g)	Dry mass earthworm sample (g)	Lipid mass earthworm sample (g)
0% biochar	Turnip, PE passive sampler, earth-worm	Control	0T1	1000.02	0	0.116	34.389		0.41	4.21	0.3635
			0T2	1000.02	0	0.119	30.433		0.41	2.48	0.2439
			0T3	1000.04	0	0.106	33.115	10	0.9	1.61	0.1799
			0T4	1000.03	0	0.11	29.659	10.15	0.22		
			0T5	1000	0	0.118	30.79	10.11	0.58	3.3	0.3066
1% rice husk		Amended	1RT1	999.99	10	0.108	33.048	10.03	0.19	3.69	0.4302
			1RT2	1000.03	9.97	0.123	32.226	9.98	0.11	5.08	0.5120
			1RT3	1000.04	9.98	0.125	32.935			4.55	0.4509
			1RT4	1000.04	10.01	0.139	32.54	10.15	0.19	4.61	
			1RT5	1000.03	10.03	0.108	29.001			4.28	0.3901
4% rice husk			4RT1	1000.01	40.01	0.11	31.627	10.09	0.35	3.83	0.3649
			4RT2	1000.04	40.02	0.117	30.537	10.05	0.19	3.61	0.4709
			4RT3	1000.02	39.99	0.103	29.26		0.19	3.15	0.3422
			4RT4	999.98	39.97	0.107	31.098		0.28	3.51	0.3476
			4RT5	1000.03	40.04	0.108	28.434	10.07	0.42	4.19	0.4103
1% mixed wood			1MT1	1000.01	9.99	0.122	35.492	10.16	0.26	4.59	0.4825
			1MT2	999.97	9.98	0.113	33.547	10.11	0.89	2.75	0.2549
			1MT3	1000.03	9.97	0.106	27.696		0.4	3.88	0.4479
			1MT4	1000.01	10.02	0.106	31.596	10.08	0.62	3.64	0.3890
			1MT5	999.98	10.01	0.13	28.896		0.55	2	0.2609
4% mixed wood			4MT1	999.98	40.05	0.116	28.635		1.69	2.51	0.2594
			4MT2	1000.05	40.02	0.119	28.465	10.08	1.23	2.24	0.2545
			4MT3	1000.04	39.96	0.099	27.793		1.52	2.55	0.2870
			4MT4	999.98	40.02	0.123	30.075	10.02	0.25	3.76	0.3614
			4MT5	1000.01	39.97	0.113	29.299		1.26	2.39	0.2660

Abbreviations in the replicate names column; T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip

Appendix IIc: Mass of phases added to unamended comparison replicate pots and mass of samples extracted

Treatment	Phases added	Replicate pot	Mass soil potting (g)	Mass biochar potting (g)	Mass PE passive sampler potting (g)	Mass earthworms potting (g)	Mass soil or soil-biochar system sample (g)	Dry mass ryegrass shoots/turnip sample (g)	Dry mass earthworm sample (g)	Lipid mass earthworm sample (g)
Spiked soil	Ryegrass	SSR1	1000.05	0				0.19		
		SSR2	1000.03	0			9.94	0.4		
		SSR3	1000.03	0			10.06	0.1		
		SSR4	1000	0			10	0.17		
	Turnip	SST1	1000.01	0			10.03	0.36		
		SST2	1000.03	0			10.02	0.56		
		SST3	1000.04	0			10.00	0.17		
		SST4	1000.06	0				0.42		
	PE passive sampler	SSPE1	1000	0	0.132		10.02			
		SSPE2	999.99	0	0.115					
		SSPE3	1000.02	0	0.107					
		SSPE4	999.99	0	0.125		10.11			
	Earthworm	SSEW1	1000.03	0		28.843			4.36	0.2874
		SSEW2	1000.03	0		28.369			4.29	0.2304
		SSEW3	1000	0		27.206	10.1		3.76	0.3481
		SSEW4	1000	0		29.615	10.05		4.65	0.4183
Non-spiked soil	Ryegrass	NSR1	1000.09	0			10.06	0.43		
		NSR2	1000.11	0			10.02	0.6		
		NSR3	1000.14	0			10.01	0.58		
		NSR4	1000.11	0				0.66		
	Turnip	NST1	1000.09	0			10.14	0.14		
		NST2	1000.11	0				0.16		
		NST3	1000.15	0			10.12	0.13		
		NST4	1000.09	0			10.01	0.05		
	PE passive sampler	NSPE1	1000.13	0	0.118					
		NSPE2	1000.12	0	0.109					
		NSPE3	1000.14	0	0.115		10.07			
		NSPE4	1000.1	0	0.122		10.09			
	Earthworm	NSEW1	1000.09	0	0	29.092			4.11	0.2458
		NSEW2	1000.11	0	0	29.871	10.02		3.71	0.2333
		NSEW3	1000.09	0	0	31.016	10.12		4.45	0.4186
		NSEW4	1000.13	0	0	23.25			3.68	0.3195

Abbreviations in the replicate names column; R: ryegrass, T: turnip, SS: spiked soil, PE: polyethylene passive sampler, EW: earthworm, NS: non-spiked soil

Appendix II: Mass of blank samples extracted

Phase	Function	Replicate pot	Mass soil or perlite sample (g)	Mass earthworm sample (g)	Dry mass earthworm sample (g)	Lipid mass earthworm sample (g)	Mass PE passive sampler (g)
Spiked soil	Blank	P-SS1	10.02				
		P-SS2	10.05				
		P-SS3	10.05				
Non-spiked soil		P-NS1	10.1				
		P-NS2	10.23				
		P-NS3	10.04				
Perlite		P-perlite1	1.3				
		P-perlite2	1.46				
		P-perlite3	1.39				
Earthworm		P-EW1		31.966	5.83	0.4290	
		P-EW3		29.936	5.68	0.4440	
		P-EW4		30.888	5.6	0.4958	
PE passive sampler		P-PE1					0.1048
		P-PE2					0.103
		P-PE-3					0.105
		Ctr-PE1					0.1046
		Ctr-PE2					0.1224
		Ctr-PE-3					0.101

Abbreviations in the replicate names column; P: potting (control) SS: spiked soil, NS: non-spiked soil, EW: earthworm, PE: polyethylene passive sampler, Ctr: control

Appendix III – Sample concentrations

Appendix IIIa: Earthworm concentrations in replicate samples of 7 PCB congeners (µg/g lipid mass)

Treatment	Phase analyzed	Other phases in pots	Function	Replicate sample	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
0% biochar	Earthworm	Turnip, PE passive samplers	Control	0T1-e	9.92	13.63	10.78	6.63	11.79	10.13	2.04
				0T2-e	7.76	11.51	9.99	6.08	10.64	9.88	1.99
				0T5-e	9.48	13.99	12.21	8.08	13.90	11.79	2.34
1% rice husk			Amended	1RT1-e	0.86	3.17	2.49	1.35	3.28	2.80	0.70
				1RT2-e	0.95	3.12	2.97	1.40	3.48	3.03	0.77
				1RT3-e	0.74	2.03	1.82	1.28	2.36	2.06	0.51
				1RT4-e	0.72	2.29	2.50	1.46	3.73	2.82	0.81
				1RT5-e	1.10	3.30	3.60	1.90	5.05	4.04	1.01
4% rice husk				4RT1-e	1.02	3.42	3.79	1.65	5.27	4.32	1.33
				4RT2-e	0.17	0.54	0.58	0.30	0.79	0.64	0.20
				4RT4-e	2.80	6.51	6.84	3.53	9.06	8.06	1.80
				4RT5-e	0.62	2.19	2.47	1.52	3.28	2.88	0.85
1% mixed wood				1MT1-e	3.52	5.46	4.81	2.74	5.14	4.85	0.96
				1MT2-e	2.14	4.44	4.44	3.18	5.54	4.45	0.91
				1MT4-e	3.21	5.81	5.41	3.28	6.19	5.00	0.95
				1MT5-e	5.01	8.45	7.03	5.15	6.84	6.39	1.33
4% mixed wood				4MT1-e	2.48	6.99	7.44	3.66	8.57	7.41	1.43
				4MT2-e	3.06	7.20	6.74	3.64	8.28	7.37	1.46
				4MT4-e	5.50	9.66	9.71	6.60	11.80	10.45	2.18
				4MT5-e	2.35	5.72	6.60	4.18	8.62	7.84	1.83
Spiked soil		None	Unamended comparison	SSEW1-e	6.58	11.13	11.87	8.06	14.11	11.85	2.74
				SSEW2-e	10.41	14.30	10.83	6.84	11.06	10.06	2.11
				SSEW3-e	7.34	15.40	11.59	13.97	12.51	9.72	0
				SSEW4-e	7.18	10.68	9.79	6.37	10.97	9.36	2.16
Non-spiked soil				NSEW1-e	0.71	1.03	0.63	0.28	0.28	0.28	0
				NSEW2-e	0.25	0.33	0	0	0	0	0
				NSEW3-e	0.14	0.19	0	0	0	0	0
Blanks		Blanks		P-EW1	0,18	0.14	0	0	0	0	0
				P-EW3	0,22	0.22	0	0	0	0	0
				P-EW4	0,24	0.24	0	0	0	0	0

Abbreviations in the replicate names column; T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip, SS: spiked soil, EW: earthworm, NS: non-spiked soil, P: potting (control)

Appendix IIIb: Ryegrass shoot or turnip concentrations in replicate samples of 7 PCB congeners (µg/g)

Treatment	Phase analyzed	Other phases in pots	Function	Replicate sample	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
0% biochar	Ryegrass shoots	PE passive samplers	Control	0R1-pl	0.162	0.325	0	0	0	0	0
				0R4-pl	0.070	0.070	0	0	0.071	0.071	0
				0R5-pl	0.059	0.059	0	0	0.118	0.059	0
1% rice husk			Amended	1RR2-pl	0	0	0	0	0.056	0.056	0
				1RR3-pl	0.033	0	0.033	0.033	0.033	0.033	0.033
				1RR4-pl	0.032	0.032	0.042	0.075	0.117	0.053	0.011
				1RR5-pl	0.117	0.078	0.039	0.079	0.158	0.079	0.039
4% rice husk				4RR1-pl	0.081	0.041	0.041	0.082	0.123	0.041	0.041
				4RR3-pl	0.041	0	0	0	0.041	0.041	0
				4RR4-pl	0.111	0.167	0	0.056	0.112	0.056	0
1% mixed wood				1MR2-pl	0.146	0.291	0.073	0.110	0.147	0.147	0.037
				1MR5-pl	0.026	0.103	0	0.052	0.052	0.052	0.026
4% mixed wood				4MR1-pl	0.044	0.088	0.044	0.044	0.089	0.044	0
				4MR5-pl	0.068	0.068	0.034	0.069	0.069	0.034	0
				4MR2-pl	0.069	0.069	0.046	0.069	0.093	0.046	0.023
				4MR3-pl	0.068	0.102	0	0.069	0.069	0.034	0
0% biochar	Turnip	PE passive samplers, earthworms	Control	0T1-pl	0.026	0.051	0.051	0.052	0.078	0.052	0
				0T2-pl	0.103	0.129	0	0	0.156	0.078	0
				0T3-pl	0.047	0.164	0.047	0.047	0.059	0.047	0.012
				0T4-pl	0.048	0.048	0	0.048	0.048	0.048	0
				0T5-pl	0.055	0.073	0.091	0.055	0.110	0.073	0.018
1% rice husk			Amended	1RT2-pl	0.192	0.192	0.096	0.097	0.097	0.097	0
				1RT4-pl	0.167	0.167	0	0.168	0.112	0.112	0
4% rice husk				4RT5-pl	0.025	0.025	0.050	0.051	0.076	0.051	0.025
1% mixed wood				1MT1-pl	0.122	0.122	0.122	0.082	0.164	0.082	0.041
				1MT2-pl	0.083	0.202	0.071	0.072	0.120	0.072	0
				1MT3-pl	0.026	0.106	0	0	0	0.027	0
				1MT5-pl	0.019	0.038	0	0.019	0.019	0.039	0.019
4% mixed wood				4MT1-pl	0.025	0.031	0.031	0.019	0.038	0.025	0.006
				4MT2-pl	0.026	0.034	0.043	0.035	0.061	0.043	0.009
				4MT3-pl	0.035	0.069	0.049	0.042	0.084	0.049	0.007
Spiked soil	Ryegrass shoots	None	Unamended comparison	SSR1-pl	0.167	0.222	0	0.168	0.168	0.056	0
				SSR2-pl	0.079	0.053	0.053	0.107	0.133	0.080	0.027
				SSR4-pl	0.186	0.497	0	0.063	0.125	0.063	0
	Turnip			SST3-pl	0.062	0.062	0.062	0.063	0.125	0.063	0.063
				SST4-pl	0.075	0.075	0	0.051	0.101	0.051	0
Non-spiked soil	Ryegrass shoots			NSR2-pl	0.053	0.035	0.035	0.071	0.089	0.053	0
				NSR3-pl	0.018	0.036	0	0.037	0.037	0.037	0
				NSR4-pl	0.048	0.032	0.032	0.065	0.081	0.032	0
	Turnip			NST1-pl	0.075	0.075	0	0.076	0.076	0.076	0
				NST4-pl	0	0	0	0	0	0	0

Abbreviations in the replicate names column; R: ryegrass, RR: rice husk biochar and ryegrass, MR: mixed wood biochar and ryegrass, T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip, SS: spiked soil, NS: non-spiked soil

Appendix IIIc: PE passive sampler concentrations in replicate samples of 7 PCB congeners (µg/g)

Treatment	Phase analyzed	Other phases in pots	Function	Replicate samples	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
0% biochar	PE passive samplers	Ryegrass	Control	0R2-pe	0.366	0.366	0.732	0.371	0.741	0.741	0.371
				0R3-pe	1.033	1.033	1.377	0.698	0.698	0.698	0.698
				0R4-pe	0.676	0.338	1.014	0.342	0.684	0.684	0.684
				0R5-pe	1.845	2.214	1.845	0.747	1.495	1.121	0.747
1% rice husk			Amended	1RR1-pe	0.329	0.329	0.329	0.333	0.333	0.666	0.333
				1RR2-pe	0.341	0.682	0.682	0.345	0.691	0.691	0.345
				1RR5-pe	0.309	0.618	0.618	0.313	0.626	0.626	0.313
4% rice husk				4RR2-pe	0	0	0.362	0	0.366	0.366	0.366
				4RR4-pe	0.320	0.320	0.320	0.324	0.324	0.324	0.324
1% mixed wood				1MR1-pe	0.952	1.586	1.269	0.642	1.285	0.964	0.642
				1MR2-pe	0.682	1.364	1.023	0.691	1.036	0.691	0.691
				1MR3-pe	0.618	1.236	0.927	0.313	0.939	0.626	0.626
				1MR4-pe	0.663	1.327	0.995	0.336	1.008	0.672	0.672
4% mixed wood				4MR2-pe	0.294	0.882	0.882	0.298	0.893	0.893	0.595
				4MR3-pe	0.329	0.657	0.657	0.333	0.666	0.666	0.333
0% biochar		Turnip, earth-worms	Control	0T1-pe	1.247	1.559	1.247	0.631	0.947	0.631	0.316
1% rice husk			Amended	1RT1-pe	0.335	0.670	0.670	0.339	0.678	0.339	0.339
				1RT3-pe	0	0	0.289	0.293	0.293	0.293	0.293
				1RT5-pe	0.335	0	0.670	0.339	0.678	0.678	0.339
4% rice husk				4RT1-pe	0.329	0.329	0.329	0	0.333	0.333	0.333
				4RT3-pe	0.351	0.351	0.351	0.356	0.356	0.356	0.356
				4RT4-pe	0.338	0.338	0.338	0.342	0.342	0.342	0.342
				4RT5-pe	0.335	0.335	0.335	0.339	0.339	0.339	0.339
1% mixed wood				1MT4-pe	1.023	1.364	1.364	0.691	1.036	0.691	0.345
4% mixed wood				4MT1-pe	0.623	0.935	1.247	0.316	0.947	0.947	0.631
				4MT2-pe	0	0	0.304	0.308	0.308	0.615	0.308
				4MT3-pe	0.365	0.730	1.096	0.370	0.740	0.740	0.370
Spiked soil		None	Unamended comparison	SSPE1-pe	0	0	0.548	0.277	0.555	0.832	0.555
				SSPE4-pe	1.157	1.446	1.157	0.586	0.879	0.879	0.586
Non-spiked soil				NSPE3-pe	0	0	0	0	0	0	0
Blanks		Blanks		P-PE1	0	0	0	0	0	0	0
				P-PE2	0	0	0	0	0	0	0
				P-PE-3	0	0	0	0	0	0	0
				Ctr-PE1	0	0	0	0	0	0	0
				Ctr-PE2	0	0	0	0	0	0	0
				Ctr-PE-3	0	0	0	0	0	0	0

Abbreviations in the replicate names column; R: ryegrass, RR: rice husk biochar and ryegrass, MR: mixed wood biochar and ryegrass, T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip, SS: spiked soil, PE: polyethylene passive sampler, NS: non-spiked soil, P: potting (control), Ctr: control

Appendix IIId: Soil, soil-biochar system or perlite concentrations in replicate samples of 7 PCB congeners (µg/g)

Treatment	Phase analyzed	Other phases in pots	Function	Replicate sample	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180
0% biochar	Soil, soil-biochar system or perlite	Ryegrass, PE passive samplers	Control	0R3-s	0.085	0.100	0.085	0.056	0.108	0.077	0.077
				0R5-s	0.005	0.005	0	0.005	0	0	0.005
1% rice husk			Amended	1RR1-s	0.105	0.115	0.135	0.092	0.169	0.118	0.123
				1RR3-s	0	0.010	0	0	0.005	0.020	0.030
4% rice husk				4RR1-s	0	0.010	0	0	0	0.010	0.021
				4RR2-s	0	0	0	0	0	0.020	0.036
				4RR3-s	0	0.005	0	0	0	0	0.010
1% mixed wood				1MR1-s	0.005	0.005	0.040	0.036	0.046	0.072	0.077
				1MR2-s	0	0.005	0.015	0.020	0.015	0.066	0.081
				1MR4-s	0.099	0.114	0.094	0.066	0.126	0.091	0.086
4% mixed wood				4MR2-s	0	0	0.005	0.010	0.005	0.061	0.071
				4MR3-s	0	0.005	0	0	0	0.021	0.041
0% biochar		Turnip, PE passive samplers, earthworms	Control	0T3-s	0.146	0.206	0.166	0.098	0.159	0.123	0.031
				0T4-s	0.074	0.089	0.069	0.046	0.081	0.056	0.056
				0T5-s	0.010	0.015	0.050	0.041	0.061	0.051	0.056
1% rice husk			Amended	1RT4-s	0	0.005	0	0	0.005	0.025	0.041
4% rice husk				4RT1-s	0.075	0.095	0.085	0.071	0.107	0.076	0.076
				4RT2-s	0.070	0.085	0.075	0.051	0.097	0.067	0.072
				4RT5-s	0.070	0.090	0.075	0.061	0.102	0.071	0.077
1% mixed wood				1MT1-s	0.005	0.025	0.020	0.010	0.020	0.020	0.010
				1MT2-s	0.129	0.144	0.119	0.081	0.158	0.107	0.112
				1MT4-s	0.020	0.020	0.085	0.066	0.102	0.082	0.082
4% mixed wood				4MT2-s	0.150	0.164	0.135	0.097	0.194	0.128	0.138
				4MT4-s	0	0.005	0.010	0.010	0.005	0.056	0.062
Spiked soil		Ryegrass	Unamended comparison	SSR2-s	0	0.005	0.015	0.026	0.016	0.083	0.098
				SSR3-s	0	0	0.010	0.015	0.010	0.077	0.087
				SSR4-s	0.111	0.131	0.116	0.077	0.134	0.103	0.103
		Turnip		SST1-s	0	0.005	0	0	0	0.005	0.010
				SST2-s	0.040	0.040	0.090	0.067	0.118	0.092	0.098
				SST3-s	0.100	0.111	0.085	0.062	0.118	0.077	0.082
		Earthworm		SSEW4-s	0.020	0.020	0.090	0.067	0.123	0.087	0.092
		PE passive sampler		SSPE1-s	0	0.005	0	0	0	0	0.015
				SSPE4-s	0.080	0.089	0.084	0.061	0.122	0.086	0.092
Non-spiked soil		Ryegrass		NSR1-s	0	0.005	0	0	0	0	0
				NSR2-s	0.005	0.005	0	0	0	0	0
				NSR3-s	0	0.005	0	0	0	0	0
		Turnip		NST3-s	0.005	0	0	0	0	0	0
				NST4-s	0.005	0.015	0	0	0	0	0
		Earthworm		NSEW2-s	0.005	0.005	0	0	0	0	0
				NSEW3-s	0	0.010	0	0	0	0	0
		PE passive sampler		NSPE3-s	0.005	0.005	0	0	0	0	0
				NSPE4-s	0.005	0.015	0	0	0	0	0
Spiked soil		Spiked soil	Blanks	P-SS1	0.080	0.085	0.095	0.067	0.128	0.087	0.098
				P-SS2	0.005	0.010	0.060	0.051	0.067	0.082	0.087
				P-SS3	0	0.005	0.020	0.031	0.015	0.072	0.077
Non-spiked soil		Non-spiked soil		P-NS1	0.005	0.025	0	0	0	0	0
				P-NS2	0	0.005	0	0	0	0	0
				P-NS3	0	0	0	0	0	0	0
Perlite		Perlite		P-Perlite 1-s	0.039	0.039	0	0	0	0	0
				P-Perlite 3-s	0.036	0.036	0	0	0	0	0

Abbreviations in the replicate names column; R: ryegrass, RR: rice husk biochar and ryegrass, MR: mixed wood biochar and ryegrass, T: turnip, RT: rice husk biochar and turnip, MT: mixed wood biochar and turnip, SS: spiked soil, PE: polyethylene passive sampler, EW: earthworm, NS: non-spiked soil, P: potting (control)

Appendix IV – Statistical analysis

Appendix IVa: Statistical analysis on mass loss data of earthworms during trial (%)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
0% biochar vs. 1% rice husk	27.1	2.577 to 51.62	Yes	0.0235
0% biochar vs. 4% rice husk	17.3	-7.223 to 41.82	No	0.3025
0% biochar vs. 1% mixed wood	9.5	-15.02 to 34.02	No	0.8717
0% biochar vs. 4% mixed wood	2.1	-22.42 to 26.62	No	>0.9999
0% biochar vs. Spiked soil	32.5	6.651 to 58.35	Yes	0.0074
0% biochar vs. Non-spiked soil	28.5	2.651 to 54.35	Yes	0.0239
1% rice husk vs. 4% rice husk	-9.8	-32.92 to 13.32	No	0.8187
1% rice husk vs. 1% mixed wood	-17.6	-40.72 to 5.52	No	0.2256
4% rice husk vs. 4% mixed wood	-15.2	-38.32 to 7.92	No	0.3807
1% mixed wood vs. 4% mixed wood	-7.4	-30.52 to 15.72	No	0.9434
Spiked soil vs. Non-spiked soil	-4	-29.85 to 21.85	No	0.9987

Apendix IVb: Statistical analysis on ryegrass shoots dry mass data (g)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
0% biochar vs. 1% rice husk	-0.1265	-0.3051 to 0.0521	No	0.296
0% biochar vs. 4% rice husk	-0.102	-0.2704 to 0.06638	No	0.4722
0% biochar vs. 1% mixed wood	-0.118	-0.2864 to 0.05038	No	0.3073
0% biochar vs. 4% mixed wood	-0.164	-0.3426 to 0.0146	No	0.0867
0% biochar vs. Spiked soil	-0.049	-0.2276 to 0.1296	No	0.9721
0% biochar vs. Non-spiked soil	-0.4015	-0.5801 to -0.2229	Yes	<0.0001
1% rice husk vs. 4% rice husk	0.0245	-0.1541 to 0.2031	No	0.9993
1% rice husk vs. 1% mixed wood	0.0085	-0.1701 to 0.1871	No	>0.9999
4% rice husk vs. 4% mixed wood	-0.062	-0.2406 to 0.1166	No	0.9173
1% mixed wood vs. 4% mixed wood	-0.046	-0.2246 to 0.1326	No	0.9796
Spiked soil vs. Non-spiked soil	-0.3525	-0.5408 to -0.1642	Yes	<0.0001

Appendix IVc: Statistical analysis on turnip dry mass data (g)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
0% biochar vs. 1% rice husk	0.2998	-0.1456 to 0.7452	No	0.3455
0% biochar vs. 4% rice husk	0.1465	-0.2392 to 0.5322	No	0.8752
0% biochar vs. 1% mixed wood	-0.04	-0.4257 to 0.3457	No	0.9999
0% biochar vs. 4% mixed wood	-0.921	-1.33 to -0.5119	Yes	<0.0001
0% biochar vs. Spiked soil	0.1265	-0.2826 to 0.5356	No	0.9485
0% biochar vs. Non-spiked soil	0.3607	-0.08472 to 0.8061	No	0.1675
1% rice husk vs. 4% rice husk	-0.1533	-0.5987 to 0.2921	No	0.917
1% rice husk vs. 1% mixed wood	-0.3398	-0.7852 to 0.1056	No	0.2182
4% rice husk vs. 4% mixed wood	-1.068	-1.477 to -0.6584	Yes	<0.0001
1% mixed wood vs. 4% mixed wood	-0.881	-1.29 to -0.4719	Yes	<0.0001
Spiked soil vs. Non-spiked soil	0.2342	-0.2316 to 0.7	No	0.6675

Appendix IVd: Statistical analysis on PCB concentration (μg/g lipid mass) data from earthworm samples

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
Earthworm PCB-28				
0% biochar vs. 1% rice husk	8.178	5.749 to 10.61	Yes	<0.0001
0% biochar vs. 4% rice husk	7.901	5.36 to 10.44	Yes	<0.0001
0% biochar vs. 1% mixed wood	5.583	3.042 to 8.124	Yes	<0.0001
0% biochar vs. 4% mixed wood	5.703	3.162 to 8.244	Yes	<0.0001
1% rice husk vs. 4% rice husk	-0.2772	-2.509 to 1.955	No	0.9949
1% rice husk vs. 1% mixed wood	-2.595	-4.827 to -0.3631	Yes	0.0192
4% rice husk vs. 4% mixed wood	-2.198	-4.551 to 0.1546	No	0.0726
1% mixed wood vs. 4% mixed wood	0.1198	-2.233 to 2.472	No	0.9998
Earthworm PCB-52				
0% biochar vs. 1% rice husk	10.26	6.537 to 13.98	Yes	<0.0001
0% biochar vs. 4% rice husk	9.881	5.986 to 13.77	Yes	<0.0001
0% biochar vs. 1% mixed wood	7.005	3.111 to 10.9	Yes	0.0005
0% biochar vs. 4% mixed wood	5.651	1.757 to 9.545	Yes	0.0034
1% rice husk vs. 4% rice husk	-0.3798	-3.8 to 3.041	No	0.9967
1% rice husk vs. 1% mixed wood	-3.255	-6.676 to 0.1649	No	0.0658
4% rice husk vs. 4% mixed wood	-4.23	-7.835 to -0.6244	Yes	0.0181
1% mixed wood vs. 4% mixed wood	-1.354	-4.959 to 2.251	No	0.7731
Earthworm PCB-101				
0% biochar vs. 1% rice husk	8.32	4.864 to 11.78	Yes	<0.0001
0% biochar vs. 4% rice husk	7.574	3.959 to 11.19	Yes	<0.0001
0% biochar vs. 1% mixed wood	5.574	1.959 to 9.189	Yes	0.002
0% biochar vs. 4% mixed wood	3.372	-0.2428 to 6.987	No	0.0732
1% rice husk vs. 4% rice husk	-0.7464	-3.921 to 2.428	No	0.9471
1% rice husk vs. 1% mixed wood	-2.746	-5.921 to 0.4291	No	0.1065
4% rice husk vs. 4% mixed wood	-4.202	-7.548 to -0.8551	Yes	0.0111
1% mixed wood vs. 4% mixed wood	-2.202	-5.549 to 1.144	No	0.2982
Earthworm PCB-118				
0% biochar vs. 1% rice husk	5.452	3.042 to 7.862	Yes	<0.0001
0% biochar vs. 4% rice husk	5.18	2.66 to 7.7	Yes	0.0001
0% biochar vs. 1% mixed wood	3.345	0.8242 to 5.865	Yes	0.0072
0% biochar vs. 4% mixed wood	2.409	-0.1114 to 4.929	No	0.0643
1% rice husk vs. 4% rice husk	-0.2719	-2.486 to 1.942	No	0.9951
1% rice husk vs. 1% mixed wood	-2.107	-4.321 to 0.1064	No	0.0658
4% rice husk vs. 4% mixed wood	-2.771	-5.104 to -0.4375	Yes	0.0166
1% mixed wood vs. 4% mixed wood	-0.9356	-3.269 to 1.398	No	0.7304
Earthworm PCB-138				
0% biochar vs. 1% rice husk	8.529	4.18 to 12.88	Yes	0.0002
0% biochar vs. 4% rice husk	7.512	2.963 to 12.06	Yes	0.0011
0% biochar vs. 1% mixed wood	6.182	1.633 to 10.73	Yes	0.0059
0% biochar vs. 4% mixed wood	2.792	-1.757 to 7.341	No	0.361
1% rice husk vs. 4% rice husk	-1.018	-5.013 to 2.978	No	0.9306
1% rice husk vs. 1% mixed wood	-2.347	-6.343 to 1.648	No	0.4017
4% rice husk vs. 4% mixed wood	-4.72	-8.932 to -0.5084	Yes	0.0247
1% mixed wood vs. 4% mixed wood	-3.391	-7.602 to 0.821	No	0.1463
Earthworm PCB-153				
0% biochar vs. 1% rice husk	7.651	3.879 to 11.42	Yes	0.0001
0% biochar vs. 4% rice husk	6.625	2.68 to 10.57	Yes	0.0009
0% biochar vs. 1% mixed wood	5.425	1.481 to 9.369	Yes	0.0054
0% biochar vs. 4% mixed wood	2.331	-1.613 to 6.275	No	0.3959
1% rice husk vs. 4% rice husk	-1.026	-4.49 to 2.438	No	0.8868
1% rice husk vs. 1% mixed wood	-2.226	-5.69 to 1.238	No	0.3192
4% rice husk vs. 4% mixed wood	-4.293	-7.945 to -0.6419	Yes	0.0178
1% mixed wood vs. 4% mixed wood	-3.094	-6.745 to 0.5578	No	0.1171
Earthworm PCB-180				
0% biochar vs. 1% rice husk	1.366	0.5212 to 2.211	Yes	0.0013
0% biochar vs. 4% rice husk	1.081	0.198 to 1.965	Yes	0.0133
0% biochar vs. 1% mixed wood	1.087	0.2036 to 1.97	Yes	0.0128
0% biochar vs. 4% mixed wood	0.402	-0.4814 to 1.285	No	0.6339
1% rice husk vs. 4% rice husk	-0.2845	-1.06 to 0.4914	No	0.7875
1% rice husk vs. 1% mixed wood	-0.2789	-1.055 to 0.497	No	0.7989
4% rice husk vs. 4% mixed wood	-0.6794	-1.497 to 0.1385	No	0.1279
1% mixed wood vs. 4% mixed wood	-0.685	-1.503 to 0.1329	No	0.1233

Appendix IVe: Statistical analysis on PCB concentration (μg/g) data from ryegrass shoot samples

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
Ryegrass PCB-28				
0% biochar vs. 1% rice husk	0.05165	-0.06411 to 0.1674	No	0.6154
0% biochar vs. 4% rice husk	0.0195	-0.1042 to 0.1432	No	0.9846
0% biochar vs. 1% mixed wood	0.01147	-0.1269 to 0.1498	No	0.9987
0% biochar vs. 4% mixed wood	0.03488	-0.08088 to 0.1506	No	0.8612
1% rice husk vs. 4% rice husk	-0.03215	-0.1479 to 0.0836	No	0.8917
1% rice husk vs. 1% mixed wood	-0.04018	-0.1714 to 0.09107	No	0.8544
4% rice husk vs. 4% mixed wood	0.01538	-0.1004 to 0.1311	No	0.9919
1% mixed wood vs. 4% mixed wood	0.02341	-0.1078 to 0.1547	No	0.9758
Ryegrass PCB-52				
0% biochar vs. 1% rice husk	0.1238	-0.09069 to 0.3383	No	0.3872
0% biochar vs. 4% rice husk	0.08217	-0.1471 to 0.3115	No	0.7733
0% biochar vs. 1% mixed wood	-0.04582	-0.3022 to 0.2105	No	0.9757
0% biochar vs. 4% mixed wood	0.0695	-0.145 to 0.284	No	0.8283
1% rice husk vs. 4% rice husk	-0.04162	-0.2561 to 0.1729	No	0.9674
1% rice husk vs. 1% mixed wood	-0.1696	-0.4128 to 0.0736	No	0.2298
4% rice husk vs. 4% mixed wood	-0.01267	-0.2272 to 0.2018	No	0.9997
1% mixed wood vs. 4% mixed wood	0.1153	-0.1279 to 0.3585	No	0.5638
Ryegrass PCB-101				
0% biochar vs. 1% rice husk	-0.02857	-0.08741 to 0.03026	No	0.5428
0% biochar vs. 4% rice husk	-0.01353	-0.07643 to 0.04937	No	0.9532
0% biochar vs. 1% mixed wood	-0.0364	-0.1067 to 0.03393	No	0.4859
0% biochar vs. 4% mixed wood	-0.03098	-0.08982 to 0.02786	No	0.4703
1% rice husk vs. 4% rice husk	0.01504	-0.04379 to 0.07388	No	0.9168
1% rice husk vs. 1% mixed wood	-0.007822	-0.07454 to 0.05889	No	0.9949
4% rice husk vs. 4% mixed wood	-0.01745	-0.07628 to 0.04139	No	0.8676
1% mixed wood vs. 4% mixed wood	0.005417	-0.0613 to 0.07213	No	0.9988
Ryegrass PCB-118				
0% biochar vs. 1% rice husk	-0.0467	-0.1206 to 0.02717	No	0.3077
0% biochar vs. 4% rice husk	-0.04601	-0.125 to 0.03295	No	0.3787
0% biochar vs. 1% mixed wood	-0.0811	-0.1694 to 0.007189	No	0.0766
0% biochar vs. 4% mixed wood	-0.06284	-0.1367 to 0.01103	No	0.1086
1% rice husk vs. 4% rice husk	0.0006885	-0.07318 to 0.07456	No	>0.9999
1% rice husk vs. 1% mixed wood	-0.0344	-0.1182 to 0.04936	No	0.6809
4% rice husk vs. 4% mixed wood	-0.01683	-0.0907 to 0.05704	No	0.9432
1% mixed wood vs. 4% mixed wood	0.01826	-0.0655 to 0.102	No	0.9511
Ryegrass PCB-138				
0% biochar vs. 1% rice husk	-0.02797	-0.1475 to 0.0916	No	0.9379
0% biochar vs. 4% rice husk	-0.02889	-0.1567 to 0.09893	No	0.9447
0% biochar vs. 1% mixed wood	-0.03633	-0.1792 to 0.1066	No	0.9183
0% biochar vs. 4% mixed wood	-0.01659	-0.1362 to 0.103	No	0.9904
1% rice husk vs. 4% rice husk	-0.0009188	-0.1205 to 0.1186	No	>0.9999
1% rice husk vs. 1% mixed wood	-0.008362	-0.1439 to 0.1272	No	0.9996
4% rice husk vs. 4% mixed wood	0.0123	-0.1073 to 0.1319	No	0.9969
1% mixed wood vs. 4% mixed wood	0.01974	-0.1158 to 0.1553	No	0.9885
Ryegrass PCB-153				
0% biochar vs. 1% rice husk	0.00972	-0.05953 to 0.07897	No	0.9892
0% biochar vs. 4% rice husk	0.0191	-0.05389 to 0.0921	No	0.9047
0% biochar vs. 1% mixed wood	-0.03436	-0.1143 to 0.04561	No	0.6332
0% biochar vs. 4% mixed wood	0.02525	-0.044 to 0.0945	No	0.7518
1% rice husk vs. 4% rice husk	0.009381	-0.05169 to 0.07045	No	0.9849
1% rice husk vs. 1% mixed wood	-0.04408	-0.1133 to 0.02517	No	0.2932
4% rice husk vs. 4% mixed wood	0.006148	-0.05492 to 0.06722	No	0.9969
1% mixed wood vs. 4% mixed wood	0.0596	-0.009644 to 0.1289	No	0.1013
Ryegrass PCB-180				
0% biochar vs. 1% rice husk	-0.02085	-0.05897 to 0.01727	No	0.4356
0% biochar vs. 4% rice husk	-0.01366	-0.05441 to 0.02709	No	0.811
0% biochar vs. 1% mixed wood	-0.03136	-0.07693 to 0.0142	No	0.2395
0% biochar vs. 4% mixed wood	-0.005791	-0.04391 to 0.03233	No	0.9866
1% rice husk vs. 4% rice husk	0.007193	-0.03093 to 0.04531	No	0.9704
1% rice husk vs. 1% mixed wood	-0.01051	-0.05373 to 0.03271	No	0.9293
4% rice husk vs. 4% mixed wood	0.00787	-0.03025 to 0.04599	No	0.9595
1% mixed wood vs. 4% mixed wood	0.02557	-0.01765 to 0.0688	No	0.3651

Appendix IVf: Statistical analysis on PCB concentration (μg/g) data from turnip samples

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
Turnip PCB-28				
0% biochar vs. 1% rice husk	-0.1236	-0.214 to -0.03331	Yes	0.0078
0% biochar vs. 4% rice husk	0.03051	-0.08777 to 0.1488	No	0.9089
0% biochar vs. 1% mixed wood	-0.006955	-0.07939 to 0.06548	No	0.9975
0% biochar vs. 4% mixed wood	0.02716	-0.05169 to 0.106	No	0.7862
1% rice husk vs. 4% rice husk	0.1542	0.02191 to 0.2864	Yes	0.0215
1% rice husk vs. 1% mixed wood	0.1167	0.02318 to 0.2102	Yes	0.0142
4% rice husk vs. 4% mixed wood	-0.003351	-0.128 to 0.1213	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.03411	-0.04835 to 0.1166	No	0.6629
Turnip PCB-52				
0% biochar vs. 1% rice husk	-0.08625	-0.2245 to 0.05202	No	0.3097
0% biochar vs. 4% rice husk	0.0679	-0.1131 to 0.2489	No	0.7333
0% biochar vs. 1% mixed wood	-0.0238	-0.1347 to 0.08706	No	0.9503
0% biochar vs. 4% mixed wood	0.04804	-0.07266 to 0.1687	No	0.6918
1% rice husk vs. 4% rice husk	0.1542	-0.04825 to 0.3566	No	0.1649
1% rice husk vs. 1% mixed wood	0.06245	-0.08067 to 0.2056	No	0.6207
4% rice husk vs. 4% mixed wood	-0.01987	-0.2107 to 0.171	No	0.9965
1% mixed wood vs. 4% mixed wood	0.07184	-0.05439 to 0.1981	No	0.3885
Turnip PCB-101				
0% biochar vs. 1% rice husk	-0.0101	-0.1372 to 0.117	No	0.9988
0% biochar vs. 4% rice husk	-0.01238	-0.1788 to 0.154	No	0.9991
0% biochar vs. 1% mixed wood	-0.01036	-0.1123 to 0.09155	No	0.9968
0% biochar vs. 4% mixed wood	-0.003036	-0.114 to 0.1079	No	>0.9999
1% rice husk vs. 4% rice husk	-0.002285	-0.1883 to 0.1838	No	>0.9999
1% rice husk vs. 1% mixed wood	-0.0002592	-0.1318 to 0.1313	No	>0.9999
4% rice husk vs. 4% mixed wood	0.009348	-0.1661 to 0.1848	No	0.9997
1% mixed wood vs. 4% mixed wood	0.007322	-0.1087 to 0.1233	No	0.9995
Turnip PCB-118				
0% biochar vs. 1% rice husk	-0.09198	-0.1776 to -0.006315	Yes	0.0343
0% biochar vs. 4% rice husk	-0.01016	-0.1223 to 0.102	No	0.998
0% biochar vs. 1% mixed wood	-0.002716	-0.0714 to 0.06596	No	>0.9999
0% biochar vs. 4% mixed wood	0.0087	-0.06607 to 0.08347	No	0.9947
1% rice husk vs. 4% rice husk	0.08181	-0.04358 to 0.2072	No	0.2733
1% rice husk vs. 1% mixed wood	0.08926	0.0005919 to 0.1779	Yes	0.0483
4% rice husk vs. 4% mixed wood	0.01886	-0.09936 to 0.1371	No	0.9826
1% mixed wood vs. 4% mixed wood	0.01142	-0.06678 to 0.08961	No	0.9875
Turnip PCB-138				
0% biochar vs. 1% rice husk	-0.01416	-0.1583 to 0.1299	No	0.9972
0% biochar vs. 4% rice husk	0.01424	-0.1744 to 0.2029	No	0.999
0% biochar vs. 1% mixed wood	0.01459	-0.1009 to 0.1301	No	0.9927
0% biochar vs. 4% mixed wood	0.02949	-0.0963 to 0.1553	No	0.9332
1% rice husk vs. 4% rice husk	0.0284	-0.1825 to 0.2394	No	0.9908
1% rice husk vs. 1% mixed wood	0.02876	-0.1204 to 0.1779	No	0.9658
4% rice husk vs. 4% mixed wood	0.01525	-0.1836 to 0.2141	No	0.9989
1% mixed wood vs. 4% mixed wood	0.01489	-0.1167 to 0.1464	No	0.9952
Turnip PCB-153				
0% biochar vs. 1% rice husk	-0.04467	-0.0952 to 0.005863	No	0.0901
0% biochar vs. 4% rice husk	0.009104	-0.05706 to 0.07527	No	0.99
0% biochar vs. 1% mixed wood	0.005048	-0.03547 to 0.04556	No	0.9931
0% biochar vs. 4% mixed wood	0.02064	-0.02347 to 0.06475	No	0.562
1% rice husk vs. 4% rice husk	0.05377	-0.0202 to 0.1277	No	0.1944
1% rice husk vs. 1% mixed wood	0.04972	-0.002589 to 0.102	No	0.0644
4% rice husk vs. 4% mixed wood	0.01154	-0.0582 to 0.08128	No	0.9802
1% mixed wood vs. 4% mixed wood	0.01559	-0.03054 to 0.06172	No	0.7968
Turnip PCB-180				
0% biochar vs. 1% rice husk	0.006042	-0.02702 to 0.03911	No	0.9717
0% biochar vs. 4% rice husk	-0.01933	-0.06262 to 0.02397	No	0.602
0% biochar vs. 1% mixed wood	-0.009046	-0.03556 to 0.01747	No	0.7915
0% biochar vs. 4% mixed wood	-0.001284	-0.03015 to 0.02758	No	0.9999
1% rice husk vs. 4% rice husk	-0.02537	-0.07377 to 0.02304	No	0.462
1% rice husk vs. 1% mixed wood	-0.01509	-0.04932 to 0.01914	No	0.6124
4% rice husk vs. 4% mixed wood	0.01804	-0.02759 to 0.06368	No	0.6966
1% mixed wood vs. 4% mixed wood	0.007763	-0.02242 to 0.03795	No	0.9098

Appendix IVg: Statistical analysis on PCB concentration (µg/g) data from PE passive samplers from ryegrass pots

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
PE ryegrass PCB-28				
0% biochar vs. 1% rice husk	0.6536	-0.2668 to 1.574	No	0.2103
0% biochar vs. 4% rice husk	0.8199	-0.2237 to 1.864	No	0.1468
0% biochar vs. 1% mixed wood	0.2511	-0.601 to 1.103	No	0.8627
0% biochar vs. 4% mixed wood	0.6686	-0.3751 to 1.712	No	0.2879
1% rice husk vs. 4% rice husk	0.1663	-0.9338 to 1.266	No	0.9858
1% rice husk vs. 1% mixed wood	-0.4025	-1.323 to 0.5179	No	0.6189
4% rice husk vs. 4% mixed wood	-0.1513	-1.356 to 1.054	No	0.9929
1% mixed wood vs. 4% mixed wood	0.4175	-0.6262 to 1.461	No	0.6881
PE ryegrass PCB-52				
0% biochar vs. 1% rice husk	0.4447	-0.819 to 1.708	No	0.7737
0% biochar vs. 4% rice husk	0.8277	-0.6052 to 2.261	No	0.3755
0% biochar vs. 1% mixed wood	-0.3907	-1.561 to 0.7793	No	0.8035
0% biochar vs. 4% mixed wood	0.218	-1.215 to 1.651	No	0.9854
1% rice husk vs. 4% rice husk	0.383	-1.127 to 1.893	No	0.9137
1% rice husk vs. 1% mixed wood	-0.8353	-2.099 to 0.4283	No	0.2631
4% rice husk vs. 4% mixed wood	-0.6097	-2.264 to 1.045	No	0.745
1% mixed wood vs. 4% mixed wood	0.6087	-0.8242 to 2.042	No	0.6422
PE ryegrass PCB-101				
0% biochar vs. 1% rice husk	0.699	-0.0371 to 1.435	No	0.0647
0% biochar vs. 4% rice husk	0.9012	0.06657 to 1.736	Yes	0.0333
0% biochar vs. 1% mixed wood	0.1884	-0.4931 to 0.8699	No	0.8868
0% biochar vs. 4% mixed wood	0.4723	-0.3623 to 1.307	No	0.3935
1% rice husk vs. 4% rice husk	0.2022	-0.6776 to 1.082	No	0.9374
1% rice husk vs. 1% mixed wood	-0.5106	-1.247 to 0.2255	No	0.227
4% rice husk vs. 4% mixed wood	-0.4289	-1.393 to 0.5349	No	0.6046
1% mixed wood vs. 4% mixed wood	0.2839	-0.5507 to 1.119	No	0.7933
PE ryegrass PCB-118				
0% biochar vs. 1% rice husk	0.209	-0.232 to 0.65	No	0.5512
0% biochar vs. 4% rice husk	0.3774	-0.1226 to 0.8775	No	0.1704
0% biochar vs. 1% mixed wood	0.04385	-0.3644 to 0.4521	No	0.9961
0% biochar vs. 4% mixed wood	0.2241	-0.2759 to 0.7242	No	0.5986
1% rice husk vs. 4% rice husk	0.1684	-0.3587 to 0.6955	No	0.8262
1% rice husk vs. 1% mixed wood	-0.1651	-0.6061 to 0.2759	No	0.7344
4% rice husk vs. 4% mixed wood	-0.1533	-0.7307 to 0.4241	No	0.9002
1% mixed wood vs. 4% mixed wood	0.1803	-0.3198 to 0.6803	No	0.7589
PE ryegrass PCB-138				
0% biochar vs. 1% rice husk	0.3546	-0.2784 to 0.9875	No	0.4025
0% biochar vs. 4% rice husk	0.5594	-0.1583 to 1.277	No	0.1513
0% biochar vs. 1% mixed wood	-0.1625	-0.7485 to 0.4234	No	0.8856
0% biochar vs. 4% mixed wood	0.125	-0.5927 to 0.8427	No	0.9761
1% rice husk vs. 4% rice husk	0.2048	-0.5517 to 0.9613	No	0.8939
1% rice husk vs. 1% mixed wood	-0.5171	-1.15 to 0.1158	No	0.1259
4% rice husk vs. 4% mixed wood	-0.4344	-1.263 to 0.3943	No	0.4619
1% mixed wood vs. 4% mixed wood	0.2876	-0.4301 to 1.005	No	0.6869
PE ryegrass PCB-153				
0% biochar vs. 1% rice husk	0.1502	-0.23 to 0.5304	No	0.6974
0% biochar vs. 4% rice husk	0.466	0.03483 to 0.8971	Yes	0.0331
0% biochar vs. 1% mixed wood	0.07295	-0.2791 to 0.425	No	0.956
0% biochar vs. 4% mixed wood	0.03159	-0.3995 to 0.4627	No	0.9991
1% rice husk vs. 4% rice husk	0.3158	-0.1387 to 0.7702	No	0.2257
1% rice husk vs. 1% mixed wood	-0.07722	-0.4574 to 0.303	No	0.9589
4% rice husk vs. 4% mixed wood	-0.4344	-0.9322 to 0.06345	No	0.0955
1% mixed wood vs. 4% mixed wood	-0.04136	-0.4725 to 0.3898	No	0.9975
PE ryegrass PCB-180				
0% biochar vs. 1% rice husk	0.2945	0.01139 to 0.5777	Yes	0.0407
0% biochar vs. 4% rice husk	0.2799	-0.0412 to 0.6009	No	0.0959
0% biochar vs. 1% mixed wood	-0.03283	-0.295 to 0.2293	No	0.993
0% biochar vs. 4% mixed wood	0.1608	-0.1602 to 0.4819	No	0.5025
1% rice husk vs. 4% rice husk	-0.01468	-0.3531 to 0.3238	No	0.9999
1% rice husk vs. 1% mixed wood	-0.3274	-0.6105 to -0.04422	Yes	0.0225
4% rice husk vs. 4% mixed wood	-0.119	-0.4898 to 0.2517	No	0.8238
1% mixed wood vs. 4% mixed wood	0.1937	-0.1274 to 0.5147	No	0.338

Appendix IVh: Statistical analysis on PCB concentration (μg/g) data from PE passive samplers from turnip pots

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
PE turnip PCB-28				
0% biochar vs. 1% rice husk	1.024	0.2104 to 1.837	Yes	0.0165
0% biochar vs. 4% rice husk	0.9087	0.1213 to 1.696	Yes	0.0256
0% biochar vs. 1% mixed wood	0.2235	-0.7725 to 1.219	No	0.9217
0% biochar vs. 4% mixed wood	0.9173	0.104 to 1.731	Yes	0.0286
1% rice husk vs. 4% rice husk	-0.1149	-0.6528 to 0.423	No	0.9332
1% rice husk vs. 1% mixed wood	-0.8001	-1.613 to 0.01311	No	0.0537
4% rice husk vs. 4% mixed wood	0.008571	-0.5293 to 0.5465	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.6938	-0.1195 to 1.507	No	0.0968
PE turnip PCB-52				
0% biochar vs. 1% rice husk	1.335	-0.04599 to 2.717	No	0.058
0% biochar vs. 4% rice husk	1.22	-0.117 to 2.558	No	0.074
0% biochar vs. 1% mixed wood	0.1941	-1.498 to 1.886	No	0.9927
0% biochar vs. 4% mixed wood	1.003	-0.378 to 2.385	No	0.1717
1% rice husk vs. 4% rice husk	-0.1149	-1.029 to 0.7987	No	0.9897
1% rice husk vs. 1% mixed wood	-1.141	-2.523 to 0.2401	No	0.1094
4% rice husk vs. 4% mixed wood	-0.2171	-1.131 to 0.6966	No	0.9063
1% mixed wood vs. 4% mixed wood	0.8092	-0.5721 to 2.191	No	0.3168
PE turnip PCB-101				
0% biochar vs. 1% rice husk	0.704	-0.5153 to 1.923	No	0.3282
0% biochar vs. 4% rice husk	0.9087	-0.2719 to 2.089	No	0.1413
0% biochar vs. 1% mixed wood	-0.1176	-1.611 to 1.376	No	0.9983
0% biochar vs. 4% mixed wood	0.3647	-0.8546 to 1.584	No	0.8162
1% rice husk vs. 4% rice husk	0.2047	-0.6018 to 1.011	No	0.8852
1% rice husk vs. 1% mixed wood	-0.8216	-2.041 to 0.3977	No	0.217
4% rice husk vs. 4% mixed wood	-0.544	-1.351 to 0.2625	No	0.2163
1% mixed wood vs. 4% mixed wood	0.4823	-0.737 to 1.702	No	0.6381
PE turnip PCB-118				
0% biochar vs. 1% rice husk	0.3077	0.1978 to 0.4175	Yes	0.0003
0% biochar vs. 4% rice husk	0.2858	0.1759 to 0.3956	Yes	0.0004
0% biochar vs. 1% mixed wood	-0.05956	-0.1941 to 0.07498	No	0.5157
0% biochar vs. 4% mixed wood	0.3003	0.1904 to 0.4101	Yes	0.0003
1% rice husk vs. 4% rice husk	-0.02191	-0.09959 to 0.05576	No	0.821
1% rice husk vs. 1% mixed wood	-0.3672	-0.4771 to -0.2574	Yes	<0.0001
4% rice husk vs. 4% mixed wood	0.01451	-0.06317 to 0.09219	No	0.949
1% mixed wood vs. 4% mixed wood	0.3598	0.25 to 0.4697	Yes	0.0001
PE turnip PCB-138				
0% biochar vs. 1% rice husk	0.3973	-0.4749 to 1.27	No	0.5247
0% biochar vs. 4% rice husk	0.6046	-0.2399 to 1.449	No	0.1799
0% biochar vs. 1% mixed wood	-0.08935	-1.158 to 0.9789	No	0.9978
0% biochar vs. 4% mixed wood	0.2822	-0.59 to 1.154	No	0.7738
1% rice husk vs. 4% rice husk	0.2073	-0.3696 to 0.7842	No	0.7075
1% rice husk vs. 1% mixed wood	-0.4867	-1.359 to 0.3856	No	0.3554
4% rice husk vs. 4% mixed wood	-0.3224	-0.8994 to 0.2545	No	0.3541
1% mixed wood vs. 4% mixed wood	0.3716	-0.5007 to 1.244	No	0.5798
PE turnip PCB-153				
0% biochar vs. 1% rice husk	0.1947	-0.3998 to 0.7891	No	0.7667
0% biochar vs. 4% rice husk	0.2889	-0.2866 to 0.8645	No	0.4428
0% biochar vs. 1% mixed wood	-0.05956	-0.7876 to 0.6684	No	0.998
0% biochar vs. 4% mixed wood	-0.1361	-0.7305 to 0.4583	No	0.9166
1% rice husk vs. 4% rice husk	0.09429	-0.2989 to 0.4875	No	0.9035
1% rice husk vs. 1% mixed wood	-0.2542	-0.8486 to 0.3402	No	0.5766
4% rice husk vs. 4% mixed wood	-0.425	-0.8182 to -0.03184	Yes	0.035
1% mixed wood vs. 4% mixed wood	-0.0765	-0.6709 to 0.5179	No	0.9887
PE turnip PCB-180				
0% biochar vs. 1% rice husk	-0.008013	-0.3927 to 0.3767	No	>0.9999
0% biochar vs. 4% rice husk	-0.02675	-0.3992 to 0.3457	No	0.9988
0% biochar vs. 1% mixed wood	-0.02978	-0.5009 to 0.4414	No	0.9993
0% biochar vs. 4% mixed wood	-0.1206	-0.5053 to 0.264	No	0.7913
1% rice husk vs. 4% rice husk	-0.01874	-0.2732 to 0.2357	No	0.9987
1% rice husk vs. 1% mixed wood	-0.02177	-0.4065 to 0.3629	No	0.9995
4% rice husk vs. 4% mixed wood	-0.0939	-0.3483 to 0.1606	No	0.6891
1% mixed wood vs. 4% mixed wood	-0.09087	-0.4756 to 0.2938	No	0.9079

Appendix IVi: Statistical analysis on PCB concentration (µg/g) data from soil samples from ryegrass pots

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
Soil ryegrass PCB-28				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.01142	-0.1272 to 0.15	No	0.9986
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.06401	-0.05698 to 0.185	No	0.4536
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.02943	-0.09155 to 0.1504	No	0.9246
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.06401	-0.0746 to 0.2026	No	0.5734
1% rice husk vs. 4% rice husk	0.05259	-0.09864 to 0.2038	No	0.7806
1% rice husk vs. 1% mixed wood	0.01802	-0.1332 to 0.1692	No	0.9942
4% rice husk vs. 4% mixed wood	0	-0.1512 to 0.1512	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.03458	-0.1167 to 0.1858	No	0.9385
Soil ryegrass PCB-52				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.02047	-0.1529 to 0.1938	No	0.9944
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.07803	-0.07327 to 0.2293	No	0.4764
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.04187	-0.1094 to 0.1932	No	0.8865
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.08052	-0.09281 to 0.2539	No	0.5683
1% rice husk vs. 4% rice husk	0.05756	-0.1316 to 0.2467	No	0.8489
1% rice husk vs. 1% mixed wood	0.02139	-0.1677 to 0.2105	No	0.9952
4% rice husk vs. 4% mixed wood	0.002493	-0.1866 to 0.1916	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.03866	-0.1505 to 0.2278	No	0.958
Soil ryegrass PCB-101				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.006366	-0.1368 to 0.1495	No	0.9999
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.07398	-0.05099 to 0.199	No	0.3541
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.02435	-0.1006 to 0.1493	No	0.9644
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.07151	-0.07166 to 0.2147	No	0.505
1% rice husk vs. 4% rice husk	0.06762	-0.0886 to 0.2238	No	0.6272
1% rice husk vs. 1% mixed wood	0.01799	-0.1382 to 0.1742	No	0.9949
4% rice husk vs. 4% mixed wood	-0.002472	-0.1587 to 0.1537	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.04716	-0.1091 to 0.2034	No	0.8525
Soil ryegrass PCB-118				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.002963	-0.08325 to 0.08918	No	>0.9999
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.04911	-0.02614 to 0.1244	No	0.2731
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.008431	-0.06682 to 0.08368	No	0.9954
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.04405	-0.04216 to 0.1303	No	0.4848
1% rice husk vs. 4% rice husk	0.04615	-0.04792 to 0.1402	No	0.5209
1% rice husk vs. 1% mixed wood	0.005468	-0.0886 to 0.09953	No	0.9996
4% rice husk vs. 4% mixed wood	-0.005062	-0.09913 to 0.089	No	0.9997
1% mixed wood vs. 4% mixed wood	0.03562	-0.05845 to 0.1297	No	0.7269
Soil ryegrass PCB-138				
0% biochar (ryegrass+turnip) vs. 1% rice husk	-0.005298	-0.1655 to 0.1549	No	>0.9999
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.08184	-0.05802 to 0.2217	No	0.3642
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.01922	-0.1206 to 0.1591	No	0.99
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.07931	-0.08092 to 0.2396	No	0.513
1% rice husk vs. 4% rice husk	0.08714	-0.08769 to 0.262	No	0.5069
1% rice husk vs. 1% mixed wood	0.02452	-0.1503 to 0.1993	No	0.9892
4% rice husk vs. 4% mixed wood	-0.002531	-0.1774 to 0.1723	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.06009	-0.1147 to 0.2349	No	0.7874
Soil ryegrass PCB-153				
0% biochar (ryegrass+turnip) vs. 1% rice husk	-0.007731	-0.1112 to 0.09573	No	0.999
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.05117	-0.03913 to 0.1415	No	0.3923
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.01497	-0.1053 to 0.07534	No	0.9801
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.02074	-0.08272 to 0.1242	No	0.9607
1% rice husk vs. 4% rice husk	0.05891	-0.05398 to 0.1718	No	0.466
1% rice husk vs. 1% mixed wood	-0.007237	-0.1201 to 0.1056	No	0.9995
4% rice husk vs. 4% mixed wood	-0.03043	-0.1433 to 0.08245	No	0.8953
1% mixed wood vs. 4% mixed wood	0.03571	-0.07718 to 0.1486	No	0.8311
Soil ryegrass PCB-180				
0% biochar (ryegrass+turnip) vs. 1% rice husk	-0.03184	-0.1104 to 0.04671	No	0.6783
0% biochar (ryegrass+turnip) vs. 4% rice husk	0.0228	-0.04577 to 0.09136	No	0.8058
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.03655	-0.1051 to 0.03201	No	0.4468
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.01106	-0.08962 to 0.06749	No	0.9891
1% rice husk vs. 4% rice husk	0.05464	-0.03107 to 0.1403	No	0.2919
1% rice husk vs. 1% mixed wood	-0.004711	-0.09042 to 0.081	No	0.9997
4% rice husk vs. 4% mixed wood	-0.03386	-0.1196 to 0.05185	No	0.6972
1% mixed wood vs. 4% mixed wood	0.02549	-0.06022 to 0.1112	No	0.8589

Appendix IVj: Statistical analysis on PCB concentration (µg/g) data from soil samples from turnip pots

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
Soil turnip PCB-28				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.06397	-0.1625 to 0.2904	No	0.8703
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.00753	-0.1585 to 0.1434	No	0.9998
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.01262	-0.1384 to 0.1636	No	0.9984
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.01078	-0.1837 to 0.1622	No	0.9995
1% rice husk vs. 4% rice husk	-0.0715	-0.3102 to 0.1672	No	0.8459
1% rice husk vs. 1% mixed wood	-0.05136	-0.2901 to 0.1873	No	0.9456
4% rice husk vs. 4% mixed wood	-0.003255	-0.192 to 0.1855	No	>0.9999
1% mixed wood vs. 4% mixed wood	-0.0234	-0.2121 to 0.1653	No	0.9925
Soil turnip PCB-52				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.07805	-0.1933 to 0.3494	No	0.8632
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.006798	-0.1877 to 0.1741	No	>0.9999
0% biochar (ryegrass+turnip) vs. 1% mixed wood	0.02007	-0.1608 to 0.2009	No	0.9951
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.001747	-0.209 to 0.2055	No	>0.9999
1% rice husk vs. 4% rice husk	-0.08484	-0.3708 to 0.2011	No	0.8502
1% rice husk vs. 1% mixed wood	-0.05797	-0.344 to 0.228	No	0.9556
4% rice husk vs. 4% mixed wood	0.005052	-0.221 to 0.2311	No	>0.9999
1% mixed wood vs. 4% mixed wood	-0.02182	-0.2479 to 0.2043	No	0.9971
Soil turnip PCB-101				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.07395	-0.1299 to 0.2778	No	0.7412
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.004199	-0.1401 to 0.1317	No	>0.9999
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.0006342	-0.1365 to 0.1352	No	>0.9999
0% biochar (ryegrass+turnip) vs. 4% mixed wood	0.001657	-0.154 to 0.1573	No	>0.9999
1% rice husk vs. 4% rice husk	-0.07815	-0.293 to 0.1367	No	0.7396
1% rice husk vs. 1% mixed wood	-0.07459	-0.2894 to 0.1403	No	0.7688
4% rice husk vs. 4% mixed wood	0.005856	-0.164 to 0.1757	No	>0.9999
1% mixed wood vs. 4% mixed wood	0.002291	-0.1676 to 0.1721	No	>0.9999
Soil turnip PCB-118				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.049	-0.08106 to 0.1791	No	0.7161
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.01227	-0.09898 to 0.07444	No	0.9878
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.003611	-0.09032 to 0.08309	No	0.9999
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.0046	-0.1039 to 0.09473	No	0.9998
1% rice husk vs. 4% rice husk	-0.06127	-0.1984 to 0.07582	No	0.5852
1% rice husk vs. 1% mixed wood	-0.05261	-0.1897 to 0.08448	No	0.7032
4% rice husk vs. 4% mixed wood	0.00767	-0.1007 to 0.1161	No	0.9991
1% mixed wood vs. 4% mixed wood	-0.0009884	-0.1094 to 0.1074	No	>0.9999
Soil turnip PCB-138				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.07684	-0.1726 to 0.3262	No	0.833
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.02023	-0.1865 to 0.146	No	0.9931
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.01142	-0.1777 to 0.1548	No	0.9992
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.0176	-0.2081 to 0.1729	No	0.9976
1% rice husk vs. 4% rice husk	-0.09707	-0.36 to 0.1658	No	0.7297
1% rice husk vs. 1% mixed wood	-0.08826	-0.3511 to 0.1746	No	0.7884
4% rice husk vs. 4% mixed wood	0.002631	-0.2052 to 0.2105	No	>0.9999
1% mixed wood vs. 4% mixed wood	-0.006178	-0.214 to 0.2016	No	>0.9999
Soil turnip PCB-153				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.03607	-0.112 to 0.1842	No	0.9182
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.01009	-0.1088 to 0.08864	No	0.9964
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.008162	-0.1069 to 0.09056	No	0.9984
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.0306	-0.1437 to 0.08251	No	0.8863
1% rice husk vs. 4% rice husk	-0.04616	-0.2023 to 0.1099	No	0.8516
1% rice husk vs. 1% mixed wood	-0.04423	-0.2003 to 0.1119	No	0.8691
4% rice husk vs. 4% mixed wood	-0.02051	-0.1439 to 0.1029	No	0.978
1% mixed wood vs. 4% mixed wood	-0.02244	-0.1458 to 0.101	No	0.9696
Soil turnip PCB-180				
0% biochar (ryegrass+turnip) vs. 1% rice husk	0.004375	-0.1269 to 0.1357	No	>0.9999
0% biochar (ryegrass+turnip) vs. 4% rice husk	-0.02999	-0.1175 to 0.05756	No	0.7768
0% biochar (ryegrass+turnip) vs. 1% mixed wood	-0.02298	-0.1105 to 0.06457	No	0.8964
0% biochar (ryegrass+turnip) vs. 4% mixed wood	-0.05476	-0.1551 to 0.04554	No	0.4112
1% rice husk vs. 4% rice husk	-0.03437	-0.1728 to 0.1041	No	0.913
1% rice husk vs. 1% mixed wood	-0.02735	-0.1658 to 0.1111	No	0.9593
4% rice husk vs. 4% mixed wood	-0.02477	-0.1342 to 0.08466	No	0.9356
1% mixed wood vs. 4% mixed wood	-0.03178	-0.1412 to 0.07765	No	0.8592

Appendix V – Mass balance

Appendix V: Mass balance calculated from mass (g) of all phases and PCB concentrations (µg/g) of all phases in the pots

Soil treatment	Phases added	Batch	Total recovery (%)							
			PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	∑ PCB-7
Spiked soil, 0% biochar	Ryegrass and PE passive sampler	Control (ryegrass)	54	63	51	37	65	46	49	52 ± 10
Spiked soil, 1% rice husk biochar	Ryegrass and PE passive sampler	Amended (ryegrass)	64	76	82	56	106	84	93	80 ± 17
Spiked soil, 4% rice husk biochar			0,04	6	0,05	0,03	0,07	13	28	7 ± 10
Spiked soil, 1% mixed wood biochar			42	50	60	49	76	93	99	67 ± 22
Spiked soil, 4% mixed wood biochar			0,07	3	3	6	3	51	70	20 ± 28
Spiked soil, 0% biochar	Turnip, PE-passive sampler and earthworm	Control (turnip)	95	129	118	76	124	96	58	100 ± 26
Spiked soil, 1% rice husk biochar	Turnip, PE-passive sampler and earthworm	Amended (turnip)	0,5	8	2	1	8	33	50	14 ± 19
Spiked soil, 4% rice husk biochar			90	114	99	77	129	91	94	99 ± 17
Spiked soil, 1% mixed wood biochar			64	79	93	65	116	87	83	84 ± 18
Spiked soil, 4% mixed wood biochar			95	108	93	69	127	118	125	105 ± 21
Spiked soil	Earthworm	Unamended comparisson	27	29	112	83	151	109	111	89 ± 46
	Ryegrass		44	54	56	47	64	105	115	69 ± 29
	Turnip		56	62	70	51	95	70	76	69 ± 14
	PE passive sampler		48	57	51	37	73	52	64	55 ± 12
Spiked soil not undergone pot trial	None	Blank	34	40	70	59	84	96	105	70 ± 27

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