THE USE OF BIOCHAR AND ACTIVATED CARBON TO MINIMIZE HYDROPHOBIC ORGANIC CONTAMINANT BIOAVAILABILITY IN SOILS

L'UTILISATION DU BIOCHAR ET DU CHARBON ACTIF AFIN DE MINIMISER LA BIODISPONIBILITE DES CONTAMINANTS ORGANIQUE HYDROPHOBE DANS LES SOLS

A Thesis Submitted

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by

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ABSTRACT

The ability of biochar to sorb hydrophobic organic contaminants (HOCs) in soil was investigated and compared to activated carbon (AC). Six biochars were comprehensively characterized using 17 physical, chemical and biological protocols outlined by the International Biochar Initiative. Only one biochar, made from construction waste was found to be unacceptable for use as a soil amendment as it inhibited plant germination (40% germination rate) and caused invertebrate avoidance (70% avoidance). Using community level physiological profiling (CLPP), microbial communities were also characterized in HOC amended soils. The results suggest that biochar helps to restore microbial function in intensely degraded Brownfield soils.

Reductions in contaminant bioavailability following biochar and activated carbon (AC) application to contaminated soils were assessed using plants (*Cucurbita pepo.* spp. *pepo*) and soil invertebrates (*Eisenia fetida*). In the first *in situ* study comparing these materials directly in soils conducted at a Brownfield site contaminated with polychlorinated biphenyls (PCBs) (71- 136 µg/g), AC and two types of biochar were statistically equal at reducing PCB uptake into plants (mean reduction of 70%). Biochar also significantly increased plant growth by up to 100% in severely degraded soil. These results suggest biochar has potential as a greener (i.e. more sustainable), lower cost alternative material to AC for HOC remediation. A complementary greenhouse study that included a bioaccumulation study of *E. fetida*, found that a mechanical mixing strategy resulted in bioavailability reductions up to 66% greater than manual mixing methods due to improved soil/sorbent homogeneity as a result of smaller biochar particle sizes and reduced resistance to mass transfer.

In soil highly contaminated with dichlorodiphenyltrichloroethane (DDT = 2.5-39 µg/g), none of the carbon amendments were successful at minimizing plant uptake or improving plant growth, and AC caused adverse effects to invertebrate health. Plant and invertebrate uptake were also compared to predicted bioavailability using an equilibrium passive sampling device (polyoxymethylene (POM)-based). The bioavailable fraction predicted by the POM samplers correlated well with measured invertebrate uptake (< 50% variability), but over-predicted plant root and shoot uptake. A literature review of DDT concentrations in *C. pepo* spp. *pepo* tissue over a range of DDT soil concentrations yielded a trend of decreasing plant uptake with increasing soil concentrations, confirming that at high DDT soil concentrations (>10 µg/g) plant uptake is limited. This threshold effect limiting DDT plant uptake appeared to impair the passive sampler's ability to adequately predict bioavailability to plants.

This thesis demonstrates biochar and AC have potential to be used as an *in situ*, non-removal management strategy for HOC contamination in soils, by minimizing bioavailability. These studies illustrate the need for careful characterization of carbon amendments prior to large scale-application to soils, and that these materials should be applied in a site-specific manner. The results also highlight the importance of including plants in bioavailability studies as the use of carbon materials for *in situ* contaminant sorption expands from a predominantly sediment to soil remediation technology.

RÉSUMÉ

La capacité du biocharbon (biochar) à adsorber les contaminants organiques hydrophobes (COH) dans les sols a été examinée et comparée au charbon actif (CA). Six biochars ont été caractérisés en détail à l'aide de 17 protocoles physiques, chimiques et biologiques dictés par l'*Initiative Biochar Internationale*. Un seul biochar, composé de déchets de construction a été jugé inacceptable pour servir à amender le sol, car il entrave la germination des plantes (taux de germination de 40%) et provoque l'évitement des invertébrés (70% d'évitement). L'étude des profils physiologiques au niveau communautaire (PPNC) a permis de caractériser des communautés microbiennes dans les sols contenant des COH. Les résultats suggèrent que le biochar aide à restaurer la fonction microbienne dans les sols de friches industrielles intensément dégradés.

Les réductions de la biodisponibilité des contaminants suivant l'application du biochar et du CA aux sols contaminés ont été évaluées à l'aide de plantes (*Cucurbita pepo* spp. *pepo*) et d'invertébrés du sol (*Eisenia fetida*). Dans la première étude *in situ*, comparant directement ces sols modifiés à une friche industrielle contaminée aux biphényles polychlorés (BPC) (71- 136 µg /g), l'utilisation d'AC et de deux types de biochars a réduit de façon statistiquement égale l'absorption de BPC dans les plantes (réduction moyenne de 70%). Le biochar a également augmenté de manière significative la croissance des plantes jusqu'à 100% dans les sols intensément dégradés. Ces résultats suggèrent que le biochar présente un potentiel intéressant comme alternative écologique au CA pour l'assainissement de COH. Une étude complémentaire en serre, incluant des essais de bioaccumulation chez le *E. fetida*, a révélé que la stratégie de mélange mécanique entraîne des réductions de biodisponibilité jusqu'à 66 % de plus que les méthodes manuelles, puisqu'elle permet d'améliorer l'homogénéité sol/adsorbant en raison de la taille réduite des particules de biochar, et d'une réduction de la résistance au transfert de masse.

Dans les sols hautement contaminés par le dichlorodiphényltrichloroéthane (DDT = 2.5 à 39 µg/g), aucun des amendements de carbone n'a réussi à réduire l'absorption par les plantes, ni à améliorer la croissance végétale. De plus, le CA a causé des effets néfastes sur la santé des invertébrés. Le degré d'absorption par les végétaux et les invertébrés a aussi été comparé à la biodisponibilité prédite en utilisant un dispositif d'échantillonnage passif équilibré (à base de polyoxyméthylène (POM)). La fraction biodisponible prédite par les échantillonneurs de POM présente une forte corrélation avec l'absorption mesurée chez les invertébrés (<50 % de la variabilité), mais a surestimé l'absorption par les racines et les pouces de plantes. Une analyse de la littérature sur les concentrations de DDT mesurées dans les tissus *C. pepo* spp. *pepo* affectés par une gamme de concentrations de DDT dans le sol révèle une diminution de l'absorption par la plante lorsque les concentrations dans le sol sont plus élevées, ce qui confirme que des concentrations DDT élevées dans le sol (> 10 µg/g) constituent une limite. Cet effet de seuil-limite pour l'absorption de DDT par les plantes semble nuire à la capacité de l'échantillonneur passif à prédire adéquatement la biodisponibilité pour les plantes.

Cette thèse démontre que le biochar et le CA peuvent potentiellement être employés dans le cadre d'une stratégie sans déplacement *in situ* de gestion des COH dans les sols, afin d'en minimiser la biodisponibilité. Ces études illustrent la nécessité d'une caractérisation minutieuse des amendements au carbone dans les sols avant de procéder à une application à grande échelle, et que ces matériaux devraient être appliquées d'une manière spécifique au site. Ces résultats mettent également en évidence l'importance d'inclure les plantes dans les études de biodisponibilité puisque l'utilisation de matériaux de carbone pour l'absorption de contaminants *in situ* permet d'élargir la portée des technologies de remédiation des sédiments au sol entier.

CO-AUTHORSHIP STATEMENT

The student's contributions to this thesis manuscript are as follows:

- Participant in the initial development of research manuscripts
- Primary researcher responsible for the successful implementation and completion of experiments, including field work at PCB-and DDT-contaminated field sites in Etobicoke, ON and Leamington, ON, greenhouse studies conducted at the Royal Military College of Canada (Kingston, ON) and Queen's University (Kingston, ON), as well as analytical work completed at both the Analytical Services Unit at Queen's University and at the Royal Military College of Canada
- Primary author on all documents

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LIST OF ABBREVIATIONS

AC	Activated carbon
ANOVA	One-way analysis of variance
ASU	Analytical Services Unit
AWCD	Average well colour development
BAF	Bioaccumulation factor =[plant, worm, POM]/[soil]
BET	Brunauer-Emmett-Teller
BSAF	Biota to sediment/soil accumulation factor
CALA	Canadian Association for Laboratory Accreditation
CBRN	Chemical Biological Radio Nuclear
CCME	Canadian Council of Ministers of the Environment
CEC	Cation exchange capacity
CEPA	Canadian Environmental Protection Act
CLPP	Community Level Physiological Profiling
CO_2	Carbon dioxide
CPOM	Concentration in the polymer (POM)
CSUP	Carbon Source Utilization Patterns
Cw	Freely dissolved porewater concentration
DCBP	Decachlorobiphenyl
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDI	Double deionized
DDT	Dichlorodiphenyltrichloroethane
ECD	electron capture detector
EPA	Environmental protection agency
GAC	Granular activated carbon
HOCs	Hydrophobic organic contaminants
IBI	International Biochar Initiative
ICP-AES	Inductively coupled plasma atomic emission spectrometer
IPA	Isopropanol
$K_{POM/w}$	Equilibrium partitioning coefficients
log K_{OW}	Octanol-water partitioning coefficient
LOI	Loss on ignition
MAE	Microwave assisted extraction
OC	Organic carbon
PAC	Powdered activated carbon
PAH	polycyclic aromatic hydrocarbons
PCA	Principal components analysis
PCBs	Polychlorinated biphenyls
PCDD/PCDF	Polychlorinated dibenzodioxins/furans

POM	Polyoxymethylene
POPs	Persistent organic pollutants
PPNP	Point Pelee National Park
PSD	Particle size distribution
QA/QC	Quality assurance/ quality control
RMC	Royal Military College of Canada
SSA	Specific surface area
TATC	Transpiration adhesion tension cohesion
USEPA	United States Environmental Protection Agency

1. INTRODUCTION

Hydrophobic organic contaminants (HOCs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) are of concern to environmental and human health due to their persistence, bioaccumulation potential and toxicity. Legacy contamination of HOCs is extensive worldwide (1) and many of these compounds have been classed as endocrine disrupters and possible carcinogens (2-4). The International Stockholm Convention signed by 152 nations including Canada, classifies many HOCs including PCBs and DDT as persistent organic pollutants (POPs). The convention seeks to protect human and environmental health and requires signatories to take measures to eliminate or reduce the release of POPs into the environment (5). Furthermore, in Canada, PCBs and DDT are classified as Track 1 substances by the Canadian Environmental Protection Act (CEPA), meaning that their virtual elimination from the environment is required (4).

Traditionally, remediation of HOCs in soil was limited to techniques such as soil excavation and transport, prior to off-site treatment and/or disposal by solvent extraction, thermal alkaline dechlorination, incineration, or landfilling (6). However, these techniques themselves can be detrimental to the environment, extremely costly and in some cases are not feasible due to the extent of contamination. Successes in green remediation technologies such as phytoextraction have been realized for both PCBs and DDT (7-9). Phytoextraction is a volume reduction technology where plants are used to mobilize the contaminants from soil and accumulate them into their above ground biomass which then can be harvested and disposed of as contaminated waste (10). However in Canada today there remain concerns that the pace of elimination via phytoextraction is too slow and the extent of contamination too great. Consequently these hydrophobic contaminants continue to enter the food chain and pose risk to environmental and human health. Thus, a risk-based remediation technology that seeks to immobilize the contamination *in situ* is particularly appealing for HOCs such as PCBs and DDT.

Carbonaceous sorbent materials, specifically biochar and activated carbon (AC), have been shown to immobilize soil-borne HOCs *in situ* and thereby minimize their bioavailability (11-19). Sorption of contaminants to organic matter is a key process that controls the toxicity, transport and fate of non-polar organic compounds such as PCBs and DDT. The low water solubility of HOCs controls their phase distribution and the extent of accumulation into organics is influenced by soil properties such as sorption capacity (e.g. specific surface area and cation exchange capacity), and contact time (e.g. aging and weathering) (20, 21). The effects of HOC exposure are not directly related to absolute concentrations in soil, but rather contaminant bioavailability (1, 20)

Biochar is the carbon-rich solid by-product produced from the thermal decomposition of organic matter under low oxygen conditions (pyrolysis) (22). The production process of biochar is different from that of AC, in that AC is further 'activated' through physical or chemical treatments to maximize the porosity, and therefore has higher associated costs and a larger carbon footprint (23). Both biochar and AC have a very high affinity and capacity for sorbing organic compounds due to their high specific surface area. Studies have concluded that sorption of organic contaminants by carbonaceous materials is a result of two separate processes: i) relatively weak and linear *ab*sorption into amorphous organic matter, and ii) relatively strong and non-linear *ads*orption onto the sorbent surface (21). Research on the ability of AC to sorb HOCs predates biochar and currently AC in an accepted form of sediment remediation in the U.S. and Europe (24).

This thesis seeks to validate the use of biochar and AC to provide an effective and sustainable *in situ* remediation strategy for reducing HOC bioavailability in contaminated soils. Chapter two provides a literature review of carbon amendments, HOCs, methods for measuring HOCs bioavailability and phytoextraction. In chapter three, the first published study investigating the potential of biochar to sorb

PCBs in soil is presented. Four biochar amendment rates are investigated in two concentrations of PCB-contaminated soil for their ability to reduce bioavailability to the known phytoextractor *Cucurbita pepo* spp. *pepo* and a soil invertebrate (*Eisenia fetida*). In chapter four, the success of two biochars to reduce PCB contaminant bioavailability is compared to activated carbon *in situ* at a PCB-contaminated Brownfield site in Etobicoke, ON. This is the first study to compare these materials directly in soils *in situ* and also reports on the importance of thorough mixing in order to achieve maximum bioavailability reductions.

Chapter five focuses on biochar production materials and pyrolysis rates, and their effects on biochar physiochemical properties. These properties are linked to the ability of biochar to successfully immobilize contaminants and overall function in the environment. This chapter seeks to demonstrate that the newly released, standardized procedures outlined by the International Biochar Initiative (IBI) for characterizing biochar are both valid and practical when characterizing biochar for the remediation of contaminated sites. It also highlights the importance of investigating biochar toxicity to soil invertebrates prior to large scale application. The focus on the environmental effects of carbon amendments continues in chapter six. Shifts in microbial communities following carbon amendment at two highly HOC-contaminated sites are investigated using Community Level Physiological Profiling (CLPP). This is the first study to report CLPP as a useful tool for observing microbial community responses, and to investigate microbial communities following carbon amendment to PCB- and DDT-contaminated soils.

Chapter seven reports on reductions in DDT bioavailability following *in situ* biochar and AC amendment to two soils at Point Pelee National Park (PPNP). This chapter also seeks to validate the use of a biomimetic method for estimating bioavailability in soils by comparing predicted bioavailability as determined by an equilibrium passive sampler to measured accumulation in plants and invertebrates. Biomimetic methods allow for cost effective assessments of remediation potential. This chapter highlights the importance of including plants in bioavailability studies as the use of carbon amendments move from predominately sediment remediation technologies to soils. Chapter eight includes a discussion of the major findings and conclusions of this thesis, as well as directions for future research. Finally, raw data and quality assurance and quality control results are included in appendices A-E.

2. LITERATURE REVIEW

2.1 HYDROPHOBIC ORGANIC CONTAMINANTS (HOCS)

Hydrophobic organic contaminants (HOCs) are organic compounds that preferentially partition to the organic phase allowing for their accumulation in living organisms. Under some circumstances, they may accumulate to hazardous levels posing risk to both environmental and human health. Although the term HOC is often used in the realm of sediment remediation technologies, persistent organic pollutant (POP) can be used interchangeably to refer to these types of contaminants. In 2004 the International Stockholm Convention was introduced and is currently signed by 152 nations to eliminate the world's most persistent, bioaccumulative and toxic substances (5). Polychlorinated biphenyls (PCBs), diclorodiphenyltrichloroethane (DDT) and its metabolites DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane) are chlorinated hydrophobic organic contaminants classified as POPs. Although the use of PCBs, DDT and other POPs is now banned in Canada, those emitted from historical sources persist in soils, sediments and waste reservoirs for extensive periods of time (decades, centuries or longer). The legacy of these contaminants represents a substantial problem from a remediation standpoint now and for future generations.

2.1.1 Polychlorinated Biphenyls

Polychlorinated biphenyls are a group of chlorinated aromatic hydrocarbons introduced into the world market in the 1930s. Since the late 1950s, over one million metric tons of PCBs have been produced, about half of those in the United States (25). Commercial PCB mixtures exhibited a broad range of physiochemical properties which resulted in their widespread use as plasticizers, pesticide extenders, adhesives, organic dilutants, dust reducing agents, flame retardants, cutting oils, heat transfer fluids, sealants, hydraulic lubricants and dielectric fluids for transformers and capacitors (25-28). Although some of these functions resulted in their immediate introduction into the environment, the majority of the environmental concern associated with PCBs resulted as a consequence of their careless disposal, accidental spills, and leakage from chemical waste disposal sites (29, 30). Due to their semi-volatile nature, PCBs are continually redistributed in a mechanism known as the ‘grasshopper effect’ or ‘global distillation’. This process involves the volatilization of PCBs in warmer climates and subsequent condensation in cooler ones. The process continues until a temperature is reached that is too cool to sustain further volatilization; hence the Arctic is a sink for PCBs (31, 32).

The Monsanto Chemical Company began manufacturing PCB mixtures in 1929 under the trade name Aroclor, and was the leading manufacturer in North America (33). Monsanto is responsible for just over half of the 1.2 million metric tons of PCBs produced world-wide (34). Aroclor mixtures were distinguished based on their degree of chlorination. For example, Aroclors 1248, 1254 and 1260 each contain 12 carbon atoms and are 48, 54, and 60% chlorine by weight, respectively (33). Similar commercial PCB combinations have been manufactured by other companies such as Prodelec in France (Phenoclors and Pyralenes), Bayer in Germany (Clophens) and Caffaro in Italy (Fenclors) (35). In the U.S., Aroclors 1254 and 1260 were used previous to 1950, while 1242 was the dominant mixture used in the 1950s and 1960s (36).

2.1.1.1 Molecular Structure of PCBs

The production of PCBs involves the chlorination of two benzene rings (a biphenyl molecule) linked by a single bond formed between two carbons that have each lost their hydrogen molecule. When a biphenyl reacts with Cl_2 in the presence of a ferric chloride catalyst, some of the hydrogen atoms are substituted by chlorine atoms (25). The extent of chlorination on the biphenyl is dependent on the length of the reaction time as well as the extent of chlorine initially present (Figure 2.1).

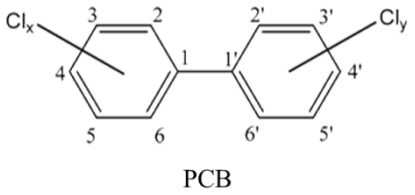

Figure 2.1 General molecular structure of a PCB molecule

The general chemical formula for any PCB congener is $C_{12}H_{10-n}Cl_n$, where n ranges from 1-10 (25, 37). Theoretically, there are 209 chlorinated biphenyl rings known as PCB congeners (25, 37). The exact proportions of the congeners depend on the ratio of chlorine to biphenyl, the reaction time, and the reaction temperature (37). The position of the chlorine atoms; *ortho*, *meta* or *para*, along with the number of chlorines determine the toxicity of the PCB congener (37). Congeners with chlorines in both *para* locations and at least two chlorines in the *meta* positions align in a single plane inducing the toxicological effects of PCBs. A less toxic PCB structure is one in which there are very few chlorines present, and they are positioned in the *ortho*-positions of the molecule.

2.1.1.2 Toxicological Information on PCBs

Exposure to PCBs and subsequent cell damage may cause endocrine disruption and neurologic damage in birds and impair reproduction of aquatic species (30, 38). In humans, PCBs may cause chloracne and liver damage, and can be transferred from mother to fetus through the placenta and from mother to infant through breast milk (38). PCBs may cause stillbirths and retard growth, and like other POPs, have been linked to reproductive disorders, birth defects and cancer (30, 38).

The method of PCB-mediated cellular dysfunction is through an increase in cellular oxidative stress (39). Co-planar PCBs induce cytochrome P450 as it acts as an aryl hydrocarbon receptor (Ah) ligand (39). Induction of cytochrome P450 may lead to generation of reactive oxygen species and thus cell damage (39, 40).

2.1.1.3 PCB Regulations and Guidelines

In 1977, as a result of human and environmental health concerns, the import, manufacture, and sale of PCBs were made illegal in Canada (41). The Canadian Council of Ministers of the Environment (CCME) has implemented soil quality guidelines for PCBs (3) (Table 2-1). These numbers represent the levels that should result in negligible risk to biota and their functions in each of the designated resource types.

Table 2-1 CCME Soil Quality Guidelines for Polychlorinated Biphenyls (3)

Land Use	PCB Soil Quality Guideline (1999) (µg/g)
Agricultural	0.5
Residential	1.3
Commercial	33
Industrial	33

In Canada, PCBs are further regulated by the Canadian Environmental Protection Act (CEPA) which states any soil containing 50 ppm or greater PCB requires immediate remedial action that cannot include being placed in a landfill (4). Remediation of PCB-contaminated sites above 50 ppm thus require soil excavation and transport, prior to off-site treatment by solvent extraction, thermal alkaline dechlorination, or incineration (6). Many of these techniques are detrimental to the environment, extremely costly and in some cases unfeasible due to the extent of contamination (42). Also, incineration of PCB contaminated soil in Canada is limited to two permanent high temperature thermal destruction units, one in Alberta and the other in Quebec (3). To date an estimated 31% of all PCBs manufactured worldwide were released into the environment prior to restrictions on their use, while 65% are still in storage or use, and only 4% have been destroyed by incineration (3).

2.1.2 Dichlorodiphenyltricloroethane (DDT)

Dichlorodiphenyltrichloroethane (DDT) is an organochlorine insecticide that was used in Canada for the control of mosquito populations for nearly 30 years until 1973 (43-45). Before DDT, common insecticides contained arsenic compounds which were very toxic, persistent, and quickly lost their effectiveness once exposed to the environment. In the 1960s, some insect populations developed a resistance to DDT and the scientific community became cognisant of its persistence and bioaccumulation in the food chain. In 1962, public awareness of the environmental problems associated with DDT increased when biologist Rachel Carson published "*Silent Spring*". The book discusses the decline of the American robin population in areas of North America where DDT was being used extensively for the control of Dutch elm disease. In 1973, the United States Environmental Protection Agency (USEPA) in response to environmental concerns, banned all DDT uses except those essential to public health (43, 45). Under the Stockholm Convention, the use of DDT is banned in Canada and the United States. Like PCBs, DDT has a low vapour pressure and travels pole-ward via the 'grasshopper effect'. Improper disposal practices and extensive historical use have led to widespread contamination in areas as 'pristine' as Point Pelee National Park (PPNP) (46, 47) and Canada's high Arctic (48).

2.1.2.1 Molecular Structure of DDT

Structurally, DDT is a substituted ethane (43, 45) (Figure 2.2). One of the carbon atoms has all three hydrogens replaced by chlorines, while the other carbon has two of the three hydrogens replaced by benzene rings. Each of the benzene rings contains a chlorine atom in the *para* position. DDT has two isomeric forms (2,4'-DDT and 4,4'-DDT), depending on the relative positioning of chlorine atoms on the two phenyl rings in its structure. In the environment, DDT can be degraded microbiologically or abiotically to DDD (1,1-dichloro-2,2-bis(chlorophenyl) ethane) or DDE (1,1-dichloro-2,2-bis(chlorophenyl) ethylene), and like DDT, these compounds each have a 2,4'- and a 4,4'- isomeric form

(45, 49). Often the terms 'total DDT' or 'ΣDDT' are used to refer to the sum of all DDT and its metabolites. Technical-grade DDT contains 65–80% 4,4'-DDT, 15–21% 2,4'-DDT, and up to 4% of 4,4'-DDD (3).

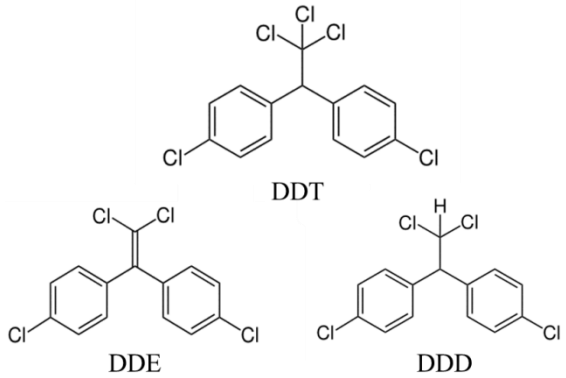

Figure 2.2 Molecular structure of ∑DDT (DDT, DDE, and DDD)

2.1.2.2 Toxicological Information on DDT, DDE and DDD

The world health organization (WHO) considers DDT not overly toxic to humans based on epidemiological data in humans, however does report on adverse effects in other species (2). The WHO recommends indoor residual spraying of DDT in countries where there are no other reasonable alternatives for malaria prevention (2) because DDT has a very strong spatial repellency and an irritant effect on malaria vectors that limits human-vector contact (2). DDT toxicity is lowest for dermal contact, however increases if ingested (2, 3). Mammalian and avian exposure to DDT has been shown to reduce longevity and cause adverse effects on reproduction, growth, and immunocompetence (2). Mutagenic and carcinogenic effects are known to occur in various species as a result of long-term dietary exposures (2, 43). Studies have confirmed that DDT is an estrogen mimic and exposure to elevated levels of DDT may reduce fertility, gestation period, fecundity, and fetal weight (2). In birds, the estrogenic effects of DDT are demonstrated via eggshell thinning and changes in mating behaviour (2, 3).

2.1.2.3 DDT Regulations and Guidelines

The CCME suggests that sites used for residential or parkland should have DDT concentrations less than 0.7 µg/g based on the soil quality guidelines for the protection of human and environmental health (43) (Table 2-2). Like PCBs, DDT is classified by CEPA as a Track 1 substance, meaning its elimination from the environment is required.

Table 2-2 CCME Soil Quality Guidelines for Dichlorodiphenyltrichloroethane and its Metabolites (43)

Land Use	DDT Soil Quality Guideline (1999) (µg/g)
Agricultural	0.7
Residential	0.7
Commercial	12
Industrial	12

2.1.3 Water Solubility

Generally, PCBs and DDT have low water solubilities, meaning that they preferentially partition to hydrophobic substances such as fats and oil (25). A 1988 study reviewed the physiochemical properties of specific Aroclor mixtures produced by the Monsanto Chemical Corporation (50). Aroclor 1248 for example, has a water solubility (at 25^{o}C) of 52 µg/L, which decreases to 12 µg/L, and 3 µg/L, for the more highly chlorinated mixtures Aroclor 1254 and Aroclor 1260, respectively. The water solubility of DDT and its metabolites are also very low ranging from 1-40 µg/L (43, 45). Octanol-water partition coefficients describe how compounds partition between octanol (plant, soil (i.e. organic)) and water phases. Octanol-water partitioning coefficients are often expressed in log form and for PCBs range across homolog groups, from 4.3 for mono-chlorinated biphenyls, to 8.3 for deca-chlorinated biphenyls. For DDT the log K_{OW} is 6.2. Log K_{OW} and water solubility data are important for estimating transport and fate, and thus environmental and human health consequences for organic contaminants. HOCs bind strongly to the organic fraction of soil, and as these compounds persist over decades, they become weathered or sequestered within the organic matter. The tendency for PCBs and DDT to sorb to the soil phase is proportional to the soil organic carbon content (51). This sequestration generally will lead to reduced bioavailability in soils and sediments and renders many *in situ* remediation strategies ineffective (52). The fate of organic contaminants in soil and aquatic systems depends on their sorption and retention characteristics which are also influenced by the extent of chlorination (25, 37, 53). The hydrophobicity of organic contaminants as determined by the large K_{OW} values (and low water solubility values), leads to their bioaccumulation in human and animal fatty tissue and their biomagnification in the food chain.

2.2 BIOCHAR

Biochar is a carbon rich by-product produced from the thermal decomposition of organic matter under very low oxygen concentrations at relatively low temperatures (< 700^{o}C) (54). Although the synthesis of biochar mirrors the technology for producing charcoal, biochar is different in that it is produced with the intent of being applied to soil as a means of sustainably sequestering carbon and improving soil function (55). Traditionally organic wastes (e.g. animal manure, crop residue, municipal biosolids) are left to decompose or are burned, releasing carbon dioxide and methane into the atmosphere (56, 57). Making biochar from these waste materials provides social benefits as it reduces greenhouse gas emissions and reduces the risk of ground and surface water contamination. Also, as it does not require biomass that could be used for human consumption, biochar's production does not create an issue with land use competition (55).

The International Biochar Initiative (IBI) is an international community of biochar researchers, promoting good industry practices, stakeholder collaboration, and environmental and ethical standards to foster economically viable biochar systems (58). The intention is to provide a knowledge platform to aid in the commercialization of biochar as a soil amendment. In 2013, the IBI released the first standardized protocol for biochar testing outlining standards for biochar physical and chemical characteristics so that it

can be safely and sustainably applied as a soil amendment (58). Worldwide, biochars are being produced by numerous companies and organizations via different pyrolysis systems and using a range of materials including (but are not limited to) woodchips, animal manure and construction wastes (59). These differences are expected to alter the biochar's physical and chemical properties, and thus their ability to improve substrates, promote long term stability and increase sorption capabilities. Also, as a result of contaminated feedstocks or inappropriate pyrolysis conditions, the biochar may become unintentionally contaminated with metals, polyaromatic hydrocarbons (PAHs) and PCBs. Therefore, before biochar can be applied on a large scale to the environment as a soil amendment it must be carefully characterized for contaminants, specific surface area (SSA), cation exchange capacity (CEC), organic carbon (OC), moisture percentage, pH, particle size distribution (PSD), earthworm avoidance, seed germination and other parameters specified by the IBI (58). Without detailing biochar physical and chemical properties in the laboratory, precise understanding of biochar function in the environment is not possible.

The molecular structure of biochar, as determined by X-ray crystallography, reveals a mostly amorphous structure containing very few and localized crystalline structures of highly conjugated aromatic compounds. The crystalline areas are visualized as stacks of flat aromatic (graphene) sheets cross-linked in a random pattern. The amorphous components of biochar are aromatic-aliphatic organic compounds of complex structure and mineral compounds such as inorganic ash (60). Biochar also contains voids, formed as macro, meso, or micro pores. Micropores (< 2 nm in diameter) contribute most to the high surface area per volume ratio of biochar and are responsible for the high sorptive capacities for molecules such as organic contaminants (61). Surface area is a very important characteristic as it influences soil functions such as nutrient cycling and cation exchange, water percolation, fertility and microbial activity. Research has shown that clay soils and sandy soils amended with large amounts of organic matter can overcome the problems of too much moisture and not enough moisture, respectfully. Due to the high carbon content in biochar and its microporosity, amendment with biochar increases the SSA of soils and thus influences the soils adsorptive characteristics (62). Macropores (> 50 nm in diameter) are vital to soil functions such as aeration and hydrology. They also contribute to the movement of roots through soil and provide habitat for soil microbes (62).

The traditional application of biochar to a soil matrix can be seen as having four main and often synergistic objectives (Figure 2.1). From a waste management perspective, exploiting organic agricultural litter as a pyrolysis resource diverts them from their regular waste streams (63, 64). The charring process produces renewable energy in the forms of heat and syngas, which also reduces the volume and weight of the waste feedstock materials. Biochar for energy production is especially important in regions of the world such as rural Africa that rely on biomass energy. Pyrolysis for bioenergy provides opportunities for more efficient energy production than wood burning, and widens the options for types of biomass that can be utilized for energy production (55). The main benefit is that pyrolysis produces clean heat, which can be used to develop cooking technologies with lower indoor smoke pollution (55). The carbon component of biochar is on a scale of 100-1000 times longer than that of regular soil organic matter and addition to soils. Biochar decomposes much more slowly than fresh plant material and thus the rate of CO_2 released back into the atmosphere is also much slower. This diverts carbon from the rapid biological cycle into a much slower biochar cycle (55). Therefore, addition of biochar to soils is a carbon sink, and can function in the mitigation of climate change. Biochar addition to agricultural soil is becoming popular, with the objective being to improve soil quality and increase crop yields (55, 65, 66). Biochar provides direct nutrient value in that most biochars contain a great deal of phosphorus and potassium (macronutrients), as well as copper (micronutrient), all of which are vital for plant growth (67). An indirect effect of improving soil quality with biochar is that nutrient leaching is reduced, ultimately leading to increased nutrient uptake by plants and higher biomass production (66, 67). Slow oxidation of the biochar surface produces carboxyl groups, increasing the CEC of the soil, which is seen as one of the most significant long term benefits (68-70). Alone or in combination, these four uses of biochar (i.e. waste management, energy production, carbon sequestration, and soil quality improvement) have social or/and financial

benefits (55). Recently due to its high SSA and CEC, biochar has shown potential for an additional application; - the sorption of contaminants for remediation of contaminated sites (Figure 2.3).

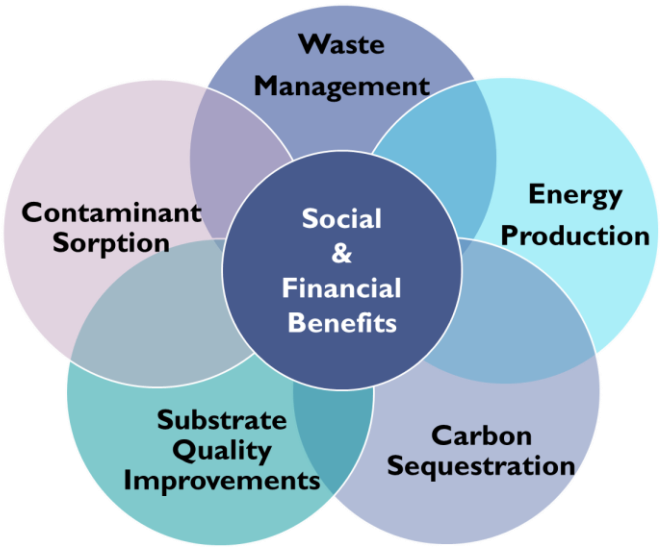

Figure 2.3 Environmental rationales for using biochar as a soil amendment

2.2.1 Sorption of Organic Contaminants

Sorption of contaminants to organic matter is a key process that controls the toxicity, transport and fate of HOCs such as PCBs and DDT. Carbonaceous sorbent materials, specifically activated carbon (AC) and more recently biochar, have been shown to strongly sorb HOCs, therefore immobilizing these contaminants and reducing their bioavailability to animals, plants and invertebrates. Research on the ability of AC to be used as an *in situ* sorbent material for the remediation of contaminated sites pre-dates biochar by at least a decade, and until now biochar research has predominantly focused on soil quality improvements and carbon sequestration potential. Application of AC is an accepted form of sediment remediation in the U.S. and Europe (24), whereas the use of biochar for remediation is considered an emerging technology that requires validation with greenhouse and field-scale trials. Activated carbon and biochar are similar carbon-rich porous materials; however AC production involves an additional step where the material is treated with steam, carbon dioxide or a chemical reagent to maximize its porosity (23). This process is complex, and can be difficult to duplicate if not carefully controlled, and therefore commercial production of AC requires expensive equipment. Also, AC is typically coal derived, whereas biochar can be made from waste materials including those from municipalities, the forestry and agriculture industries (crop and animal) (63). Once validated, biochar may offer a lower cost, greener (i.e. more sustainable) alternative to AC, as an *in situ* sorbent material for the remediation of contaminated sites.

Sorption of organic contaminants by biochar and AC is a result of two separate processes; i) relatively weak and linear *ab*sorption into amorphous organic matter, and ii) relatively strong and non-linear *ad*sorption onto the sorbent surface (21, 71). At low contaminant concentrations the sorbent surface is responsible for the sorption, however, as the contaminant concentration increases, these surface sites will become saturated and thus the amorphous organic matter phase will become the dominant sorption mechanism. Biochar and AC are also capable of sorbing inorganic contaminants (e.g. Hg, Cd,

Pb, and Zn) and nutrients by adsorption onto the surface, as a result of the abundance of carboxyl and hydroxyl groups (i.e. CEC). Slow oxidation of the biochar surface increases these functional groups, and therefore contaminant sorption capacity increases over time. In some cases AC and biochar have been 'activated' with functional groups to target specific cations, facilitating more effective sorption.

Interest in biochar has increased greatly in recent years, however there are still only a limited number of studies published examining its use to minimize the bioavailability of contaminants (52, 72-76), and most of these studies are laboratory based. Similarly, there are studies available in which AC is applied to soils in the greenhouse (7, 77) and also in the field (18, 24, 78-81) for sorption of contaminants. However, very few studies compare the *in situ* efficiency of biochar and AC directly (13, 82), and most have utilized laboratory based biomimetic methods (1, 79, 83-85).

Important lessons regarding contaminant sorption have been drawn from successful *in situ* applications of AC to sediments (24) such as the case study of the Hunters Point Shipyard in San Francisco Bay, California. In the first field-scale application, AC was added *in situ* to PCB-contaminated sediment at 3% (by weight) nearly 10 years ago and monitoring has been in place since then. The authors showed that field scale AC amendment reduced the bioavailability of PCBs to biota without causing adverse effects to the natural benthic community of macroinvertebrates or releasing PCBs into overlying water. These studies also demonstrated that mixing carbon amendments *in situ* generally results in lower bioavailability reductions compared to laboratory based methods, as it may result in spatially heterogeneous AC particles, minimizing contaminant contact and delaying treatment benefits (86, 87). Smaller particle sizes of biochar/AC are favorable as this they will increase the external surface area, and thus the ability of a contaminant to access pore space for binding (88). Additionally, smaller particle sizes are favored for sorption as there are then a greater number of particles per unit volume of sediment/soil (89). Using passive sampling efforts and kinetic modeling however, it has been suggested that over time even poorly mixed systems will achieve significant PCB immobilization by AC (81). Following numerous publications, it has been concluded that AC sorbent mixed with sediment is a cost-effective, *in situ*, management strategy for reducing risk and the bioavailability of HOCs (14, 17, 18, 24, 79-81, 86-98).

2.2.2 Methods for Measuring Hydrophobic Organic Contaminant Bioavailability

The hydrophobicity of HOCs underlines that their distribution is closely influenced by soil properties such as percent organic matter and carbon, CEC and weathering (21). These influences dictate that risk is not solely related to chemical concentration, and therefore predicting and measuring contaminant bioavailability is a critical step in risk assessment of HOC-contaminated soils (20). Over the past decade extensive efforts have been made to validate biomimetic methods for estimating HOC bioavailability (1, 99). Bioavailability can be measured by two fundamentally different parameters, accessible quantity and chemical activity (1, 99). The accessible quantity is a measurement of the HOC fraction that is weakly or reversibly sorbed and can undergo rapid desorption from the solid phase to the aqueous phase in given time (1, 21, 99). It represents the fraction that can become available for biodegradation (1, 21, 99), as it is thought that to be degraded by bacteria, HOCs must first enter the water phase. Bioaccessibility is determined using partial extraction methods (such as mild solvent extraction or cyclydextrin extraction or Tenax desorption) (99). On the other hand, chemical activity is the spontaneous ability for a chemical to undergo diffusion or partitioning (i.e. a physiochemical process). The link between chemical activity and bioavailability (i.e. the potential of HOCs to partition into organisms) is explained by the equilibrium partitioning theory originally proposed by Di Toro et al., (100). The theory dictates that at equilibrium, the chemical activity is equal in every phase compartment (e.g. soil, water, biota), and therefore so is the HOC concentration. Equilibrium passive sampling devices such as those based on the polymer, polyoxymethylene (POM), can be used to determine the chemical activity, and thus bioavailability, of HOCs (15, 79, 81, 83, 90). Once the relative relationship between two phases is known, the HOC concentration in one phase (i.e. concentration in the polymer (C_{POM})) can

be used with a partition coefficient (K_{oc}) to predict the concentration in another phase. In 2011, Endo et al. provided a practical application to the theory by publishing the experimentally derived equilibrium partition coefficients ($K_{POM/w}$) of many HOCs including DDT (83). In environmental systems, chemical activity is expressed by the freely dissolved porewater concentration (C_w) and is often used as an analogue for invertebrate bioaccumulation (18, 84, 94, 101, 102), who accumulate HOCs via diffusion (1, 99).

$$C_w = C_{POM} / K_{POM/w}$$

Polyoxymethylene (POM)-based equilibrium passive sampler devices are commonly deployed in sediment–pore water systems as this polymer has good chemical and physical stability. Due to its hard, but smooth, surface it is less susceptible to biofouling compared to other types of materials (85). Also, in contrast to other types of samplers, POM has relatively large partition coefficients for PCBs and other POPs, allowing for the determination of C_w at pg/L levels (83). The chemical activity of a contaminant decreases with increasing sorption; therefore the POM-based biomimetic method can measure the effectiveness of carbon amendments at sorbing HOCs and minimizing bioavailability (21, 99). A 2008 study by Sun and Ghosh (102) showed that POM derived sediment pore water values were related to PCB congener concentrations in *Lytechinus variegatus,* a freshwater oligochaete for both AC treated and untreated sediments. The relationship was linear for tetra- and penta- chlorinated congeners (19, 102). The authors concluded that this biomimetic method provided a "convenient and accurate" method for monitoring sediment remediation via AC amendment. Other studies further supported the biological basis for using passive samplers to monitor the success of AC remediation in PCB-contaminated sediments in freshwater (79, 103) and soil (19) invertebrates. The use of POM-passive sampling has not been validated in soils, and its ability to accurately predict plant accumulation is unknown. However if validated, this type of sampling would provide a meaningful, new, convenient and cost effective tool for measuring risk at contaminated sites. Currently, the lack of field-scale studies using naturally contaminated systems, and comparison of accumulation into higher trophic levels are hindering wide scale acceptance.

2.3 Phytotechnologies and the Remediation of Organic Pollutants

The term phytotechnologies describes the use of vegetation to contain, sequester, remove or degrade organic and inorganic contaminants in soils, sediments and water (104). As the definition implies, this technology can involve complex processes by soil organisms including protein production and symbiotic relationships with the vegetation itself (10, 104), as well as functions such as water uptake, root exudation, evapotranspiration and bio-metabolism. A fundamental constituent of these processes is the interaction that occurs between plants and microbes at the root-soil interface known as the rhizosphere. Many of the mechanisms that are essential for phytotechnologies occur in this ~1-3 mm zone surrounding the roots (104-106).

Advantages of phytotechnologies are that they can be implemented *in situ* or *ex* situ, are aesthetically pleasing, have a nonintrusive nature and are applicable at remote sites (10, 48). Furthermore, it is estimated that phytotechnologies cost two to four times less than traditional remediation technologies such as excavation, landfilling and incineration (42, 107). However, disadvantages exist, and include that phytotechnologies are only effective to the depth of the plant root, long periods of time may be required to reach clean up targets, and the effectiveness depends on the success of plant growth, which can be affected by unpredictable variations in weather patterns (10).

2.3.1 Mechanisms of Phytoremediation

As illustrated in Figure 2.4, plants can remediate contaminants via the following five mechanisms. Phytostabilization is defined as the immobilization or sequestration of contaminants in soil, on the root surfaces or within the root tissues (108). During rhizodegradation the contaminants are degraded by subsurface microorganisms that are supported or enhanced by the presence of vegetation (109, 110). The rate limiting factor in this technology is the availability of electron acceptors such as dissolved oxygen. Phytodegradation is closely related to rhizodegradation except the degradation occurs within the plant via enzymes or cofactors produced by the plant (111). Contaminants may also be vaporized by the plant following uptake by plant via transevaporation in a process called phytovolatilization (112, 113). Phytoextraction is a volume reduction technology, where plants are used to accumulate significant amounts of a contaminant from the soil and store it in the plant biomass, which can then be harvested and treated as contaminated waste (114-117).

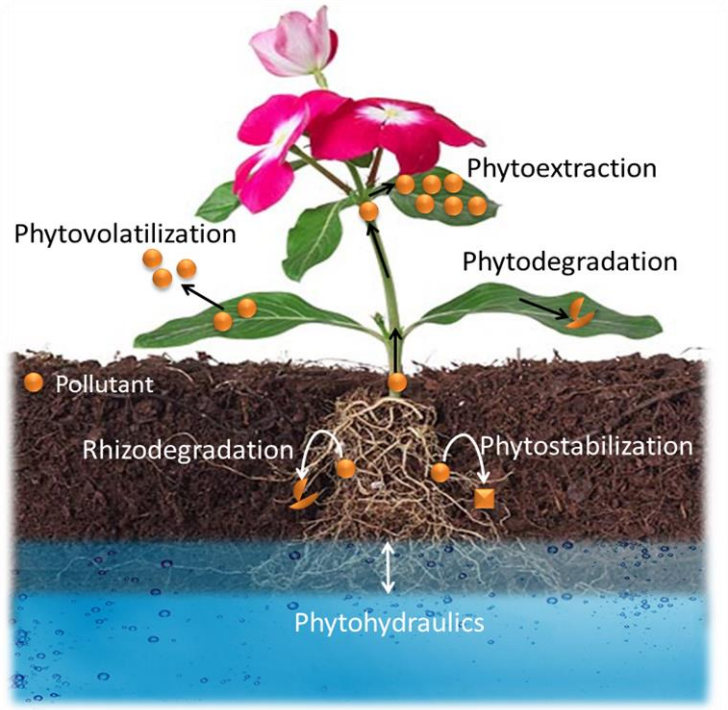

Figure 2.4 Mechanisms of phytotechnologies

2.3.2 Phytoextraction of Organic Contaminants

The goal of organic contaminant phytoextraction is to concentrate the contaminant into plant biomass to a level where it is more economical to treat the contaminated plant material than to excavate and treat the soil. The success of phytoextraction depends on two key components; i) the nature of the contaminated soil, and ii) the plant species. Influential soil factors include the degree of contamination, the bioavailability of the contaminant for uptake into the roots, and the nutrient status of the soil (118). Key plant characteristics include the ability of the roots to interact with the contaminant and subsequently translocate it to the shoots, as well as the toxicity tolerance of the plant species to the contaminant (104).

The rhizosphere plays an important role in the mobility of HOCs in soils. Proteins exuded by plant roots are thought to increase the solubility of HOCs such as PCBs, and DDT, and facilitate uptake. Plant roots can take up organic contaminants via passive diffusive partitioning (i.e. apoplastic) and/or active (i.e. symplastic) processes, depending on the properties of the organic contaminant and the plant species. Ryan et al. (119) attributes the absorption of organic compounds by roots and subsequent translocation to the shoots of plants to be a passive, diffusive, partitioning and nonmetabolic process. The process is known as transpiration adhesion tension cohesion (TATC), where the transpiration of water from the leaves creates a pressure differential that pulls fluids (held together by cohesion) up from the roots (120). Despite much work to date, the mechanisms for HOC uptake by plant roots and translocation in plants still remains unclear, in particular, whether active transport is involved in root uptake, and what the proportion of active transport to the total uptake by roots is.

Organics that are most likely to be phytoextracted by plants are moderately hydrophobic compounds with octanol-water partition coefficients ranging from 0.5 to 3 (121). Researchers have suggested that substances with a $\log K_{ow} > 3.5$ are not available for uptake by plants because if a chemical is too hydrophobic it sorbs strongly to soil particles, thus becoming less bioavailable to the plant. However, many researchers have published results contradictory to this, demonstrating efficient phytoextraction of PCBs ($\log K_{ow} > 4$) (9, 114, 122) and DDT ($\log K_{ow} \sim 6$) (7, 48). Although not completely understood, it is thought that proteins secreted by the plants in root exudates are responsible for acting as surfactants and increasing the solubility of these HOCs.

Studies as early as 1959 indicated that phytoextraction of PCBs and other organic contaminants from soil were possible. Lichtenstein published on the uptake and translocation of chlorinated hydrocarbon insecticides (aldrin and heptachlor) into roots and shoots of species such as cucumber (*Cucumis sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), and soybeans (*Glycine max*) (123). Suzuki et al. (124) reported the uptake and translocation of PCBs in soybeans (*Glycine max*); Iwata and Gunther (125) in carrots (*Daucus carota*); and Sawhney and Hankin (126) in beets (*Beta vulgaris*), turnips (*Barssica rapa L.*), and beans (*Phaseolus vulgaris*). In each case, the researchers reported that lower chlorinated congeners were found more abundantly in the shoots than the roots of the various plant species. In more recent years, studies published at the Royal Military College of Canada (RMCC) have documented the successful phytoextraction of PCBs and DDT (48, 114, 122, 127-129). A 2004 study by Lunney (48), examined the ability of five plant species (zucchini, tall fescue, alfalfa, rye grass and pumpkins) to mobilize and translocate DDT. This study determined that the pumpkin species (*Curcurbita pepo* ssp *pepo* cv. Howden) extracted the highest absolute amount of DDT, with 4.3 µg/g accumulated into the shoot tissue (48). Hülster et al. (130) found that plants of the genus *Cucurbita* (e.g. pumpkin and zucchini) show a propensity to take up polychlorinated dibenzodioxins/furans (PCDD/PCDF) over plants from different genera. For example, they reported that pumpkin fruit had a contaminant concentration two orders of magnitude higher than other fruits and vegetables examined. Hülster provided convincing evidence that root uptake and subsequent translocation was the main uptake pathway of PCDDs/PCDFs in *Cucurbita* plants. The above studies provide evidence that the uptake of compounds similar to PCBs is possible and that *C. pepo* is a promising species for the phytoremediation of HOCs. Zeeb et al. (122) confirmed this in a greenhouse study examining the potential of phytoextraction of PCB contaminated soil using nine species of plants. The results of this greenhouse study indicated that varieties of C. *pepo* were more effective at phytoextracting PCBs than other plants screened. A 2008 field study (9) reported that pumpkins grown in PCB contaminated soil (soil concentration of 21 µg /g) accumulated 11 and 8.9 µg/g PCBs into their stem and leaf material, respectively. The authors also reported that the lower portions of the plant stem had PCB concentrations as high as 43 µg/g. A paper published in 2010 by Ficko et al. screened 27 species of weeds from two locations in Ontario contaminated with different Aroclors at different concentrations. The study found that vetch (*Vicia cracca)* accumulated 35 µg/g in shoot tissue, while lambs quarters (*Chenopodium album)* only accumulated 0.42 µg/g in shoot tissue. Maximum shoot extractions in this study were found to be 420 µg/g by Canada goldenrod (*Solidago*

canadenis) at a site contaminated with Aroclor 1254/1260 and 120 µg by oxeye daisy (*Chrysanthemum leucanthemum)* at a site contaminated with Aroclor 1248 (131). Using published optimal planting densities, red clover (*Trifolium pratense*) could potentially extract 110,000 µg PCB/m^2, which is much greater than the determined 2100 µg/m^2 by pumpkins. Thus several species of plants have exhibited successful phytoextraction of organic contaminants.

The success of phytoextraction can be measured using a bioaccumulation factor (BAF):

$$BAF = [HOC]_{plant\ tissue}/[HOC]_{soil}$$

Roots consistently have higher BAFs than shoots of plants, however, roots comprise a relatively small amount of total plant biomass (~2.5%) compared to shoots (~97.5%) (122). Therefore, the above ground biomass (shoot) typically contributes more than the roots to the total effectiveness of phytoextraction. Shoot BAFs generally increase as the soil HOC concentration decreases (129). Therefore, the feasibility of phytoextraction may be enhanced at lower soil contaminant concentrations. When employing phytoextraction as a remediation approach, a higher BAF is desired as it equates to a lower clean-up cost; with an economic break-even point reached at a shoot BAF of 1, unless other methods (such as composting) to concentrate the contaminant are used (9)

Despite some successes in phytoextraction, concerns remain that the pace of contaminant removal is too slow and consequently these HOCs continue to enter the food chain and pose risk to environmental and human health. Thus, a contaminant immobilization strategy such as biochar and AC amendments that seek to minimize risk by reducing contaminant bioavailability is exceptionally appealing for the remediation of HOCs.

3. THE USE OF BIOCHAR TO REDUCE SOIL PCB BIOAVAILABILITY TO *CUCURBITA PEPO* AND *EISENIA FETIDA*

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3.1 ABSTRACT

Biochar is a carbon rich by-product produced from the thermal decomposition of organic matter under low oxygen concentrations. Currently many researchers are studying the ability of biochar to improve soil quality and function in agricultural soils while sustainably sequestering carbon. This research focuses on a novel but complimentary application of biochar- the reduced bioavailability and phytoavailability of organic contaminants in soil, specifically polychlorinated biphenyls (PCBs). In this greenhouse experiment, the addition of 2.8% (by weight) biochar to soil contaminated with 136 and 3.1 µg/g PCBs, reduced PCB root concentration in the known phytoextractor *Cucurbita pepo* ssp. *pepo* by 77% and 58%, respectively. At 11.1% biochar, even greater reductions of 89% and 83% were recorded, while shoot reductions of 22% and 54% were observed. PCB concentrations in *Eisenia fetida* tissue were reduced by 52% and 88% at 2.8% and 11.1% biochar, respectively. In addition, biochar amended to industrial PCB-contaminated soil increased both aboveground plant biomass, and worm survival rates. Thus, biochar has significant potential to serve as a mechanism to decrease the bioavailability of organic contaminants (e.g. PCBs) in soil, reducing the risk these chemicals pose to environmental and human health, and at the same time improve soil quality and decrease CO_2 emissions.

3.2 INTRODUCTION

In recent years, the popularity of biochar as a soil amendment has substantially increased, mostly in response to increased global carbon emissions and deterioration of agricultural soil quality. Biochar is a carbon rich by-product produced from the pyrolysis of organic matter under zero oxygen concentrations at relatively low temperatures (< 700^{o}C) (54). Due its high porosity (132), specific surface area (66, 133) and carbon content (134), biochar decreases nutrient and water leaching loss (135), increases soil cation exchange (69, 133, 136, 137), sustainably sequesters carbon and improves the overall sorption capacity of soil (69).

Persistent organic pollutants (POPs) are organic compounds with low water solubility and resistant to environmental degradation by biological, photoylic and chemical processes (138). Research has suggested that carbon rich, charcoal-like materials such as biochar and activated carbon (AC) have the ability to sorb POPs and thus limit their bioavailability in sediments and soil. However, little data exists on the potential of using biochar, which as a consequence of its production, is a greener and more cost effective material than activated carbon. The production process of biochar is different from that of AC, in that AC is further 'activated' through physical or chemical treatments to maximize the porosity (23). Commercial production of activated carbon requires expensive equipment, and as a result AC has much higher associated costs than biochar. Biochar production is also more sustainable than the production of AC as it does not require chemical reagents and biochar can be made from waste materials including those from municipalities, the forestry and agriculture industries (crop and animal) (63).

Sorption studies utilizing activated carbon predate those of biochar, and currently there is substantially more information available on ability of AC (7, 11, 14, 18, 24, 77, 90, 97, 102, 139, 140) to sorb contaminants. A few studies have suggested that biochar amended to soil may function in the remediation of organic pollutants such as polyaromatic hydrocarbons (PAHs) (11, 76) and pesticides (73, 74, 141-143), and to sediments for polychlorinated biphenyls (PCBs) (21). A recent study found that biochar produced from pine needles under a high pyrolytic temperature (700°C) increased the sorption of PAHs in agricultural soils (76). Another 2011 study reported a 91% suppression of dieldrin uptake into cucumber plants with biochar produced from wood chips (73). Zheng et al. (144) reported that biochar (produced from greenwaste (plant pruning mixture of maple, elm, and oak woodchips and barks) at 450°C) exhibited a high sorption affinity to atrazine and simazine, and was effective at removing these pesticides from aqueous solution. Xu et al. (145) proposed that biochar made from bamboo added to soil at 5% (w/w) could be used as *in situ* sorbents for pentachlorophenyl and thus minimize the contaminants' bioavailability to earthworms. Thus the addition of biochar to soil or sediment has potential to function as a mitigation technology for a variety of POPs.

The sorption of organic contaminants by biochar is a result of two separate processes: i) relatively weak and linear *ab*sorption into amorphous organic matter, and ii) relatively strong and non-linear *ads*orption onto the biochar surface (21, 71, 146-148). The sorption and subsequent immobilization of POPs to carbon materials would control their toxicity and fate, and decrease the potential adverse health effects associated with their bioaccumulation through the food web (24, 77, 96, 145, 149).

Soil and sediment contamination of PCBs in particular, is widespread as a result of extensive use, improper storage facilities and accidental releases (29). Traditionally, the remediation of PCBs involved soil excavation and transport, prior to off-site treatment by solvent extraction, thermal desorption, incineration, or landfilling (6). However, these techniques themselves can be detrimental to the environment, extremely costly and in some cases infeasible, due to the extent of contamination (42). The use of phytoextraction, a volume reduction technology in which plants (e.g. *Cucurbita pepo* spp. *pepo*) are used to mobilize and accumulate significant amounts of the contaminant from the soil, has been a successful *in situ* green remediation strategy for POPs (9, 114, 130, 131, 150, 151). However phytoextraction has been shown to have limited effectiveness as contaminant concentration increases

(122, 152, 153) and the high cost of traditional remediation technologies usually dictates that low concentrations of PCBs are left on site. Despite many successes in both high cost traditional and low cost green remediation technologies, there are still concerns that significant PCB contamination remains in the soils at Brownfield sites, and consequently PCBs continue to enter the food chain and pose environmental and human health risks (154).

The current greenhouse study provides an evaluation of the ability of biochar to minimize the uptake of PCBs by the known PCB phytoextractor *Cucurbita pepo* ssp. *pepo* cv. Howden (pumpkin) and a common invertebrate species, *Eisenia fetida* (redworm). The reduced uptake of organic contaminants due to biochar soil additions would provide significant social benefits by reducing or eliminating the potential adverse effects of these substances entering the food chain. In addition, minimizing the bioavailability of organic contaminants in soil may alleviate some of the financial burden associated with the remediation of contaminated sites while reducing greenhouse gas emissions and improving soil quality.

3.3 Materials and Methods

3.3.1. Greenhouse Soil Preparation

Weathered soils contaminated with commercial Aroclors 1254 and 1260 were collected from a contaminated site in Etobicoke, Ontario (Canada). The site is a former manufacturing facility for electrical transformers. Soils were collected from two areas on site, and were determined to have PCB concentrations of 136 ± 15.3 and 3.1 ± 0.75 μg/g, respectively. Using the sodium acetate method for cation exchange capacity (CEC) described by Laird and Fleming (155), the PCB-contaminated soil had an average CEC of 10.22 cmol/kg (n=3) and the pH of the soil was 7.72. Previously this soil was characterized (9, 114) as being coarse-grained and sandy with a total organic carbon content of 3.5%. The soils were dried, sieved to 16 mm, and then homogenized separately using the Japanese pie-slab mixing method (156).

3.3.2 Experimental Design and Sample Collection for *Cucurbita pepo* Tissue

The two soils (136 and 3.1 μg/g) were amended in triplicate (A, B, and C) with 0, 0.2, 0.7, 2.8 or 11.1% (w/w) biochar obtained from Burt's Greenhouses in Odessa, ON. The biomass feedstock of this biochar consisted of wood waste, mostly from used shipping pallets and construction waste. The temperature within the pyrolysis equipment reached 700^{o}C, and occurred over ~30 mins. Each treatment (n=10) was tumbled at 30 rpm for 24 h in a leachate soil tumbler at the Analytical Services Unit located at Queen's University. Vermiculite (density = 0.11 g mL^{-1} Schultz^{TM}, Brantford, ON,) was added to all treatments in a 2:1 v/v soil:vermiculite ratio to increase soil aeration. The soil/biochar/vermiculite mixture (total weight per planter of 2.25 kg) was placed in bottom perforated 8-inch diameter planting pots (n=30) lined with aluminum foil.

Each planter received three pumpkin (*Cucurbita pepo* ssp. *pepo* cv. Howden) seeds purchased from the 'Ontario Seed Company' (Waterloo, ON), however extra seedlings were removed such that each planter contained only one growing plant. Pumpkin plants were grown in the greenhouse located at the Royal Military College of Canada (RMCC), measured on a weekly basis and harvested at 50 days. Greenhouse temperature was maintained at 27ºC (± 6ºC) and the pumpkins were grown under a 14:10 h (day:night) fluorescent photoperiod. Planters were top and bottom watered to maintain ~35% soil moisture.

A 30 g composite soil sample was collected from replicates for all treatments both immediately after soil tumbling with and without (i.e control) biochar and after 50 days. All soils remained frozen until analysis. Particle size distribution by sieving performed on oven-dried samples (95 to 125 °C, 16 hours) and pH of freshly tumbled treatments were analysed by the Analytical Sciences Group at the Royal

Military College of Canada (Appendix A, Figure A.1, Table A-7). Cation exchange capacity of Burt's biochar, PCB-contaminated soil, and PCB-contaminated soil with biochar additions, all aged 50 days was calculated via the sodium acetate method outlined by Laird and Fleming (155).

After 50 days, plants were harvested by cutting the shoot of the pumpkin with acetone rinsed scissors as close to the soil surface as possible. The soil in the planter was then emptied onto a tray (cleaned and rinsed with acetone between samples) and the root tissues collected. Air-monitoring of the greenhouse indicated PCB concentrations were below detectable limits (< 0.1 μg/g), therefore aerial deposition on the plant tissues was considered insignificant. Plant tissues (root and shoot) were washed using running water, patted dry, and weighed to the nearest hundredth of a gram. They were then placed in individually labelled Whirlpak® bags and frozen until analysis.

3.3.3 Experimental Design and Sample Collection for *Eisenia fetida* Tissue

Following plant harvest, redworms (*Eisenia fetida)* (n=50 worms, average weight=20 ± 1.0 g) purchased from 'The Worm Factory' (Westport, ON), were added to the biochar treatments (i.e. 0, 0.2, 0.7, 2.8, 11.1%) in the 136 μg/g PCB-contaminated soil. The planters were covered with perforated aluminum foil and the worms were removed from the soil after 50 days. Soil moisture was maintained ~35% moisture. Deceased earthworms were not included for PCB analysis as they could not be depurated.

Surviving worms were collected by emptying the soil from each planter onto a tray (cleaned and rinsed with acetone between samples). Collected worms were then counted, washed using a container of clean water, weighed, depurated for 72 hours at 4ºC, dried for 24 hours at 25ºC and stored in individually labelled Whirlpak® bags and frozen until analysis.

3.3.4 Analytical Procedures

3.3.4.1. PCB Aroclors in soil, plant, and worm samples

Plant root and shoot samples were analysed by microwave-assisted extraction (MAE) at the RMC. Microwave-assisted extraction was performed at a temperature of 120°C for 35 min in 30 mL of 1:1 hexane:acetone mixture using a Milestone Ethos SEL microwave extraction system. Following extraction, sample extracts were concentrated using a Syncore, the solvent exchanged for hexane, and then extracts were applied to a Florisil column for cleanup.

PCB concentrations in soil and worm tissues were analyzed via Soxhlet extraction, based on the methods described by (114) and performed at the Analytical Services Unit located at Queen's University. Briefly, worm samples were finely chopped using metal scissors (rinsed with acetone between samples) and homogenized. Soil and chopped worm samples were dried overnight in a vented oven at 25°C for approximately 12–18 h, and then ground with sodium sulphate and Ottawa sand. Decachlorobiphenyl (DCBP) was used as an internal surrogate standard. All soil and worm samples were extracted in a Soxhlet apparatus for 4 h at 4–6 cycles per hour in 250 mL of dichloromethane. The use of both extraction methods was validated by (9).

Plant, worm and soil extracts were analyzed for total Aroclors, using an Agilent 6890 Plus gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/μECD), a SPB™-1 fused silica capillary column (30 m, 0.25 mm ID × 0.25 μm film thickness) and HPChem station software. The carrier gas was helium, at a flow rate of 1.6 mL/min. Nitrogen was used as the makeup gas for the electron capture detector (ECD). Detection limits were 0.1 μg/g. All values were reported as μg/g dry weight.

3.3.4.2. Quality Assurance/Quality Control (QA/QC)

One analytical blank, one control and one analytical duplicate sample were prepared and analyzed for every nine samples analyzed by Soxhlet or MAE. The control sample was spiked with a known

amount of either Aroclor 1254 or 1260. Decachlorobiphenyl (DCBP) was added to each sample as a surrogate standard prior to extraction. None of the analytical blanks contained any PCB congeners at concentrations above detection limits (0.1 μg/g for total Aroclors) and all control samples were between 80–110% of the expected value. Relative standard deviations between the samples and their analytical duplicate were below 24% for all results and the average surrogate recovery for samples analyzed for total Aroclor was 98%.

3.3.5 Statistical Analyses

PCB concentrations (soil and tissue) are reported on a dry-weight basis. The tissue concentration data were analyzed by one-way analysis of variance (ANOVA) (dependant variable: shoot, root or worm PCB concentration; independent variable: percent biochar) followed by a post hoc Tukey comparison (levels of: percent biochar). Shoot and worm wet weights were compared between soil types (i.e. high or low level of PCB contamination) using a two-way ANOVA. Percent reductions in shoot, root and worm tissue among biochar percentages were also compared between types of biochar using a two-way ANOVA. All residuals of the data were determined to be normally distributed as determined by a Kolmogorov Smirnov test for normality. When data failed to meet the assumptions, data were $\log_{10}$-transformed. A significance level of α=0.05 was used for all tests, and results were recorded with the standard error of the mean. All statistical analyses were performed using SPLUS 8.0.

3.4 Results and Discussion

3.4.1 Plant Shoot and Worm Harvestable Biomass

PCB concentrations in both control soils did not vary from the beginning (136 ± 15.3 and 3.1 ± 0.75 μg/g) to the end (153 ± 3.4 and 3.4 ± 0.29 μg/g) of the experiment. Traditionally, biochar amendments have been used as a method to increase plant productivity in agriculture (67, 136). Pumpkin shoot weights significantly increased in size by 85 and 90% in the 136 μg/g PCB-contaminated soil, with biochar additions of 2.8 and 11.1%, respectively ($p < 0.05$) (Figure 3.1).

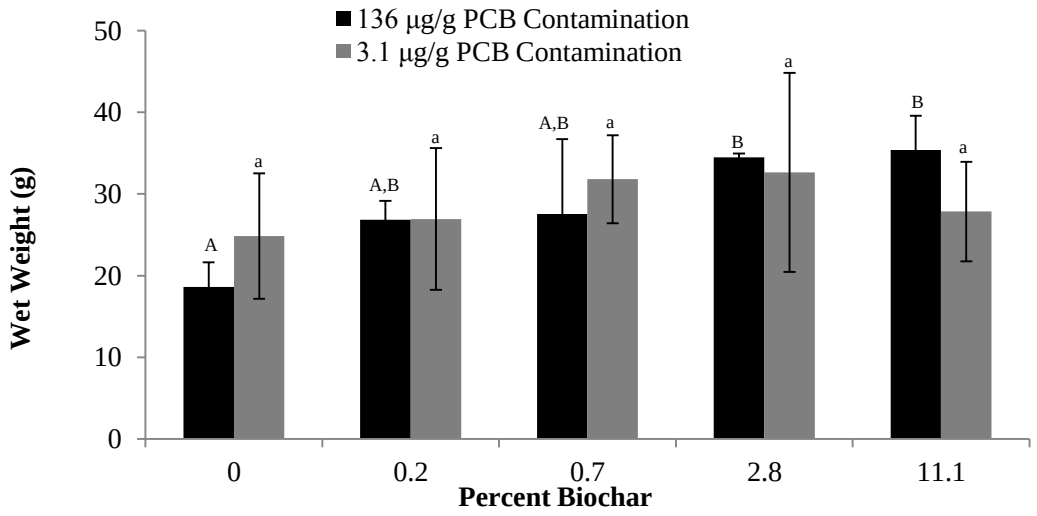

Figure 3.1 Harvested wet weight of *Cucurbita pepo* shoots in unamended PCB-contaminated industrial soil and soil amended with a range of biochar concentrations. Error bars represent one standard deviation. Upper-case (136 μg/g PCB-contaminated soil) and lower-case letters (3.1 μg/g PCB-contaminated soil) indicate statistically significant differences between treatments ($p < 0.05$).

Pumpkin shoot wet weights did not differ among the two levels (136 μg/g and 3.1 μg/g) of soil contamination at any biochar application rate (Figure 3.1). Whitfield et al (9, 114) documented that *Cucurbita pepo* accumulated significant concentration of PCBs in plant shoots without jeopardizing plant health. Increase in shoot biomass could be due to biochar's ability to maintain soil moisture (137, 157, 158) and provide macronutrients (potassium, phosphorous) and micronutrients (copper) (67, 137) Also oxidation of the biochar surface creates carboxyl groups which contribute to a higher cation exchange capacity (CEC) than in unamended control soil (69, 70, 136). CEC is a measure of the negatively charged sites on a biochar or soil particle and is important as soil with a high CEC is better able to retain nutrients (e.g. Ca^{2+}, K^{+}, and Mg^{2+}) to replenish those removed from the soil water by plant uptake (Liang et al. 2006). The Burt's biochar used in this study had a CEC (determined by the sodium acetate method) of 24.2 cmol/kg, whereas, the PCB-contaminated soil (136 μg/g) had a CEC of 10.4 cmol/kg. Upon addition of 2.8 and 11.1% Burt's biochar to the 136 μg/g PCB-contaminated soil the CECs were only slightly higher at 12.8 and 10.8 cmol/kg, respectively after 50 days. The small difference could be due to the short duration of our experimental design (i.e. 50 days) as well as the soil and biochar heterogeneity. Future studies should analyze the CEC of the soil after several months or many years of biochar amendment to determine the long term benefits to soil CEC and seek further statistical significance.

Another soil improvement ability of biochar is that it can reduce the overall tensile strength of the soil (159). Reductions in tensile strength may be especially important for revegetation of contaminated sites where the soil quality if often intensely degraded (7). Biochar addition to soils at contaminated sites to lower tensile strength may alleviate root elongation and proliferation problems, allow seeds to germinate more easily, and allow invertebrates to move more readily through the soil. After 50 days of pumpkin growth, the 136 μg/g PCB-contaminated soil in the control treatment had become hard and thus it was more difficult to harvest the root tissue. Roots were easily harvested with gentle force from the soil treated with 2.8% and 11.1% biochar. Biochar additions to the 3.1 μg/g PCB-contaminated soil did not significantly increase plant growth. This area of the PCB-contaminated site has been revegetated for many years and subsequently is not as degraded as the soil collected in the area of higher contamination. It is not uncommon to observe greater yield improvements as a result of biochar soil amendments in degraded soils, as was the case in this study (158, 160, 161).

The presence of earthworms is considered a useful indicator of soil health (162). When collecting the 136 μg/g PCB-contaminated soil it was observed that earthworms of any species were absent from the site, however there were some occupying the area contaminated with 3.1 μg/g PCBs. Thus, the soil contaminated with the higher amount of PCB contamination was selected for the earthworm study. If biochar is to improve soil functions at Brownfield sites it must allow for re-habitation of the earthworm population and not have an adverse effect on the earthworms that occupy the soil. *Eisenia fetida* were specifically chosen for this study because Langlois et al. (77) reported no significant differences in worm weights between those exposed to PCB-contaminated soil (> 50 μg/g), or PCB-contaminated soil amended with granular activated carbon (GAC) after 2 months. The PCB concentration used in this study (136 μg/g) is not acutely toxic to *Eisenia fetida*, which has an Aroclor 1254 LD_{50} of 4500 μg/g (163). However, soil at Brownfield sites are typically intensely degraded (i.e. lack essential nutrients, substrate quality, and/or vegetative cover) which may not allow for earthworm habitation. *E. fetida* in this study exposed to the control treatment had only a 4 ± 2% survival rate (n=3). In this greenhouse experiment, the addition of 2.8% biochar to industrial PCB-contaminated soil (136 μg/g) was optimal, significantly increasing the rate of worm survivorship by 17.5 times the control (Figure 3.2) ($p < 0.5$). It is noteworthy to also mention that addition of 0.7 and 11.1% biochar to the PCB-contaminated soil also increased worm survivorship by 7.7 and 8.8 times the control, respectively. Increases in worm survivorship resulted in up to 2.1 times greater worm weights (at 2.8% biochar addition) at harvest time (50 days) compared to the controls (Figure 3.2).

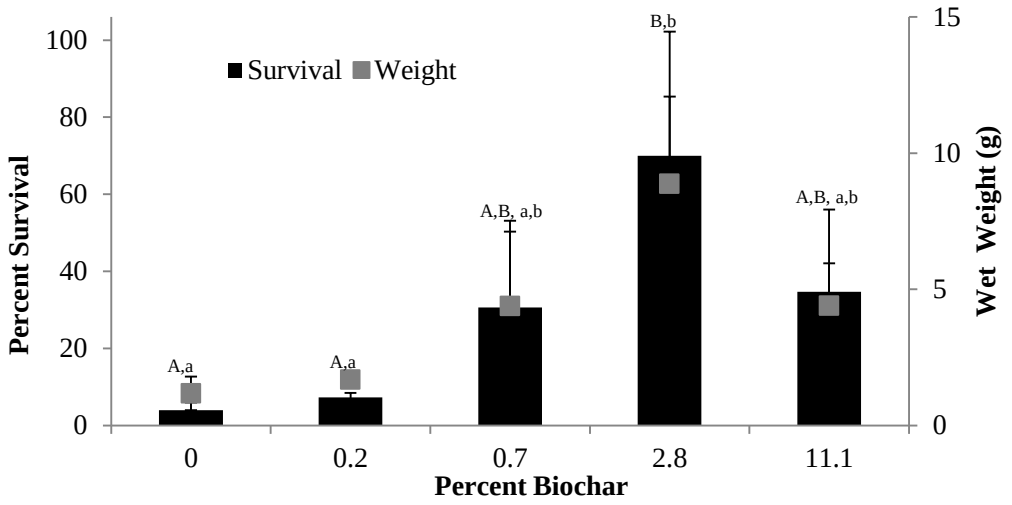

Figure 3.2 Percent survival and wet weight of *Eisenia fetida* after 50 days of exposure to 136 µg/g unamended PCB-contaminated soil and soil amended with a range of biochar concentrations. Error bars represent one standard deviation. Upper case letters indicate statistically significant differences in worm survival between treatments and lower case letters indicate significant difference in worm weights between treatments ($p < 0.05$).

Thus biochar additions can improve the health of soil invertebrates even in Brownfield soil highly contaminated with PCBs. This result, along with the significant increases in plant growth provide optimism for contaminated sites- in that with biochar additions, revegetation and the return of mesofauna are probable and thus the overall soil health and functionality may also be restored.

3.4.2 PCB Concentrations in *Cucurbita pepo*

Cucurbita pepo was chosen to study the effects of biochar on the phytoavailability of PCBs because it has been widely documented as an efficient species at phytoextracting PCBs and other organic pollutants (8, 9, 114, 130, 151, 164). The translocation and deposition of PCB congeners through the shoot tissue of *C. pepo* occurs via transport in the xylem sap (127). Whitfield et al. (9) reported that contaminant transfer pathways such as direct soil contamination, atmospheric deposition and volatilization from soil and subsequent redeposition on shoot tissue were negligible. Thus, if the addition of biochar to the soil reduced PCB uptake by *C. pepo*, it is likely to also reduce uptake by other plant species. As expected, root and shoot tissue of *C. pepo* accumulated substantial amounts of PCBs in the two control treatments (Figure 3.3a and b). The extent of PCB bioaccumulation, as determined by a bioaccumulation factor (BAF= $[PCB]_{tissue}/[PCB]_{soil}$) in this study (0.11) was comparable to that of Whitfield et al. (114) (0.15) who determined there was potential for *in situ* phytoextraction of PCBs. Generally shoot BAFs decrease as the soil concentration increases (122). The soil concentration in the current study was roughly three times higher than that of Whitfield et al. (114).

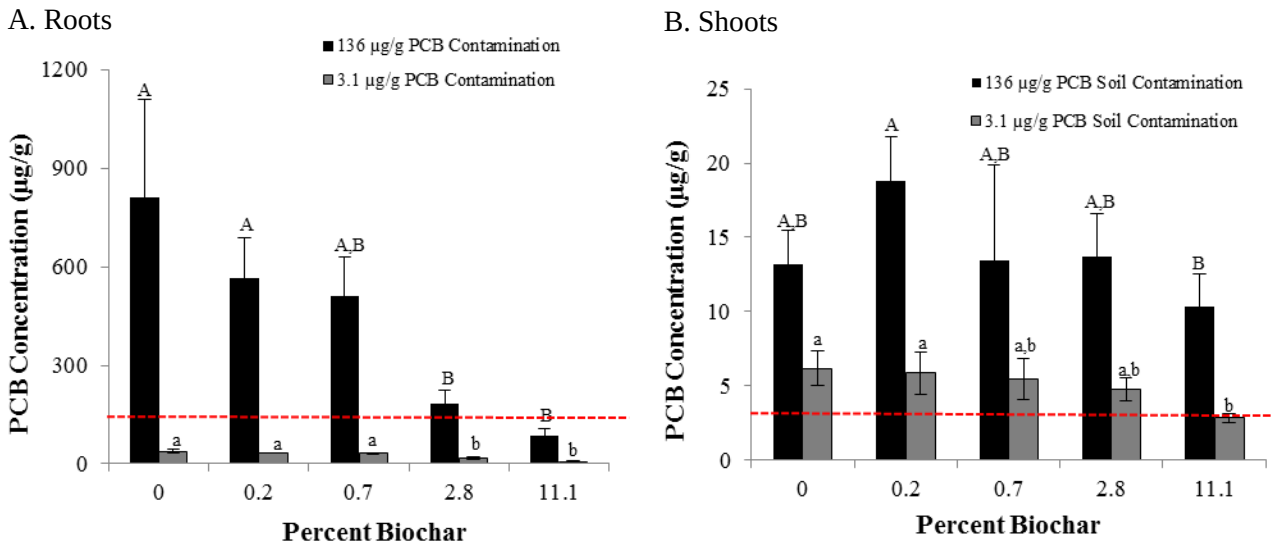

Figure 3.3 Polychlorinated biphenyl (PCB) concentrations in A) root and B) shoot tissue of *Cucurbita pepo* grown in unamended PCB-contaminated industrial soil and soil amended with a range of biochar concentrations. Error bars represent one standard deviation. Upper-case (136 µg/g PCB-contaminated soil) and lower-case letters (3.1 µg/g PCB-contaminated soil) indicate statistically significant differences between treatments ($p < 0.05$). The line represents A) the high PCB-contaminated soil concentration of 136 µg/g and B) the low PCB-contaminated soil concentration of 3.1 µg/g.

In both soils, the addition of biochar significantly reduced PCB levels in the plant roots. In soil with 136 µg/g PCB-contamination, the PCB concentration in root tissue decreased by 77% and 89% ($p < 0.05$) with 2.8% and 11.1% biochar amendment, respectively (Figure 3.3a). In soil with 3.1 µg/g PCB-contamination, biochar amendment at 2.8% and 11.1% reduced the concentration of PCBs in *C. pepo* root tissue 58% and 83%, respectively; $p < 0.05$) (Figure 3.3a).

The addition of biochar had less of an effect on PCB uptake into the plant shoots. At an 11.1% rate of biochar amendment in 3.1 µg/g PCB-contaminated soil, a significant 54% reduction in shoot tissue was observed ($p < 0.05$) (Figure 3.3b). Although not significant ($p = 0.058$), biochar amendment at a rate of 11.1%, to 136 µg/g PCB-contaminated soil, reduced the shoot concentration of *C. pepo* by 22%. Significant reductions were not seen for plant shoots in soil amended with lower concentrations of biochar. These results are consistent with a study by Langlois et al. (77) which determined 12.5% AC amendment reduced the PCB concentration (Aroclor 1254) in root tissue of *C. pepo* by 97%, but only by 63% in shoot tissue. Lunney et al. (7) demonstrated that uptake of DDT into shoots and roots was eliminated with the addition of high levels of AC to soils contaminated with 1100 ppb DDT.

The significant reductions in PCB concentrations into *C. pepo* root and shoot tissue observed, are consistent with Graber et al. (165), Mesa and Spokas (166), Nag et al. (167) and Yu et al. (168), which have stated biochar soil amendment may also lead to decreased efficacy of soil-applied herbicides. Thus, although biochar amendment to minimize the phytoavailibility of organic compounds such as PCBs has a profound positive effect from a remediation point of view; it may have a negative effect from an agricultural standpoint. Hence, careful consideration of site specific characteristics is necessary before applying biochar amendment, on a large scale.

3.4.3 PCB Concentrations in *Eisenia fetida*

The greatest reductions in PCB uptake by *C. pepo* were observed in the 136 µg/g PCB-contaminated soil, thus biochar treatments at this concentration were chosen for worm exposure. Worms exposed to 136 µg/g PCB-contaminated soil had PCB concentrations of 2440 µg/g. This 18-fold (Figure 3.4) increase in tissue concentration illustrates the ability of PCBs to bioaccumulate within an organism (BAF = 18.0 ± 2.9), and the potential for them to biomagnify through the food chain (14, 18). Treatment of 136 µg/g PCB-contaminated soil with 2.8% and 11.1% biochar, significantly ($p < 0.05$) reduced the bioaccumulation of PCBs into the worm tissue by tissue by 53% and 88%, respectively. Worms in the 0.2% and 0.7% amendments had PCB concentrations that were not significantly different from the control. Biochar is a porous material consisting mostly of micropores (< 2 nm) that provide surface area for contaminant binding (21, 56, 143, 144, 169). It is possible that biochar adsorbed the PCB molecules so strongly that the contaminant-biochar complex cannot be broken down by digestive enzymes and microbial flora as it passes through the gut of *E. fetida* (77), resulting in reduced worm PCB concentrations.

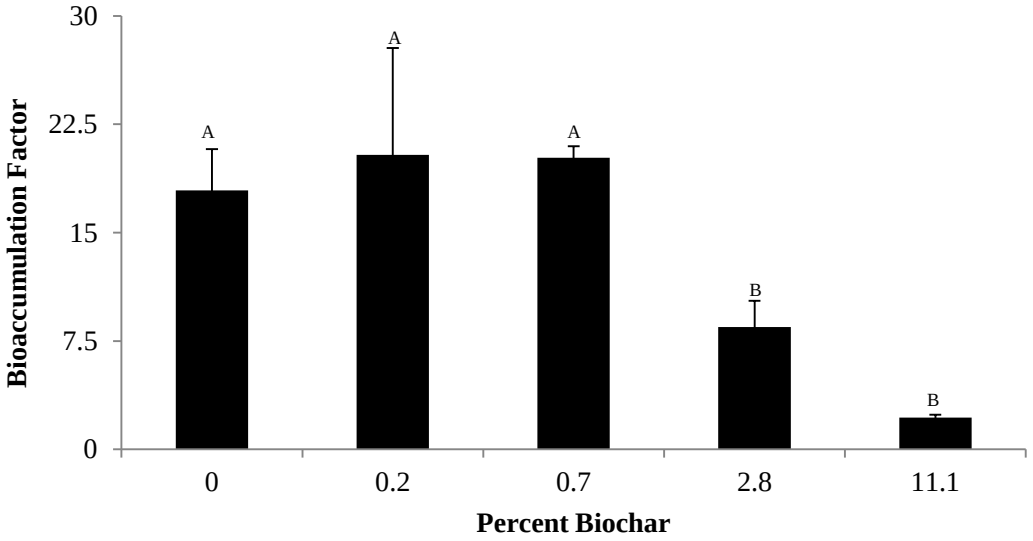

Figure 3.4 Bioaccumulation factor of polychlorinated biphenyls (PCBs) into *Eisenia fetida* exposed to an unamended (control) 136 µg/g PCB-contaminated industrial soil and soil amended with a range of biochar concentrations. Error bars represent one standard deviation. Upper-case letters indicate statistically significant ($p < 0.05$) differences between treatments.

These large reductions of the bioavailability of PCBs to the earthworm *E. fetida* are consistent with Xu et al. (145) who used a chemical extraction method using methanol to represent bioavailability of pentachlorophenyl to earthworms. In this study the authors found that compared to the control, the concentration of pentachlrophenyl extracted by methanol decreased by 56% in the soil amended with 5% (w/w) bamboo biochar. The high efficacy of biochar to reduce PCB bioaccumulation in invertebrates can be compared to the efficiency of activated carbon. Langlois et al. (77) determined that an addition of 12.5% AC to soil significantly reduced PCB bioaccumulation in *E. fetida* by 99%. It will be useful in future studies to include activated carbon as a positive control to directly compare the efficiency of biochar and activated carbon to minimize the bioavailability of organic contaminants.

Sorption of contaminants is a key process that controls the toxicity, transport and fate of non-polar organic compounds such as PCBs (21, 24, 71). In the past few years much work has been published as a result of laboratory kinetic testing, that organic contaminants are adsorbed onto the surfaces and absorbed into the organic matter of biochar (21, 56, 143, 144, 169). In comparison, this study demonstrates sorption and hence immobilization of PCBs by biochar in a complex scenario with biological components such as weathered PCB-contaminated soil, earthworms and plants. This study provides evidence that biochar has significant potential to serve as a mechanism to sequester PCBs in the soil, thereby, minimizing their bioavailability and potential to enter the food chain. This technology, possibly in combination with bioaccessibility assays to determine appropriate cleanup levels, based on environmental and human health risks (e.g. (170)), could be used during Brownfield site closure, where traditional remediation approaches or phytoextraction have been exhausted, yet levels of residual contamination remain.

Biochar is produced by the pyrolysis of organic matter; however, many types of organic matter can be used, varying from sawdust to corn stalks to chicken manure to construction wastes, under different pyrolysis conditions. These differences are expected to alter the biochar's physiochemical properties and its sorption capabilities (171). Care must be taken to ensure the biomass itself does not contain any contaminants (e.g heavy metals, PAHs, PCBs). Thus, before this technology can be implemented *in situ*, careful characterization of the biochar including, contaminants, sorption capacity, specific surface area, cation exchange and those suggested by the International Biochar Initiative (IBI) should be conducted.

In this greenhouse experiment biochar produced the greatest percent reductions in *C. pepo* shoot and root material as well as *E. fetida* tissue when added at 11.1% (w/w). However, statistically significant reductions in PCB concentration in root and worm tissues were achieved at 2.8% (w/w), which is a much more realistic application rate for large-scale experiments such as at a PCB-contaminated Brownfield sites, and this concentration is currently recommended by some researchers for activated carbon amendment (77, 91, 172). Thus, future work should focus on field-relevant application rates and direct comparisons between the efficiency of different biochars with activated carbon at ca. 3%. Many groups have investigated the potential of activated carbon to sorb PCBs in aquatic sediments and terrestrial soils; - this study is the first to present reductions in PCB phytoavailability and bioavailability in weathered PCB-contaminated soil. Given that biochar costs are typically 50-75% less than the cost the activated carbon, and the additional agricultural and environmental benefits, this is a promising new application of biochar.

4. *IN SITU* APPLICATION OF ACTIVATED CARBON AND BIOCHAR TO PCB-CONTAMINATED SOIL AND THE EFFECTS OF MIXING REGIME

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4.1 ABSTRACT

The *in situ* use of carbon amendments such as activated carbon (AC) and biochar to minimize the bioavailability of organic contaminants is gaining in popularity. In the first *in situ* experiment conducted at a Canadian PCB-contaminated Brownfield site, GAC and two types of biochar were statistically equal at reducing PCB uptake into plants. PCB concentrations in *Cucurbita pepo* root tissue were reduced by 74%, 72% and 64%, with the addition of 2.8% GAC, Burt's biochar and BlueLeaf biochar, respectively. A complementary greenhouse study which included a bioaccumulation study of *Eisenia fetida* (earthworm), found mechanically mixing carbon amendments with PCB-contaminated soil (i.e. 24 hrs at 30 rpm) resulted in shoot, root and worm PCB concentrations 66%, 59% and 39% lower than in the manually mixed treatments (i.e. with a spade and bucket). Therefore, studies which mechanically mix carbon amendments with contaminated soil may over-estimate the short-term potential to reduce PCB bioavailability.

4.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of chlorinated organic contaminants characterized as being persistent, bioaccumulative and anthropogenic in nature. Recent studies have shown that both plants and earthworms can accumulate up to six and eighteen times the soil PCB concentration, respectively (173). In Canada, soils containing PCBs are regulated and may require remedial action, including costly techniques such as soil excavation and incineration (4). Therefore, there is a considerable need for cost effective alternative remediation strategies which also minimize the bioavailability of PCBs.

The use of carbon-rich, charcoal like materials such as biochar and activated carbon (AC) for the *in situ* stabilization of organic contaminants in sediments and soils has received increasing attention in recent years (19, 24, 52, 173, 174). The addition of these materials to soils has been shown to immobilize organic contaminants thereby reducing their bioavailability to plants (7, 77, 78, 173), invertebrates (19, 77, 88, 173), and fish (174). Biochar is a charcoal like material produced from the pyrolysis of organic matter under very low oxygen conditions (22), while AC is a more processed form of charcoal which has higher associated costs. Both biochar and AC have high sorptive capacities as a result of their chemical structures, high porosity and large surface areas (12, 13, 23).

In recent years, Europe and the United States have implemented pilot testing of the *in situ* use of AC as a sediment amendment (24). Many studies (18, 24, 96, 97, 149, 174, 175) have shown that as a result of high AC sorption capacity, the porewater hydrophobic organic contaminant (HOC) concentration and the bioaccumulation of HOCs in benthic organisms are decreased. Additionally, studies by Hale et al. (13) and Langlois et al. (77) suggested that AC may be suitable for soil remediation of pyrene and PCBs, respectively.

While AC research has focused on soil and sediment remediation as this product was considered to have the strongest sorption potential (24, 176), biochar research has focused on soil quality improvements and carbon sequestration potential. Biochar offers additional agronomic and environmental benefits such as increases in soil cation exchange capacity (CEC) (133), water holding capacity (177) and decreased fertilizer requirements, leading to increased crop yields at lower costs. Additionally, the carbon component of biochar is stable, and thus may sequester atmospheric carbon (59, 178) and function in the mitigation of climate change. Biochar is quickly gaining in popularity, however there are still only a limited number of studies published examining the use of biochar to minimize the bioavailability of contaminants (72, 73, 143, 173, 179) and most of these studies are laboratory based. Similarly, there are studies available in which AC is applied to soils in the greenhouse (7, 77) and also in the field (11, 18, 24, 78) for sorption of contaminants. However, very few studies compare the *in situ* efficiency of biochar and AC directly (13, 82) and most have utilized laboratory based sorption methods (21, 75, 76, 144, 145).

The present study investigates and compares the performances of two types of biochar and granular activated carbon (GAC) as *in situ* stabilization amendments for PCB-contaminated Brownfield soils under both field and greenhouse conditions. In addition, the effects of an agronomic/environmentally-relevant mixing regime on the ability to reduce PCB uptake of both biochar and AC are compared to standard sorption literature mixing methods.

4.3 MATERIALS AND METHODS

4.3.1 Soil and Materials

The *in situ* experiments were carried out at a Brownfield site in Etobicoke, Ontario (Canada) that was a former manufacturing facility for electrical transformers. The soil was contaminated with commercial Aroclors 1254 and 1260, and has weathered in place over a period of approximately 50 years. It was determined to have a PCB concentration of 71.4 ± 10.8 μg/g (n=5). Using the sodium acetate method for cation exchange capacity (CEC) described by Laird and Fleming (155), the PCB-

contaminated soil had an average CEC of 9.5 ± 1.3 cmol/kg (n=2) and a pH of 7.7. Previously this soil was characterized (114) as being coarse-grained and sandy with a total organic carbon content of 3.5%. Soil was collected from the Brownfield site for both greenhouse experiments.

Two types of biochar and one GAC were used for all field and greenhouse experiments (Table 4-1). Cation exchange capacity for all carbon amendments were determined using the sodium acetate method for CEC described by Laird and Fleming (155). The Brunauer–Emmett–Teller (BET) surface area of the GAC and biochars were measured by N_2 gas sorption analysis at 77 K in a relative pressure range from 0.01 to 0.10 using an ASAP 2000 surface area analyzer (Micromeritics, USA) after degassing at 120°C for a minimum of 2 h. Burt's biochar (Burt's Greenhouses, Odessa ON) was produced from used shipping pallets and construction waste. The temperature within the pyrolysis equipment reached 700^{o}C over 30 mins. BlueLeaf biochar (BlueLeaf Inc, Drummondville, QB) was created via pyrolysis of softwood material at a temperature of 450°C for 2.5 hours. GAC obtained from A.C. Carbone Inc. (St. Jean sur Richelieu, QC) was produced from bituminous coal. The percent organic matter and moisture of the carbon amendments were determined using the loss on ignition procedure outlined by Nelson and Sommers (180), and pH was determined as outlined in Ahmedna et al.(181). Burt's biochar was determined to have a moisture content of 20%, be 63% organic matter as determined by loss on ignition and have a pH of 9. BlueLeaf biochar was determined to have a moisture content of 3.8%, be 97% organic matter as determined by loss on ignition and have a pH of 10. The GAC used in this study had a moisture content of 6.8%, be 39% organic matter as determined by loss on ignition and had a pH of 9. Particle size distribution of all materials was determined via progressive dry sieving (in triplicate) adapted from ASTM D5158-98 (182) using seven U.S. Standard sieves (4.7, 2.0, 1.0, 0.50, 0.25, 0.15, and 0.0075 mm).

Table 4-1 Origin and characteristics of GAC, Burt's biochar and BlueLeaf biochar

Treatment	Source Material	Pyrolysis Temperature (^{o}C)	Moisture (%)	Loss on Ignition (%)	pH	Cation Exchange Capacity (cmol/kg)	Specific Surface Area (m^2/g)	Coarse Particles (%)	Fine Particles (%)
GAC	Bituminous Coal	700	6.8	39	9.2	5.0	808.5	96	4
Burt's biochar	Shipping Pallets/ Construction Waste	700	20	63	9.0	34	373.6	52	48
BlueLeaf biochar	Softwood	450	3.8	97	10	18	54.7	86	14

4.3.2 Field Experimental Design

PCB-contaminated soil was tilled extensively using a 22" garden tiller. The four treatments included in this experiment were i) control (0% carbon amendment), ii) 2.8% (w/w) GAC, iii) 2.8% (w/w) Burt's biochar, and iv) 2.8% (w/w) BlueLeaf biochar. The amendment percentage of 2.8% (w/w) was based on the work of Langlois et al. (77) and Denyes et al. (173). Four plots, 400 cm long by 50 cm wide were dug to a depth of 30 cm, spaced 50 cm apart from each other. The carbon treatments were mixed into their corresponding plot using the following method. Half of the amount of carbon material needed for each plot was added into the plot, covered with half the soil and tilled four times. The remainder of

the measured carbon material and soil were added to the plot and tilled another four times. This method ensured thorough mixing and mimicked agronomic practices.

Each plot received nine pumpkin (*Cucurbita pepo* ssp. *pepo* cv. Howden) seeds purchased from the 'Ontario Seed Company' (Waterloo, ON), however extra seedlings were removed at ca. three weeks, such that each plot contained only three growing plants, evenly spaced at one per square meter. Pumpkin plants were measured for growth on a weekly basis and harvested at 50 days. Average daily temperatures and total precipitation in this region for the duration of the experiment (i.e. June to August 2011), were 21.8^{o}C and 163.6 mm, respectively (183). Plants were watered three times a week regardless of precipitation.

4.3.3 Greenhouse Experiments

4.3.3.1 Initial Greenhouse Experiment

PCB-contaminated soil was collected from the field site immediately prior to initiation of the field experiment for both this greenhouse experiment and the subsequent one described below. The soil was dried, sieved to 16 mm, and then homogenized using the Japanese pie-slab mixing method (156). The soil was amended in triplicate (A, B, and C) with 0% and 2.8% (w/w) GAC, Burt's biochar, or BlueLeaf biochar as in the field experiment). Treatments were manually mixed together (10 turns/replicate/treatment) using a bucket and spade, then the amendment mixture was placed in bottom perforated 6-inch diameter planting pots (total soil weight per planter of 1000 g).

4.3.3.2 Greenhouse Comparison of Mechanically Tumbled vs. Manually Mixed Carbon Amendments

For this second greenhouse experiment, PCB-contaminated soil was either manually mixed or mechanically tumbled in triplicate (A, B and C). Manual mixing was identical to that performed in the initial greenhouse experiment as described above (Section 4.3.3.1). Mechanical tumbling occurred at 30 rpm for 24 h (173). The soil/carbon amendment mixture was placed in the same sized planting pots as the initial greenhouse experiment (Section 4.3.3.1), however the mixture was half the weight (total soil weight per planter of 500 g).

For both greenhouse experiments, each planter received three pumpkin seeds (*Cucurbita pepo* ssp. *pepo* cv. Howden) and extra seedlings were removed such that each planter contained only one growing plant. Pumpkin plants were grown in the greenhouse located at the Royal Military College of Canada (RMC), measured for growth on a weekly basis and harvested at 50 (4.3.3.1) or 36 days (4.3.3.2). Greenhouse temperature was maintained at 27^{o}C (±6^{o}C) and the pumpkins were grown under a 14:10 h (day:night) fluorescent photoperiod. Planters were top and bottom watered to maintain sufficient moisture.

A 30 g composite soil sample was collected from replicates for all treatments for both greenhouse and field experiments, immediately after mechanically mixing or manual mixing with each carbon amendment and after the pumpkins were harvested. A 200 g soil sample was collected from each of the mechanically or manually mixed treatments and analysed for particle size distribution via progressive dry sieving (in triplicate) adapted from ASTM D5158-98 (182) using seven U.S. Standard sieves (4.7, 2.0, 1.0, 0.50, 0.25, 0.15, and 0.0075 mm). All soils were frozen until analysis. Plants were harvested by cutting the shoot of the pumpkin with acetone rinsed scissors as close to the soil surface as possible. Root samples were then collected and rinsed clean with water. Both shoot and root tissues were patted dry, and weighed to the nearest hundredth of a gram. They were then placed in individually labelled Whirlpak® bags and frozen until analysis. Air-monitoring of the greenhouse and field site indicated PCB concentrations were below detectable limits (< 0.1 µg/g), therefore, aerial deposition on the plant tissues was considered negligible.

4.3.4 Experimental Design and Sample Collection for *Eisenia fetida* Tissue

The worm experiment was performed in the RMC greenhouse, post pumpkin harvest. Twenty-five redworms (*Eisenia fetida)* purchased from 'The Worm Factory' (Westport, ON) were added to each pot for 36 days in both the mechanically and manually mixed treatments. The pots were covered with perforated aluminum foil. Soil moisture was maintained ~35% moisture (19, 75, 184).

Surviving worms were collected by emptying the soil from each planter onto a tray (cleaned and rinsed with acetone between samples). Worms were counted, washed using a container of clean water, weighed, depurated for 72 hours at 4ºC, dried for 24 hours at 25ºC and stored in individually labelled Whirlpak® bags and frozen until analysis.

4.3.5 Analytical Procedures

4.3.5.1 PCB Aroclors in soil, plant, and worm samples

All samples were dried at 25°C for 24 h immediately prior to analysis. Plant root and shoot samples were analysed by microwave-assisted extraction (MAE) at RMC. Microwave-assisted extraction was performed at a temperature of 120°C for 35 min in 30 mL of 1:1 hexane:acetone mixture using a Milestone Ethos SEL microwave extraction system. Following extraction, sample extracts were concentrated using a Syncore, the solvent exchanged for hexane, and then extracts were applied to a Florisil column for cleanup (9).

PCB concentrations in soil and worm tissues were analyzed via Soxhlet extraction, based on the methods described by Whitfield Åslund et al. (114) and performed at the Analytical Services Unit located at Queen's University. Briefly, worm samples were finely chopped using metal scissors (rinsed with acetone between samples) and homogenized. Chopped worm samples were dried overnight in a vented oven at 25°C for approximately 12–18 h, and then soil and worm samples were ground with sodium sulphate and Ottawa sand. Decachlorobiphenyl (DCBP) was used as an internal surrogate standard. All soil and worm samples were extracted in a Soxhlet apparatus for 4 h at 4–6 cycles per hour in 250 mL of dichloromethane. The use of both extraction methods was previously validated by Whitfield Åslund et al. (9).

Plant, worm and soil extracts were analyzed for total Aroclors, using an Agilent 6890 Plus gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/μECD), a SPB™-1 fused silica capillary column (30 m, 0.25 mm ID × 0.25 μm film thickness) and HPChem station software. The carrier gas was helium, at a flow rate of 1.6 mL/min. Nitrogen was used as the makeup gas for the electron capture detector (ECD). Detection limits were 0.1 μg/g. All values were reported as μg/g dry weight.

4.3.5.2 Quality Assurance/Quality Control (QA/QC)

One analytical blank, one control and one analytical duplicate sample were prepared and analyzed for every nine samples analyzed by Soxhlet or MAE. The control sample was spiked with a known amount of either Aroclor 1254 or 1260. Decachlorobiphenyl (DCBP) was added to each sample as a surrogate standard prior to extraction. None of the analytical blanks contained PCBs at concentrations above detection limits (0.1 μg/g for total Aroclors) and all control samples were between 80–110% of the expected value. Relative standard deviations between the samples and their analytical duplicate were below 18% for all results and the average surrogate recovery for samples analyzed for total Aroclor was 98%.

4.3.6 Statistical Analyses

PCB concentrations (soil and tissue) are reported on a dry-weight basis. The tissue concentration data were analyzed by one-way analysis of variance (ANOVA) (dependant variable: shoot, root or worm PCB concentration; independent variable: carbon amendment) followed by a post hoc Tukey comparison (levels of: carbon amendment). The particle size distribution of the i) PCB-contaminated soil, ii) the carbon amendments and iii) the 2.8% soil/carbon mixtures were compared between manually mixed and mechanically mixed treatments using a one-way ANOVA. All residuals of the data were determined to be normally distributed as determined by a Kolmogorov Smirnov test for normality. A significance level of α=0.05 was used for all tests, and results were recorded with the standard error of the mean. All statistical analyses were performed using SPLUS 8.0.

4.4 Results and Discussion

4.4.1 Field and Greenhouse Experiments

In the field experiment all carbon amendments significantly reduced the uptake of PCBs into the root tissue of *C. pepo* ($p < 0.05$). Activated carbon reduced *C. pepo* root PCB uptake by 74%, and Burt's biochar and BlueLeaf biochar reduced PCB uptake by 72% and 64%, respectively (Figure 4.1a). There were no significant differences between the GAC, Burt's biochar and BlueLeaf biochar treatments in either tissue type implying that all carbon amendments performed equally in terms of their ability to minimize PCB phytoavailability *in situ* (Figure 4.1a and b). When this experiment was replicated in the greenhouse, the results were quite different with only the GAC amendment significantly reducing the PCB uptake into *C. pepo* root tissue (Figure 4.1a). In the field experiment, *C. pepo* shoot tissue PCB concentration was not significantly reduced as a result of any carbon amendment (Figure 4.1b) whereas in the greenhouse the addition of BlueLeaf biochar did significantly reduce shoot uptake (55%). As in Denyes et al. (173) and Langlois et al. (77), *Cucurbita pepo* spp. *pepo* were chosen to study PCB phytoavailability because this species has been widely documented to accumulate significant amounts of organic contaminants (9, 114, 122, 130, 185) , including PCBs from the soil into root and shoot tissues via transport in the xylem sap (127)

The shoot tissue of *C. pepo* plants grown *in situ* in the control treatment, i.e. PCB-contaminated soil alone, grew well, being on average 2.5 meters long and weighing 1013 g. This result was expected as Whitfield Åslund et al. (9, 114) demonstrated that *C. pepo* can accumulate significant concentration of PCBs in plant shoots without jeopardizing plant health. In the greenhouse, pumpkins grown in PCB-contaminated soil amended with 2.8% Burt's or BlueLeaf biochar grew even larger (97% and 100%, respectively) than the control pumpkins ($p < 0.05$). These treatments also produced *C. pepo* plants that were 72% and 75% larger than the *C. pepo* plants grown in the 2.8% GAC amendment. These greenhouse results are expected as one of the agronomic benefits of adding biochar to soil is that it can reduce the overall tensile strength of the soil (159) which will in turn alleviate root elongation and proliferation problems, allow seeds to germinate more easily, and invertebrates to move more readily through the soil (135, 136). Pumpkin plants grown *in situ* in PCB-contaminated soil amended with BlueLeaf biochar were 92% larger than the controls and 28% larger than the *C. pepo* plants grown in GAC amendment, however due to large standard deviations that are often associated with field data, these results are not significant. These results are further discussed in Section 4.4.2.

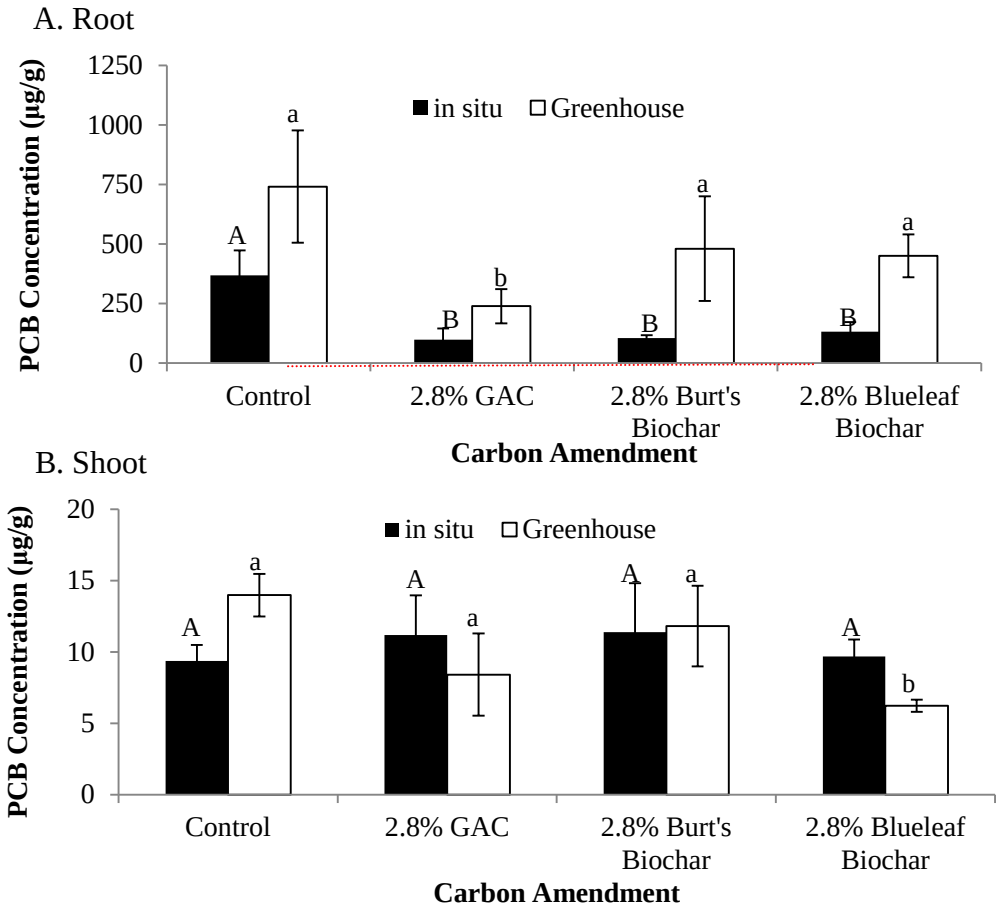

Figure 4.1 Polychlorinated biphenyl (PCB) concentrations in A) root and B) shoot tissue of *Cucurbita pepo* grown in unamended PCB-contaminated Brownfield soil and Brownfield soil amended with 2.8% GAC or biochar, carbon amendments. Error bars represent one standard deviation. Upper-case (*in situ*) and lower-case letters (greenhouse) indicate statistically significant differences between treatments ($p < 0.05$). The line represents (A) the high PCB-contaminated soil concentration of 71 µg/g.

Decreases in contaminant uptake and increases in plant growth can be explained by strong sorption of the PCB molecules onto the AC/biochar particles and the relative improvements to substrate quality these materials offer to the intensely degraded Brownfield soil. Results of *in situ* carbon amendment showed that AC and biochar statistically performed equally in terms of their abilities to minimize the phytoavailability of PCBs to *C. pepo*. However, biochar outperformed AC with respect to improving substrate quality and increasing plant growth. These results are consistent with the recent findings of Oleszczuk et al. (12, 176), who reported that AC was more effective than biochar in reducing sewage sludge toxicity, while biochar was more effective in improving *Lepidium sativum* growth. Given that in this study, the biochars offered additional agronomic and environmental benefits and performed statistically as well as AC in terms of ability to minimize PCB uptake, biochar may offer a lower cost, greener (i.e. sustainable) alternative to AC amendment for soil remediation.

Although substantial, the effects of all carbon soil amendments observed both *in situ* and in the greenhouse were less than expected and there were significant differences in the greenhouse and field results. Upon careful review of the literature, and comparison to our previous study (173), one difference between this study and others, that report up to 99% reductions in PCB bioavailability (18, 19, 77), is the

mixing strategy. The study described here attempted to mimic agronomic methods which are relevant from an engineering perspective for the *in situ* stabilization of PCBs, however most published studies mix the carbon amendment and soil mechanically for hours or days (13, 75, 77, 173). Mass kinetic modelling has also been used to explain PCB immobilization in sediments as a result of AC amendments using different mixing approaches (81, 95, 172). Generally *in situ* mixing has lower short term efficiencies compared to laboratory based experiments, as it may result in spatially heterogeneous AC particles, minimizing PCB contact and delaying treatment benefits (140, 149). Using passive sampling efforts and kinetic modeling however, it has been suggested that over-time even poorly mixed systems will achieve significant PCB immobilization by AC (81).

To quantify the effect of mixing at our PCB-contaminated Brownfield site, an additional greenhouse experiment to directly compare mixing methods was performed in which the carbon amendments were either manually mixed with a spade and bucket, or mechanically mixed in a soil tumbler for 24 hours at 30 rpm.

4.4.2 Manual versus. Mechanical Mixing Regimes

4.4.2.1 Particle Size Distribution

In this study, particle size distribution was analyzed in an effort to understand differences in sorption and growth between manually and mechanically mixing carbon amendments with soil. Amendment of AC/biochar to Brownfield soils alters the particle size distribution which in turn may change the soil structure, texture, and porosity. These changes are expected to improve Brownfield substrate quality by altering tensile strength and oxygen content, water storage capacity, and nutritional status of the soil within the plant rooting zone (135). Currently, few studies are available linking biochar sorption potential with particle size distribution (75, 144, 169, 186), while even fewer report on soil substrate effects (187, 188). From a sorption potential perspective, smaller particle sizes of biochar/AC are favorable as this is expected to increase the external surface area, and thus ability of the contaminant to access pore space for binding (88). Additionally smaller particle sizes are favored for sorption as there are then a greater number of particles per unit volume of soil (89). However, larger particle sizes may favorably increase soil aeration and prevent biochar/AC movement into the subsoil over time. Kasozi et al. (169), Cabrera et al. (186) and Chai et al. (75) reported higher sorption of catechol, fluometuron and polychlorinated dibenzo-p-dioxins/dibenzofurans, respectively, to fine particle sized biochar versus coarse biochar.

Activated carbon is generally available as two types, powdered (PAC) or granular (GAC). These two types of AC have different particles sizes and generally PAC particles are around 20 μm in size while GAC particles are in the range of 300-1700 μm (13, 75, 77, 78, 97, 139). Studies examining biochar sorption often homogenize the biochar to pass through a 250 μm sieve (or similar size) (75, 76, 189, 190) which may enhance sorption potential, however, not be feasible for large scale application and may overestimate the inherent sorption potential.

Based on the studies by Kasozi et al. (169) and Cabrera et al (186) which defined fine particle sizes as those < 0.25 mm and < 0.2 mm, respectively in this study, fine particles are defined as those $\leq$ 0.25 mm and coarse particles as $\geq$ 0.5 mm. The carbon amendments used increased in their relative proportions of coarse particles from Burt's biochar (52%) < BlueLeaf biochar (86%) < GAC (96%) (Table 4-1). Burt's and BlueLeaf biochars were more uniformly distributed among all sieve sizes, while 95% of the GAC particles were retained in the 1 mm and 0.5 mm sieves only. Although GAC is considered the most 'coarse' carbon amendment in this study, both Burt's and BlueLeaf biochar contain higher proportions of larger particle sizes (4.7 and 2 mm).

When the carbon amendments were manually mixed into the contaminated soil, the average percent of fine particles in the soil/carbon mixture were 32%, 32% and 31%, for the amendment of GAC,

Burt's biochar and BlueLeaf biochar, respectively (Figure 4.2a). However these percentages significantly increased for all carbon amendments to 47%, 50% and 49%, respectively, when the soil was mechanically mixed ($p < 0.05$).

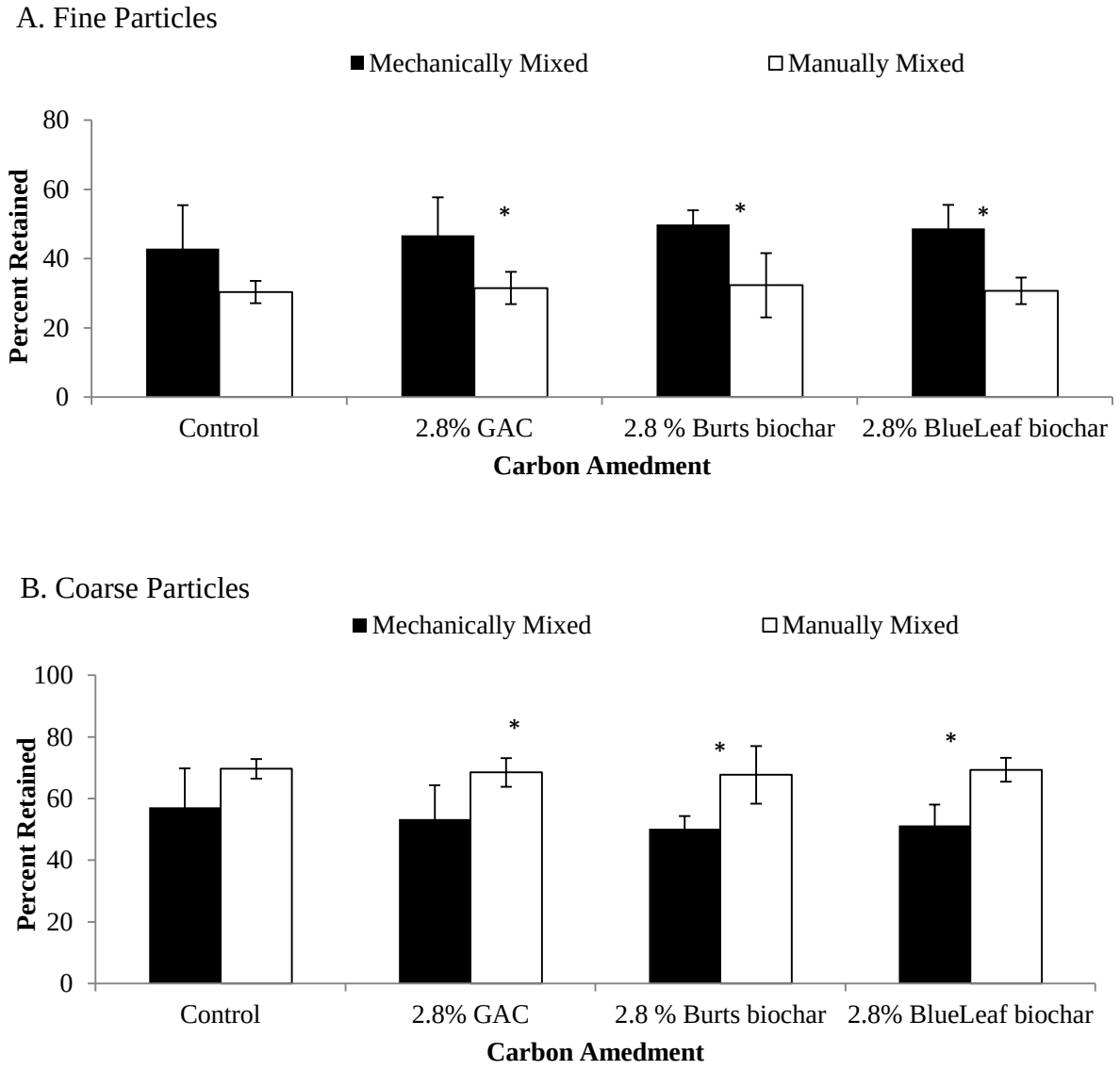

Figure 4.2 Particle size distribution of biochar and activated carbon amendments mechanically or manually mixed with PCB-contaminated Brownfield soil at 2.8% (by weight). Fine particles (A) are those 0.25 mm and smaller and coarse particles are 0.5 mm or larger (B). Error bars represent one standard deviation. * indicate statistically significant differences between mixing methods ($p < 0.05$).

4.4.2.2 The Effect of Mixing Regime on Plant and Worm PCB Concentrations

When AC and biochar were manually mixed into PCB-contaminated soil there were no significant reductions in PCB concentration in *C. pepo* root and shoot tissues (Figure 4.3a and 4.3b), and the only significant reduction in *E. fetida* tissue was seen in AC-amended soil (54% reduction) (Figure 4.3c). This result is similar to the initial greenhouse study where only GAC and BlueLeaf reduced root and shoot uptake, respectively. However, when the same treatments were mechanically mixed (i.e. 24 hrs at 30 rpm) PCB uptake became significantly reduced. Activated carbon mechanically mixed into soil at 2.8% reduced the PCB concentration in *C. pepo* root and shoot tissue by 74% and 66%, respectively ($p < 0.05$) (Figure 4.3a and b) and *E. fetida* tissue by 72% (Figure 4.3c). Burt's biochar amendment at 2.8% reduced the uptake of PCBs into *C. pepo* root tissue and *E. fetida* tissue by 53% and 44%, respectively ($p < 0.05$). BlueLeaf biochar amendment (2.8%) also reduced the uptake of PCBs into *C. pepo* root tissue by 66% and *E. fetida* tissue by 44%. These results are more consistent with our previous work (173) which found that 2.8% Burt's biochar addition to PCB-contaminated soil decreased the uptake of PCBs to *E. fetida* by 53% and *C. pepo* roots by 77%.

This is the first study demonstrating PCB bioavailability reductions in soils under environmentally relevant mixing methods. On average, when PCB-contaminated soil was mechanically mixed for 24 hrs with biochar or AC, PCB concentrations in shoot, root and worm tissues were 66%, 59% and 39% lower than the manually mixed treatments (i.e. with a spade and bucket). In our work the percent reductions in earthworm PCB concentration after AC amendment increased from 54% (manual mixing) to 72% (mechanical mixing), respectively. This is consistent with an earlier study by Sun and Ghosh (88), which showed larger PCB reductions (14%) in *L. variegatus* after mixing AC and sediment for longer times, compared to short-term mixing. These authors also stressed that under identical conditions with the same GAC dosage and mixing time, reduction in AC particle size decreased PCB bioaccumulation. In the current study, particle size decreased by as much as 18% as a result of mechanical mixing, offering an explanation for the greater PCB reductions. This 24 hour mixing strategy increased the soil/carbon amendment contact time, improved the homogeniety of the mixture and offered a greater number of particles per unit volume of soil.

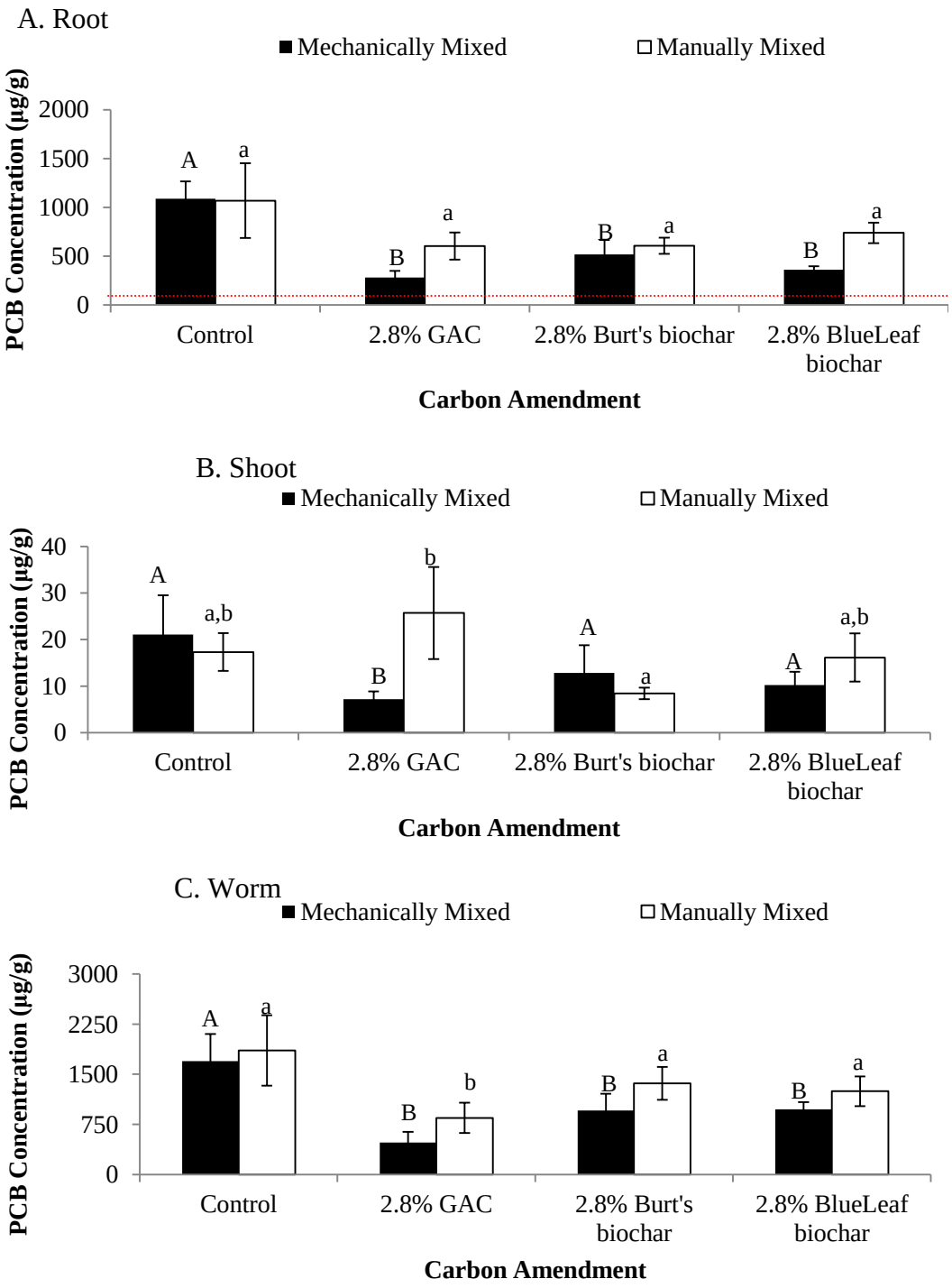

Figure 4.3 Polychlorinated biphenyl (PCB) concentrations in A) root B) shoot and C) worm tissue of *Cucurbita pepo* and *Eisenia fetida* grown in unamended PCB-contaminated Brownfield soil and Brownfield soil amended with 2.8% GAC or biochar, carbon amendments. Upper-case (mechanically mixed) and lower-case letters (manually mixed) indicate statistically significant differences between treatments ($p < 0.05$). The line represents (A) the high PCB-contaminated soil concentration of 71 µg/g.

A biota to sediment/soil accumulation factor (BSAF) (BSAF= $[PCB_{tissue}/[PCB]_{soil}$) is often used to quantify the bioavailability of organic contaminants to plants and earthworms. The mean BSAF reduction for all mechanically mixed carbon amendments in earthworms was 90 ± 3% and 58 ± 13% in plants (roots and shoots). The mean BSAF reductions for all manually mixed carbon amendments for earthworms (38 ± 15%) and plants (roots and shoots) (21 ± 38%) (Table 4-2), observed in this study was closely related to the results of Jakob et al.(78). These authors performed *in situ* amendment of GAC to PAH-contaminated soils using an excavator, which may be considered an agronomic mixing method similar to that in our study. The study reported that GAC (particle size range: 300-800 μm) reduced BSAFs of PAHs by an average of 47 ± 44% in earthworms and 46 ± 35% in plants. These authors also found that powdered activated carbon (PAC), i.e. AC with particles smaller than 45 μm, performed better than GAC, reducing earthworm BSAF by on average 72 ± 19%.

It appears from the large reductions in PCB uptake by both plants and worms as a result of mechanical mixing, that mixing regime and particle size may explain the differences in contaminant uptake reported in literature (18, 19, 77, 173) and in the present study. Based on the shift towards smaller grain sizes as a result of mechanical mixing, it is evident that this type of mixing achieved a more homogeneous distribution of AC/biochar in the soil and increased the accessible surface area (95), again resulting in lower PCB uptake by plants and worms. This effect was demonstrated by Werner et al. (95) using passive samplers and Sun and Ghosh (88) using worms, but this is the first study to report on the effects in soils on plant and worm uptake. Both the *in situ* and mixing experiments presented were conducted over a relatively short periods of time (60 and 40 days, respectively). Based on mass transfer kinetic modeling and a five year field study of PCB-contaminated sediment in California, it has been suggested that over-time the lower treatment effects of poorly mixed systems will diminish and treatment goals will eventually be achieved, although it may take many years longer than in a homogeneous system (81, 95). Therefore, a monitoring component should be included in future work to determine if this phenomenon also holds true in soil systems. Also, from a soil remediation perspective to achieve the desired reduction in risk, carbon amendments with larger particle size distributions should be ground and thoroughly mixed into the soil with agronomic methods in mind.

Table 4-2 Bioaccumulation factor of polychlorinated biphenyls (PCBs) into *Cucurbita pepo* and *Eisenia fetida* grown in/exposed to unamended PCB-contaminated Brownfield soil and Brownfield soil amended with 2.8% GAC or biochar, carbon amendments with two mixing methods

		Biota to Soil Accumulation Factors		
		C. pepo ssp. *pepo*		*E. fetida*
Mixing Type	Treatment	Root	Shoot	
Manually Mixed	Control	15 ± 5.1	0.2 ± 0.1	26 ± 7.4
	2.8% GAC	8.6 ± 1.9	0.4 ± 0.1	12 ± 3.2*
	2.8% Burt's biochar	8.5 ± 1.2	0.1 ± 0.0	19 ± 3.4
	2.8% BlueLeaf biochar	10 ± 1.5	0.2 ± 0.1	17 ± 3.1
Mechanically Mixed	Control	15 ± 2.5	0.3 ± 0.1	25 ± 5.7
	2.8% GAC	3.9 ± 1.0*	0.1 ± 0.0	6.1 ± 2.3*
	2.8% Burt's biochar	7.2 ± 2.1*	0.2 ± 0.1	13 ± 3.5*
	2.8% BlueLeaf biochar	5.0 ± 0.5*	0.1 ± 0.0	14 ± 1.5*

* indicate statistically significant differences from the corresponding control.

Mixing regime also affected the biomass of both *C. pepo* shoot and *E. fetida* tissues. On average, *C. pepo* plants grown in soil that was manually mixed with biochar had 28% more biomass than plants grown in soil that was mechanically mixed, a result likely linked to improved substrate texture due to larger particle sizes. However, as in the *in situ* experiment, GAC amendment which was manually mixed did not increase *C. pepo* biomass. Soil amendment with GAC is not expected to offer comparable soil quality improvements to biochar. The slow oxidation of the biochar surface increases soil cation exchange capacity (69), while its macroporosity (> 50 nm) decreases nutrient and water leaching losses (135). Activated carbon is produced with the objective of maximizing microporosity (< 2 nm), which is important for contaminant sorption and allows it to remain stable over long periods of time. The average biomass increase of *E. fetida* as a result of AC/biochar soil additions was significantly larger in the mechanically mixed treatment (34 ± 11%) than the manually mixed treatment (1 ± 7%) ($p < 0.05$). In all mechanically mixed treatments and all but GAC manually mixed treatments, earthworm biomass increased over the respective controls, suggesting that biochar amendments did not cause adverse ecotoxicological effects. The reduction in particle size and subsequent increase in external surface area and mixture homogeneity as a result of mechanically mixing likely explain why AC/biochar treatments were more effective at minimizing the uptake of PCBs to *C. pepo* and *E. fetida* than when manually mixed and the ensuing reduced toxicity may then explain why *E. fetida* biomass was greater in the mechanically mixed treatments.

4.5 Conclusions

This is the first study to investigate and directly compare the effects of *in situ* biochar and AC amendments to a PCB-contaminated Brownfield soil. It was determined that both biochars performed equally to GAC at decreasing phytoavailability of PCBs to pumpkin roots. When biochar was added to the degraded PCB-contaminated Brownfield soil, pumpkin *C. pepo* plants grew larger than plants grown in the controls and AC treatments and neither biochars caused adverse ecotoxicological effects to soil invertebrates. This study also shows that laboratory based mixing may exaggerate the sorptive capacities of both AC and biochar, at least in the short-term. Although similar work has been conducted using kinetic modelling or passive samplers in PCB-contaminated sediment systems, our work is novel in approaching the effects of mixing in PCB-contaminated soil (71.4 µg/g) systems to both plants and earthworms. Reductions in *E. fetida* tissue as a result of biochar addition were only significant when the soil was mechanically mixed for 24 hrs at 30 rpm, and under greenhouse conditions. Neither AC nor biochar when manually mixed were capable of significantly reducing PCB uptake by *C. pepo*. Sorption and subsequent reductions in bioavailability of organic contaminants as a result of carbon amendments, are important in mitigating risks posed to both environmental and human health. Thus although AC and biochar show significant potential to serve as sorbents for the *in situ* stabilization of organic contaminants, future research should focus on environmentally relevant application methods to better determine the actual remediation potential of these materials.

5. PHYSICAL, CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF SIX BIOCHARS PRODUCED FOR THE REMEDIATION OF CONTAMINATED SITES

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5.1 ABSTRACT

The physical and chemical properties of biochar vary based on feedstock sources and production conditions, making it possible to engineer biochars with specific functions (e.g. carbon sequestration, soil quality improvements, or contaminant sorption). In 2013, the International Biochar Initiative (IBI) made publically available their Standardized Product Definition and Product Testing Guidelines (Version 1.1) which set standards for physical and chemical characteristics for biochar. Six biochars made from three different feedstocks and at two temperatures were analyzed for characteristics related to their use as a soil amendment. The protocol describes analyses of the feedstocks and biochars and includes: cation exchange capacity (CEC), specific surface area (SSA), organic carbon (OC) and moisture percentage, pH, particle size distribution, and proximate and ultimate analysis. Also described in the protocol are the analyses of the feedstocks and biochars for contaminants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), metals and mercury as well as nutrients (phosphorous, nitrite and nitrate and ammonium as nitrogen). The protocol also includes the biological testing procedures, earthworm avoidance and germination assays. Based on the quality assurance/ quality control (QA/QC) results of blanks, duplicates, standards and reference materials, all methods were determined adequate for use with biochar and feedstock materials. All biochars and feedstocks were well within the criterion set by the IBI and there were little differences among biochars, except in the case of the biochar produced from construction waste materials. This biochar (referred to as Old biochar) was determined to have elevated levels of arsenic, chromium, copper, and lead, and failed the earthworm avoidance and germination assays. Based on these results, Old biochar would not be appropriate for use as a soil amendment for carbon sequestration, substrate quality improvements or remediation.

5.2 INTRODUCTION

Biochar is a carbon-rich by-product produced during the pyrolysis of organic matter (59). Interest, both publicly and academically, in adding biochar to soils, stems from its ability to improve soil quality and plant growth (173, 191), sustainably sequester carbon (192), and sorb harmful contaminants (12, 13, 24, 173, 191) whilst simultaneously offering alternatives for waste management and energy production by pyrolysis.

Biochars are being produced by numerous companies and organizations worldwide via different pyrolysis systems. Materials used for biochar production include (but are not limited to) woodchips, animal manure and construction wastes (59). These differences are expected to alter the biochars' physical and chemical properties and thus their ability to improve substrates, promote long term stability and increase sorption capabilities. Additionally, during the pyrolysis process the biochar may become unintentionally contaminated with metals, PAHs and PCBs as a result of contaminated feedstocks or inappropriate pyrolysis conditions. Therefore, before biochar can be applied on a large scale to the environment as a soil amendment, careful characterization of the biochar for contaminants, specific surface area, cation exchange capacity, earthworm avoidance and germination and others suggested by the International Biochar Initiative (IBI) must be conducted. In 2013, the first Standardized Product Definition and Product Testing Guidelines for Biochar which sets standards for biochar physical and chemical characteristics, was published and made publically available.

Research has shown that biochar produced at a commercial greenhouse in Odessa, ON Canada has the ability to significantly improve plant growth in intensely degraded soils and sorb persistent organic pollutants (POPs) such as PCBs (173, 191). This biochar has been produced from three different feedstocks (i.e. organic matter sources) via a boiler system where the heat generated is used to warm their greenhouse operation during winter months.

This study provides characterization data pertinent to the production of biochar in a biomass boiler, and the use of biochar as a soil amendment. The objective of this study is to thoroughly characterize the physical, chemical and biological characteristics of six biochars according to standards set by the IBI in their Standardized Product Definition and Product Testing Guidelines (Version 1.1) (2013). These characteristics will be linked, where possible, to the performance of each biochar as agricultural amendments and their ability to sorb contaminants.

5.3 PROTOCOL

Note: Chemical analyses were conducted at the Analytical Services Unit (ASU) in the School of Environmental Studies at Queen's University (Kingston, ON). The ASU is accredited by the Canadian Association for Laboratory Accreditation (CALA) for specific tests listed in the scope of accreditation. Other analyses, including greenhouse trials, were conducted at The Royal Military College of Canada (Kingston, ON) in the Department of Chemistry and Chemical Engineering.

5.3.1 General Considerations

5.3.1.1 To ensure quality assurance and quality control, analyze an analytical blank and an analytical duplicate, a sample duplicate and a standard reference material with each batch of samples (maximum batch size 10) for the methods in the protocol.

5.3.1.2 Establish duplicate samples when sub-sampling from the original sample and go through the same preparation as the unknown samples. Ensure that duplicate values are within 20% of each other or repeat the analysis. Ensure that analysis outcomes of the blanks are below detection limits for the corresponding method. Standard reference material limits depend on the individual method but ensure that they are generally within 15 to 30% of the expected value.

5.3.1.3 In many of the methods described in the protocol, details are included on the suggested order of sample analysis including calibrants, blanks, high and low standards, and unknown samples. This is to ensure no cross contamination between samples and ensure a high standard to QA/QC.

Note: Six biochars were produced at a commercial greenhouse and analyzed for chemical, physical and biological parameters. The names of each biochar reflect their production parameters or feedstock source (Table 5-1).

5.3.2 Test Category A: Basic Biochar Utility Properties

5.3.2.1 Moisture and Organic Matter Content

5.3.2.1.1 Use the loss on ignition procedure outlined by Nelson and Sommers (1996).

5.3.2.1.2 Include a sample duplicate and standard reference material (Ottawa Sand) for every 10 unknown samples.

5.3.2.1.3 Label 50-mL beakers with heat resistant marker, oven dry them at 105^{o}C, allow them to cool then record weight.

5.3.2.1.4 Weigh 2 g of air-dried sample into the oven-dried beaker. Dry sample at 105^{o}C for 24 hours, then remove from the oven and allow to cool.

5.3.2.1.5 Once cool, weigh the beaker and the sample (X = weight of dried sample - weight of beaker)

5.3.2.1.6 Place the sample in the muffle furnace and heat for 16 hours covering at 420^{o}C. Remove the sample from the furnace and allow to cool. Weigh the beaker with sample again and record the weight (Y = weight of ashed sample - weight of beaker).

5.3.2.1.7 Perform the following calculations:

i) Loss on Ignition = X-Y

ii) % Moisture = ((Sample Weight – X)/Sample Weight) x 100%

iii) % Organic Matter = (Loss on Ignition/X) x 100%

5.3.2.2 Proximate and Ultimate Analysis

Note: For proximate/ultimate analysis, four samples were analyzed: Low, High, Standard Fuel and High 2. PAH analysis was carried out on Low, High, and Standard Fuel. These were chosen as representative of the biochars produced since 2012.

5.3.2.2.1 Conduct Proximate and Ultimate analyses at a commercial facility based on methods: ASTM D3172-13(193) and D3176-09, Standard Practice for Proximate and Ultimate (194) Analysis of Coal and Coke, respectively.

5.3.2.3 pH

5.3.2.3.1 Calibrate the pH probe daily before use with calibration standards

5.3.2.3.2 Add 0.25 g biochar to 25 mL distilled, deionized water

5.3.2.3.3 Shake manually for 2 mins, then centrifuge for 3000 x g for 5 mins

5.3.2.3.4 Collect supernatant into glass test tube and measure pH.

5.3.2.4 Particle Size Distribution

5.3.2.4.1 Analyze all samples in triplicate via progressive dry sieving adapted from ASTM D5158-98 (182) using seven U.S. Standard sieves and pan (4.7, 2.0, 1.0, 0.50, 0.25, 0.15, and 0.0075 mm)

5.3.2.4.2 Record the weight of each empty sieve and stack the sieves in order from pan to 4.7 mm with the 4.7 mm sieve being at the top.

5.3.2.4.3 Place 60 g of biochar in the 4.7 mm sieve, place the lid on top and secure the stack of sieves on the shaker.

5.3.2.4.4 Shake for 10 min and record the weight of each sieve. Report the data in an excel file as percent remaining in each sieve.

Table 5-1 Feedstock type, pyrolysis temperature and physical characteristics of the six biochars

Sample	Feedstock	Pyrolysis Temperature (°C)	Organic Matter (LOI) (%)	pH	CEC (cmol/kg)	PSD Coarse (%)	PSD Fine (%)	SSA (m^2/g)
Old	1	>700	63.2	9.3	34.8	51.7	48.3	373.6
New	2	700	97.8	9	16	98.7	1.3	324.6
Low Temp	2	500	96.7	8.7	15.9	86.2	13.8	336.9
High Temp	2	>700	97.9	8.4	11.1	98.1	1.9	419.5
Third Feedstock	3	700	96.2	9.6	13.2	97.6	2.4	244.4
High Temp-2	3	>700	97.1	9.1	17.1	97.9	1.9	428

LOI: Loss on Ignition, CEC: Cation Exchange Capacity, PSD: Particle Size Distribution, SSA: Specific Surface Area

5.3.3 Test Category B: Toxicant Reporting

5.3.3.1 Germination Tests

5.3.3.1.1 Use the seed germination testing method outlined by Solaiman et al. (2012) (195).

5.3.3.1.1.1 Use filter paper and potting soil as positive controls. Note: Without a substrate such as filter paper in the petri dishes the seeds will rot.

5.3.3.1.1.2 Ensure that the respective weights of each treatment is 3 g of biochar, 10 g of potting soil, and 1 piece of filter paper. Note: These values are based on volume in the Petri dish so that each dish is ~50% full (by volume).

5.3.3.1.1.3 Into the Petri dishes (8.5 cm in diameter), place five *Cucurbita pepo* spp. *pepo* (pumpkin) seeds and 50 *Medicago sativa* (alfalfa) seeds into each treatment.

5.3.3.1.1.4 Using a graduated cylinder add 15 mL of water to all Petri dishes, then cover them with their respective lids.

5.3.3.1.1.5 Place the Petri dishes for germination under a 14:10 h (day:night) fluorescent photoperiod and maintain temperature at 27ºC (±6ºC).

5.3.1.1.6 After seven days record the number of seeds germinated. Report results as % germinated per Petri dish. Measure the root length of germinated seeds using a ruler. Report root lengths as a sum for each Petri dish (cm/Petri dish).

5.3.3.2 Earthworm Avoidance

5.3.3.2.1 Store *Eisenia fetida* in a healthy soil matrix comprised of peat moss and potting soil and maintain soil moisture at ~30%.

5.3.3.2.2 Use earthworm avoidance method described by Li *et al*. (2011). Choose worms ranging from 0.3-0.6 g in size.

5.3.3.2.2.1 For this assay, use six avoidance wheels (Figure 5.1) or similar structure to those outlined in Environment Canada's Acute Avoidance Test (Environment Canada, 2004).

5.3.3.2.2.2 Mix biochars separately using a spade and bucket with potting soil at a rate of 2.8% (by weight).

5.3.3.2.2.3 Fill each of the six compartments with 120 g of soil or soil/biochar mixture, with every other compartment serving as an unamended control (Figure 5.1) i.e. soil without biochar. Add 10 worms to the round middle compartment.

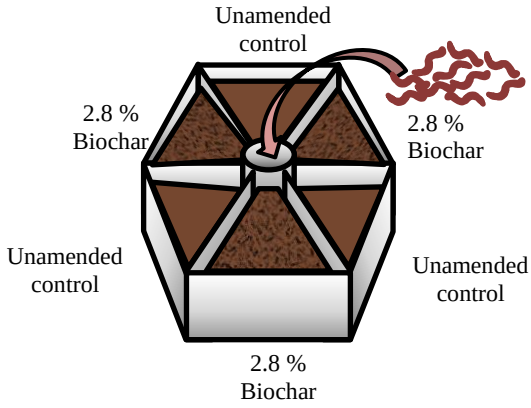

Figure 5.1 Earthworm avoidance wheel. The wheels are produced from steel and the worms are allowed to move throughout the compartments via multiple holes which are approximately 5 cm in diameter.

5.3.3.2.2.4 Expose the worms for 48 hours keeping the avoidance wheel covered with aluminum foil to prevent worm escape. Maintain temperature conditions for the avoidance wheels between 20-25°C. Monitor the soil moisture and maintain at ~30%.

5.3.3.2.2.5 After 48 hours remove the worms and record their location in the avoidance wheel, i.e. if they are in the i) amended or ii) unamended compartments. Do not reuse worms for future testing.

5.3.3.3 Polycyclic Aromatic Hydrocarbons (PAHs)

5.3.3.3.1 Analyze PAHs by solvent extraction and GC-MS based on EPA 8270(196).

5.3.3.4 Polychlorinated Biphenyls (PCB) Concentration

5.3.3.4.1 Dry samples (10 g) overnight at 25°C for 18-24 hours then grind them to a fine powder (particle size < 0.15 mm) with 10 g sodium sulphate and 10 g Ottawa sand.

5.3.3.4.2 Include one analytical blank (Ottawa sand), one control (a known amount of PCB standard) and one analytical duplicate sample for every 10 unknown samples.

5.3.3.4.3 Place 2 g sample into Soxhlet thimble and add 100 µL decachlorobiphenyl (DCBP) as an internal surrogate standard.

5.3.3.4.4 Extract samples in a Soxhlet apparatus for 4 h at 4–6 cycles per hour in 250 mL of dichloromethane.

5.3.3.4.5 Using a gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/µECD), a fused silica capillary column (30 m, 0.25 mm ID × 0.25 µm film thickness) and appropriate software analyze biochar extracts for total Aroclors. Use helium as the carrier gas at a flow rate of 1.6 mL/min. Use Nitrogen as the makeup gas for the electron capture detector (ECD). Report values as µg/g dry weight.

5.3.3.5 Metal Analysis

5.3.3.5.1 Air-dry samples for 18-24 hours and grind into a fine powder (particle size < 0.15 mm) with a mortar and pestle.

5.3.3.5.2 Using reagent grade concentrated acids, heat 0.5 g of the sample in 2 mL 70% (w/w) nitric acid and 6 mL 38% (w/w) hydrochloric acid, until the volume is reduced to 1-2 mL. Then make-up the solution to 25 mL in a volumetric flask using distilled, deionized water, filtered through a Whatman No. 40 filter paper.

5.3.3.5.3 Analyze samples using a simultaneous inductively coupled plasma atomic emission spectrometer (ICP-AES) with the following standards/controls (see step 3.5.3.1). Analyze multi-element ICP standards and check % error and correlation coefficients of the calibration curves. Standards are purchased in custom blends with many elements in each standard. Each element has a 3 point calibration curve (for example cadmium is run at 0, 0.1, 1.0 and 5 ppm). Verify curves with calibration check standards. Recalibrate approximately every 18 samples.

5.3.3.5.3.1 Add internal standards (indium and scandium) ‘on line’ with samples to verify instrument stability. Analyze samples with additional quality control standards including certified reference materials (Bush, Branches and Leaves; White Cabbage and Spinach), method blanks (add acids to an empty digestion tube and treat them as described in 5.3.3.5.2 above), analytical duplicates, and field duplicates.

5.3.3.6 Mercury

5.3.3.6.1 Ensure the instrumentation meets the criteria outlined in US EPA Method 7473 and allows for direct mercury measurement

5.3.3.6.2 Weigh 100 mg of ground air-dried biochar (particle size < 0.15 mm) into quartz or nickel weigh boats.

5.3.3.6.3 Use an ICP-AES stock solution of 1000 µg/mL Hg and 5% hydrochloric acid in double deionized water (DDI) to make working stocks (5 µg/mL, 1 µg/mL , 0.1 µg/mL , 0.01 µg/mL) and calibration standards.

5.3.3.6.4 Use a cleaned empty boat as a method blank. Analyze samples starting with a Method blank, Low QC (20 ng Hg – 20 uL of 1 µg/mL Hg), Blank, High QC (200 ng Hg – 40 uL of 1 µg/mL Hg), Blank, Blank, Standard Reference Material (MESS-3), Blank, MESS-3, Blank, Sample 1, Blank, Sample 2, Blank, Sample 2 dup, Blank, Sample 3, Blank etc.

5.3.3.6.5 Place the boats in the instrument chamber where the sample will thermally decompose in a continuous flow of oxygen. Note: The combustion products will then be carried off in the oxygen flow and then further decomposed in a hot catalyst bed. Mercury vapors will be trapped on a gold amalgamator tube and subsequently desorbed for spectrophotometric quantitation at 254 nm.

5.3.4 Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties

5.3.4.1 Ammonium as Nitrogen

Note: The method makes use of the Berthelot reaction wherein ammonium salts in the solution react with phenoxide. Addition of sodium hypochlorite causes the formation of a green-colored compound. Sodium nitroprusside is added to intensify the color.

5.3.4.1.1 Weigh 5 g of ground air-dried sample (particle size < 0.15 mm) into a 125-mL Erlenmeyer flask. Add 50 mL of 2 M (0.01% (v/v) KCl. Put the flasks on a rotating shaker for 1 hr at 200 rpm. After shaking is complete, filter the samples through Whatman No. 42 filter paper into 100-mL plastic vials.

5.3.4.1.2 Prepare Reagent Solutions:

5.3.4.1.2.1 Alkaline Phenol- measure 87 mL of liquefied phenol into 1-L volumetric filled 2/3 with double deionized (DDI) water. Add 34 g NaOH, make up to volume with DDI water.

5.3.4.1.2.2 Hypochlorite Solution- using 100-mL graduated cylinder measure 31.5 mL of commercial bleach (5-10%) and fill to 100 mL with DDI water. Transfer to bottle and add 1.0 g of NaOH pellets and allow them to dissolve.

5.3.4.1.2.3 EDTA solution- dissolve 32 g of di-sodium EDTA and 0.4 g NaOH in a 1-L volumetric filled 2/3 with DDI water. Add 0.18 g nitroprusside and dissolve by shaking. Make up to volume with DDI water and add 3 ml Triton (10%).

5.3.4.1.3 Make calibration standards (0.1, 0.2, 0.3, 0.5, 1.0, and 2.0 µg/mL N Concentration) using reagent grade NH_4Cl and DDI water. Prepare QC reference standard from a reagent grade source of ammonium chloride different from the source used to make the standards. Use double deionized water as the blanks.

5.3.4.1.4 Begin running the autoanalyzer. Design each run to start with the High Standard (2.0 µg/mL N) x 2, Calibration Standards (high to low), Method Blank, High Standard, Low Standard (0.1 µg/mL N) x 2, Wash Water, QC Reference Sample x 2, Samples, Sample duplicate, and High Standard., and Wash Water. Note: The autoanalyzer software will automatically calculate concentrations in the extract.

5.3.4.1.5 Calculate the Biochar Concentration = (Extract Concentration X 50 mL (KCl)) / 5 g Biochar Sample.

5.3.4.2 KCl Extractable Nitrite and Nitrate by Autoanalyzer

Note: The Griess Ilosvay colorimetric method utilizes the reaction of nitrite ions with sulfanilaminde under acidic conditions to form a diazo compound. The compound further reacts with N-1-naphthylethylenediamine dihydrochloride to form a magenta azo dye. Nitrate in the sample is converted to nitrite through exposure to a reducing agent (in this case a copper-cadmium reducing column). This gives a measure of the nitrate + nitrite concentration in the sample.

5.3.4.2.1 Weigh 5 g of ground air-dried sample (particle size < 0.15 mm) into 125-mL Erlenmeyer flask. Add 50 mL of 2 M (0.01% (V/V)) KCl. Put the flasks on a rotating shaker for 1 hr at 200 rpm. After shaking is complete, filter the samples through Whatman No. 42 filter paper into 100-mLplastic vials.

5.3.4.2.2 Allow reagents (Ammonium chloride and Color Reagent) to warm to room temperature.

5.3.4.2.3 Turn on colorimeter to let the lamp warm up. Stored within the auto analyzer are reagent lines labeled Ammonium chloride, Color Reagent and Water; start the pump and allow water to run through the system, check all pump-tubing lines for proper function.

5.3.4.2.4 Once the system has equilibrated, place lines in the respective reagents and allow to run for 5-10 mins. Turn on the chart recorder. Wait for baseline to stabilize, and set to the 10^{th} chart unit.

5.3.4.2.5 Prepare 100 µg/mL nitrate and nitrite QC Stock Standards from KNO_3 and $NaNO_2$ and DDI water, respectively. To make a 10 µg/mL Intermediate Standard, add 5 mL of 100 µg/mL stock solution to 50-mL volumetric flask and make up to volume with 0.01% KCl. To make Calibration Standards combine 0.01% KCl and the 10 µg/mL intermediate standard prepared in 25-mL volumetric flasks to make calibration standards (0.05, 0.2, 0.5, 1.0, 1.5, 2 µg/mL NO_3 or NO_2). Use KCl for method blanks.

5.3.4.2.6 Prepare spikes using 5 g of Ottawa sand (inert material) and add 0.05 mL of the appropriate 1000 µg/mL QC standard for an end result of 10 mg N/kg sample. Make a combined NO_3 + NO_2 spike by spiking a single sample with 0.025 mL of each 1000 µg/mL QC standard stock. Prepare one sample spike per run by spiking 5.0 g of the unknown biochar sample with 0.025 mL of the appropriate 1000 µg/mL QC standard stock.

5.3.4.2.7 Begin running analysis. Include a full set of calibration standards, two QC Reference Samples, at least two KCl blanks, and at least two Nitrite Standards, a set of Ottawa Sand Spikes and blanks and a Sample Spike in each run.

Note: Standards may be rerun as markers between every 5 unknown samples and to verify the values for preparation of the standard curve.

5.3.4.2.8 Repeat the 2.0 µg/mL standard at the end of each run. Run duplicate samples at a minimum rate of 10%. Run Nitrite + Nitrate analysis first, followed by the Nitrite analysis.

5.3.4.2.9 Record on the nitrite nitrate worksheet peak heights of all standards, QC checks and samples. Use the number of chart units as the measurement of height. To calibrate the instrumentation, use the relative heights of the standards. Ensure that the R^2 value lies above 0.99, if not re-run the standards.

5.3.4.2.10 Calculate the concentration of the samples using the formula:

Extract Concentration = (Peak Height- Intercept of the Calibration Curve/Calibration Curve Slope) x Dilution

Biochar Concentration = (Extract Concentration X 50 mL(KCl)) / 5 g Biochar Sample

5.3.4.2.11 Subtract the estimated nitrite concentration from the nitrate plus nitrite concentration to calculate nitrate.

5.3.4.3 Extractable Phosphorus (2% Formic Acid Extraction)

Note: The auto analyzer software automatically calculates concentrations. The software reports calibration information, goodness of fit of the calibration curve, concentrations for all samples, calibrants, blanks and QC samples that have been run.

5.3.4.3.1 Prior to analysis store samples in a clean glass container or sterile plastic bag. Keep samples refrigerated and analyze within two weeks or keep frozen for up to one year.

5.3.4.3.2 Make all standards and QC standard with the same extraction fluid that is used for the samples. Use Estuarine Sediment as a standard reference material and in every bath of samples include two blanks to be extracted.

5.3.4.3.3 Using a 1L volumetric filled to 750 mL with DDI water, add 20 mL (98-99%) formic acid and fill to volume with DDI water.

5.3.4.3.4 Add 1.0 g of ground air-dried sample (particle size < 0.15 mm) into a 125-mL Erlenmeyer flask. Add 50 mL of 2% formic acid solution. Put the flasks on sonicator for 10 mins, then transfer onto

rotating shaker for 1 hr at 200 rpm. After shaking, filter samples using Whatman No. 42 filter paper into another set of 125-mL Erlenmeyer flasks.

5.3.4.3.5 Prepare Standards and Spikes:

5.3.4.3.5.1 Prepare a 1000 µg/mL QC Stock Standard from potassium dihydrogen orthophosphate and DDI water. Use the QC Stock Standard to make the Calibration Standards (5 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.2 µg/mL , 0.1 µg/mL). Use 0.100 mL of the QC Standard to make the QC Spike. To make a QC Standard Check, add 0.100 mL of the QC Stock Standard to a 50-mL volumetric flask and make it up to volume with KCl. Note: This is a 0.2 µg/mL dilution concentration.

5.3.4.3.5.2 Use Estuarine sediment as a QC Reference Sample. Use 0.01% KCl as the method blank.

5.3.4.3.6 Analyze on the autoanalyzer system. Set samples up as Primer (High Standard (0.5 µg/mL), Calibrants (5 µg/mL , 1 µg/mL , 0.5 µg/mL , 0.2 µg/mL , 0.1 µg/mL), Blank, Null, High Standard (0.5 µg/mL), Low Standard (0.1 µg/mL), Low Standard (0.1 µg/mL), Null, QC (Reference Sample/ Estuarine Sediment), QC (Reference Sample/Estuarine Sediment), Method Blank, Sample 1, Sample 2, Sample 2 Dup, Sample 3 etc., High Standard, Null.

5.3.4.3.7 In every batch of samples also extract two blanks: one is a calibration blank and it is to be placed in the standard rack of the autosampler, the other is a method blank and it is to be placed in the sample tray.

5.3.4.4 Specific Surface Area

Note: Analysis for Brunauer-Emmett-Teller (BET) surface area was conducted in the Chemical Biological Radio Nuclear (CBRN) Protection Lab at RMC. The method utilizes N_2 gas sorption analysis at 77 K in a relative pressure range from 0.01 to 0.10 after degassing at 120°C for a minimum of 2 h. A duplicate sample was analysed for every 6 unknown samples. Samples are not ground into powdered form prior to analysis.

Note: Degassing times and pressures are specific to instrument manufacturer and the method provided has been validated previously with high temperature activated carbons.

5.3.4.5 Cation Exchange Capacity (CEC)

5.3.4.5.1 Follow the sodium acetate method for CEC described by Laird and Fleming (2008) to calculate CEC.

5.3.4.5.2 Include one analytical blank (DDI water), standard reference material (Ottawa Sand) and duplicate for every 10 samples.

5.3.4.5.3 Prepare saturating solution (1 M NaOAc pH 8.2) by dissolving 136.08 g of $NaOAc{\cdot}3H_2O$ in 750 mL distilled, deionized water. Adjust the pH to 8.2 by adding acetic acid or sodium hydroxide. Dilute to 1 L with DDI water.

5.3.4.5.4 Prepare first rinsing solution (80% isopropanol (IPA)) by combining 800 mL IPA with 200 mL distilled, deionized water. Then prepare the second rinsing solution (100% IPA).

5.3.4.5.5 Prepare the replacing solution (0.1 M NH_4Cl) by dissolving 5.35 g NH_4Cl into 1 L distilled, deionized water.

5.3.4.5.6 Weigh 0.2 g of sample (air dried, not ground) into a 30-mL centrifuge tube. At the same time, weigh 0.5 g of the same air dried sample into a pre-weighed aluminum drying pan. Place the sample in the aluminum drying pan in the oven at 200^{o}C for 2 hours, cool it in a desiccator and then weigh again to determine the water content of the air-dried sample. Use this sample to calculate the water content correction factor, F (step 5.3.4.5.10).

5.3.4.5.7 Add 15 mL of the saturating solution, vortex, then centrifuge at 3000 x g for 5 mins. Decant and carefully discard the supernatant to ensure no sample is lost. Repeat this step two more times.

5.3.4.5.8 Add 15 mL of the first rinsing solution. Vortex and centrifuge at 3000 x g for 5 mins. Decant and carefully discard the supernatant. Repeat this step several times, each time measuring the electrical conductivity of the supernatant solution. When the conductivity of the supernatant drops below the conductivity of NaOAc saturated with IPA (~6 µS/cm), switch to the second rinsing solution. Continue to rinse the sample until the conductivity of the supernatant drops below 1 µS/cm.

5.3.4.5.9 Allow the sample to air dry in a fume hood, then add 15 mL of the replacing solution. Vortex and centrifuge at 3000 x g for 5 mins. Decant and save the supernatant into a 100-mL volumetric flask. Repeat this step three more times, each time saving the supernatant into the same volumetric flask. Then bring the volumetric to 100 mL with distilled, deionized water.

5.3.4.5.10 Analyze the sodium content via inductively coupled plasma-atomic emission spectrometry (ICP-AES) as previously described.

5.3.4.5.11

Perform the following calculations:

F= (weight of oven dried, air dried sample - weight of air dried sample)

C= Na concentration (mg/L) in the 100-mL volumetric flask

W= weight (g) of air-dry sample added to centrifuge tube

CEC= (C x 0.435)/(W x F)(cmol/kg)

5.3.5 Statistical Analysis

Data were analysed using one-way ANOVA. All residuals of the data were determined to be normally distributed as determined by a Kolmogorov Smirnov test for normality. A significance level of α=0.05 was used for all tests, and results were recorded with the standard error of the mean. All statistical analyses were performed using SPLUS 8.0.

5.4 Representative Results

A summary of all results including a comparison to the criteria set by the IBI (58) can be found in Tables 5-1 (summary), 5-2 (New, High, Low, Third Feedstock and High-2 biochars) and 5-3 (Old biochar). All biochars and feedstocks used in 2012 and 2013 (Table 5-2) were well within the criterion set by the IBI and there were little differences among biochars. Old biochar (Table 5-3), the first biochar submitted for testing, was made from used shipping pallets and construction wastes and was determined to have elevated levels of the metals arsenic, chromium, copper, and lead. Old biochar also had the lowest levels of organic carbon (63.2%) as determined by loss on ignition. This biochar had the highest levels of extractable phosphorus (850 mg/kg) and CEC (34.8 cmol/kg), as well as the highest percentage of fine particles (< 0.5 mm, 48%). Old biochar was also the only biochar to fail the germination test (Figure 5.3) and it was determined that *Eisenia fetida* (soil invertebrate) significantly avoided the 2.8% Old biochar amendment, whereas they preferred the 2.8% amendment of the New biochar (Figure 5.2).

Table 5-2 Summary Criteria and Characteristics for New, High, Low, Third and High-2 Biochars and Feedstocks.

Requirement	IBI Criteria	Biochar Range	Feedstock Range	Unit
Test Category A- Basic Biochar Utility Properties - Required for All Biochars				
Moisture	Declaration	< 0.1-4.3		%
Organic Carbon	Class 1 ≥ 60% Class 2 ≥ 30% Class 3 ≥ 10 < 30%	96.2-97.8 (LOI) 92.44-97.93(Pro/Ult)		%
$H:C_{org}$	0.7 max	0.01-0.02		Ratio
Total Ash	Declaration	1.38-2.26		%
Total N	Declaration	0.28-1.06		%
pH	Declaration	8.4-9.6		pH
Particle Size Distribution	Declaration	86-98 1.3-14		% Coarse % Fine
Test Category B: Toxicant Reporting- Required for All Feedstocks				
Germination	Pass/Fail	Pass		
Earthworm Avoidance	Declaration	No Avoidance		
Polyaromatic Hydrocarbons	6-20	<2.0		mg/kg
Polychlorinated Biphenyls	0.2-0.5	<0.1		mg/kg
Arsenic	12-100	<1.0	<1.0	mg/kg
Cadmium	1.4-39	<1.0	<1.0	mg/kg
Chromium	64-1200	<2.0	<2.0-2.6	mg/kg
Cobalt	40-150	<1.0	<1.0	mg/kg
Copper	63-1500	3.6-6.5	<2.0-5.9	mg/kg
Lead	70-500	<2.0-2.7	<2.0-8.1	mg/kg
Mercury	1000-17000	<5.0-294		ng/g
Molybdenum	5-20	<2.0	<2.0	mg/kg
Selenium	1-36	<10	<10	mg/kg
Zinc	200-7000	5.6-56.2	7.8-30.5	mg/kg
Chlorine	Declaration			mg/kg
Sodium	Declaration	137-878	<75-770	mg/kg
Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties- Optional for All Biochars				
Mineral N (Ammonium and Nitrate)	Declaration	<0.2-6.1		mg/kg
Total Phosphorus	Declaration	69.5-276	52.5-74	mg/kg
Available Phosphorus	Declaration	9-80		mg/kg
Volatile Matter	Declaration	12.47-19.09		%
Specific Surface Area	Declaration	244-428		m^2/g
Cation Exchange Capacity	Declaration	11.1-17.1		cmol/kg

Note: All biochars listed in Table 5-2 are produced from similar feedstocks at the same pyrolysis facility. Declaration implies that the IBI has no set criteria for that method and results only need to be declared.

Table 5-3 Summary Criteria and Characteristics for Old Biochar and Feedstock.

Requirement	IBI Criteria	Biochar Range	Feedstock Range	Unit
Test Category A- Basic Biochar Utility Properties - Required for All Biochars				
Moisture	Declaration	20		%
Organic Carbon	Class 1 ≥ 60% Class 2 ≥ 30% Class 3 ≥ 10 < 30%	63.2 (LOI)		%
$H:C_{org}$	0.7 max			Ratio
Total Ash	Declaration			%
Total N	Declaration			%
pH	Declaration	9.3		pH
Particle Size Distribution	Declaration	52 48		% Coarse % Fine
Test Category B: Toxicant Reporting- Required for All Feedstocks				
Germination	Pass/Fail	Fail		
Earthworm Avoidance	Declaration	Avoided		
Polyaromatic Hydrocarbons	6-20			mg/kg
Polychlorinated Biphenyls	0.2-0.5	1.2		mg/kg
Arsenic	12-100	167	<1.0	mg/kg
Cadmium	1.4-39	<1.0	<1.0	mg/kg
Chromium	64-1200	206	<20	mg/kg
Cobalt	40-150	5.3	<5.0	mg/kg
Copper	63-1500	558	<5.0	mg/kg
Lead	70-500	314	<10	mg/kg
Mercury	1000-17000	<5.0		ng/g
Molybdenum	5-20	<2.0	<2.0	mg/kg
Selenium	1-36	<10	<10	mg/kg
Zinc	200-7000	498	<15	mg/kg
Chlorine	Declaration			mg/kg
Sodium	Declaration	6460	<75	mg/kg
Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties- Optional for All Biochars				
Mineral N (Ammonium and Nitrate)	Declaration	2.6		mg/kg
Total Phosphorus	Declaration			mg/kg
Available Phosphorus	Declaration	850		mg/kg
Volatile Matter	Declaration			%
Specific Surface Area	Declaration	373.6		m^2/g
Cation Exchange Capacity	Declaration	34.8		cmol/kg

Note: Declaration implies that the IBI has no set criteria for that method and results only need to be declared

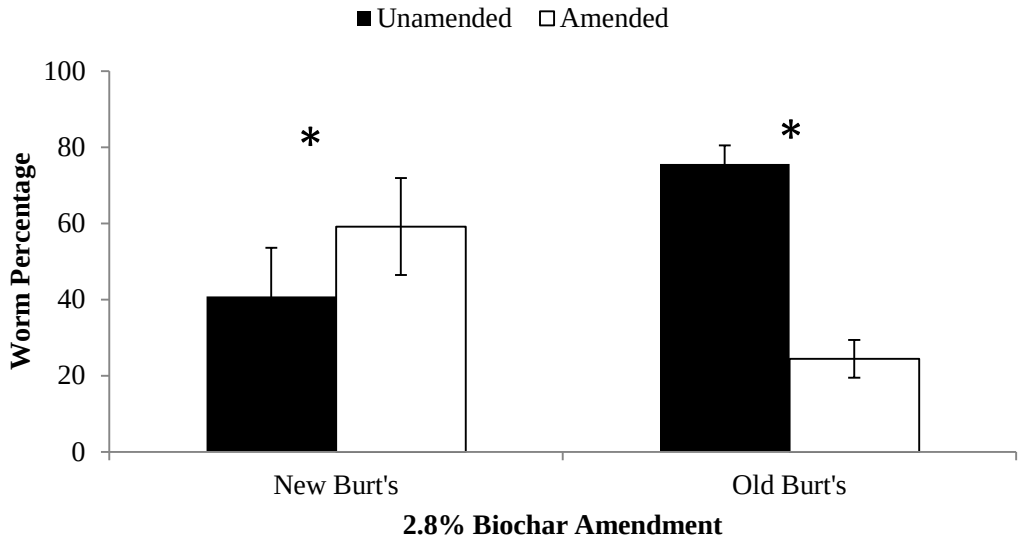

Figure 5.2 Earthworm avoidance assay of Old and New Burt's biochars.* indicates a significant difference between unamended potting soil and potting soil amended with 2.8% of either biochar ($p < 0.05$).

5.4.1 Test Category A: Basic Biochar Utility Properties

Biochar production via pyrolysis is essentially the carbonization of biomass. The carbonization process allows for the transformation of structured organic molecules of wood and cellulose materials into carbon, or carbon-containing residues, which are often aromatic in nature (197-201). Carbonization is obtained through the elimination of water and volatile substances from the biomass feedstock, due to the action of heat during the pyrolysis process (202). All of the biochars produced at the commercial greenhouse contained a relatively low moisture percentage (< 5%) with the exception of Old biochar. All biochars are categorized by the IBI as Class A (> 60%) in terms of their composition of organic carbon as a result of complete carbonization of the feedstock material via pyrolysis. Thus due to the high percentage of organic carbon, all biochars produced have a low percentage of ash (< 2.5%), which is the inorganic or mineral component of the biochar (58). Although these low ash biochars do not provide substantial amounts of nutrients directly to the soil as do their high-ash biochar (often made from manures and bones) counterparts; the carbon content of these biochars is much higher and therefore they have higher long-term nutrient retention abilities (203-205).

The hydrogen to carbon ratio (H:C) is a term often used to measure the degree of aromaticity and maturation of the biochar, which has been linked to their long term stability in the environment (201). For biomass feedstock containing cellulose and lignin, the H:C ratios are approximately 1.5. However, pyrolysis of these materials at temperatures greater than 400^{o}C is expected to produce biochars with H:C ratios < 0.5. It has been reported that an H:C ratio < 0.1 indicates a graphite-like structure in the biochar (206). All biochars in this report have H:C ratios less than 0.02, indicating that these biochars are highly aromatic in nature and will have long term stability in the environment.

Soil pH is a measure of soil acidity, and unfortunately many agricultural soils in Canada and worldwide are acidic (pH < 7), meaning that they are not ideal for crop growth. Biochars with an alkaline

pH (> 7), such as those being produced at the greenhouse, can be added to acidic soils to increase the soil pH to levels that are more appropriate for plant growth.

Another important soil characteristic for plant growth is particle size distribution (PSD). Biochars that have a higher percentage of coarse particles may favorably increase soil aeration and prevent biochar movement into the subsoil over time, thereby increasing the length of time biochar offers benefits to plant growth (135). However, smaller particle sizes are favored for biochars that are being produced for remediation purposes with the intent to sorb contaminants and minimize their bioavailability, as contaminants are more easily able to access pore space for binding (94, 191)·(101)· Also smaller particles sizes increase the number of biochar particles per unit volume of soil which is favorable for contaminant sorption (89). As in a previous study (191), fine particles are defined as those $\leq$ 0.25 mm and coarse particles as $\geq$ 0.5 mm. The biochars named New-, High- and Third Feedstock have a high proportion of coarse particles (~98%), and a low proportion of fine particles (~2%). The biochar produced at a slightly lower temperature, had 89% coarse and 11% fine particles sizes. All of these biochars may offer substantial improvements to soil texture and aeration especially in degraded or clay type soils. The Old biochar had a PSD that differed substantially from the others, having 52% coarse and 48% fine particles. A biochar with this PSD may be preferable for use at contaminated sites, where contaminant sorption is the primary focus.

5.4.2 Test Category B: Toxicant Reporting

Biological testing of biochar is important to assess the toxicity (if any) of these materials to soil invertebrates and plants. To date, there is little existing literature on the potential impact of biochar on terrestrial organisms and their associated response, and often the literature that does exist presents conflicting results. Exposure to contaminants may inhibit earthworms ability to perform essential soil functions such as decomposition, nutrient mineralization, and soil structure improvements (207). New biochar showed no detrimental effects on the earthworm *Eisenia fetida* as assessed by earthworm avoidance, however worms significantly avoided Old biochar (Figure 5.2). Germination assays are a technique used to evaluate the toxicity of a particular material to plants. Potting soil served as a better control than filter paper as the filter paper often encouraged mold formation. Pumpkin and alfalfa seeds germinated well with 67% ± 12% and 81% ± 6% germination, respectively. Roots also proliferated well with average lengths after seven days being 14 cm ± 0.6 cm and 55 cm ± 8 cm for pumpkins and alfalfa, respectively. As with the earthworm avoidance studies Old biochar showed toxicity to plants and all other biochars evaluated showed no detrimental effects to seed germination as measured by percent germination and root length after seven days (Figure 5.3).

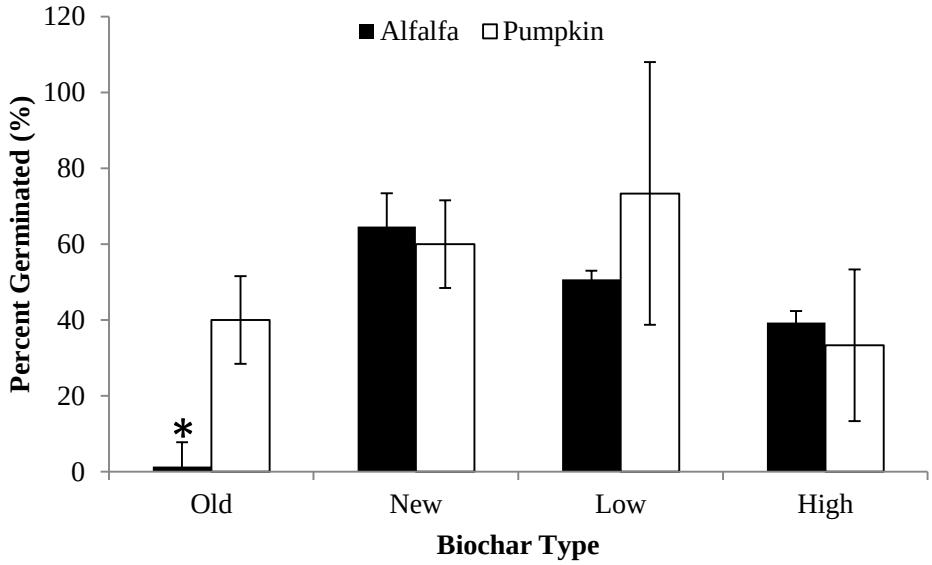

Figure 5.3 Percent germination of two different plant species; pumpkin (*Cucurbita pepo* spp. *pepo*) and alfalfa (*Medicago sativa*) grown in triplicate in various Burt's biochars for seven days. * indicates significantly difference from the controls (potting soil and filter paper).

Although some types of biochar have the potential to sorb organic contaminants and reduce their toxicity in the environment, careful characterization of the biochar is required to ensure that it does not contain harmful contaminants such as PAHs, PCBs, and metals as a result of contaminated feedstocks or pyrolysis conditions. None of the biochars produced at the greenhouse had PAH concentrations exceeding IBI guidelines. Old biochar was determined to have elevated levels of PCBs and the metals arsenic, chromium, copper, and lead, however none of the biochars produced from the other two biomass materials contained metals above IBI guidelines. Old biochar was produced from used shipping pallets and construction wastes which is likely the source of the metal contamination. Although Old biochar would not be suitable for use in agricultural soils or home gardens, all other biochars could be used for these purposes.

5.4.3 Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties

Biochars containing a high concentration of ammonium and nitrate may be applied to agricultural soils to offset the requirements for synthetic fertilizers. However, if biochar contains an excess of these nitrogen compounds then application on a large scale could increase the atmospheric N_2O concentration and contaminate drinking water sources with nitrates. None of the biochars studied contained elevated amounts of ammonium or nitrate.

Phosphorus is an essential component for many physiological processes related to proper energy utilization in both plants and animals. Biochars with moderate amounts of available phosphorus will act as important plant fertilizers. In Ontario, soils containing 15-30 mg/kg phosphorus are considered low, 31-60 mg/kg moderate, and 61-100 mg/kg high. Old biochar was highest in available phosphorus at 850 mg/kg and may not be suitable for adding to soils already classified as high in phosphorus. However, all

other biochars tested had a much lower amount of available phosphorus and would not be expected to cause problems when added at rates up to 10% (w/w).

The components of biochar (except moisture) that are released during pyrolysis are referred to as volatile matter. These components are typically a mix of short and long chain hydrocarbons, aromatic hydrocarbons with minor amounts of sulfur. Volatile matter was determined via proximate analysis which also determines the moisture and ash content of biochars (section 2.2). The volatile content affects the stability of the material (208), N availability and plant growth (209). In theory, biochars high in volatile matter are less stable and have a higher proportion of labile carbon that provides energy for microbial growth and limits the availability of nitrogen necessary for plant growth. A study by Deenik et al., (210) considered 35% volatile matter to be high (inducing nitrogen deficiency), and 10% volatile matter to be low. All biochar in this report contained less than 20% volatile matter, and hence would not be expected to limit plant growth. Proximate analysis determination of volatile matter is most important for biochars with low ash concentrations such as those produced at the commercial greenhouse.

Specific surface area (SSA) is a measure of the porosity of a biochar. It includes not only the external biochar surface area, but also the surface area within the pore spaces and is an important characteristic used to predict the ability of a biochar to sorb organic contaminants. Contaminant sorption has been attributed to π-π interactions (attractive, non-covalent binding) between the aromatic ring(s) of the contaminant and those of the biochar (211). Activated carbon (AC) is a charcoal-like material that is treated during its production to maximize its porosity and therefore has higher SSAs than most biochars. Although all the of biochars presented in this report have SSAs in the 300 m^2/g range (i.e. much less than that of AC; ~800 m^2/g), as reported in Denyes et al., 2012 and 2013, the biochars, Old and New, have both shown significant potential to serve as a soil amendment for the remediation of PCBs.

Cation exchange capacity (CEC) is a measure of the number of cations (positively charged ions) that a soil particle is capable of holding at a given pH. The ability of the soil to hold cations is due to electrostatic interactions with negatively charged sites on the surface of a particle, such as hydroxyl (OH^-) and carboxyl (COO^-) groups (70, 212). The CEC of the soil can be linked to the ability of the soil to hold nutrients and retain cations from fertilizers which are essential for plant growth. Also, many environmental contaminants such as lead, cadmium and zinc have positive charges; therefore soils with a high CEC may function to prevent the leaching of these contaminants into drinking water sources. Biochars have been reported to increase the CEC of soils, due to the slow oxidation of the biochar surface which increases the number of negatively charged sites, and therefore may reduce fertilizer requirements and immobilize positively charged contaminants in soils (70). Typically, sandy soils have a CEC between 1-5 cmol/kg, loam soils 5-15 cmol/kg, clay type soils > 30 cmol/kg and organic matter 200-400 cmol/kg. The methods for determining the CEC of biochar are still in their infancy and therefore should be considered in relative terms. The CEC of the biochars produced at the greenhouse are higher than the CEC of PCB-contaminated soils (Denyes et al., 2012), but lower than compost amended soils.

5.5 DISCUSSION

All of the methods listed in the protocol have been carefully validated and extensively used for soils. As biochar characterization is still in its infancy, the effectiveness of these methods for the carbon-rich substrate was largely unknown. Hence, although these methods themselves are not novel, their application to routinely characterize biochar is. In terms of quality assurance/ quality control, there were no issues among any of the methods with respect to the blanks being below detection limits or the recoveries being correct for the standard reference materials. This indicates that these methods are suitable to be used for the characterization of biochar and other charcoal-like materials. Many different methods have been used to characterize biochars in the literature (158, 203, 213-219) however, as biochar becomes increasingly accepted as a soil additive, routine methods are required.

Cation exchange capacity was the only method in which difficulty arose. The method for calculating the CEC of a sample is dependent on the weight of sample and the concentration of sodium in that given weight. Biochar has a very low density and therefore does not pelletize at the bottom of the tube after centrifugation, as soil does. Therefore, when decanting and discarding the supernatant in steps 6 and 7 of the method (4.4), it is important to not lose any of the biochar sample. Pipetting the solution from the centrifuge was required to avoid any sample loss.

Other analytical methods were easily adapted from soil methods. Ultimate and proximate analysis is specific to biochar and similar products such as coal, and hence is not normally available in laboratories which routinely analyze soils. Another method (ASTM D1762) is available, for the determination of moisture, volatile matter, and ash in charcoal made specifically from wood. This method would also have also been suitable for proximate analysis. When determining loss on ignition for percent organic matter and percent moisture some may choose to perform these analyses at temperatures greater than 420^{o}C, especially if the biochars in question are produced via very high temperatures of pyrolysis. In the case this particular study 420^{o}C was sufficient to completely ash all biochars, and although not discussed this temperature was sufficiently high to ash even activated carbon.

Working with biological organisms such as plants and worms can often be challenging. Selecting the appropriate study organisms is of particular importance. The soil invertebrate *Eisenia fetida* is used frequently as a terrestrial organism model in contamination experiments because this species is capable of surviving at high concentrations of organic contaminants, is very well researched, and is ecologically relevant in many areas of the globe (19, 72, 173, 207, 220-222). Soil invertebrates play an important role in the soil matrix, as they degrade organic matter, cycle nutrients, and transfer water. The plant species' alfalfa (*M. sativa*) and pumpkin (*C. pepo*) were chosen for the germination assays as they are commonly grown in Canada and have been used in our complimentary work on contaminant remediation (173, 191, 223). Greenhouse conditions for germinating seeds need to be carefully monitored to ensure proper functioning of lighting and to avoid extreme temperature fluctuations.

The characterization of biochar is essential to its successful application as measured parameters will indicate the effectiveness of different biochars for different applications (i.e. whether a biochar is appropriate for contaminant sequestration, soil quality improvement, contaminant remediation etc.). Because the methods detailed here are widely available for soil analysis, they are a cost-effective means for characterization of biochars, and should be widely employed prior to large-scale application of biochar in the field.

6. SHIFTS IN MICROBIAL COMMUNITIES FOLLOWING BIOCHAR AND ACTIVATED CARBON ADDITIONS TO SOILS CONTAMINATED WITH PERSISTENT ORGANIC POLLUTANTS

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6.1 ABSTRACT

Biochar and granular activated carbon (GAC) were added *in situ* to soils contaminated with persistent organic pollutants (POPs) at 2.8% (by weight) with the intent to minimize contaminant bioavailability. Microbial community metabolic profiles from amended and un-amended soils contaminated with either polychlorinated biphenyls (PCBs) or dichlorodiphenyltrichloroethane (DDT) were characterized using community level physiological profiling (CLPP). In PCB-contaminated soils, principal components analysis (PCA) of carbon source utilization patterns (CSUP) revealed distinctly different groupings for un-amended highly contaminated soils, versus highly contaminated with PCBs soils amended with biochar or AC. These results suggest that *in situ* remediation with carbon amendments may assist in restoring the microbial community at intensely degraded Brownfield sites. In DDT-contaminated soil, CLPP showed no impact on the soil microbial community even 16 months post application. This study demonstrates CLPP as an effective method for detecting changes in soil biota following amendment with carbon sorbent materials, and illustrates the importance of applying these materials in a site specific manner.

6.2 INTRODUCTION

There is a developing trend towards risk based approaches in the remediation of persistent organic pollutants (POPs). Traditional remediation approaches such as excavation and landfilling are detrimental to the environment, costly and, in some cases, not feasible due to the extent of contamination. Consequently these hydrophobic contaminants continue to enter the food chain and pose risk (42). Recently, studies have shown carbonaceous sorbent materials such as biochar and activated carbon (AC) to be efficient at minimizing the bioavailability of POPs in soils (11, 15, 19, 77, 97, 173, 191, 224, 225). Minimizing POP bioavailability limits their accumulation in terrestrial organisms and hence the potential risk these contaminants pose to environmental and human health. Biochar and AC are carbon-rich materials that have high sorptive capabilities as a result of their abundance of micropores. Application of biochar offers a smaller carbon footprint relative to AC because it is a waste by-product of energy production without an 'activation' step to maximize porosity. At present, there is a general lack of research on the use of carbon amendments in contaminated soils at the field level, and as a result their effects on soil the microbial community function are unknown.

Microbial communities play a vital role in soil ecosystems through the regulation of carbon and nitrogen cycling, the degradation of organic matter and subsequent nutrient availability to plants (226). Amendment of soil with biochar has been demonstrated to increase plant yield, improve chemical and physical properties and modify the soil physical habitat for microbial colonisation, altering the community structure (192, 227, 228). The effects of biochar amendments in soil are not yet fully understood due to the wide range of biochar types, application rates, soil types and plant responses (192). Increases in microbial activity and biomass have been reported with biochar addition (229), along with changes in microbial community composition and abundance (228, 230). Suggested reasons for these changes include that biochar is: i) providing a habitat for microbial colonisation, ii) protecting microbes from predation by soil microarthropods, and iii) in the short term, providing organic carbon as a source of energy (227, 230, 231). Conversely microbial activity inhibiting compounds have also been identified in biochar (209) or in soils after its introduction (232).

Community-level physiological profiling (CLPP) is a technique used to obtain information regarding mixed microbial community function, and functional adaptations over space and time (233). The technique has proven successful for examining microbial diversity in a number of different ecosystems including soils (234) and wetlands (235, 236) and has been used to characterize microbial communities post biochar addition in non-contaminated soils (230). This is the first study examining the use of the CLPP technique at sites highly contaminated with POPs and undergoing remediation by contaminant immobilization via activated carbon and biochar amendments. The aim of this study is to use the CLPP method to observe microbial community metabolic responses following carbon amendments (i.e. activated carbon and biochar) in POP-contaminated soil. This study complements the results of earlier work by the authors (191) and Denyes et al. (*in prep* (238)) which found that AC and two types of biochar significantly reduced contaminant uptake into plants and invertebrates, and therefore have potential for risk-based remediation.

6.3 MATERIALS AND METHODS

6.3.1 Field Site Description

Two POP contaminated sites which both contained areas of high and low concentrations of the respective contaminant were chosen for analysis. The first site is a PCB-contaminated Brownfield located in Etobicoke, Ontario. Soils were previously characterized as being weathered, coarse-grained and sandy with a total organic carbon content of 3.5 %, pH of 7.1 (9), and with a cation exchange capacity (CEC) of 9.5 cmol/kg (173). The mean PCB concentration in contaminated soils collected was 71.4 μg/g (191). Immediately adjacent to the highly contaminated soil, an area exists with a residual amount of

PCB contamination (3.1 µg/g) (173). This latter site is lower than the CCME soil quality guideline for the protection of environmental health at commercial and industrial sites (33 µg/g) (3).

The second site is a DDT-contaminated parkland located in Leamington, ON. The soil is classified as sandy and contains levels of DDT higher than the CCME recommendation for parklands of 0.7 µg/g. The contamination is composed predominantly of 4,4'- DDE and 4,4'- DDT, which has weathered in place for over 40 years. Sample collection occurred over two consecutive growing seasons. In the first year a plot was established in soil containing a mean total DDT (∑DDT) of 2.5 ± 0.03 µg/g and is referred to as the low level area. The soil had a cation exchange capacity of 11.2 cmol/kg, a pH of 7.7 and contained 3.5% organic matter. In the second year, another plot was established in high level DDT-contaminated soil, with a mean ∑DDT of 39 ± 1.8 µg/g. This soil had a CEC of 5.8, a pH of 7.9 and contained 3.1% organic matter.

6.3.2 Plot Design and Sample Collection

Two types of biochar and one granular activated carbon (GAC) were purchased for *in situ* addition to the contaminated soil (Table 6-1). Biochar and AC were thoroughly characterized according to the methods outlined in our previous work (237). Full carbon amendment methodology can be found in our earlier studies (191, 238) for the PCB and DDT-contaminated sites, respectfully. Briefly carbon amendments were mixed into the 71.4 µg/g PCB-contaminated soil and the 2.5 µg/g and 39 µg/g DDT-contaminated soil at 2.8% (w/w). Pumpkins (*Cucurbita pepo* spp. *pepo*) were grown in triplicate in each treatment, control (0%), 2.8% AC, 2.8% Burt's biochar, and 2.8% BlueLeaf biochar for 50 days. *Cucurbita pepo* spp. *pepo* was chosen to study the effects of biochar and AC on the bioavailability to plants as it has been widely documented as an efficient species at phytoextracting PCBs and DDT (e.g. (9, 114, 122, 130, 151, 164).

Table 6-1 Details of the carbonaceous sorbent materials.

Carbonaceous Material	Feedstock Material	Pyrolysis Conditions	Moisture Content (%)	Organic Matter (%)	pH
Burt's Biochar (Burt's Greenhouses, Odessa, ON)	Hardwood wastes	700°C, 30 mins	4.3-20	63-98	9.0-9.3
BlueLeaf Biochar (BlueLeaf Inc., Drummondville, QB)	Softwood	450°C, 2.5 hrs	3.8	97	9.9
Granular Activated Carbon (A.C. Carbone, St. Jean sur Richelieu, QC)	Bituminous Coal	> 700°C	6.8	39	9.2

Root (5 g) and soil (10 g) samples were collected from the control (un-amended POP-contaminated soil), and POP-contaminated soils amended with 2.8% Burt's biochar, BlueLeaf biochar, or GAC. Rhizosphere (10 g) samples were also collected from all treatments in DDT-contaminated soil. All samples were collected in triplicate in DDT-contaminated soil, whereas composite samples from the triplicate plants were collected from each carbon treatment in PCB-contaminated soil. Sampling in PCB-contaminated soil occurred at ~50 days post amendment, and sampling in DDT-contaminated soil occurred twice. During the first, samples were collected at day 60 day post amendment, and the second at day 120 and 16 months post amendment.

6.3.3 Community Level Physiological Profiling

Community level physiological profiling (CLPP) provides information relating to mixed microbial function and functional adaptations over space and time. Heterotrophic microbial communities are compared and classified based on sole carbon source utilization patterns (CSUPS) gathered using BIOLOG™ microplates (233). In this study, BIOLOG™ ECO plates containing 31 carbon sources and a control well in triplicate were used (see Weber and Legge (239) for a full list of carbon sources with classification).

A suspended mixed microbial sample (set to an optical density of 0.19 at 420 nm) was obtained by adding the individual soil or root samples to 100 mL of phosphate buffer (10 mM with 8.5 g/L NaCl) and orbitally shaking at 100 rpm for 3 h. Suspensions representing a single sample were then inoculated into each of the 96 wells (125 µL per well) on a BIOLOG™ ECO plate and the absorbance was read at 595 nm at periodic intervals for seven days.

6.3.4 Data analysis

CLPP data was analyzed according to Weber and Legge (2010) (233) using average well colour development (AWCD), substrate richness, substrate diversity, and principal components analysis (PCA) using carbon source utilisation patterns (CSUPs). AWCD refers to the absorbance value (corrected by the blank well) averaged for all 31 wells giving an assessment of overall catabolic activity.

$$AWCD = \frac{1}{31}\sum_{i=1}^{31}\left(A_i - A_0\right)$$

Where

AWCD – average well colour development

A_i – absorbance reading of well *i*

A_0 – absorbance reading of the blank well (inoculated, but without a carbon source).

Where there is very little response in a well, negative values of standardized absorbance may occur and, as this is physically meaningless, they are coded as zeros for further analysis.

Substrate richness is a measure of the number of different carbon sources utilised by a microbial population, and is calculated as the number of wells with a corrected absorbance greater than 0.25 AU. Diversity is expressed here in terms of the Shannon index.

A single time point was selected according to Weber and Legge (2010) (233) for the evaluation of all plate data based on a combination of greatest variance between well responses and least number of absorbance values above 2 (as these are above the linear absorbance range).

Following Weber, et al. (2007) (240) the data were assessed for normality, homoscedasticity, and linear correlations between variables yielding a recommended Taylor power law transform for principle components analysis. Principal components were extracted and ordinations created from the covariance

matrix of the data using Statistica 8.0. Following an ANOVA, a post hoc Tukey comparison was performed to assess differences in metabolic responses (activity, richness, diversity) based on the type of carbon amendment.

6.4 Results and Discussion

6.4.1 PCB-Contaminated Soil

Clear differences between the root and soil microbial communities at the investigated sites were observed for all metrics (Table 6-2). AWCD was typically higher in root samples than in soils and was consistently in the range of 0.8 – 1.2 (Table 6-2). AWCD varied to a greater degree in soils (from 0.3 – 0.8) with the lowest values found in the low level PCB-contaminated soil (3.1 μg/g). Microbial activity is expected to be high in the rhizosphere where readily degradable substances are exuded from plant roots and are reported to significantly support microbial activity (241).

The AWCD was not significantly different among any of the carbon amended soils. The AWCD of the soil sample collected from the highly PCB-contaminated soil amended with 2.8% BlueLeaf biochar was similar to that of the non-amended low-PCB contaminated soil. Burt's biochar and AC amendments to the highly contaminated PCB soil resulted in AWCDs significantly higher than in the un-amended low level PCB-contaminated soil ($p < 0.05$). Dempster, et al. (2012) (230) report that the addition of a Eucalyptus biochar to a coarse sandy soil led to changes in microbial community functional diversity but not increases in mass, activity or structural diversity. Likewise, in the present study, the addition of biochar or AC to the contaminated soil did not lead to a significant increase in microbial activity measured using CLPP. AWCD of root samples were not significantly different between any treatment including from plots amended with biochar or AC.

Similar trends were observed in the data for substrate richness (the number of different carbon sources utilised) and diversity with higher values for all root samples compared to soils. Carbon amended highly PCB-contaminated soils had richness and diversity values larger than the soils with the low level of PCB contamination, and similar to that of the non-amended highly PCB-contaminated soil ($p < 0.05$). The type of carbon amendment used had an effect on microbial diversity in soils, with Burt's biochar and AC being significantly higher than BlueLeaf biochar amended soils. In the root samples however, there were no marked differences in microbial richness and diversity between the types of biochar and AC. Greater variance in both richness and AWCD was seen in roots from amended plots compared to soils from amended plots, which suggests that the influence of sample type (i.e. soil or root) defined the richness and activity to a greater degree.

Table 6-2 Sample details and CLPP data from PCB-contaminated soils.

Treatment	Sample Type	PCB Soil Concentration (µg/g)	AWCD (49.5 hrs)		Richness		Diversity	
			mean	st. dev. ($\times 10^{-2}$)	mean	st. dev. ($\times 10^{-2}$)	mean	st. dev. ($\times 10^{-2}$)
					Soils			
Control	Low [PCB] Soil	3.1	0.37	3.3	14	1.5	2.4	12
Control	Low [PCB] Soil	3.1	0.28	6.2	10	1.7	2.7	1.1
Control	Low [PCB] Soil	3.1	0.59	2.6	16	1.2	2.8	4.6
Control	High [PCB] Soil	71.4	0.84	5.2	17	0.58	2.8	2.3
Control	High [PCB] Soil	71.4	0.60	2.2	15	0.58	2.8	1.9
Control	High [PCB] Soil	71.4	0.66	3.9	17	1.2	2.9	6.1
Burt's Biochar Amended	High [PCB] Soil	71.4	0.80	12	19	2.0	2.9	5.8
BlueLeaf Biochar Amended	High [PCB] Soil	71.4	0.62	2.5	15	0.58	2.7	5.9
GAC Amended	High [PCB] Soil	71.4	0.76	6.5	19	1.5	2.9	3.1
					Roots			
Control	High [PCB] Root	71.4	1.1	3.4	26	0.58	3.2	2.3
Control	High [PCB] Root	71.4	1.1	0.52	27	0.58	3.3	1.8
Control	High [PCB] Root	71.4	1.2	1.9	28	0.00	3.3	1.1
Burt's Biochar Amended	High [PCB] Root	71.4	1.2	2.3	25	0.00	3.0	0.83
BlueLeaf Biochar Amended	High [PCB] Root	71.4	1.1	1.7	26	0.58	3.2	1.5
GAC Amended	High [PCB] Root	71.4	1.2	1.0	26	0.58	3.2	1.3

PCA ordinations from the CLPP results were used to compare the carbon source utilisation patterns (CSUPs) of the microbial communities from the sampled plots (Figure 6.1). Whereas AWCD is an averaged measure of activity, and richness is a measure of the number of carbon sources utilised, the CSUPs give an overall idea of the function based on carbon source utilisation patterns, or in other words, the proportions of each carbon source utilised in relation to one another. Two distinct groupings are evident for soil and root samples corresponding with the differences observed in AWCD, richness and diversity between these sample types (Figure 6.1A). The remaining samples falling outside of these two groups are the soils containing only a residual amount of PCB contamination and samples from the high level of PCB-contaminated soil amended with biochar or AC. When soils are plotted alone (Figure 6.1B) these groupings become more obvious with one group containing samples from the unamended highly PCB-contaminated area and another with samples from the remediated soil (3.1 µg/g), and the highly PCB-contaminated soil amended with 2.8% BlueLeaf biochar and 2.8% GAC. The highly contaminated soil amended with 2.8% Burt's biochar outlies both of these groups and can be explained by differences in source material and pyrolysis conditions. These groupings suggest CSUPs for highly PCB-contaminated soils amended with either BlueLeaf biochar or GAC at 2.8%, are comparable to those at a remediated site (i.e. the site containing only a residual amount of PCB contamination).

Groupings are less pronounced within root samples alone (data not shown). Root samples from the carbon-amended plots did not group together and again Burt's biochar appeared to outlie all other sample types. This suggests that the effects of amending contaminated soils with biochar or AC are more pronounced in the soil than in the roots, which may in part be due to the pronounced effect the rhizosphere has on defining microbial community function.

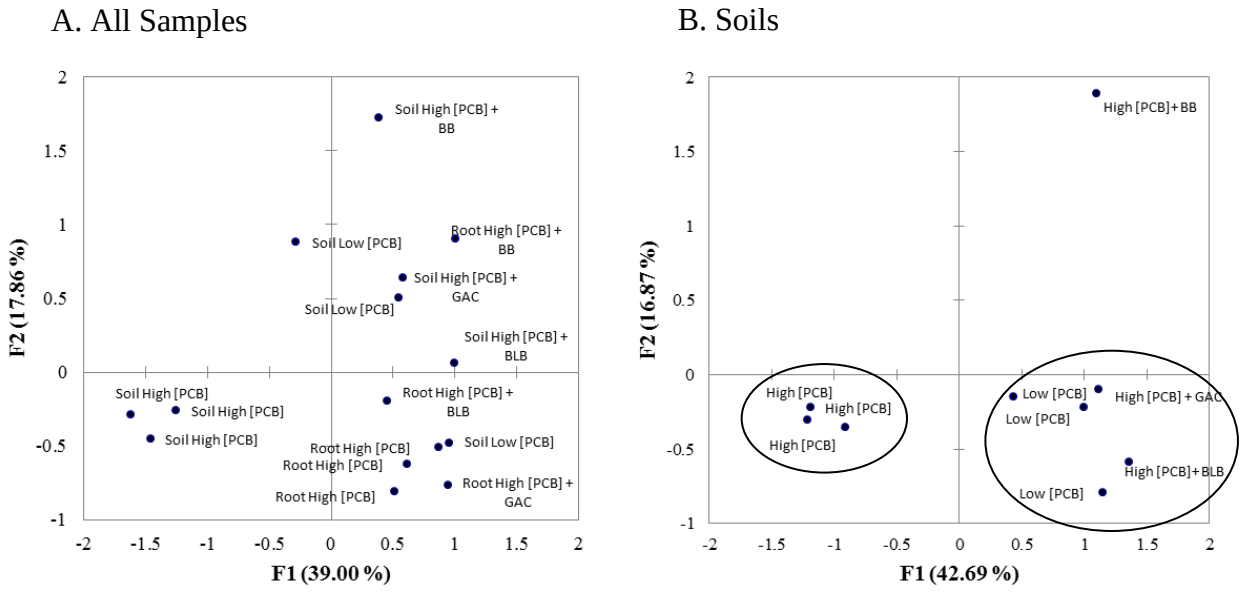

Figure 6.1 A) CLPP Taylor transformed (b=1.032) PCA results for all samples in high and low PCB-contaminated soil and highly PCB-contaminated soil amended with 2.8% Burt's Biochar (BB), BlueLeaf Biochar (BLB) or Granular Activated Carbon (GAC); **B)** CLPP Taylor transformed (b=1.039) PCA results for soil samples. Circles show distinct groupings.

6.4.2 DDT-Contaminated Site

Consistent with the PCB-contaminated soil, clear differences between root, rhizosphere and soil microbial communities were observed in all metrics (AWCD, diversity, richness and evenness) for both sampling time points (only AWCD is shown, Table 6-3). Values were highest in the rhizosphere samples indicative of the highly active microbial community commonly found here (Table 6-3). The AWCD was significantly higher in the highly DDT-contaminated soil compared to the low level of DDT-contaminated soil ($p < 0.05$). This was the only significant result among all rhizosphere, root and soil samples from both the low and high DDT-contaminated plots. At PPNP, even the highly DDT-contaminated soil matrix was healthy enough to sustain plant growth and may explain the lack of significant effects. None of the carbon amendments caused significant changes to AWCD, substrate richness and substrate diversity at the 2.5 µg/g DDT-contaminated site (16 months post amendment) and the 39 µg/g DDT-contaminated site, and there were no differences between soil concentrations.

Table 6-3 Sample details and average well colour development (AWCD) of soil, root and rhizosphere samples collected from the DDT-contaminated soils.

		Average Well Colour Development (AWCD)		
Sample Type		Soil	Root	Rhizosphere
	ΣDDT Concentration (µg/g)		Day 60 (88 hrs)	
Control	39	0.3 ± 0.3	0.8 ± 0.0	1.1 ± 0.3
GAC Amended	39	0.4 ± 0.4	0.8 ± 0.1	1.2 ± 0.1
Burt's Biochar Amended	39	0.3 ± 0.2	0.7 ± 0.1	1.2 ± 0.0
BlueLeaf Biochar Amended	39	0.4 ± 0.3	0.7 ± 0.1	1.2 ± 0.1
Control	2.5	0.0 ± 0.0		
			Day 120 (89 hrs)	
Control	39	0.3 ± 0.1*	0.6 ± 0.3	1.1 ± 0.2
GAC Amended	39	0.6 ± 0.5	0.9 ± 0.1	1.0 ± 0.1
Burt's Biochar Amended	39	0.3 ± 0.2	0.5 ± 0.0	0.7 ± 0.3
BlueLeaf Biochar Amended	39	0.5 ± 0.4	0.7 ± 0.1	0.8 ± 0.1
			16 Months Post Amendment	
Control	2.5	1.0 ± 0.1*	0.8 ± 0.1	0.6 ± 0.2
GAC Amended	2.5	0.6 ± 0.3		
Burt's Biochar Amended	2.5	0.5 ± 0.2		
BlueLeaf Biochar Amended	2.5	0.7 ± 0.1		

Values represent the average of three replicates ± their respective standard deviations., * represent a significant difference ($p < 0.05$) between treatments

Furthermore, the PCA analyses revealed no distinct grouping in root, rhizosphere or soil samples between carbon amendments in either soil concentration of DDT (Figure 6.2a-d)

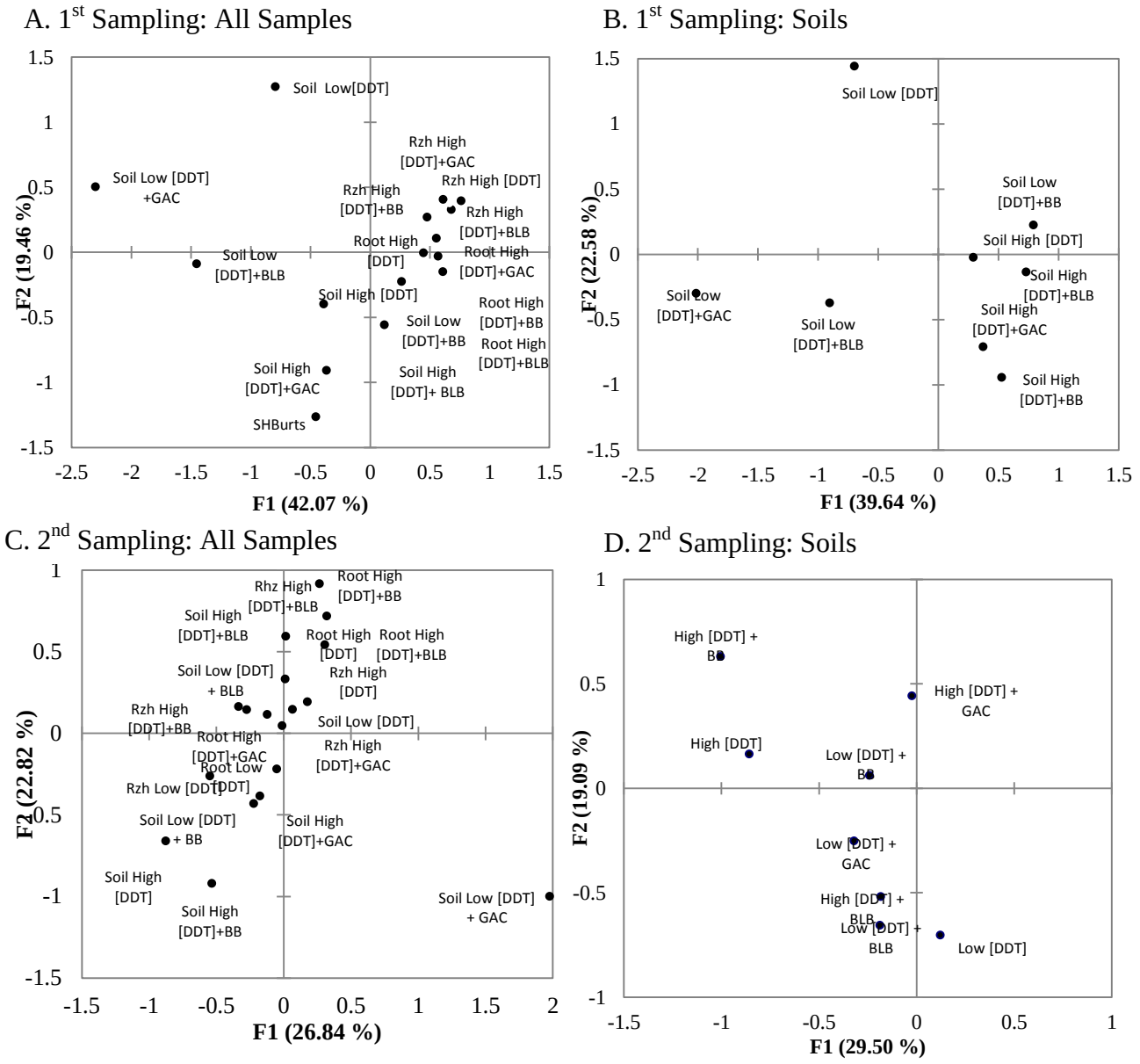

Figure 6.2 A) CLPP Taylor transformed (b=0.7803) PCA results for all samples collected during the first sampling in DDT-contaminated soil and highly DDT-contaminated soil amended with 2.8% Burt's Biochar (BB), BlueLeaf Biochar (BLB) or Granular Activated Carbon (GAC); **B)** PCA results for soil samples; **C)** CLPP Taylor transformed (b=1.8125) PCA results for all samples collected during the second sampling and **D)** PCA results for soil samples.

The specific effects carbon amendments have on the soil microbial community is still poorly understood and few studies exist that report potentially negative (230, 242) or short lived beneficial (243)

effects on the microbial activity as a result of biochar amendment. In this study, even 16 months post amendment, the use of biochar and AC as *in situ* sorbents for the remediation of DDT-contaminated soil at PPNP had no effect on the microbial community metabolic profiles.

The microbial communities in soils at contaminated sites play an essential role in both the biodegradation of POPs and uptake by accumulating plants. Application of CLPP revealed important information about the metabolic profiles of microbial communities at PCB and DDT remediation plots. Metabolic activity, substrate richness and substrate diversity were higher in the root tissues of plants than in soils highlighting the importance of the rhizosphere in defining microbial community function during remediation in POP-contaminated soils.

6.5 CONCLUSIONS

From a remediation perspective with the aim to restore full ecological function, there appear to be benefits from both the presence of plant roots and the addition of carbon amendments with increased microbial activity, richness and diversity in the rhizosphere. There is currently a lack of research focused on the potential effect of different biochars and AC amendments on soil microbes (229). The results here are insufficient to speculate in detail on the effects of biochar source material and pyrolysis conditions on microbial activity, but are useful in highlighting how such differences can be measured with the CLPP method and the importance of understanding the different effects of varying biochar/AC amendments on soil microbial activity. When using biochar/AC amendments in PCB-contaminated soils, the metabolic profiles of microbial communities in highly contaminated soils become more similar to those soils containing only a residual amount of contamination. Combining the findings of this study with that of our earlier study (191), it appears that not only do biochar and AC have the potential to restore the microbial community function of a site, but they simultaneously minimize PCB bioavailability and may therefore be useful as a site closure technology. The addition of carbon amendments to DDT-contaminated soil did not result in the same positive outcome observed in the PCB study; however neither biochar nor AC caused detrimental effects to the soil microbial community. The results of this study illustrate the importance of using carbon amendments in a site-specific manner for POPs.

7. COMPARISON OF POM-PREDICTED AND MEASURED PLANT AND INVERTEBRATE BIOAVAILABILITY FOLLOWING APPLICATION OF CARBON AMENDMENTS TO DDT-CONTAMINATED SOIL

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7.1 ABSTRACT

Granular activated carbon (GAC) and two biochars were added (2.8% w/w) *in situ* and *ex situ* to soil contaminated with dichlorodiphenyltrichloroethane (DDT) (39 µg/g). Biochar significantly reduced DDT accumulation in *Eisenia fetida* (by up to 49%) and showed no detrimental effects to invertebrate health. In contrast, addition of GAC to soil contaminated with DDT caused significant toxic effects (invertebrate avoidance, decreased survivorship and decreased weight) and did not significantly reduce the accumulation of DDT into invertebrate tissue. None of the carbon amendments reduced plant uptake of DDT. Bioaccumulation of 4,4'DDT and 4,4'-DDE in plants (*Cucurbita pepo* spp. *pepo*) and invertebrates (*E. fetida*) was assessed and compared to predicted bioavailability using the freely-dissolved porewater obtained from a polyoxymethylene (POM) equilibrium biomimetic method. The bioavailable fraction predicted by the POM samplers correlated well with measured invertebrate uptake (< 50% variability), but was different from plant root uptake by 134%. A literature review of *C. pepo* BAFs across DDT soil contamination levels was performed, and these BAFs as well as BAFs from a 2.5 µg/g DDT-contaminated field plot were compared to the POM-derived BAFs. The results suggest a POM-based biomimetic method does not provide an accurate representation of the contaminant fraction available to plants in high soil DDT concentrations, and illustrates the need to include plants in bioavailability studies as the use of carbon materials for *in situ* contaminant sorption moves from predominantly sediment to soil remediation technologies.

7.2 INTRODUCTION

Soils contaminated with persistent, bioaccumulative, and toxic organic chemicals occur worldwide and pose a significant challenge to environmental risk assessment and management. Alternative 'greener' (i.e. sustainable) remediation approaches than traditional soil excavation and transport for hydrophobic organic contaminants (HOCs) are being sought in order to reduce risk to both the environment and human health. Carbon amendments such as activated carbon (AC) and biochar have been successful in immobilizing contaminants in sediment (18, 24, 81, 93, 174), and more recently soil (75, 77, 191), systems when added at ca 3% (w/w). Immobilization of organic contaminants *in situ* reduces bioaccumulation of these compounds in plants, invertebrates and fish, reducing risk to higher trophic organisms. Contaminant toxicity decreases as a consequence, and the overall health of the ecosystem improves as measured by increased plant and invertebrate biomass (191).

Over the past few decades, extensive work has been conducted on measuring bioavailability via biomimetic methods. Equilibrium passive sampling devices such as those based on the polymer, polyoxymethylene (POM), can be used to determine the chemical activity and thus bioavailability of HOCs (15, 79, 81, 83, 90). In environmental systems, chemical activity is expressed by the freely dissolved porewater concentration and is often used as an analogue for invertebrate bioaccumulation, who accumulate HOCs via diffusion. Biomimetic methods such as POM-based samplers have been used to measure the effect of activated carbon on contaminant bioavailability. A 2008 study by Sun and Ghosh (102) showed that POM derived sediment porewater values were related to PCB congener concentrations in *Lytechinus variegatus*, a freshwater oligochaete for both AC treated and untreated sediments. The relationship was linear for tetra- and penta- chlorinated congeners over a range of 0.33-84.7 µg/g (102). These authors concluded that this biomimetic method provided a "convenient and accurate" method for monitoring sediment remediation via AC amendment. Other studies further supported the biological basis for using passive samplers to monitor the success of AC remediation in PCB-contaminated sediments in freshwater (79, 103) and soil (19) invertebrates.

In a recent review of methods to assess bioavailability (1), the following gaps in literature were identified: i) a lack of studies exploring naturally contaminated sediments and soils, ii) field scale scenarios, and iii) accumulation in higher trophic levels. The current study investigates the effects of field scale AC and biochar addition to soils that have been contaminated (and naturally weathered) with high levels (39 µg/g) of the organochlorine insecticide dichlorodiphenyltrichloroethane (DDT). Bioavailability to higher tropic organisms is assessed using the plant species *Cucurbita pepo* spp. *pepo*, a plant known to accumulate DDT without jeopardizing plant health (8, 9, 48, 128, 151, 244-247), and *Eisenia fetida*, a common soil invertebrate. The primary objective of the current study is to compare *C. pepo* and *E. fetida* uptake to a POM-based biomimetic method. For plant studies, bioaccumulation data from an area with a lower DDT soil concentration (2.5 µg/g) is included as well as a literature review of *C. pepo* bioaccumulation factors (BAFs) across various DDT soil concentrations.

7.3 MATERIALS AND METHODS

7.3.1 Site Details

In situ experiments were conducted at Point Pelee National Park (PPNP) located immediately south of the town of Leamington, Ontario, Canada. The area has historical significance as Canada's first National Park and is comprised of a unique Carolinian ecosystem making it renowned worldwide for its influx of endangered migratory birds (248, 249). As a result of PPNP's former use as orchard land, legacy DDT contamination exists at levels greater than the agricultural guideline of 0.7 µg/g set by the Canadian Council of Ministers of the Environment (46, 248). Due to the historical significance of the park, as well as the sensitivity of many species of birds (248, 249) traditional remediation approaches such as soil excavation and off site transport are not viable options due to their detrimental effects on the ecosystem.

7.3.2 Soil and Materials

The soil at PPNP is classified as sandy and contains DDT contamination, composed predominantly of 4,4'- DDE (dichlorodiphenyldichloroethylene) and 4,4'- DDT (90% ± 12%), which have weathered in place for over 40 years. Experimentation was conducted in June-September 2012 and 2013 at two former agricultural areas of the park. The 2012 plot was established in soil containing an average total DDT concentration (∑DDT) of 2.5 ± 0.03 μg/g. This soil had a cation exchange capacity (CEC) of 11.2 cmol/kg, a pH of 7.7 and contained 3.5% organic matter. In 2013, plots were established in soil with a mean total DDT concentration of 39 ± 1.8 μg/g, a CEC of 5.8, a pH of 7.9 and 3.1% organic matter.

Two types of biochar and one granular activated carbon (GAC) were obtained for experimentation. Full details of each carbon amendment can be found in Denyes et al. (2013) (191). Briefly, Burt's biochar, BlueLeaf biochar and GAC were determined to have organic matter contents of 63%, 97%, and 29%, respectively and BET-N_2 specific surface areas of 372, 55 and 809, respectively.

All plant experiments were conducted in both soil concentrations, however invertebrate and POM-based studies were conducted in the 39 μg/g DDT-contaminated soil only.

7.3.3 Plant Experiments

For both field sites (2012 and 2013) native vegetation was removed and four plots, 200 cm long by 50 cm wide and 20 cm deep were established manually using a shovel. Plots were a minimum 50 cm apart. The corresponding carbon amendment (0% (control), 2.8% GAC, 2.8% Burt's biochar, or 2.8% BlueLeaf biochar) was then added to the plot and mixed thoroughly. Each plot received nine pumpkin (*Cucurbita pepo* ssp. *pepo* cv. Howden) seeds purchased from the 'Ontario Seed Company' (Waterloo, ON) in June, however extra seedlings were removed at ca. three weeks, such that each plot contained only three growing plants, evenly spaced. Pumpkin plants were harvested at 65 days. The 2012 (2.5 ug/g DDT) site was replanted just with control pumpkins in 2013. In the 2013 experimental design only, the plots were planted again with another nine pumpkin seeds, and plants were grown in triplicate for another 60 days. Plants were watered two times per week regardless of precipitation.

The *in situ* field experiment in 2013 was replicated in triplicate in the greenhouse located at the Royal Military College of Canada (RMC) using DDT-contaminated soil collected from site. The amendment mixture was placed in bottom perforated six-inch diameter planting pots (total soil weight per planter of 500 g). Pumpkin plants were measured for plant growth on a weekly basis and harvested at 60 days. Greenhouse temperature was maintained at 27ºC (±7ºC) and the pumpkins were grown under a 14:10 h (day:night) fluorescent photoperiod. Planters were top and bottom watered to maintain sufficient moisture.

All pumpkin plants (field and greenhouse) were harvested by cutting the shoot of the pumpkin with acetone rinsed scissors as close to the soil surface as possible. Root samples were then collected and roots and shoots rinsed clean with water. Plant tissues (shoots and roots) were patted dry, weighed, and biomass was used to assess plant health. Plant samples were then placed in individually labelled Whirlpak® bags and frozen prior to analysis for DDT concentration.

7.3.4 Soil Invertebrate (Worm) Experiments

Worms (*Eisenia fetida*) purchased from "The Worm Factory" (Westport, ON), were tested for DDT bioaccumulation, toxicity and avoidance in the Phytotechnology Laboratory located at the Royal Military College of Canada. In all cases worms were maintained in dark aluminium containers, at a temperature of 21ºC (±3ºC), at approximately 35% moisture.

Two toxicity experiments were performed in $[DDT]_{soil}$=39 µg/g mixed with 0%, 2.8% GAC, 2.8% Burt's biochar and 2.8% BlueLeaf biochar amendments. Worms were added to the soil/amendment mixtures and the number of surviving worms counted and weighed at ~ 50 days. Surviving worms from the second experiment were washed using a container of clean water and depurated for 72 hours at 4ºC, dried for 24 hours at 25ºC and stored in individually labelled Whirlpak® bags and frozen until analyzed for DDT concentration. Worms from the first experiment were not analysed for DDT.

Selection for invertebrate avoidance assays was based on the method described by Li et al. (2011) (207), and worms weighing 0.3-0.6 g were used. Six avoidance wheels were constructed using a modified design from Environment Canada's Acute Avoidance Test (220, 237). Each of the six compartments was filled with 120 g of DDT-contaminated soil or DDT-contaminated soil/carbon amendment mixture, with every other compartment serving as an unamended control. During testing, wheels were covered with aluminum foil to prevent worm escape and to maintain moisture. Testing was done in triplicate for each amendment and worms were exposed for a period of 48 hours.

7.3.5 Polyoxymethylene (POM) Passive Sampling Experiment

A thin sheet of POM (76 µm thick) was purchased from CS Hyde Company (Lake, Villa, IL) as this product has been commonly used to determine porewater concentrations of HOCs (15, 16, 90). The partition coefficients (K_{POM}) for 4,4-DDT and 4,4-DDE were previously determined by Endo et al. (2011) (83). Using the K_{POM} value, the soil pore water concentration (C_w) was calculated based on the equation, $C_w = C_{POM}/K_{POM}$, where C_{POM} is the calculated concentration in the polymer. The POM sheets were cut into 9 x 2 cm strips (200 mg each) and cleaned via immersion in a series of hexane, methanol and double distilled water containing 200 mg/L NaN_3 (a biocide) with gentle shaking (100 rpm) for 24 hours each. All carbon amendments were added at 2.8% (w/w) to DDT-contaminated soil (39 µg/g) and mixed for 1 hr at 30 rpm. Treatments were tested in triplicate by adding 10 g (dry wt.) of soil or soil/amendment to 300 mL amber glass vials. Thirty millilitres of water (25 mg/L NaN_3) and 200 mg POM were added to each vial. Bottles were shaken on an orbital shaker at 25 rpm for 28 days. POM strips were removed, rinsed with double deionized water, gently wiped dry and extracted as described below.

7.3.6 Analytical Procedures

7.3.6.1 DDT Concentrations in Soil, Plant, Worm and POM samples

All soil, plant and worm samples were dried at 25°C for 24 h immediately prior to analysis. POM samplers were patted dry with clean tissue. Plant root and shoot samples were analysed by microwave-assisted extraction (MAE) at RMC. MAE was performed at a temperature of 120°C for 35 min in 30 mL of 1:1 hexane:acetone mixture using a Milestone Ethos SEL microwave extraction system. Following extraction, sample extracts were concentrated using a Syncore, the solvent exchanged for hexane, and then extracts were applied to a Florisil column for cleanup.

DDT concentrations in soil and worm tissues and POM samplers were analyzed via Soxhlet extraction, at the Analytical Services Unit of Queen's University. Worm samples were finely chopped using metal scissors (rinsed with acetone between samples) and homogenized. Chopped worm samples were dried at room temperature for approximately 12–18 h, and then soil and worm samples were ground with sodium sulphate and Ottawa sand. Soil and worm and POM samples were extracted in a Soxhlet apparatus for 4 h at 4–6 cycles per hour in 250 mL of dichloromethane and 250 mL of a 1:1 hexane:acetone mixture, respectively.

Sample extracts were analyzed for DDT and its key metabolites, using an Agilent 6890 Plus gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/µECD), a SPB™-1 fused silica capillary column (30 m, 0.25 mm ID × 0.25 µm film thickness) and HPChem station software. The carrier gas was helium, at a flow rate of 1.6 mL/min. Nitrogen was used as the makeup gas for the electron

capture detector (ECD). Detection limits were 1.0 ng/g. All values were reported as μg/g dry weight, and DDT concentration unless otherwise specified refers to the sum of DDT and its metabolites.

7.3.6.2 Quality Assurance/Quality Control (QA/QC)

One analytical blank, one control and one analytical duplicate sample were prepared and analyzed for every nine samples analyzed by Soxhlet or MAE. The control sample was spiked with a known amount of Supelco Appendix IX pesticide mixture. Decachlorobiphenyl (DCBP) was added to each sample as a surrogate standard prior to extraction. None of the analytical blanks contained DDT at concentrations above detection limits (1.0 ng/g for total DDT) and all control samples were between 80–110% of the expected value. Relative standard deviations between the samples and their analytical duplicate were below 14% for all results and the average surrogate recovery for samples was 89%.

7.3.7 Statistical Analyses

The tissue concentration data were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc Tukey comparison. All residuals of the data were determined to be normally distributed by a Kolmogorov Smirnov test. A significance level of α=0.05 was used for all tests, and results were recorded with the standard error of the mean.

7.4 Results and Discussion

7.4.1 Plants

Plants grew well in soils contaminated with 2.5 and 39 μg/g DDT, showing no signs of toxicity, and there were no significant differences in plant biomass between treatments. Earlier studies by Denyes et al. (173, 191) reported increased plant growth as a result of the addition of biochar to intensely degraded Brownfield soil. In this study the PPNP soil was not intensely degraded and therefore substrate improvements such as CEC, increased particle size distribution and nutrient additions as a result of biochar amendment were negligible.

Significant differences ($p < 0.05$) in plant shoot and root bioaccumulation factors (BAF= $[DDT]_{plant/worm/POM}$/[DDT]soil) were observed between the two study sites. Plants grown in 2.5 μg/g DDT-contaminated soil had mean shoot and root BAFs of 4.6 and 13.1 (2012) and 2 and 15 (2013), respectively. These were significantly higher than the shoot and root BAFs of the plants grown in 39 μg/g DDT-contaminated soil which were 0.26 and 0.92, 0.002 and 0.99, and 0.06 and 1.95 for the first and second field harvests and the greenhouse study, respectively. In Figure 7.1, BAF results from this study are plotted with those reported in literature (7, 48, 151, 244, 246, 247, 250-252). A trend emerges from the combined data showing decreasing BAFs with increasing DDT soil concentrations, particularly in plant shoots (Figure 7.1a). Plots of the $\log BAF_{root}$ and $\log BAF_{shoot}$ versus $\log[DDT]_{soil}$ (Supporting information Figure E.10.1) show linear correlations having R^2 values of 0.4 and 0.6, respectfully. The significant difference in plant uptake between sites suggests that the ability of plants to accumulate high levels of DDT is dependent on soil concentration and may be indicative of a concentration threshold effect.

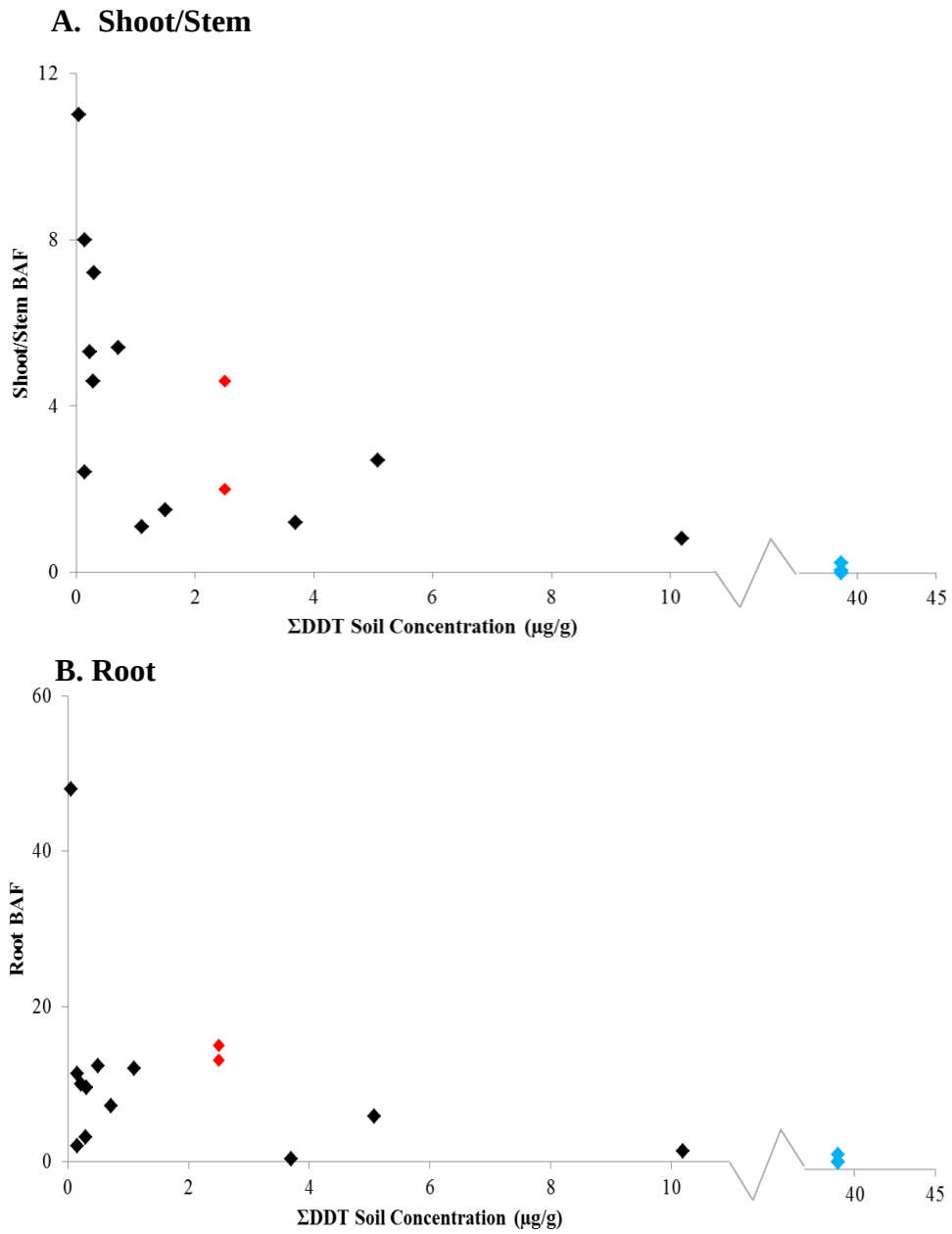

Figure 7.1 Literature reported ΣDDT bioaccumulation factors (BAFs) and BAFs from the current study in *Cucurbita pepo* spp. *pepo* A) shoot/stem and B) root tissues in soils with various concentrations of ∑DDT (µg/g) (n=18) (7, 48, 151, 244, 246, 247, 250-252). The red and blue markers indicate the BAFs from the current study from soil contaminated with 2.5 µg/g and 39 µg/g ΣDDT, respectively.

No significant reductions in plant DDT uptake as a result of AC and biochar amendments in either soil concentration were achieved. These results were not expected as the same three carbon amendments at the same addition rate were previously reported to reduce PCB (Aroclor 1254/1260) uptake to *C. pepo* by up to 74% (191). In the soil containing 39 µg/g DDT, this may be explained by the

low BAFs in the amended and control pumpkins. In the 2.5 µg/g DDT-contaminated soil, the inability of the carbonaceous sorbents to effectively immobilize the DDT contamination may be related to the unique ability of *C. pepo* to facilitate DDT uptake via root exudates, a process known to be even more effective than in PCB-contaminated soils (7, 191, 246). Also, incomplete mixing of the carbon amendments with the DDT-contaminated soil has been shown to delay treatment benefits (191) due to spatially heterogeneous AC particles, minimizing contaminant contact and delaying treatment benefits (17, 89, 140).

7.4.2 Invertebrates

Worms (*Eisenia fetida*) exposed to the 2.8% GAC in the 39 µg/g DDT-contaminated soil had the lowest survival rate (93%) and were 33% smaller ($p < 0.05$) at the end of the experiment than at the beginning (Figure 7.2). It has been suggested that carbon amendments may cause adverse effects such as weight loss to soil/sediment invertebrates (14, 92, 253), as a result of strong nutrient sorption to the AC/biochar particle, and particle interference within the gut of the organisms. The weights and survival percentage of worms exposed to the unamended DDT-contaminated soil (control), as well as Burt's and BlueLeaf biochars did not change significantly throughout the experiment.

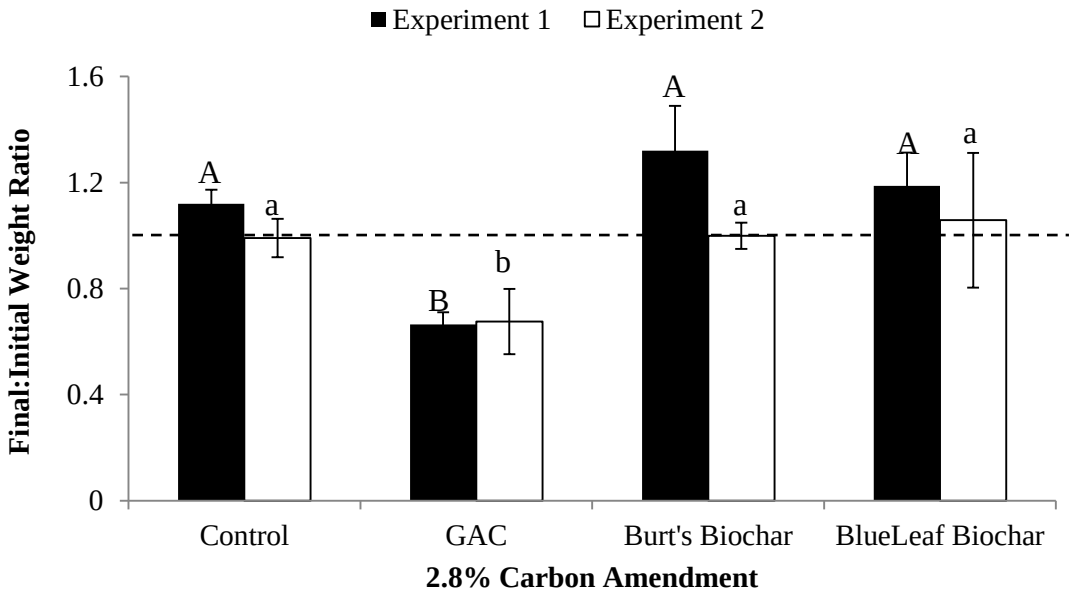

Figure 7.2 Ratio of final to initial weights (g) of *Eisenia fetida* exposed to unamended DDT-contaminated soil (control) or 2.8% (w/w) GAC, Burt's biochar or BlueLeaf biochar for both toxicity experiments. Values < the dashed 1.0 line represent a loss in worm weight. Error bars represent one standard deviation. Upper-case letters (first experiment) and lower-case letters (second experiment) indicate statistically significant differences between treatments ($p < 0.05$).

In the avoidance study, 84% and 80% of *E. fetida* significantly avoided the 2.8% GAC and BlueLeaf amended soil, respectively, similar to other studies (92, 254). However, the worms preferred the 2.8% Burt's biochar amended soil over the unamended soil highly contaminated with DDT (60% preference, $p < 0.05$). Soil invertebrates are essential for maintaining soil health (255) and represent an

important food source for many avian species at PPNP. Hence, maintaining healthy soil invertebrates while minimizing contaminant uptake is especially important at PPNP.

In contrast to the plant experiments, all of the carbon amendments reduced the bioaccumulation of DDT into worm tissue. Burt's and BlueLeaf biochar significantly reduced DDT uptake by 49% and 36% respectively, and although not significant, GAC reduced DDT bioavailability by 29%. Bioavailability reductions of HOCs to invertebrates as a result of carbonaceous amendment are well reported (14, 15, 17-19, 72, 77, 88, 94, 173, 191, 256), and are explained by strong sorption of the contaminant molecule to the carbonaceous sorbent particle.

7.4.3 Comparison of Accumulation in Plants and Invertebrates with Predicted Bioavailability using Passive Samplers in 39 µg/g DDT-Contaminated Soil

Soil porewater concentrations were calculated from the POM based passive samplers using partition coefficients for 4,4'-DDT and 4,4'-DDE from Endo et al., (83) given that PPNP soil is predominately composed of these metabolites. The proportion of these two compounds relative to total DDT extracted was 84.7% ± 4.9%, and did not differ significantly between sample type (i.e. soil, shoot, root, worm and POM). Reductions in the soil porewater concentrations of the combined total of 4,4'-DDT and 4,4'-DDE, as a result of AC and biochar soil amendments, are compared to the corresponding reductions in worms, plant roots and plant shoots in Figure 7.3. Porewater concentrations significantly decreased by 29% and 31% as a result of GAC and BlueLeaf biochar amendments, respectfully. The POM-biomimetic method adequately predicted reductions in worm accumulation in all three carbon amendments, as also shown by Chai et al., (15). None of the carbon amendments reduced uptake of 4,4'-DDT and 4,4'-DDE into the plant roots or shoot and soil porewater concentrations determined by a POM based passive sampler did not successfully predict treatment effectiveness of carbon amendments to plants.

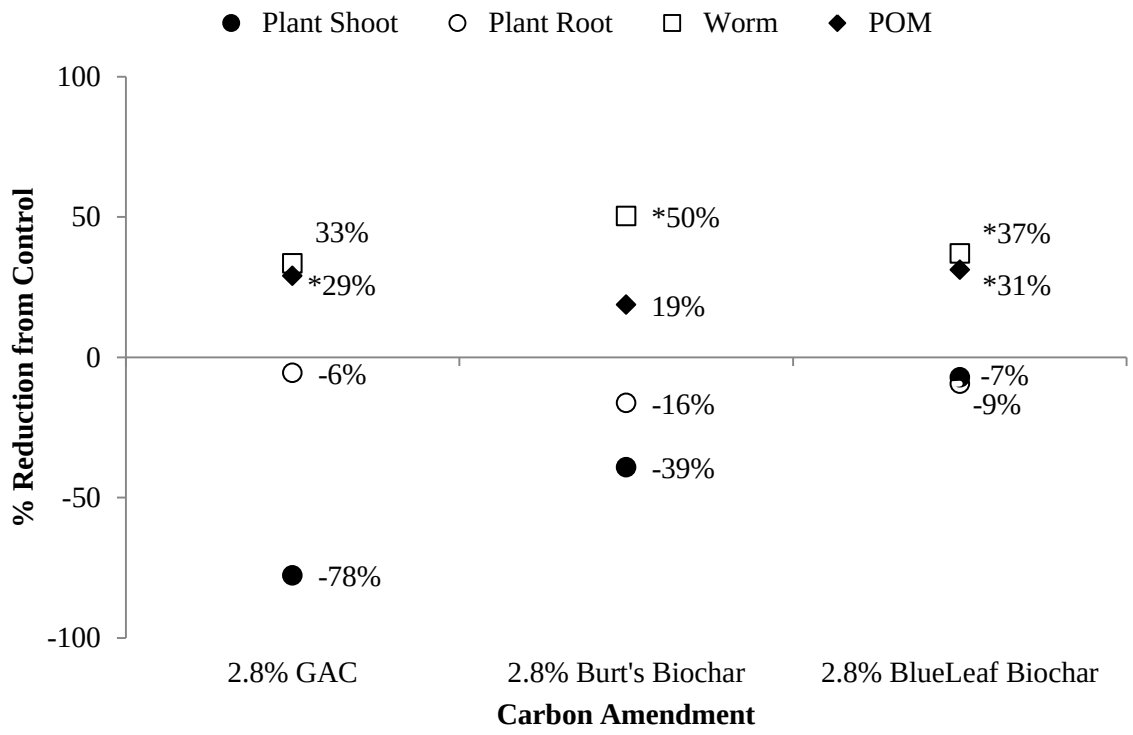

Figure 7.3 Measured plant and invertebrate- and POM-predicted bioavailability reductions of ∑4,4'-DDE and 4,4'-DDT following 2.8% carbonaceous amendment to DDT-contaminated soil (39 µg/g). Data labels present the percent reduction from the relative control in each experiment as a result of that particular AC or biochar amendment. * indicate a significant reduction ($p < 0.05$). Negative values represent no reduction in plant, invertebrate or POM DDT uptake.

In Figure 7.4a-c POM BAFs (determined in amended and unamended 39 ppm total DDT soils) are compared to the corresponding worm, shoot and root BAFs as per Gomez et al. (84). Soil porewater concentrations generally underestimated the concentration of 4,4'DDT and 4,4'-DDE accumulated into worm tissue (Figure 7.4a) but mean worm BAFs for unamended and amended soils were within 50% of the POM-derived BAFs.

Studies have shown better POM-predicted and measured bioavailability correlations for invertebrates in sediment systems using linear regression models ($R^2 \sim 0.9$) (19, 79, 102) in compounds with similar levels of chlorination. However, in soil systems contamination is more heterogeneous than in sediments potentially limiting mass transfer (89, 191). Gomez et al. (84) have suggested one order of magnitude error in the biomimetic method (Figure 7.4) is appropriate due to soil heterogeneity.

The POM biomimetic method clearly over-predicted the actual accumulation in plant tissues (Figure 7.4b and 7.4c). As expected bioavailability in roots is better predicted and is within an order of magnitude (134 ± 18%). Gomez et al. (84) observed an under-prediction of PAH bioavailability in plants via POM-based extraction methods based on the ability of root exudates to act as biosurfactants and increase the mobilization of PAHs from the soil matrix. A similar result was expected in this study as DDT is readily mobilized by exudates of *C. pepo*.

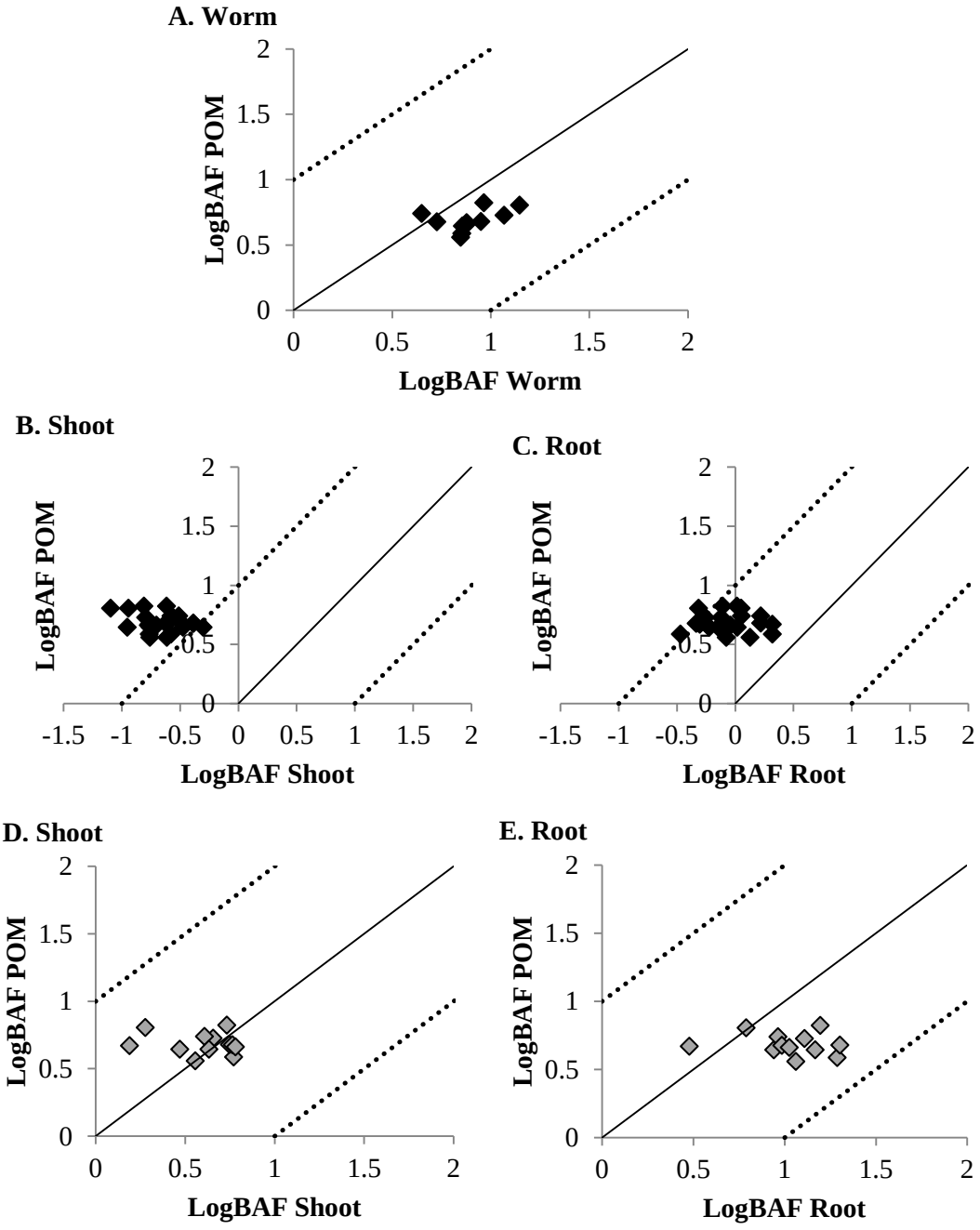

Figure 7.4 Relationship between predicted bioavailability of 4,4'-DDT and 4,4'-DDE as determined by POM bioaccumulations factors (BAFs) and BAFs in A) Worm, B) Shoot, and C) Root tissues from experiments performed in [DDT]soil= 39 µg/g, and relationships with D) Shoot and E) Root tissue BAFs from experiments conducted in $[DDT]_{soil}$=2.5 µg/g (grey markers). Plant data is based on one and two field studies in 2.5 µg/g and 39 µg/g DDT contamination, respectfully. The solid black line indicates 1:1 relationship, whereas the dotted black lines delimit one order of magnitude deviation intervals.

There are a lack of studies comparing equilibrium aqueous concentrations and bioavailability in higher trophic levels such as plants (1, 84) especially in highly contaminated systems. This is the first study to report an effect of contaminant concentration on that of POM passive sampler's ability to accurately predict plant bioavailability. Plant shoot and root 4,4'-DDT and 4,4'-DDE concentrations did not differ significantly between soil concentrations, despite having significantly higher BAFs when grown in lower DDT contamination ($p < 0.05$). This indicates that plant uptake is inhibited at high soil DDT concentrations, and a possible threshold effect is occurring. In Figures 7.4d and 7.4e the POM BAF from the 39 ppm total DDT soils are compared to the shoot and root BAFs in the 2.5 ppm total DDT soils and illustrates a potential for improved correlation at lower soil concentrations. Hence the use of POM-equilibrium passive samplers for calculating soil porewater concentrations of DDT may have potential for predicting risk at sites with a lower level of DDT contamination (i.e. < 10). Future experiments of POM samplers in a range of soils having lower DDT contamination are required to confirm this. Also, a POM-based biomimetic sampling method may be appropriate for use in contaminated soils where plant uptake is not controlled by soil contaminant concentration.

Further research is required to determine if the POM-biomimetic method can predict the effect of carbon amendments on contaminant bioavailability in plants.

8. OVERALL CONCLUSIONS

Over the past few decades research has significantly improved our understanding of how soil and sediment chemistry control contaminant bioavailability (1). Carbon-rich charcoal-like materials such as AC and biochar are highly porous materials capable of strongly binding HOCs in soil, thereby minimizing their bioavailability, exposure and subsequent risk. In sediment systems, pilot projects of AC amendments have been in place for more than 10 years (17, 24), and have contributed greatly to expanding this technology into soil systems. Prior to the onset of this thesis, very little work had been conducted in soils (and sediments) on the ability of biochar to serve as an alternative to AC to sorb HOCs. A few of these studies were by authors well versed in applying AC to sediments, expanding their research to include biochar and sorption to PAHs (13, 76) and PCBs (21), however all were laboratory-based. Other biochar sorption-based studies developed as a result of concerns in the agriculture sector that the application of biochar to improve crop yields could have a negative impact on pesticide efficiency (74, 141-143, 145, 257).

Over the past four years, the studies outlined in this thesis have provided many of the first lines of evidence to support the use of carbonaceous sorbents in soils, and address gaps in literature that were hindering their large-scale application. With each study undertaken and completed, new lessons were learned and new questions were identified. Although similar work has been conducted using kinetic modelling or passive samplers in HOC-contaminated sediment systems, the work in this thesis is novel in that it investigates the effects of carbonaceous amendments on bioavailability in soil systems to plants, invertebrates and soil microbes. This environmentally-relevant approach (i.e. the use of soils naturally weathered and highly contaminated with PCBs or DDT), and the combination of field and greenhouse studies, give this work strength and sets it apart from others in the field.

In this thesis, biochar and AC were assessed for their abilities to reduce the PCB bioavailability in highly contaminated soils. Biochar made from construction waste materials at a local greenhouse (Burt's Greenhouses, Odessa, ON), successfully reduced the uptake of PCBs into the known PCB phytoextractor *Cucurbita pepo* ssp. *pepo* cv. Howden (pumpkin) and a common invertebrate species, *Eisenia fetida* (worm). The reductions were greatest using the highest amendment application; however significant reductions were achieved at 2.8% (by weight) which corresponds to current protocol standards for sediment remediation using AC (24). When applied at 2.8% *in situ*, AC and two types of biochar were equally effective at reducing PCB uptake into plants (~70% reduction), and both biochars outperformed AC with respect to improving substrate quality and increasing plant growth. Given that biochar is a waste by-product, reduces greenhouse gases and costs between 50-75% (173) less than AC, it has significant potential as an alternative to AC for the sorption of PCBs.

The effect of mixing techniques on carbonaceous amendments was evaluated in PCB-contaminated soil as the AC was less effective in field applications than expected based on bioavailability reductions reported in literature (18, 24, 77). *In situ* mixing is expected to have lower short term efficiencies compared to laboratory based experiments due to spatially heterogeneous AC particles minimizing PCB contact and delaying treatment benefits (89, 95). Mechanical mixing of the amendments with PCB-contaminated soil increases the soil/carbon amendment contact time, improves the homogeneity of the mixture, and offers a greater number of particles per unit volume of soil. These changes resulted in bioavailability reductions up to 66% greater than manual mixing methods. The results of these field and greenhouse studies demonstrate that AC and biochar show significant potential to serve as sorbents for the *in situ* stabilization of organic contaminants.

In addition to the work on PCB soils, carbon amendments and biomimetic methods were investigated using soils contaminated with DDT at Point Pelee National Park. Reductions in contaminant bioavailability following biochar and AC application to contaminated soils were again assessed using *C. pepo* spp. *pepo* and *E. fetida*, and in addition were compared to predicted bioavailability using a

polyoxymethylene (POM)-based equilibrium passive sampling device. In contrast to the findings in PCB-contaminated soil, none of the carbon amendments significantly reduced plant DDT uptake despite thorough mixing, and in these soils AC demonstrated toxic effects to soil invertebrates. These results demonstrate the importance of adding biochar and AC in a site-specific manner, as these materials perform differently in terms of their abilities to minimize HOC bioavailability and improve soil quality depending on the site characteristics. Plant DDT accumulation was significantly higher in soils contaminated with only 2.5 µg/g DDT than with 39 µg/g DDT. When these results were plotted with literature values over a range of DDT soils concentrations, a linear trend emerged suggesting that plant uptake of DDT is inhibited at high (> 10 µg/g) soil concentrations. As expected based on numerous publications from sediment research (27, 44-46), the bioavailable fraction predicted by the POM samplers correlated well with measured invertebrate uptake (< 50% variability). However, due to the concentration threshold effect limiting plant uptake, the predicted bioavailability was greater than actual plant root accumulation by 134%. The POM samplers did not correctly predict the effect of carbon amendments on root or shoot bioavailability. Currently this study is one of only two reporting on the potential to use POM-equilibrium passive samplers to predict plant bioavailability, and the first in DDT-contaminated soil. These results highlight the importance of including plants in bioavailability studies of this kind.

The differences in bioavailability reductions, plant growth and toxicity effects observed between the two soils contaminated with HOCs also highlights the importance of careful characterization of the carbon amendments prior to use. Feedstock materials and pyrolysis conditions influence physical, chemical and biological properties, and therefore affect the product's end use as a soil amendment (i.e. carbon sequestration, substrate improvements, sorption of contaminants). This knowledge enables the potential to produce 'designer biochars' with properties engineered for a specific function. On the other hand, the use of contaminated feedstock materials or inappropriate pyrolysis conditions may create a material that causes adverse biological effects and is therefore unacceptable for use as a soil amendment. This was the case for one biochar that was produced from construction wastes and was found to impair plant germination and cause invertebrate avoidance. These results demonstrate that the standardized protocols outlined by the International Biochar Initiative are effective and practical when analysing biochars for the remediation of contaminated sites. Results of community level physiological profiling (CLPP) imply that carbon amendments cause no effects to the microbial communities when substrate quality is fair and some biochars may help to restore microbial function in intensely degraded Brownfield soils.

Overall, the results presented in this thesis demonstrate that biochar or AC mixed with soil is potentially an effective *in situ*, management strategy for reducing the bioavailability of HOCs in highly contaminated soils. These studies show that biochar, when deemed safe for use, is as effective as AC at minimizing HOC bioavailability and does so at a lower cost, with a smaller carbon footprint, while improving substrate quality, and without adverse effects to soil invertebrates. Currently the main disadvantage of using carbon amendments as a remediation technology is that soil contamination is only stabilized, not removed. However, as the trend in remediation technologies continues to move towards more risk-based strategies and life cycle analysis becomes an integral part of remediation plans, carbon amendments are expected to increase in acceptability. Future studies should include plants other than *C. pepo*, such as those native or naturalized to the contaminated site. As well, the microbial characterization should be expanded to include sequencing such that shifts in microbial community structure and function could be identified at the genus level, rather than solely based on carbon source utilization patterns. Finally, since these studies have shown great potential for the use of carbonaceous sorbent materials for the *in situ* immobilization of PCBs and DDT in soils, future research should look at the interaction with other HOCs, and build on the important lessons learned.

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10. APPENDICES

APPENDIX A: RAW DATA AND QA/QC FOR CHAPTER 3

Table A-1 PCB Concentrations (µg/g) in Plant Shoot Tissue

Shoot				
Soil Concentration	Data File	Rep	Burt's Biochar (%)	PCB Concentration (µg/g)
Low	101119	A	0	4.86
Low	101119	B	0	7.22
Low	101119	C	0	7.05
Low	101119	A	0.2	5.06
Low	101119	B	0.2	5.04
Low	101119	C	0.2	7.54
Low	101119	A	0.7	4.38
Low	101119	B	0.7	5.04
Low	101119	C	0.7	7.06
Low	101124	A	2.8	4.66
Low	101124	B	2.8	5.25
Low	101124	C	2.8	5.50
Low	101124	A	11.1	2.55
Low	101124	B	11.1	3.13
Low	101124	C	11.1	2.88
High	101124	A	0	9.35
High	101124	B	0	13.5
High	101124	C	0	21.0
High	101130	A	0.2	16.3
High	101130	B	0.2	19.2
High	101130	C	0.2	17.1
High	101130	A	0.7	20.6
High	101130	B	0.7	8.34
High	101130	C	0.7	11.4
High	101208	A	2.8	13.2
High	101208	B	2.8	18.0
High	101208	C	2.8	11.9
High	101208	A	11.1	13.0
High	101208	B	11.1	6.81
High	101208	C	11.1	11.1

Table A-2 PCB Concentrations (µg/g) in Plant Root Tissue

Root				
Soil Concentration	Data File	Rep	Burt's Biochar (%)	PCB Concentration (µg/g)
Low	101201	A	0	46.9
Low	110119	B	0	41.5
Low	110120	C	0	30.3
Low	101201	A	0.2	34.6
Low	110119	B	0.2	34.1
Low	110120	C	0.2	32.6
Low	101201	A	0.7	33.9
Low	110119	B	0.7	29.7
Low	110120	C	0.7	31.7
Low	101201	A	2.8	11.7
Low	110119	B	2.8	17.7
Low	110120	C	2.8	18.7
Low	101201	A	11.1	5.27
Low	110120	B	11.1	5.21
Low	110120	C	11.1	9.01
High	101208	A	0	896
High	110119	B	0	1180
High	101210	C	0	482
High	101208	A	0.2	587
High	110119	B	0.2	691
High	101210	C	0.2	393
High	101208	A	0.7	439
High	110119	B	0.7	592
High	101210	C	0.7	386
High	101210	A	2.8	140
High	110119	B	2.8	2020
High	101210	C	2.8	194
High	101210	A	11.1	82.1
High	110119	B	11.1	111
High	101210	C	11.1	69.4

Table A-3 PCB Concentrations (µg/g) in Worm Tissue

Worm *(Eisenia fetida)*				
Soil Concentration	Data File (ASU)	Rep	Burt's Biochar (%)	PCB Concentration (µg/g)
Low	13507	A	0	48.0
Low	13507	B	0	46.7
Low	13507	C	0	28.3
High	13507	A	0	2340
High	13507	B	0	3040
High	13507	C	0	2650
High	13507	A	0.2	2730
High	13507	B	0.2	2190
High	13507	C	0.2	4240
High	13507	A	0.7	2980
High	13507	B	0.7	2770
High	13507	C	0.7	3410
High	13507	A	2.8	1130
High	13507	B	2.8	1060
High	13507	C	2.8	1530
High	13507	A	11.1	270
High	13507	B	11.1	306
High	13507	C	11.1	321

Table A-4 PCB Concentrations (µg/g) in Soils

Soil				
Soil Concentration	Data File	Rep	Burt's Biochar (%)	PCB Concentration (µg/g)
Low	110103	A	0	0.74
Low	110103	B	0	5.2
Low	101222	C	0	4.1
Low	110103	A	0.2	4.3
Low	110103	A	0.7	4.4
Low	110103	A	2.8	4.2
Low	110103	A	11.1	3.9
High	101222	A	0	6.7
High	101222	A	0.2	6.3
High	101222	A	0.7	6.0
High	101222	A	2.8	7.9
High	101222	A	11.1	6.9

Table A-5 QA/QC Chapter 3

Data File	[PCB] Spike (ug/g)	% of Target	[PCB] Blank (ug/g)	RSD	Average Efficiency (%)	Matrix
13507	3.90	78.0	< 0.1	9.66	102	worm
101119	-	-	< 0.1	10.5	105	shoot
101124	5.30	106	< 0.1	14.8	87.1	shoot
101130	5.03	101	< 0.1	23.6	78.6	shoot
101208	5.18	104	< 0.1	7.74	97.7	shoot & root
101201	4.38	87.6	< 0.1	3.86	95.4	root
101210	4.25	85.0	< 0.1	11.9	102	root
110119	5.29	106	< 0.1	4.38	103	root
110120	4.47	89.4	< 0.1	1.20	104	root
110203	5.70	114	< 0.1	1.48	91.2	soil
101222	4.70	94.0	< 0.1	0.17	114	soil
Average	4.82	96.4	< 0.1	8.11	98.3	

Table 10-6 Plant and Invertebrate Harvest Data

	Shoot Weights		Invertebrates	
	Low Contaminated	High Contaminated	Weight (g)	% Survival
Control	33.4	16.9	1.20	4
Control	18.5	16.7	1.79	6
Control	22.7	22.1	0.55	2
0.2% Burt's Biochar	18.8	24.7	1.85	8
0.2% Burt's Biochar	36.1	26.6	1.78	8
0.2% Burt's Biochar	25.9	29.3	1.42	6
0.7% Burt's Biochar	27.2	19.8	1.55	6
0.7% Burt's Biochar	30.4	37.7	7.01	50
0.7% Burt's Biochar	37.7	25.1	4.60	36
2.8% Burt's Biochar	30.5	34.2	8.20	80
2.8% Burt's Biochar	21.7	44.2	12.4	96
2.8% Burt's Biochar	45.7	34.8	6.06	34
11.1% Burt's Biochar	31.5	40.2	3.68	16
11.1% Burt's Biochar	20.8	32.9	3.32	30
11.1% Burt's Biochar	31.3	33.0	6.19	58

APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 3

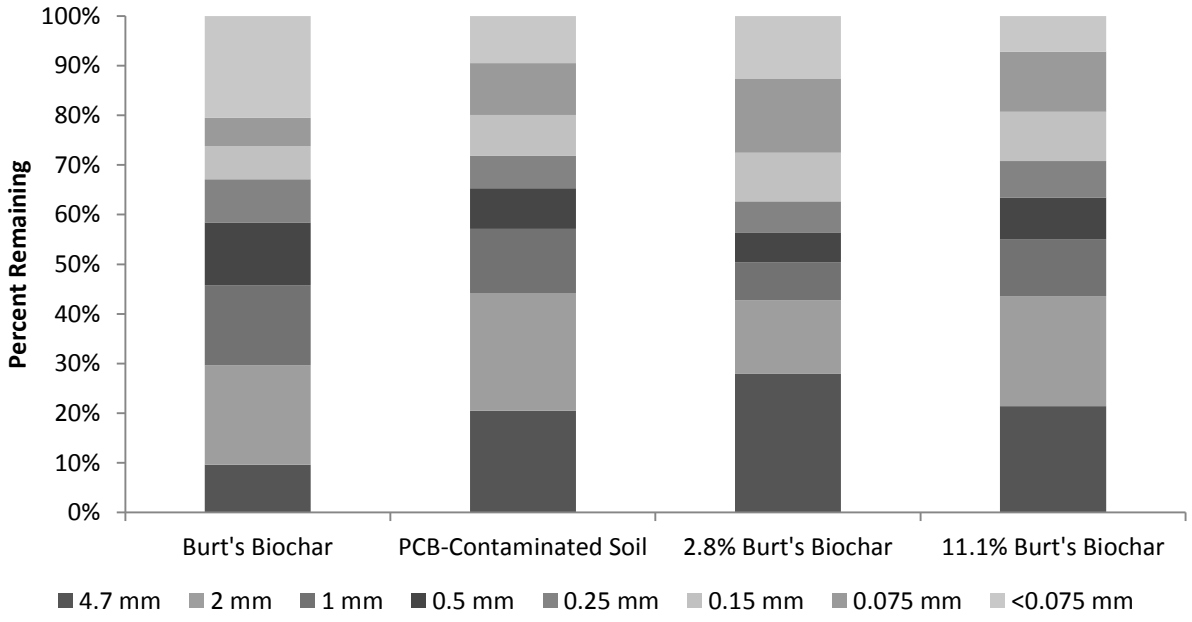

Figure A.10.1 Particle size distribution of Burt's biochar, PCB-contaminated soil and freshly tumbled PCB-contaminated soil amended with 2.8 and 11.1% Burt's biochar.

Table A-7 Particle size distribution and pH of Burt's biochar, PCB-contaminated soil and PCB – contaminated soil amended with freshly tumbled Burt's biochar

		Particle Size Distribution: Percent Remaining							
		Sieve Opening							
Sample	pH	4.7 mm	2 mm	1 mm	0.5 mm	0.25 mm	0.15 mm	0.075 mm	<0.075 mm
Burt's Biochar	9.30	9.71	20.27	16.27	12.77	8.79	6.77	5.76	20.69
PCB-Contaminated Soil	7.72	20.52	23.63	13.02	8.18	6.55	8.21	10.45	9.52
2.8% Burt's Biochar	8.27	28.07	14.76	7.62	6.07	6.25	9.83	14.96	12.61
11.1% Burt's Biochar	8.26	21.46	22.21	11.51	8.42	7.37	9.97	12.12	7.18

APPENDIX B: RAW DATA AND QA/QC FOR CHAPTER 4

Table B-1 PCB Concentrations (µg/g) in Plant Shoot Tissue (Field Data)

Shoot							
				PCB Concentration (µg/g)			
Study	Data File (ASU)	Rep	Carbon Amendment	Base	Mid	Tip	Average
Field	120214	A	Control	21.1	6.94	2.73	10.2
Field	120214	B	Control	20.5	2.60	6.22	9.78
Field	120214	C	Control	20.1	2.30	2.10	8.13
Field	120214	A	2.8% GAC	17.9	4.76	3.21	8.63
Field	120214	B	2.8% GAC	25.4	5.52	1.45	10.8
Field	120214	C	2.8% GAC	30.7	5.86	5.92	14.2
Field	120214	A	2.8% BlueLeaf	22.9	2.68	1.65	9.08
Field	120214	B	2.8% BlueLeaf	21.9	3.73	1.26	8.95
Field	120214	C	2.8% BlueLeaf	24.0	4.96	4.19	11.1
Field	120214	A	2.8% Burt's	18.6	7.74	1.72	9.35
Field	120214	B	2.8% Burt's	35.4	6.13	4.50	15.4
Field	120214	C	2.8% Burt's	23.8	3.33	1.26	9.47

Table B-2 PCB Concentrations (µg/g) in Plant Root Tissue (Field Data)

Root				
Study	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Field	111031	A	Control	427
Field	111031	B	Control	430
Field	111031	C	Control	247
Field	111027	A	2.8% GAC	59.8
Field	111027	B	2.8% GAC	81.1
Field	111027	C	2.8% GAC	151
Field	111027	A	2.8% BlueLeaf	177
Field	111027	B	2.8% BlueLeaf	105
Field	111027	C	2.8% BlueLeaf	110
Field	111031	A	2.8% Burt's	116
Field	111031	B	2.8% Burt's	105
Field	111031	C	2.8% Burt's	93.3

Table B-3 PCB Concentrations (µg/g) in Plant Shoot Tissue (Greenhouse Data)

Shoot				
Study	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Greenhouse	120214	A	Control	12.3
Greenhouse	120214	B	Control	14.8
Greenhouse	120214	C	Control	14.9
Greenhouse	120214	A	2.8% GAC	11.8
Greenhouse	120214	B	2.8% GAC	6.97
Greenhouse	120214	C	2.8% GAC	6.55
Greenhouse	120214	A	2.8% BlueLeaf	6.72
Greenhouse	120214	B	2.8% BlueLeaf	6.10
Greenhouse	120214	C	2.8% BlueLeaf	5.89
Greenhouse	120214	A	2.8% Burt's	13.0
Greenhouse	120214	B	2.8% Burt's	13.9
Greenhouse	120214	C	2.8% Burt's	8.59

Table B-4 PCB Concentrations (µg/g) in Plant Root Tissue (Greenhouse Data)

Root				
Study	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Greenhouse	120223	A	Control	1010
Greenhouse	120223	B	Control	587
Greenhouse	120223	C	Control	622
Greenhouse	120223	A	2.8% GAC	314
Greenhouse	120223	B	2.8% GAC	171
Greenhouse	120223	C	2.8% GAC	231
Greenhouse	120229	A	2.8% BlueLeaf	732
Greenhouse	120229	B	2.8% BlueLeaf	331
Greenhouse	120229	C	2.8% BlueLeaf	376
Greenhouse	120229	A	2.8% Burt's	520
Greenhouse	120229	B	2.8% Burt's	348
Greenhouse	120229	C	2.8% Burt's	481

Table B-5 PCB Concentrations (µg/g) in Plant Shoot Tissue (Mixing Experiment)

Shoot				
Mixing Method	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Mechanical	120327	A	Control	30.8
Mechanical	120328	B	Control	15.5
Mechanical	120329	C	Control	16.9
Mechanical	120330	A	2.8% GAC	6.89
Mechanical	120331	B	2.8% GAC	5.63
Mechanical	120332	C	2.8% GAC	8.96
Mechanical	120333	A	2.8% BlueLeaf	12.8
Mechanical	120334	B	2.8% BlueLeaf	10.7
Mechanical	120335	C	2.8% BlueLeaf	7.21
Mechanical	120329	A	2.8% Burt's	19.7
Mechanical	120329	B	2.8% Burt's	9.58
Mechanical	120329	C	2.8% Burt's	9.08
Manual	120412	A	Control	12.7
Manual	120412	B	Control	20.5
Manual	120412	C	Control	18.7
Manual	120412	A	2.8% GAC	36.1
Manual	120412	B	2.8% GAC	24.8
Manual	120412	C	2.8% GAC	16.3
Manual	120412	A	2.8% BlueLeaf	11.1
Manual	120412	B	2.8% BlueLeaf	15.9
Manual	120412	C	2.8% BlueLeaf	21.4
Manual	120412	A	2.8% Burt's	9.07
Manual	120412	B	2.8% Burt's	9.20
Manual	120412	C	2.8% Burt's	7.02

Table B-6 PCB Concentrations (µg/g) in Plant Root Tissue (Mixing Experiment)

Root				
Method	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Mechanical	120403	A	Control	930
Mechanical	120403	B	Control	1060
Mechanical	120403	C	Control	1270
Mechanical	120403	A	2.8% GAC	215
Mechanical	120403	B	2.8% GAC	274
Mechanical	120403	C	2.8% GAC	354
Mechanical	120403	A	2.8% BlueLeaf	344
Mechanical	120403	B	2.8% BlueLeaf	334
Mechanical	120403	C	2.8% BlueLeaf	401
Mechanical	120405	A	2.8% Burt's	423
Mechanical	120405	B	2.8% Burt's	688
Mechanical	120405	C	2.8% Burt's	439
Manual	120417	A	Control	627
Manual	120417	B	Control	1280
Manual	120417	C	Control	1290
Manual	120417	A	2.8% GAC	453
Manual	120417	B	2.8% GAC	725
Manual	120417	C	2.8% GAC	632
Manual	120417	A	2.8% BlueLeaf	812
Manual	120417	B	2.8% BlueLeaf	785
Manual	120417	C	2.8% BlueLeaf	618
Manual	120424	A	2.8% Burt's	513
Manual	120424	B	2.8% Burt's	634
Manual	120424	C	2.8% Burt's	670

Table B-7 PCB Concentrations in Worm Tissue (Mixing Experiment)

			Worm	
Mixing Method	Data File (ASU)	Rep	Carbon Amendment	PCB Concentration (µg/g)
Mechanical	14086	A	Control	24560
Mechanical	14086	B	Control	1470
Mechanical	14086	C	Control	1640
Mechanical	14086	A	2.8% GAC	844
Mechanical	14086	B	2.8% GAC	1070
Mechanical	14086	C	2.8% GAC	621
Mechanical	14086	A	2.8% BlueLeaf	1080
Mechanical	14086	B	2.8% BlueLeaf	1500
Mechanical	14086	C	2.8% BlueLeaf	1160
Mechanical	14086	A	2.8% Burt's	1650
Mechanical	14086	B	2.8% Burt's	1240
Mechanical	14086	C	2.8% Burt's	1210
Manual	14086	A	Control	2140
Manual	14086	B	Control	1340
Manual	14086	C	Control	1600
Manual	120718	A	2.8% GAC	303
Manual	120718	B	2.8% GAC	393
Manual	120718	C	2.8% GAC	616
Manual	120718	A	2.8% BlueLeaf	1003
Manual	120718	B	2.8% BlueLeaf	860
Manual	120718	C	2.8% BlueLeaf	1070
Manual	120718	A	2.8% Burt's	1220
Manual	120718	B	2.8% Burt's	932
Manual	120718	C	2.8% Burt's	722

Table B-8 PCB Concentrations (µg/g) in Soils

Soils		
Data File	Sample Name	PCB Concentration (µg/g)
13840	GH-2011-Pre	75.9
13840	GH-2011-Post	75.7
13840	Field-2011-Pre	84.7
13840	Field-2011-Post	61.9
13840	HC Schneider Soil	58.8

Table B-9 Chapter 4 QA/QC: Field, Greenhouse and Mixing Experiments

Data File	[PCB] Blank (ug/g)	RSD	Average Efficiency (%)	Matrix	Study
111024	< 0.1	0.26	99.1	shoot	Field
111014	< 0.1	9.69	94.2	shoot	Field
111011	< 0.1	2.61	101	shoot	Field
111020	< 0.1	5.39	92.4	shoot	Field
111031	< 0.1	2.46	93.1	root	Field
111027	< 0.1	3.98	92.1	root	Field
120214	< 0.1	11.2	80.0	shoot	Greenhouse
120229	< 0.1	7.82	95.0	root	Greenhouse
120223	< 0.1	18.3	88.5	root	Greenhouse
120327	< 0.1	3.39	93.3	shoot	Mixing
120329	< 0.1	10.1	79.8	shoot	Mixing
120403	< 0.1	2.91	91.7	root	Mixing
120405	< 0.1	8.96	95.3	root	Mixing
120412	< 0.1	5.11	81.0	shoot	Mixing
120413	< 0.1	2.77	79.7	shoot	Mixing
120417	< 0.1	2.3	97.2	root	Mixing
120424	< 0.1	4.77	95.5	root	Mixing
14086	< 0.1	14.8	99.3	worm	Mixing
14086	< 0.1	4.97	96.7	worm	Mixing
120717	< 0.1	3.60	106	worm	Mixing
120718	< 0.1	2.73	100	worm	Mixing
13840	< 0.1	12.4	122	soil	-
13840	< 0.1	8.83	148	soil	-

Table B-10 Plant Harvest Data (Field)

Plants					
	Shoot Weight (g)		Shoot Length (cm)	Root Length (cm)	Field Mass (g)
	Greenhouse	Field	Field	Field	Field
Control	19	981	179	67	50
Control	30.9	927	278	48	45
Control	15.7	1130	254	62	42
2.8% GAC	29.1	575	166	45	32
2.8% GAC	35.78	2140	365	62	103
2.8% GAC	16.8	2260	454	78	61
2.8% Burt's Biochar	38.3	1960	235	68	68
2.8% Burt's Biochar	49.9	497	124	59	25
2.8% Burt's Biochar	40.7	546	130	41	27
2.8% BlueLeaf Biochar	41.1	1760	293	90	73
2.8% BlueLeaf Biochar	43.7	2090	353	79	85
2.8% BlueLeaf Biochar	46.1	1970	395	75	70

Table B-11 Invertebrate Data

Invertebrates								
	Mechanically Mixed				Manually Mixed			
	Pre Exp		Post Exp		Pre Exp		Post Exp	
	n	weights (g)	n	weight (g)	n	weights (g)	n	weight (g)
Control	25	4.08	21	1.65	25	5.48	20	1.74
Control	25	2.82	21	0.99	25	6.18	10	1.13
Control	25	3.87	22	1.42	25	4.98	19	1.48
2.8% GAC	25	4.73	23	2.12	25	3.16	23	1.32
2.8% GAC	25	4.18	23	1.84	25	4.53	19	1.41
2.8% GAC	25	4.29	21	1.63	25	3.57	25	1.83
2.8% Burt's Biochar	25	5.61	25	2.6	25	4.66	20	1.05
2.8% Burt's Biochar	25	4.89	23	2.08	25	3.52	14	1.00
2.8% Burt's Biochar	25	4.59	16	1.05	25	5.02	21	1.99
2.8% BlueLeaf Biochar	25	5.14	19	1.94	25	3.88	20	1.73
2.8% BlueLeaf Biochar	25	4.83	20	1.93	25	4.38	21	1.28
2.8% BlueLeaf Biochar	25	3.84	15	1.05	25	4.89	19	1.57

Table B-11 Plant Harvest Data (Mixing Experiment)

	Plants							
	Mechanically Mixed				Manually Mixed			
	Shoot		Root		Shoot		Root	
	length (cm)	weight (g)	length (cm)	weight (g)	length (cm)	weight (g)	length (cm)	weight (g)
Control	10	1.83	9	0.11	18.5	4.65	12	0.76
Control	16	2.71	6.5	0.52	22.5	5.85	19	1.68
Control	20	4.99	18	1.97	17	4.25	20	1.74
2.8% GAC	23	2.71	13	0.75	10	2.86	11	0.87
2.8% GAC	19	5.00	24	1.63	19	3.47	19	1.05
2.8% GAC	24	5.86	25	1.79	24	2.94	21	1.02
2.8% Burt's Biochar	18	3.76	13	0.65	19	4.38	11	0.49
2.8% Burt's Biochar	17	4.22	13	0.75	26	6.74	16	0.95
2.8% Burt's Biochar	18	4.23	10.5	0.4	26.5	5.88	17	0.96
2.8% BlueLeaf Biochar	13	4.16	18	1.13	31	5.96	13.5	1.1
2.8% BlueLeaf Biochar	20	5.64	16	1.02	26	8.01	17	1.05
2.8% BlueLeaf Biochar	24	5.26	10	1.12	12	3.63	27	0.8

Table B-12 Particle Size Distribution

	Particle Size Distribution						
	Treatment	**Sieve Number**	**Sieve Opening**	**Mass of Empty Sieve**	**Mass of Sieve + Soil**	**Sample Mass in Sieve**	**% Retained**
Activated Carbon	GAC	4	4.7	536	536	0.11	0.22
		10	2	472	472	0.01	0.02
		18	1	412	437	24.8	49.5
		35	0.5	399	422	22.8	45.5
		60	0.25	365	367	2.02	4.04
		100	0.15	362	362	0.1	0.2
		200	0.075	354	355	0.04	0.08
		Pan	<0.075	343	343	0.19	0.38
Biochar	Old Burt's Biochar	4	4.7	536	540	4.37	8.67
		10	2	472	480	8.24	16.4
		18	1	413	420	7.64	15.2
		35	0.5	399	406	7.43	14.8
		60	0.25	365	370	5.16	10.2
		100	0.15	361	365	3.75	7.44
		200	0.075	354	358	3.13	6.21
		Pan	<0.075	343	354	10.7	21.2
Biochar	New Burt's Biochar	4	4.7	536	580	44.2	90.8
		10	2	464	466	2.64	5.42
		18	1	411	412	1.5	3.08
		35	0.5	399	399	0.08	0.16
		60	0.25	365	365	-0.09	-0.18
		100	0.15	362	362	0.13	0.27
		200	0.075	355	355	0.08	0.16
		Pan	<0.075	343	343	0.16	0.33
Biochar	BlueLeaf Biochar	4	4.7	536	563	26.4	50.5
		10	2	472	481	8.92	17
		18	1	413	416	2.88	5.5
		35	0.5	399	400	1.32	2.52
		60	0.25	365	366	0.71	1.36
		100	0.15	361	362	0.67	1.28
		200	0.075	354	355	0.5	0.96
		Pan	<0.075	343	354	10.9	20.8
Mechanically Mixed	Control	4	4.7	536	543	6.55	12.9
		10	2	472	486	13.9	27.4

		18	1	412	420	7.88	15.5
		35	0.5	399	403	3.66	7.2
		60	0.25	365	369	3.85	7.57
		100	0.15	361	366	4.9	9.64
		200	0.075	354	361	6.04	11.9
		Pan	<0.075	343	347	4.03	7.93
Mechanically Mixed	2.8% GAC	4	4.7	536	542	6.41	12.7
		10	2	472	484	12.3	24.4
		18	1	412	419	6.94	13.7
		35	0.5	399	403	4.07	8.06
		60	0.25	365	369	3.46	6.85
		100	0.15	362	366	4.92	9.74
		200	0.075	355	361	6.47	12.8
		Pan	<0.075	343	349	5.93	11.7
Mechanically Mixed	2.8% Burt's Biochar	4	4.7	537	542	5.29	10.5
		10	2	464	468	11.6	23.0
		18	1	412	422	9.57	18.9
		35	0.5	399	403	4.23	8.37
		60	0.25	365	369	4.12	8.16
		100	0.15	362	367	5.33	10.6
		200	0.075	355	361	6.24	12.4
		Pan	<0.075	343	347	4.13	8.18
Mechanically Mixed	2.8% BlueLeaf Biochar	4	4.7	536	545	8.57	16.8
		10	2	464	474	10.9	21.3
		18	1	412	419	7.01	13.9
		35	0.5	399	403	3.94	7.73
		60	0.25	365	369	3.89	7.64
		100	0.15	362	366	4.5	8.83
		200	0.075	355	361	5.94	11.7
		Pan	<0.075	342	348	6.24	12.3
Manually Mixed	Control	4	4.7	536	549	12.9	26.0
		10	2	472	481	9.06	18.2
		18	1	412	419	7.07	14.2
		35	0.5	399	403	4.09	8.21
		60	0.25	365	369	3.76	7.54
		100	0.15	361	365	3.83	7.68
		200	0.075	354	359	4.29	8.61
		Pan	<0.075	343	348	4.8	9.63
Manually	2.8% GAC	4	4.7	536	541	5.12	10.8

Mixed							
		10	2	472	480	7.36	15.5
		18	1	412	422	9.77	20.6
		35	0.5	399	406	7.22	15.2
		60	0.25	365	372	6.62	13.9
		100	0.15	361	366	4.62	9.73
		200	0.075	354	358	3.92	8.26
		Pan	<0.075	343	346	2.83	5.96
Manually Mixed	2.8% Burt's Biochar	4	4.7	536	542	5.29	10.6
		10	2	472	481	9.11	18.3
		18	1	412	421	8.76	17.6
		35	0.5	399	406	6.73	13.5
		60	0.25	365	372	6.72	13.5
		100	0.15	361	367	5.35	10.7
		200	0.075	354	359	4.56	9.13
		Pan	<0.075	343	347	3.4	6.81
Manually Mixed	2.8% BlueLeaf Biochar	4	4.7	536	555	18.6	37.0
		10	2	472	482	10.5	20.8
		18	1	412	418	5.86	11.7
		35	0.5	399	402	3.08	6.13
		60	0.25	365	368	2.69	5.35
		100	0.15	362	365	3.02	6.01
		200	0.075	354	357	2.90	5.77
		Pan	<0.075	343	347	3.68	7.32

APPENDIX C: RAW DATA AND QA/QC FOR CHAPTER 5

Table C-1 Physical Properties of Burt's Biochars

Sample	pH	Organic Matter %	Cation Exchange (CEC) cmol/kg	Moisture %
ASU 14347 Burts Old	9.3	63.2	34.8	20
ASU 14347 Burts New	9.0	97.8	16.0*	4.3
ASU 14538 Burts high temp	8.7	96.7	15.9	<0.1
ASU 14538 Burts low temp	8.4	97.9*	11.1	1.0
ASU 14565 Standard Fuel Char	9.6	96.2	13.2*	0.3
ASU 14573 High 2	9.1	97.1	17.1	0.2
ASU 14565 Ash	-	-	22.0	-
ASU 14455 Feedstock summer 2012 wood	-	99.8	-	-
ASU 14455 Feedstock October 2012 wood	-	99.9*	-	-
ASU 14564 Coarse wood	-	99.6	-	-
ASU 14564 Fine wood	-	99.4*	-	-
Laboaratory QA/QC				
ASU 14347 Burts New	-	-	19.9	
ASU 14347 Burts New	-	-	12.0	
ASU 14538 Burts low temp	-	97.9	-	
ASU 14538 Burts low temp	-	97.8	-	
ASU 14565 Standard Fuel Char	-	-	5.6	
ASU 14565 Standard Fuel Char	-	-	20.8	
ASU 14455 Feedstock October 2012 wood	-	99.9	-	
ASU 14455 Feedstock October 2012 wood	-	99.9	-	
ASU 14564 Fine wood	-	99.2	-	
ASU 14564 Fine wood	-	99.6	-	

* Average result of duplicates

Table C-2 PCB and Mercury Analysis of Burt's Biochars

Sample	PCB µg/g	Mercury ng/g
ASU 14347 Burts Old	1.2*	<5.0
ASU 14347 Burts New	<0.1	<5.0
ASU 14538 Burts high temp	<0.1	24.2*
ASU 14538 Burts low temp	<0.1	112*
ASU 14565 Standard Fuel Char	<0.1*	17.1*
ASU 14573 High 2	<0.1*	294*
Laboaratory QA/QC		
Blank	<0.1 ; <0.1 ; <0.1 ; <0.1	<5.0 ; <5.0 ; <5.0
Control	4.7 ; 4.7 ; 4.0 ; 4.6	91.2 ; 87.9 ; 92.1
Control Target	5.0	91
ASU 14347 Burts Old	0.2	-
ASU 14347 Burts Old	2.1	-
ASU 14538 Burts high temp	-	34.3
ASU 14538 Burts high temp	-	9.1
ASU 14538 Burts high temp	-	25.7
ASU 14538 Burts high temp	-	27.8
ASU 14538 Burts low temp	-	9.2
ASU 14538 Burts low temp	-	22.8
ASU 14538 Burts low temp	-	304
ASU 14565 Standard Fuel Char	<0.1	18.0
ASU 14565 Standard Fuel Char	<0.1	16.1
ASU 14573 High 2	<0.1	76.6
ASU 14573 High 2	<0.1	16.4
ASU 14573 High 2	-	18.2
ASU 14573 High 2	-	754
ASU 14573 High 2	-	607
* Average result of replicates		

Table C-3 ICP 30 Element Burt's Biochar

Sample	ASU 14347 Old Burt	ASU 14347 New Burt	ASU 14538 Low Temp.	ASU 14538 High Temp. *	ASU 14565 Standard Fuel char	ASU 14573 High 2*
Ag	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Al**	5450	<100	50.5	35.1	526	49.1
As	167	<1.0	<1.0	<1.0	<1.0	<1.0
B**	489	34.6	23.1	<20	22.4	<20
Ba	216	61.8	52.9	54.9	63.1	60.3
Be	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	45000	5550	4840	4870	15600	5220
Cd	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Co	5.3	<1.0	<1.0	<1.0	2.2	<1.0
Cr	206	<2.0	<2.0	<2.0	<2.0	<2.0
Cu	558	4.4	5.0	3.6	6.5	4.7
Fe	7370	61.6	106	235	902	92.1
K	6860	2150	1850	1840	2850	2230
Mg	5850	848	853	871	1560	864
Mn	799	752	760	658	307	718
Mo	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Na	6460	164	298	137	878	256
Ni	10.5	<2.0	<2.0	<2.0	<2.0	<2.0
P	731	69.5	89.0	54.0	276	72.2
Pb	314	<2.0	2.7	<2.0	2.4	<2.0
S	5380	79.9	88.4	70.1	262	72.3
Sb	16.1	<2.0	<2.0	<2.0	<2.0	<2.0
Se	<10	<10	<10	<10	<10	<10
Sn**	<10	<10	2.9	6.9	33.9	3.0
Sr	175	23.3	20.6	25.9	42.1	24.1
Ti	847	<10	<10	<10	945	<10
Tl	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10	<10
V	12.4	<10	<10	<10	<10	<10
Zn	498	5.6	18.5	9.6	56.2	17.9

** Detection limits raised due to interferences

* Average result of duplicate analysis

Laboratory QA/QC

Sample	Blank	Blank	Control 1	Control 1 Target Range	Control 2	Control 2	Control 2 Target Range
Ag	<2.0	<2.0	<2.0	-	<2.0	<2.0	-
Al**	<100	<100	945	136-1150	163	198	75.4-214
As	<1.0	<1.0	1.4	0.45-2.2	<1.0	<1.0	-
B**	<25	<25	70.2	14.9-72.0	37.7	39.2	18.6-69.4
Ba	<5.0	<5.0	13.7	-	<5.0	6.2	-
Be	<4.0	<4.0	<4.0	-	<4.0	<4.0	-
Ca	<50	<50	15900	8800-22200	13000	10800	10810-16890
Cd	<1.0	<1.0	<1.0	0.46-0.91	2.0	2.2	2.0-2.9
Co	<1.0	<1.0	<1.0	0.22-0.69	<1.0	<1.0	0.12-0.69
Cr	<2.0	<2.0	<2.0	0.73-1.9	<2.0	<2.0	-
Cu	<2.0	<2.0	7.9	3.6-11.3	11.6	13.5	9.9-15.8
Fe	<20	<20	1060	641-1170	227	259	170-276
K	<20	<20	8860	6760-10600	22700	18800	17660-30620
Mg	<20	<20	4590	3500-5060	7280	7940	6760-9040
Mn	<1.0	<1.0	65.1	47.0-73.9	67.9	74.9	60.8-80.3
Mo	<2.0	<2.0	<2.0	-	<2.0	<2.0	0.0-0.7
Na	<75	<75	18100	12800-23000	15000	16500	12250-18900
Ni	<2.0	<2.0	<2.0	1.9-2.2	<2.0	2.0	0.30-0.38
P	<20	<20	998	744-1100	4290	3700	3600-5590
Pb	<2.0	<2.0	44.9	27.0-63.0	<2.0	<2.0	<2.0
S	<25	<25	6480	4920-7170	2670	2130	1670-5230
Sb	<2.0	<2.0	<2.0	-	<2.0	<2.0	-
Se	<10	<10	<10	-	<10	<10	-
Sn**	<10	<10	<10	-	6.9	3.1	-
Sr	<5.0	<5.0	236	150-	48.5	52.5	43.9-

				281			55.9
Ti	<10	<10	34.6	-	10.3	10.7	-
Tl	<1.0	<1.0	<1.0	-	<1.0	<1.0	-
U	<10	<10	<10	-	<10	<10	-
V	<10	<10	<10	-	<10	<10	-
Zn	<5.0	<5.0	53.1	41.9-65.8	71.2	75.5	56.2-90.2

Table C-4 ICP 30 Element Burt's Biochar Feedstocks

Sample	ASU 14455 Feedstock Oct 2012	ASU 14455 Feedstock Summer 2012*	ASU 14564 Coarse Wood*	ASU 14564 Fine Wood
Ag	<2.0	<2.0	<2.0	<2.0
Al**	27.9	<20	60.2	100
As	<1.0	<1.0	<1.0	<1.0
B**	<20	<20	<20	<20
Ba	24.5	9.7	17.2	22.6
Be	<4.0	<4.0	<4.0	<4.0
Ca	1290	914	3240	1760
Cd	<1.0	<1.0	<1.0	<1.0
Co	<1.0	<1.0	<1.0	<1.0
Cr	<2.0	<2.0	<2.0	2.6
Cu	<2.0	<2.0	<2.0	5.9
Fe	45.1	<20	106	350
K	674	417	667	678
Mg	204	110	376	344
Mn	45.1	86.8	92.1	71.3
Mo	<2.0	<2.0	<2.0	<2.0
Na	770	<75	183	341
Ni	4.4	<2.0	2.2	3.4
P	48.3	27.6	105	79.8
Pb	<2.0	<2.0	<2.0	8.1
S	106	30.9	94.6	111
Sb	<2.0	<2.0	<2.0	<2.0
Se	<10	<10	<10	<10
Sn**	5.3	<10	4.2	2.4
Sr	9.9	<5.0	12.1	8.3
Ti	<10	<10	<10	<10
Tl	<1.0	<1.0	<1.0	<1.0

U	<10	<10	<10	<10
V	<10	<10	<10	<10
Zn	17.8	7.8	16.7	30.5

** Detection limits raised due to interferences

* Average result of duplicates

Sample	**Blank**	**Blank**	**Control 1*** **	**Control 1*** **
Ag	<2.0	<2.0	<2.0	<2.0
Al**	<100	<20	198	163
As	<1.0	<1.0	<1.0	<1.0
B**	<25	<20	39.2	37.7
Ba	<5.0	<5.0	6.2	<5.0
Be	<4.0	<4.0	<4.0	<4.0
Ca	<50	<50	10800	13000
Cd	<1.0	<1.0	2.2	2.0
Co	<1.0	<1.0	<1.0	<1.0
Cr	<2.0	<2.0	<2.0	<2.0
Cu	<2.0	<2.0	13.5	11.6
Fe	<20	<20	259	227
K	<20	<20	18800	22700
Mg	<20	<20	7940	7280
Mn	<1.0	<1.0	74.9	67.9
Mo	<2.0	<2.0	<2.0	<2.0
Na	<75	<75	16500	15000
Ni	<2.0	<2.0	2.0	<2.0
P	<20	<20	3700	4290
Pb	<2.0	<2.0	<2.0	<2.0
S	<25	<25	2130	2670
Sb	<2.0	<2.0	<2.0	<2.0
Se	<10	<10	<10	<10
Sn**	<10	<10	3.1	6.9
Sr	<5.0	<5.0	52.5	48.5
Ti	<10	<10	10.7	10.3
Tl	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10
V	<10	<10	<10	<10
Zn	<5.0	<5.0	75.5	71.2

*** Refer to table C-3 for Control 1 limits

Table C-5 Available Nitrogen and Phosphorus

Sample	Ammonia (N) µg/g	Nitrate (N) µg/g	Nitrite (N) µg/g	Formic Acid Extraction Phosphorus µg/g	Olsen Extraction Phosphorus µg/g
ASU 14347 Burts Old	<2.0	2.3	<2.0	850	190
ASU 14347 Burts New	<2.0	3.0	<2.0	31	<5*
ASU 14538 Burts high temp	<2.0	6.1	<2.0	9.0	-
ASU 14538 Burts low temp	<2.0	<2.0	<2.0	28	-
ASU 14565 Standard Fuel Char	<2.0*	<2.0	<2.0	80	-
ASU 14573 High 2	<2.0	<2.0	<2.0	28*	-
Laboaratory QA/QC					
Blank	<2.0	<2.0	<2.0	<5	<5
Control	9.5	4.5	3.4	65	70
Control Target	10.0	5.0	5.0	95	93
ASU 14347 Burts New	-	-	-	31	<5
ASU 14347 Burts New	-	-	-	31	<5
ASU 14565 Standard Fuel Char	<2.0	<2.0	<2.0	-	-
ASU 14565 Standard Fuel Char	<2.0	<2.0	<2.0	-	-
ASU 14573 High 2	-	-	-	33	-
ASU 14573 High 2	-	-	-	23	-
* Average result of replicates					

Table C-6 Burt's Biochar PAH Analysis

PAH** µg/g dry weight	ASU 14538 Low Temp.	ASU 14538 High temp.	ASU 14505 Standard Fuel	Blank	Control	Control Target
Acenaphthene	<0.06	<0.06	<0.06	<0.06	0.098	0.167
Acenaphthylene	<0.06	<0.06	<0.06	<0.06	0.091	0.167
Anthracene	<0.06	<0.06	<0.06	<0.06	0.140	0.167
Benzo[a]anthracene	<0.06	<0.06	<0.06	<0.06	0.185	0.167
Benzo[a]pyrene	<0.06	<0.06	<0.06	<0.06	0.140	0.167
Benzo[b]fluoranthene	<0.06	<0.06	<0.06	<0.06	0.214	0.167
Benzo[g,h,i]perylene	<0.06	<0.06	<0.06	<0.06	0.131	0.167
Benzo[k]fluoranthene	<0.06	<0.06	<0.06	<0.06	0.181	0.167
1,1-Biphenyl	<0.06	<0.06	<0.06	<0.06	-	-
Chrysene	<0.06	<0.06	<0.06	<0.06	0.195	0.167
Dibenzo[a,h]anthracene	<0.06	<0.06	<0.06	<0.06	0.134	0.167
Fluoranthene	<0.06	<0.06	<0.06	<0.06	0.145	0.167
Fluorene	<0.06	<0.06	<0.06	<0.06	0.111	0.167
Indeno[1,2,3-cd]pyrene	<0.06	<0.06	<0.06	<0.06	0.130	0.167
1-Methylnaphthalene	<0.06	<0.06	0.07	<0.06	0.085	0.167
2-Methylnaphthalene	<0.06	<0.06	<0.06	<0.06	0.091	0.167
Methylnaphthalene (1&2)	<0.12	<0.12	0.13	<0.12	-	-
Naphthalene	<0.03	<0.03	0.03	<0.03	0.090	0.167
Phenanthrene	<0.06	<0.06	<0.06	<0.06	0.148	0.167
Pyrene	<0.06	<0.06	<0.06	<0.06	0.151	0.167

** Analysis subcontracted

Table C-7 Proximate and Ultimate Analysis

Analyte**	Unit	ASU 14538 High Results as received	ASU 14538 High Results Dry Wt. Corrected	ASU 14538 Low Results as received	ASU 14538 Low Results Dry Wt. Corrected
Moisture	wt %	4.98	-	6.98	-
Ash	wt %	1.54	1.62	1.36	1.46
Carbon	wt %	92.35	97.19	90.3	97.0
Hydrogen	wt %	1.99	1.51	2.08	1.40
Nitrogen	wt %	0.35	0.37	0.28	0.30
Oxygen by Difference	wt %	3.76	<0.10	6.00	<0.10
Fixed Carbon	wt %	79.92	84.11	77.8	83.6
Sulphur	wt %	0.02	0.02	0.02	0.02
Volatile Matter	wt %	13.56	14.27	13.89	14.94
Low Heat Value	BTU/lb	13180	13870	12990	13970
High Heat Value	BTU/lb	13370	14070	13190	14180

Analyte**	Unit	ASU 14565 Standard Fuel Results as received	ASU 14565 Standard Fuel Results Dry Wt. Corrected	ASU 14573 High 2 Results as received	ASU 14573 High 2 Results Dry Wt. Corrected
Moisture	wt %	5.36	-	4.42	-
Ash	wt %	2.14	2.26	1.32	1.38
Carbon	wt %	87.5	92.4	93.6	97.9
Hydrogen	wt %	2.45	1.96	1.70	1.26
Nitrogen	wt %	1.00	1.06	0.34	0.35
Oxygen by Difference	wt %	6.89	2.25	3.03	<0.10
Fixed Carbon	wt %	74.4	78.7	82.3	86.2
Sulphur	wt %	0.02	0.02	0.02	0.02
Volatile Matter	wt %	18.1	19.1	11.9	12.5
Low Heat Value	BTU/lb	12600	13310	13500	14130
High Heat Value	BTU/lb	12830	13550	13660	14290

Table C-8 Particle Size Distribution

	Percent Retained							
Sieve Size (mm)	4.7	2	1	0.5	0.25	0.15	0.0075	
Sieve Number	4	10	18	35	60	100	200	pan
GAC A	0.22	0.02	49.54	45.52	4.04	0.20	0.08	0.38
GAC B	-0.07	0.18	55.16	41.22	2.72	0.10	0.04	0.66
GAC C	0.00	0.25	53.63	41.34	2.99	0.49	0.45	0.85
Old Burts A	8.67	16.36	15.16	14.75	10.24	7.44	6.21	21.16
Old Burts B	8.69	15.14	10.95	10.74	14.48	21.19	4.20	14.61
Old Burts C	10.52	13.91	13.77	16.39	8.92	6.42	5.41	24.66
New Burts A	90.76	5.42	3.08	0.16	-0.18	0.27	0.16	0.33
New Burts B	72.04	23.40	2.97	1.26	0.40	-0.79	-1.26	1.98
New Burts C	80.23	13.21	1.11	2.33	0.37	0.37	0.48	1.90
BlueLeaf A	50.52	17.04	5.50	2.52	1.36	1.28	0.96	20.83
BlueLeaf B	71.19	21.39	4.20	0.83	0.19	-0.19	0.23	2.16
BlueLeaf C	39.59	28.26	12.36	5.29	2.85	2.22	1.33	8.10
Low Burts A	65.15	28.63	2.66	0.57	0.54	0.51	0.76	1.19
Low Burts B	74.96	20.54	2.07	0.37	0.48	0.54	0.23	0.82
Low Burts C	60.83	28.71	5.97	1.10	0.64	0.84	0.61	1.30
High Burts A	94.00	3.77	0.45	0.28	0.28	0.39	0.28	0.53
High Burts B	96.28	2.11	0.26	0.24	0.26	0.37	0.21	0.26
High Burts C	80.82	13.71	1.35	0.89	0.79	0.63	0.56	1.25
Standard Fuel A	54.30	33.67	8.76	1.19	0.26	0.26	0.37	1.19
Standard Fuel B	51.06	38.52	7.03	0.91	0.33	0.38	0.36	1.41
Standard Fuel C	73.10	21.49	2.27	0.38	0.45	0.38	0.34	1.59

Table C-9 Worm Avoidance Data

Treatment	Number of Worms in Compartment					
Wheel	Unamended	2.8%	Unamended	2.8%	Unamended	2.8%
Burt's Biochar (New)						
A	3	4	2	2	1	1
B	2	2	3	4	1	3
C	0	2	3	1	1	4
Activated Carbon						
D	3	2	5	1	4	1
E	2	4	1	1	2	0
F	5	2	0	2	3	0
BlueLeaf Biochar						
D	2	4	0	2	2	0
E	1	2	3	1	0	3
F	3	1	0	0	4	2
Burt's Biochr (Old)						
A	2	0	2	1	2	2
B	1	0	8	1	0	0
C	6	3	1	0	0	0

Table C-10 Germination Data

	Pumpkin		after 7 days	Alfalfa		after 7 days
	germinated	planted	length (cm)	germinated	planted	Length (cm)
Potting soil	3	5	15	43	50	62
Potting soil	3	5	14	42	50	55
Potting soil	4	5	14	37	50	47
Filter Paper	1	5	3	44	50	39
Filter Paper	2	5	0	37	50	33
Filter Paper	1	5	11	36	50	35
High	0	5	5	10	50	48
High	3	5	6	26	50	61
High	2	5	6	23	50	41
Crushed New	4	5	17	36	50	36
Crushed New	3	5	12	38	50	38
Crushed New	2	5	15	41	50	41
BlueLeaf	2	5	21	25	50	61
BlueLeaf	4	5	20	27	50	73
BlueLeaf	5	5	14	31	50	34
Low	2	5	8	25	50	70
Low	5	5	26	27	50	90
Low	4	5	11	24	50	87
New	4	5	21	31	50	60
New	3	5	12	34	50	61
New	2	5	12	32	50	65
ABRI-Tech	1	5	6	30	50	15
ABRI-Tech	1	5	10	26	50	8
ABRI-Tech	3	5	24	32	50	12
GAC	4	5	29	34	50	20
GAC	5	5	26	36	50	25
GAC	4	5	20	43	50	25
Old	3	5	7	0	50	3
Old	3	5	15	2	50	7
Old	0	5	22	0	50	4
Standard Fuel	1	5	1	11	24	12
Standard Fuel	2	5	0	10	25	25
Standard Fuel	2	5	0	20	35	45
High-2	5	5	13.5	57	50	114
High-2	2	5	8.5	48	50	115
High-2	2	5	13	53	50	115

APPENDIX D: RAW DATA AND QA/QC FOR CHAPTER 6

Table D-1 Community Level Physiological Profiling Data for PCB Contaminated Site

Soil Contamination	Sample Type	Sample Name	Treatment	Sampling Date	AWCD	Richness	Eveness	Diversity
Low PCB	soil	SL1	Control	Aug-11	0.38	14.0	2.72	2.37
Low PCB	soil	SL1	Control	Aug-11	0.39	15.0	2.79	2.37
Low PCB	soil	SL1	Control	Aug-11	0.33	12.0	2.56	2.37
Low PCB	soil	SL2	Control	Aug-11	0.33	11.0	2.65	2.54
Low PCB	soil	SL2	Control	Aug-11	0.21	8.0	2.67	2.96
Low PCB	soil	SL2	Control	Aug-11	0.29	11.0	2.65	2.55
Low PCB	soil	SL3	Control	Aug-11	0.61	15.0	2.77	2.35
Low PCB	soil	SL3	Control	Aug-11	0.59	17.0	2.85	2.32
Low PCB	soil	SL3	Control	Aug-11	0.56	15.0	2.77	2.36
High PCB	soil	SHB	2.8% Burt's Biochar	Aug-11	0.67	17.0	2.84	2.31
High PCB	soil	SHB	2.8% Burt's Biochar	Aug-11	0.89	21.0	2.93	2.22
High PCB	soil	SHB	2.8% Burt's Biochar	Aug-11	0.85	19.0	2.95	2.30
High PCB	soil	SHBL	2.8% BlueLeaf Biochar	Aug-11	0.63	15.0	2.75	2.34
High PCB	soil	SHBL	2.8% BlueLeaf Biochar	Aug-11	0.64	15.0	2.69	2.28
High PCB	soil	SHBL	2.8% BlueLeaf Biochar	Aug-11	0.59	14.0	2.63	2.30
High PCB	soil	SHAC	2.8% GAC	Aug-11	0.83	21	2.95	2.23
High PCB	soil	SHAC	2.8% GAC	Aug-11	0.73	18	2.89	2.30
High PCB	soil	SHAC	2.8% GAC	Aug-11	0.71	19	2.92	2.28
High PCB	soil	SHP1	Control	Aug-11	0.89	18	2.87	2.29
High PCB	soil	SHP1	Control	Aug-11	0.83	19	2.86	2.24
High PCB	soil	SHP1	Control	Aug-11	0.79	18	2.83	2.25
High PCB	soil	SHP2	Control	Aug-11	0.61	15	2.79	2.37
High PCB	soil	SHP2	Control	Aug-11	0.57	16	2.78	2.31
High PCB	soil	SHP2	Control	Aug-11	0.61	15	2.82	2.40
High PCB	soil	SHP3	Control	Aug-11	0.71	16	2.83	2.35
High PCB	soil	SHP3	Control	Aug-11	0.65	18	2.93	2.33
High PCB	soil	SHP3	Control	Aug-11	0.63	16	2.81	2.34
High PCB	Root	RHP1	Control	Aug-11	1.10	26	3.24	2.29
High PCB	Root	RHP1	Control	Aug-11	1.04	25	3.20	2.29
High PCB	Root	RHP1	Control	Aug-11	1.05	26	3.21	2.27
High PCB	Root	RHP4	Control	Aug-11	1.15	27	3.27	2.29
High PCB	Root	RHP4	Control	Aug-11	1.14	27	3.26	2.28
High PCB	Root	RHP4	Control	Aug-11	1.14	26	3.24	2.29
High PCB	Root	RHP5	Control	Aug-11	1.17355	28	3.28	2.27

High PCB	Root	RHP5	Control	Aug-11	1.19797	28	3.28	2.27
High PCB	Root	RHP5	Control	Aug-11	1.16087	28	3.27	2.26
High PCB	Root	RHB	2.8% Burt's Biochar	Aug-11	1.15	25	3.20	2.29
High PCB	Root	RHB	2.8% Burt's Biochar	Aug-11	1.20	25	3.21	2.29
High PCB	Root	RHB	2.8% Burt's Biochar	Aug-11	1.16	25	3.19	2.28
High PCB	Root	RHBL	2.8% BlueLeaf Biochar	Aug-11	1.08	25	3.22	2.30
High PCB	Root	RHBL	2.8% BlueLeaf Biochar	Aug-11	1.10	26	3.21	2.27
High PCB	Root	RHBL	2.8% BlueLeaf Biochar	Aug-11	1.07	26	3.19	2.25
High PCB	Root	RHAC	2.8% GAC	Aug-11	1.20	26	3.23	2.28
High PCB	Root	RHAC	2.8% GAC	Aug-11	1.19	27	3.21	2.24
High PCB	Root	RHAC	2.8% GAC	Aug-11	1.18	26	3.20	2.26

Table D-2 Community Level Physiological Profiling Data for First Sample Collection at DDT-Contaminated Site

Soil Contamination	Sample Type	Sample Name	Treatment	AWCD	Richness	Eveness	Diversity
High DDT	root	RH ControlA	Control	0.83	23	2.28	3.11
High DDT	root	RH ControlB	Control	0.78	21	2.30	3.04
High DDT	root	RH ControlC	Control	0.81	25	2.27	3.17
High DDT	root	RHGACA	2.8% GAC	0.80	24	2.25	3.13
High DDT	root	RHGACB	2.8% GAC	0.84	23	2.28	3.11
High DDT	root	RHGACC	2.8% GAC	0.68	20	2.33	3.01
High DDT	root	RHBlueA	2.8% BlueLeaf Biochar	0.53	19	2.32	2.99
High DDT	root	RHBlueB	2.8% BlueLeaf Biochar	0.64	20	2.35	3.05
High DDT	root	RHBlueC	2.8% BlueLeaf Biochar	0.90	22	2.26	3.04
High DDT	root	RHBurtsA	2.8% Burt's Biochar	0.66	22	2.28	3.08
High DDT	root	RHBurtsB	2.8% Burt's Biochar	0.74	22	2.28	3.08
High DDT	root	RHBurtsC	2.8% Burt's Biochar	0.57	19	2.35	2.99
High DDT	rhizosphere	RZH Control A	Control	0.99	23	2.28	3.09
High DDT	rhizosphere	RZH Control B	Control	1.47	28	2.24	3.23
High DDT	rhizosphere	RZH Control C	Control	0.82	22	2.25	3.04
High DDT	rhizosphere	RZHGACA	2.8% GAC	1.32	27	2.21	3.18
High DDT	rhizosphere	RZHGACB	2.8% GAC	1.32	27	2.25	3.22

High DDT	rhizosphere	RZHGACC	2.8% GAC	1.07	23	2.28	3.12
High DDT	rhizosphere	RZHBlueA	2.8% BlueLeaf Biochar	1.25	25	2.25	3.15
High DDT	rhizosphere	RZHBlueB	2.8% BlueLeaf Biochar	1.25	23	2.31	3.14
High DDT	rhizosphere	RZHBlueC	2.8% BlueLeaf Biochar	1.06	22	2.31	3.08
High DDT	rhizosphere	RZH BurtsA	2.8% Burt's Biochar	1.17	25	2.28	3.17
High DDT	rhizosphere	RZH BurtsB	2.8% Burt's Biochar	1.19	25	2.27	3.17
High DDT	rhizosphere	RZH BurtC	2.8% Burt's Biochar	1.14	24	2.28	3.13
High DDT	soil	SH ControlA	Control	0.21	7	2.94	2.47
High DDT	soil	SH ControlB	Control	0.71	17	2.29	2.84
High DDT	soil	SH ControlC	Control	0.05	2		2.61
High DDT	soil	SHGACA	2.8% GAC	0.32	12	2.61	2.75
High DDT	soil	SHGACB	2.8% GAC	0.07	5	3.93	2.46
High DDT	soil	SHGACC	2.8% GAC	0.80	19	2.31	2.95
High DDT	soil	SHBlueA	2.8% BlueLeaf Biochar	0.63	17	2.29	2.84
High DDT	soil	SHBlueB	2.8% BlueLeaf Biochar	0.09	3		2.53
High DDT	soil	SHBlueC	2.8% BlueLeaf Biochar	0.45	15	2.34	2.77
High DDT	soil	SHBurtsA	2.8% Burt's Biochar	0.09	2	5.97	2.01
High DDT	soil	SHBurtsB	2.8% Burt's Biochar	0.16	9	2.85	2.66
High DDT	soil	SHBurtsC	2.8% Burt's Biochar	0.50	11	2.34	2.46
Low DDT	soil	SLControl1	Control	-0.01	0		3.67
Low DDT	soil	SLControl2	Control	0.00	0		
Low DDT	soil	SLControl3	Control	0.01	1		3.68
Low DDT	soil	SLGAC1	2.8% GAC	0.02	0		2.57
Low DDT	soil	SLGAC2	2.8% GAC	0.02	0		2.69
Low DDT	soil	SLGAC3	2.8% GAC	0.03	1		2.50
Low DDT	soil	SLBlue1	2.8% BlueLeaf Biochar	0.07	3	6.34	2.35
Low DDT	soil	SLBlue2	2.8% BlueLeaf Biochar	0.03	1		2.42
Low DDT	soil	SLBlue3	2.8% BlueLeaf Biochar	0.02	0		3.84
Low DDT	soil	SLBurts1	2.8% Burt's Biochar	0.09	3	3.80	1.63
Low DDT	soil	SLBurts2	2.8% Burt's Biochar	0.82	18	2.36	2.96
Low DDT	soil	SLBurts3	2.8% Burt's Biochar	0.90	24	2.26	3.12
Low DDT	root	RLControl1	Control	-	-	-	-
Low DDT	root	RLControl2	Control	-	-	-	-
Low DDT	root	RLControl3	Control	-	-	-	-

Low DDT	rhizosphere	RZH Control1	Control	-	-	-	-
Low DDT	rhizosphere	RZH Control2	Control	-	-	-	-
Low DDT	rhizosphere	RZH Control3	Control	-	-	-	-

Table D-3 Community Level Physiological Profiling Data for Second Sample Collection at DDT-Contaminated Site

Soil Contamination	Sample Type	Sample Name	Treatment	AWCD	Richness	Eveness	Diversity
High DDT	root	RHControlA	Control	0.25	7.3	2.70	2.33
High DDT	root	RHControlB	Control	0.70	21.7	2.33	3.11
High DDT	root	RHControlC	Control	0.78	25.0	2.26	3.16
High DDT	root	RHGACA	2.8% GAC	0.79	21.7	2.33	3.10
High DDT	root	RHGACB	2.8% GAC	0.96	24.0	2.26	3.12
High DDT	root	RHGACC	2.8% GAC				
High DDT	root	RHBlueA	2.8% BlueLeaf Biochar	0.68	21.0	2.33	3.07
High DDT	root	RHBlueB	2.8% BlueLeaf Biochar	0.58	19.3	2.32	2.98
High DDT	root	RHBlueC	2.8% BlueLeaf Biochar	0.69	23.0	2.27	3.10
High DDT	root	RHBurtsA	2.8% Burt's Biochar	0.47	19.3	2.28	2.93
High DDT	root	RHBurtsB	2.8% Burt's Biochar	0.49	17.0	2.31	2.83
High DDT	root	RHBurtsC	2.8% Burt's Biochar				
High DDT	rhizosphere	RZHControlA	Control	1.01	22.7	2.28	3.08
High DDT	rhizosphere	RZHControlB	Control	0.92	20.3	2.32	3.03
High DDT	rhizosphere	RZHControlC	Control	1.22	27.3	2.22	3.19
High DDT	rhizosphere	RZHGACA	2.8% GAC	1.19	26.7	2.24	3.19
High DDT	rhizosphere	RZHGACB	2.8% GAC	1.02	21.7	2.29	3.05
High DDT	rhizosphere	RZHGACC	2.8% GAC	0.91	18.0	2.32	2.91
High DDT	rhizosphere	RZHBlueA	2.8% BlueLeaf Biochar	0.92	22.0	2.27	3.04
High DDT	rhizosphere	RZHBlueB	2.8% BlueLeaf Biochar	0.74	19.3	2.29	2.95
High DDT	rhizosphere	RZHBlueC	2.8% BlueLeaf Biochar	0.86	20.7	2.30	3.02
High DDT	rhizosphere	RZHBurtsA	2.8% Burt's Biochar	0.37	10.3	2.61	2.63
High DDT	rhizosphere	RZHBurtsB	2.8% Burt's Biochar	0.66	18.7	2.27	2.88
High DDT	rhizosphere	RZHBurtC	2.8% Burt's	1.03	22.3	2.25	3.04

			Biochar				
High DDT	soil	SHControlA	Control	0.12	6.7	3.50	2.64
High DDT	soil	SHControlB	Control	0.23	7.0	2.73	2.31
High DDT	soil	SHControlC	Control	0.36	13.0	2.58	2.84
High DDT	soil	SHGACA	2.8% GAC	1.18	26.0	2.26	3.20
High DDT	soil	SHGACB	2.8% GAC	0.26	9.0	2.84	2.70
High DDT	soil	SHGACC	2.8% GAC	0.43	13.0	2.51	2.78
High DDT	soil	SHBlueA	2.8% BlueLeaf Biochar	0.90	21.0	2.27	2.99
High DDT	soil	SHBlueB	2.8% BlueLeaf Biochar	0.14	5.7	3.28	2.35
High DDT	soil	SHBlueC	2.8% BlueLeaf Biochar	0.52	17.7	2.34	2.91
High DDT	soil	SHBurtsA	2.8% Burt's Biochar	0.10	1.3		2.74
High DDT	soil	SHBurtsB	2.8% Burt's Biochar	0.26	9.7	2.44	2.40
High DDT	soil	SHBurtsC	2.8% Burt's Biochar	0.53	13.3	2.36	2.64
Low DDT	soil	SLControl1	Control	0.97	19.7	2.32	3.00
Low DDT	soil	SLControl2	Control	0.96	19.3	2.31	2.98
Low DDT	soil	SLControl3	Control	1.05	23.0	2.26	3.07
Low DDT	soil	SLGAC1	2.8% GAC	0.87	23.0	2.24	3.05
Low DDT	soil	SLGAC2	2.8% GAC	0.76	22.0	2.29	3.07
Low DDT	soil	SLGAC3	2.8% GAC	0.24	12.0		
Low DDT	soil	SLBlue1	2.8% BlueLeaf Biochar	0.71	18.3	2.32	2.92
Low DDT	soil	SLBlue2	2.8% BlueLeaf Biochar	0.77	19.0	2.31	2.95
Low DDT	soil	SLBlue3	2.8% BlueLeaf Biochar	0.53	17.7	2.29	2.84
Low DDT	soil	SLBurts1	2.8% Burt's Biochar	0.63	18.7	2.39	3.01
Low DDT	soil	SLBurts2	2.8% Burt's Biochar	0.63	15.3	2.31	2.71
Low DDT	soil	SLBurts3	2.8% Burt's Biochar	0.35	10.3	2.55	2.56
Low DDT	root	RLControl1	Control	0.75	23.3	2.25	3.08
Low DDT	root	RLControl2	Control	0.66	22.0	2.30	3.08
Low DDT	root	RLControl3	Control	0.94	22.0	2.30	3.08
Low DDT	rhizosphere	RZHControl1	Control	0.30	11.7	2.38	2.53
Low DDT	rhizosphere	RZHControl2	Control	0.71	18.7	2.26	2.87
Low DDT	rhizosphere	RZHControl3	Control	0.66	18.7	2.35	2.97

APPENDIX E: RAW DATA AND QA/QC FOR CHAPTER 7

Table E-1 DDT Concentrations (ng/g) in Plant Shoots (Field Data-Harvest 1)

Shoot							
Field Study: Harvest 1 (August 2013)				Concentration (ng/g)			
Data File (ASU)	Rep	Treatment	Compound	Base	Mid	Tip	Average
131022	A	Control	2,4 DDE	85.1	14.0	< 1.0	33.1
131022	A	Control	4,4 DDE	9160	795	97.1	3350
131022	A	Control	2,4 DDD	107	31.0	< 1.0	45.9
131022	A	Control	4,4 DDD	95.9	37.2	18.2	50.4
131022	A	Control	2,4 DDT	2080	442	58.3	861
131022	A	Control	4,4 DDT	2400	763	103	1090
131022	***A Total***	***Control***	***∑DDT***	***13900***	***2080***	***276***	**5430**
131022	B	Control	2,4 DDE	165	42.0	7.29	71.4
131022	B	Control	4,4 DDE	18200	3440	115	7260
131022	B	Control	2,4 DDD	140	61.4	8.32	69.9
131022	B	Control	4,4 DDD	123	82.7	29.3	78.4
131022	B	Control	2,4 DDT	2830	966	137	1310
131022	B	Control	4,4 DDT	4360	1870	260	2160
131022	***B Total***	***Control***	***∑DDT***	***25800***	***6470***	***557***	**10900**
131022	C	Control	2,4 DDE	267	42.0	14.4	108
131022	C	Control	4,4 DDE	12300	813	128	4420
131022	C	Control	2,4 DDD	139	33.3	13.3	61.7
131022	C	Control	4,4 DDD	127	37.5	20.9	61.8
131022	C	Control	2,4 DDT	3520	610	283	1470
131022	C	Control	4,4 DDT	4310	830	380	1840
131022	***C Total***	***Control***	***∑DDT***	***20700***	***2370***	***840***	**7970**
131106	A	2.8% GAC	2,4 DDE	194	43.9	6.37	81.5
131106	A	2.8% GAC	4,4 DDE	12800	1570	89.2	4810
131106	A	2.8% GAC	2,4 DDD	345	100	8.64	151.1
131106	A	2.8% GAC	4,4 DDD	336	110	8.72	151.5
131106	A	2.8% GAC	2,4 DDT	6180	1400	249	2610
131106	A	2.8% GAC	4,4 DDT	14200	3680	656	6180
131106	***A Total***	***2.8% GAC***	***∑DDT***	***34000***	***6920***	***1020***	**14000**
131106	B	2.8% GAC	2,4 DDE	151	64.2	12.0	75.7
131106	B	2.8% GAC	4,4 DDE	7320	2100	532	3320
131106	B	2.8% GAC	2,4 DDD	311	198	25.0	178
131106	B	2.8% GAC	4,4 DDD	75.7	120	26.0	73.9
131106	B	2.8% GAC	2,4 DDT	6390	2340	555	3100
131106	B	2.8% GAC	4,4 DDT	5050	3720	1460	3410

131106	***B Total***	***2.8% GAC***	***∑DDT***	***19300***	***8540***	***2610***	**10200**
131106	C	2.8% GAC	2,4 DDE	190	42.9	16.3	83.2
131106	C	2.8% GAC	4,4 DDE	13700	1990	303	5340
131106	C	2.8% GAC	2,4 DDD	334	96.5	16.3	149
131106	C	2.8% GAC	4,4 DDD	158	93.5	27.9	93.2
131106	C	2.8% GAC	2,4 DDT	5660	1370	514.	2510
131106	C	2.8% GAC	4,4 DDT	7930	3310	1270	4170
131106	***C Total***	***2.8% GAC***	***∑DDT***	***28000***	***6900***	***2140***	**12400**
131028	A	2.8% BlueLeaf Biochar	2,4 DDE	107	25.9	7.16	46.8
131028	A	2.8% BlueLeaf Biochar	4,4 DDE	7700	891	65.7	2890
131028	A	2.8% BlueLeaf Biochar	2,4 DDD	155	50.4	11.2	72.3
131028	A	2.8% BlueLeaf Biochar	4,4 DDD	130	56.2	10.2	65.4
131028	A	2.8% BlueLeaf Biochar	2,4 DDT	2550	638	99.6	1100
131028	A	2.8% BlueLeaf Biochar	4,4 DDT	3040	1180	170	1460
131028	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***13700***	***2840***	***364***	**5630**
131028	B	2.8% BlueLeaf Biochar	2,4 DDE	140	27.5	7.15	58.2
131028	B	2.8% BlueLeaf Biochar	4,4 DDE	13300	1420	61.0	4930
131028	B	2.8% BlueLeaf Biochar	2,4 DDD	285	73.9	19.8	126
131028	B	2.8% BlueLeaf Biochar	4,4 DDD	271	105	32.7	136
131028	B	2.8% BlueLeaf Biochar	2,4 DDT	4380	991	151	1840
131028	B	2.8% BlueLeaf Biochar	4,4 DDT	6180	1910	300	2800
131028	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***24583.36***	***4526.50***	***571.40***	**9893.75**
131028	C	2.8% BlueLeaf Biochar	2,4 DDE	167	45.2	9.64	73.8
131028	C	2.8% BlueLeaf Biochar	4,4 DDE	19700	3590	190	7840
131028	C	2.8% BlueLeaf Biochar	2,4 DDD	156	60.0	11.9	75.9
131028	C	2.8% BlueLeaf Biochar	4,4 DDD	112	55.0	20.9	62.7
131028	C	2.8% BlueLeaf Biochar	2,4 DDT	3300	843	186	1440
131028	C	2.8% BlueLeaf Biochar	4,4 DDT	3370	1190	373	1650
131028	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***26900***	***5780***	***790***	**11100**
131029	A	2.8% Burt's Biochar	2,4 DDE	202	43.3	6.73	83.9
131029	A	2.8% Burt's Biochar	4,4 DDE	12600	1580	115	4770
131029	A	2.8% Burt's Biochar	2,4 DDD	277	64.1	24.9	122
131029	A	2.8% Burt's Biochar	4,4 DDD	124	40.6	8.91	57.7
131029	A	2.8% Burt's Biochar	2,4 DDT	3960	817	223	1700
131029	A	2.8% Burt's Biochar	4,4 DDT	4180	1330	379	1960
131029	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	***21300***	***3870***	***758***	**8660**
131029	B	2.8% Burt's Biochar	2,4 DDE	288	77.9	7.64	124
131029	B	2.8% Burt's Biochar	4,4 DDE	18600	3290	130	7330
131029	B	2.8% Burt's Biochar	2,4 DDD	407	130	23.7	187
131029	B	2.8% Burt's Biochar	4,4 DDD	277	113	10.5	133

131029	B	2.8% Burt's Biochar	2,4 DDT	5750	1690	249	2560
131029	B	2.8% Burt's Biochar	4,4 DDT	9490	3790	758	4680
131029	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	***34800***	***9080***	***1180***	**15000**
131029	C	2.8% Burt's Biochar	2,4 DDE	239	44.6	9.64	7.94
131029	C	2.8% Burt's Biochar	4,4 DDE	12700	1400	190	149
131029	C	2.8% Burt's Biochar	2,4 DDD	312	67.9	11.9	23.8
131029	C	2.8% Burt's Biochar	4,4 DDD	321	74.5	20.9	9.18
131029	C	2.8% Burt's Biochar	2,4 DDT	5300	1190	186	2967
131029	C	2.8% Burt's Biochar	4,4 DDT	10200	2640	373	665
131029	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	***29200***	***5420***	***790***	**11800**

Table E-2 DDT Concentrations (ng/g) in Plant Roots (Field- Harvest 1)

Root				
Field Study: Harvest 1 (August 2013)				
Data File (ASU)	Rep	Treatment	Compound	Concentration (µg/g)
131107	A	Control	2,4 DDE	108
131107	A	Control	4,4 DDE	16400
131107	A	Control	2,4 DDD	110
131107	A	Control	4,4 DDD	64.0
131107	A	Control	2,4 DDT	2460
131107	A	Control	4,4 DDT	2620
131107	***A Total***	***Control***	***∑DDT***	**21800**
131107	B	Control	2,4 DDE	270
131107	B	Control	4,4 DDE	34400
131107	B	Control	2,4 DDD	202
131107	B	Control	4,4 DDD	151
131107	B	Control	2,4 DDT	4920
131107	B	Control	4,4 DDT	6330
131107	***B Total***	***Control***	***∑DDT***	**46200**
131107	C	Control	2,4 DDE	271
131107	C	Control	4,4 DDE	17700
131107	C	Control	2,4 DDD	134
131107	C	Control	4,4 DDD	108
131107	C	Control	2,4 DDT	3430
131107	C	Control	4,4 DDT	4040
131107	***C Total***	***Control***	***∑DDT***	**25700**
131107	A	2.8% GAC	2,4 DDE	262
131107	A	2.8% GAC	4,4 DDE	23500

131107	A	2.8% GAC	2,4 DDD	611
131107	A	2.8% GAC	4,4 DDD	392
131107	A	2.8% GAC	2,4 DDT	9230
131107	A	2.8% GAC	4,4 DDT	1730
131107	***A Total***	***2.8% GAC***	***∑DDT***	**51300**
131107	B	2.8% GAC	2,4 DDE	118
131107	B	2.8% GAC	4,4 DDE	9320
131107	B	2.8% GAC	2,4 DDD	340
131107	B	2.8% GAC	4,4 DDD	129
131107	B	2.8% GAC	2,4 DDT	4680
131107	B	2.8% GAC	4,4 DDT	3970
131107	***B Total***	***2.8% GAC***	***∑DDT***	**18560**
131107	C	2.8% GAC	2,4 DDE	195
131107	C	2.8% GAC	4,4 DDE	22900
131107	C	2.8% GAC	2,4 DDD	380
131107	C	2.8% GAC	4,4 DDD	182
131107	C	2.8% GAC	2,4 DDT	6470
131107	C	2.8% GAC	4,4 DDT	8900
131107	***C Total***	***2.8% GAC***	***∑DDT***	**39100**
131107	A	2.8% BlueLeaf Biochar	2,4 DDE	134
131107	A	2.8% BlueLeaf Biochar	4,4 DDE	18800
131107	A	2.8% BlueLeaf Biochar	2,4 DDD	180
131107	A	2.8% BlueLeaf Biochar	4,4 DDD	95.0
131107	A	2.8% BlueLeaf Biochar	2,4 DDT	5000
131107	A	2.8% BlueLeaf Biochar	4,4 DDT	4650
131107	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**2890**
131107	B	2.8% BlueLeaf Biochar	2,4 DDE	181
131107	B	2.8% BlueLeaf Biochar	4,4 DDE	24900
131107	B	2.8% BlueLeaf Biochar	2,4 DDD	319
131107	B	2.8% BlueLeaf Biochar	4,4 DDD	219
131107	B	2.8% BlueLeaf Biochar	2,4 DDT	7730
131107	B	2.8% BlueLeaf Biochar	4,4 DDT	8000
131107	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**41300**
131107	C	2.8% BlueLeaf Biochar	2,4 DDE	173
131107	C	2.8% BlueLeaf Biochar	4,4 DDE	28300
131107	C	2.8% BlueLeaf Biochar	2,4 DDD	197
131107	C	2.8% BlueLeaf Biochar	4,4 DDD	120
131107	C	2.8% BlueLeaf Biochar	2,4 DDT	4740
131107	C	2.8% BlueLeaf Biochar	4,4 DDT	4430
131107	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**38000**

131107	A	2.8% Burt's Biochar	2,4 DDE	188
131107	A	2.8% Burt's Biochar	4,4 DDE	15800
131107	A	2.8% Burt's Biochar	2,4 DDD	311
131107	A	2.8% Burt's Biochar	4,4 DDD	114
131107	A	2.8% Burt's Biochar	2,4 DDT	4560
131107	A	2.8% Burt's Biochar	4,4 DDT	3710
131107	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**24700**
131107	B	2.8% Burt's Biochar	2,4 DDE	361
131107	B	2.8% Burt's Biochar	4,4 DDE	33100
131107	B	2.8% Burt's Biochar	2,4 DDD	473
131107	B	2.8% Burt's Biochar	4,4 DDD	194
131107	B	2.8% Burt's Biochar	2,4 DDT	9020
131107	B	2.8% Burt's Biochar	4,4 DDT	11100
131107	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**54300**
131107	C	2.8% Burt's Biochar	2,4 DDE	285
131107	C	2.8% Burt's Biochar	4,4 DDE	20700
131107	C	2.8% Burt's Biochar	2,4 DDD	336
131107	C	2.8% Burt's Biochar	4,4 DDD	192
131107	C	2.8% Burt's Biochar	2,4 DDT	7060
131107	C	2.8% Burt's Biochar	4,4 DDT	10200
131107	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**38700**

Table E-3 DDT Concentrations (ng/g) in Plant Shoots (Field-Harvest 2)

Shoot				
Field Study: Harvest 2 (October 2013)				
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
131217	A	Control	2,4 DDE	84.7
131217	A	Control	4,4 DDE	2450
131217	A	Control	2,4 DDD	51.4
131217	A	Control	4,4 DDD	46.8
131217	A	Control	2,4 DDT	937
131217	A	Control	4,4 DDT	679
131217	***A Total***	***Control***	***∑DDT***	**4250**
131217	B	Control	2,4 DDE	75.0
131217	B	Control	4,4 DDE	4520
131217	B	Control	2,4 DDD	80.8
131217	B	Control	4,4 DDD	126.3
131217	B	Control	2,4 DDT	1090

131217	B	Control	4,4 DDT	1515
131217	***B Total***	***Control***	***∑DDT***	**7400**
131217	C	Control	2,4 DDE	101
131217	C	Control	4,4 DDE	8440
131217	C	Control	2,4 DDD	115
131217	C	Control	4,4 DDD	156
131217	C	Control	2,4 DDT	1690
131217	C	Control	4,4 DDT	2330
131217	***C Total***	***Control***	***∑DDT***	**12800**
131217	A	2.8% GAC	2,4 DDE	176
131217	A	2.8% GAC	4,4 DDE	7420
131217	A	2.8% GAC	2,4 DDD	161
131217	A	2.8% GAC	4,4 DDD	189
131217	A	2.8% GAC	2,4 DDT	2390
131217	A	2.8% GAC	4,4 DDT	5626
131217	***A Total***	***2.8% GAC***	***∑DDT***	**16000**
131217	B	2.8% GAC	2,4 DDE	60.4
131217	B	2.8% GAC	4,4 DDE	3840
131217	B	2.8% GAC	2,4 DDD	132
131217	B	2.8% GAC	4,4 DDD	329
131217	B	2.8% GAC	2,4 DDT	1930
131217	B	2.8% GAC	4,4 DDT	6510
131217	***B Total***	***2.8% GAC***	***∑DDT***	**12800**
131217	C	2.8% GAC	2,4 DDE	99.9
131217	C	2.8% GAC	4,4 DDE	9130
131217	C	2.8% GAC	2,4 DDD	236
131217	C	2.8% GAC	4,4 DDD	400
131217	C	2.8% GAC	2,4 DDT	2200
131217	C	2.8% GAC	4,4 DDT	6870
131217	***C Total***	***2.8% GAC***	***∑DDT***	**18900**
131217	A	2.8% BlueLeaf Biochar	2,4 DDE	67.7
131217	A	2.8% BlueLeaf Biochar	4,4 DDE	5170
131217	A	2.8% BlueLeaf Biochar	2,4 DDD	141
131217	A	2.8% BlueLeaf Biochar	4,4 DDD	171
131217	A	2.8% BlueLeaf Biochar	2,4 DDT	1420
131217	A	2.8% BlueLeaf Biochar	4,4 DDT	1660
131217	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**8600**
131217	B	2.8% BlueLeaf Biochar	2,4 DDE	65.3
131217	B	2.8% BlueLeaf Biochar	4,4 DDE	4460
131217	B	2.8% BlueLeaf Biochar	2,4 DDD	104

131217	B	2.8% BlueLeaf Biochar	4,4 DDD	179
131217	B	2.8% BlueLeaf Biochar	2,4 DDT	1230
131217	B	2.8% BlueLeaf Biochar	4,4 DDT	2160
131217	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**8200**
131217	C	2.8% BlueLeaf Biochar	2,4 DDE	70.7
131217	C	2.8% BlueLeaf Biochar	4,4 DDE	4960
131217	C	2.8% BlueLeaf Biochar	2,4 DDD	154
131217	C	2.8% BlueLeaf Biochar	4,4 DDD	159
131217	C	2.8% BlueLeaf Biochar	2,4 DDT	1500
131217	C	2.8% BlueLeaf Biochar	4,4 DDT	1830
131217	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**8700**
131217	A	2.8% Burt's Biochar	2,4 DDE	76.9
131217	A	2.8% Burt's Biochar	4,4 DDE	3460
131217	A	2.8% Burt's Biochar	2,4 DDD	110
131217	A	2.8% Burt's Biochar	4,4 DDD	155
131217	A	2.8% Burt's Biochar	2,4 DDT	1500
131217	A	2.8% Burt's Biochar	4,4 DDT	2340
131217	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**7600**
131217	B	2.8% Burt's Biochar	2,4 DDE	81.8
131217	B	2.8% Burt's Biochar	4,4 DDE	4190
131217	B	2.8% Burt's Biochar	2,4 DDD	101
131217	B	2.8% Burt's Biochar	4,4 DDD	161
131217	B	2.8% Burt's Biochar	2,4 DDT	1590
131217	B	2.8% Burt's Biochar	4,4 DDT	2880
131217	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**9000**
131217	C	2.8% Burt's Biochar	2,4 DDE	117
131217	C	2.8% Burt's Biochar	4,4 DDE	6940
131217	C	2.8% Burt's Biochar	2,4 DDD	194
131217	C	2.8% Burt's Biochar	4,4 DDD	335
131217	C	2.8% Burt's Biochar	2,4 DDT	2530
131217	C	2.8% Burt's Biochar	4,4 DDT	5230
131217	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**15400**

Table E-4 DDT Concentrations (ng/g) Plant Root (Field- Harvest 2)

Root				
Field Study: Harvest 2 (October 2013)				
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
131224	A	Control	2,4 DDE	285
131224	A	Control	4,4 DDE	40000
131224	A	Control	2,4 DDD	265
131224	A	Control	4,4 DDD	129
131224	A	Control	2,4 DDT	3180
131224	A	Control	4,4 DDT	4170
131224	***A Total***	***Control***	***∑DDT***	**48000**
131224	B	Control	2,4 DDE	191
131224	B	Control	4,4 DDE	27200
131224	B	Control	2,4 DDD	348
131224	B	Control	4,4 DDD	106
131224	B	Control	2,4 DDT	3020
131224	B	Control	4,4 DDT	3400
131224	***B Total***	***Control***	***∑DDT***	**34300**
131224	C	Control	2,4 DDE	191
131224	C	Control	4,4 DDE	25900
131224	C	Control	2,4 DDD	273
131224	C	Control	4,4 DDD	67.7
131224	C	Control	2,4 DDT	3750
131224	C	Control	4,4 DDT	3830
131224	***C Total***	***Control***	***∑DDT***	**34000**
131230	A	2.8% GAC	2,4 DDE	277
131230	A	2.8% GAC	4,4 DDE	22100
131230	A	2.8% GAC	2,4 DDD	691
131230	A	2.8% GAC	4,4 DDD	342
131230	A	2.8% GAC	2,4 DDT	10500
131230	A	2.8% GAC	4,4 DDT	10700
131230	***A Total***	***2.8% GAC***	***∑DDT***	**44600**
131230	B	2.8% GAC	2,4 DDE	408.0
131230	B	2.8% GAC	4,4 DDE	40010
131230	B	2.8% GAC	2,4 DDD	1076
131230	B	2.8% GAC	4,4 DDD	560
131230	B	2.8% GAC	2,4 DDT	21400
131230	B	2.8% GAC	4,4 DDT	41600
131230	***B Total***	***2.8% GAC***	***∑DDT***	**105000**
131230	C	2.8% GAC	2,4 DDE	362
131230	C	2.8% GAC	4,4 DDE	39600

131230	C	2.8% GAC	2,4 DDD	1030
131230	C	2.8% GAC	4,4 DDD	805
131230	C	2.8% GAC	2,4 DDT	12800
131230	C	2.8% GAC	4,4 DDT	25300
131230	***C Total***	***2.8% GAC***	***∑DDT***	**79800**
131230	A	2.8% BlueLeaf Biochar	2,4 DDE	98.8
131230	A	2.8% BlueLeaf Biochar	4,4 DDE	16500
131230	A	2.8% BlueLeaf Biochar	2,4 DDD	159
131230	A	2.8% BlueLeaf Biochar	4,4 DDD	45
131230	A	2.8% BlueLeaf Biochar	2,4 DDT	2810
131230	A	2.8% BlueLeaf Biochar	4,4 DDT	1620
131230	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**21300**
131230	B	2.8% BlueLeaf Biochar	2,4 DDE	155
131230	B	2.8% BlueLeaf Biochar	4,4 DDE	22300
131230	B	2.8% BlueLeaf Biochar	2,4 DDD	355
131230	B	2.8% BlueLeaf Biochar	4,4 DDD	181
131230	B	2.8% BlueLeaf Biochar	2,4 DDT	4800
131230	B	2.8% BlueLeaf Biochar	4,4 DDT	5700
131230	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**33500**
131230	C	2.8% BlueLeaf Biochar	2,4 DDE	197
131230	C	2.8% BlueLeaf Biochar	4,4 DDE	28800
131230	C	2.8% BlueLeaf Biochar	2,4 DDD	379
131230	C	2.8% BlueLeaf Biochar	4,4 DDD	162
131230	C	2.8% BlueLeaf Biochar	2,4 DDT	7060
131230	C	2.8% BlueLeaf Biochar	4,4 DDT	6850
131230	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**43400**
131230	A	2.8% Burt's Biochar	2,4 DDE	422
131230	A	2.8% Burt's Biochar	4,4 DDE	38200
131230	A	2.8% Burt's Biochar	2,4 DDD	696
131230	A	2.8% Burt's Biochar	4,4 DDD	291
131230	A	2.8% Burt's Biochar	2,4 DDT	12300
131230	A	2.8% Burt's Biochar	4,4 DDT	14500
131230	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**66400**
131230	B	2.8% Burt's Biochar	2,4 DDE	296
131230	B	2.8% Burt's Biochar	4,4 DDE	36500
131230	B	2.8% Burt's Biochar	2,4 DDD	611
131230	B	2.8% Burt's Biochar	4,4 DDD	218
131230	B	2.8% Burt's Biochar	2,4 DDT	9940
131230	B	2.8% Burt's Biochar	4,4 DDT	12100
131230	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**59700**

131230	C	2.8% Burt's Biochar	2,4 DDE	541
131230	C	2.8% Burt's Biochar	4,4 DDE	59900
131230	C	2.8% Burt's Biochar	2,4 DDD	966
131230	C	2.8% Burt's Biochar	4,4 DDD	561
131230	C	2.8% Burt's Biochar	2,4 DDT	13300
131230	C	2.8% Burt's Biochar	4,4 DDT	20800
131230	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**96100**

Table E-5 DDT Concentration (ng/g) in Plant Shoot Tissue (2012 Study)

Shoot							
Field Study: Point Pelee National Park 2012				Concentration (ng/g)			
Data File (ASU)	Rep	Treatment	Compound	Base	Mid	Tip	Average
130117	A	Control	2,4 DDE	110	25.1	< 1.0	45.1
130117	A	Control	4,4 DDE	10900	16200	35.1	4200
130117	A	Control	2,4 DDD	133	45.6	< 1.0	59.4
130117	A	Control	4,4 DDD	76.7	33.6	7.79	39.4
130117	A	Control	2,4 DDT	1280	256	31.2	525
130117	A	Control	4,4 DDT	1130	418	52.6	533
130117	***A Total***	***Control***	***∑DDT***	***13700***	***241***	***127***	**5400**
130117	B	Control	2,4 DDE	320	89.1	47.7	152
130117	B	Control	4,4 DDE	28500	5450	983	11600
130117	B	Control	2,4 DDD	403	102	85.7	197
130117	B	Control	4,4 DDD	269	90.9	129	163
130117	B	Control	2,4 DDT	3110	771	983	1620
130117	B	Control	4,4 DDT	2980	1000	1640	1880
130117	***B Total***	***Control***	***∑DDT***	***35600***	***7500***	***3870***	**15600**
130117	C	Control	2,4 DDE	268	140	85.7	164
130117	C	Control	4,4 DDE	19800	8210	1690	9900
130117	C	Control	2,4 DDD	246	135	211	197
130117	C	Control	4,4 DDD	116	90.5	245	150
130117	C	Control	2,4 DDT	2480	1120	1230	1610
130117	C	Control	4,4 DDT	1600	1000	1720	1440
130117	***C Total***	***Control***	***∑DDT***	***24500***	***10700***	***5170***	**13650**
130121	A	2.8% GAC	2,4 DDE	305	66.8	3.31	125
130121	A	2.8% GAC	4,4 DDE	14500	1350	87.6	5290
130121	A	2.8% GAC	2,4 DDD	301	121	15.4	146
130121	A	2.8% GAC	4,4 DDD	189	121	9.22	106
130121	A	2.8% GAC	2,4 DDT	3120	868	124	1370

130121	A	2.8% GAC	4,4 DDT	4640	1460	225	2110
130121	***A Total***	***2.8% GAC***	***∑DDT***	***23000***	***3980***	***465***	**9150**
130121	B	2.8% GAC	2,4 DDE	423	94.8	0	172
130121	B	2.8% GAC	4,4 DDE	33100	3960	78.1	12400
130121	B	2.8% GAC	2,4 DDD	495	92.6	11.8	200
130121	B	2.8% GAC	4,4 DDD	582	96.7	5.61	228
130121	B	2.8% GAC	2,4 DDT	4040	1120	18.5	1730
130121	B	2.8% GAC	4,4 DDT	5230	1880	35.5	2380
130121	***B Total***	***2.8% GAC***	***∑DDT***	***43900***	***7250***	***150***	**17100**
130121	C	2.8% GAC	2,4 DDE	500	88.5	1.57	197
130121	C	2.8% GAC	4,4 DDE	34300	2410	127	12300
130121	C	2.8% GAC	2,4 DDD	473	117	10.6	200.3
130121	C	2.8% GAC	4,4 DDD	186	79.7	14.6	93.4
130121	C	2.8% GAC	2,4 DDT	4370	885	107	1790
130121	C	2.8% GAC	4,4 DDT	3890	1060	165	1710
130121	***C Total***	***2.8% GAC***	***∑DDT***	***43700***	***4640***	***426***	**16300**
130121	A	2.8% BlueLeaf Biochar	2,4 DDE	332	102	< 1.0	144
130121	A	2.8% BlueLeaf Biochar	4,4 DDE	21300	4160	55.9	8510
130121	A	2.8% BlueLeaf Biochar	2,4 DDD	3630	129	2.95	165
130121	A	2.8% BlueLeaf Biochar	4,4 DDD	308	145	10.1	154
130121	A	2.8% BlueLeaf Biochar	2,4 DDT	4470	1250	40.9	1920
130121	A	2.8% BlueLeaf Biochar	4,4 DDT	49000	1800	65.2	2250
130121	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***31700***	***7580***	***175***	**13200**
130121	B	2.8% BlueLeaf Biochar	2,4 DDE	245	221	240	235
130121	B	2.8% BlueLeaf Biochar	4,4 DDE	17600	12100	5550	11700
130121	B	2.8% BlueLeaf Biochar	2,4 DDD	278	222	264	255
130121	B	2.8% BlueLeaf Biochar	4,4 DDD	130	103	257	163
130121	B	2.8% BlueLeaf Biochar	2,4 DDT	2890	2320	4570	3260
130121	B	2.8% BlueLeaf Biochar	4,4 DDT	1914	2060	6230	3400
130121	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***23020.35***	***17000***	***17100***	**19100**
130121	C	2.8% BlueLeaf Biochar	2,4 DDE	316	136	< 1.0	151
130121	C	2.8% BlueLeaf Biochar	4,4 DDE	15800	3710	44.1	6500
130121	C	2.8% BlueLeaf Biochar	2,4 DDD	305	127	< 1.0	144
130121	C	2.8% BlueLeaf Biochar	4,4 DDD	305	123	6.80	145
130121	C	2.8% BlueLeaf Biochar	2,4 DDT	3610	1470	68.2	1720
130121	C	2.8% BlueLeaf Biochar	4,4 DDT	4740	2680	157	2530
130121	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	***25000***	***8250***	***276***	**11200**
130125	A	2.8% Burt's Biochar	2,4 DDE	80.1	41.7	1.93	41.2
130125	A	2.8% Burt's Biochar	4,4 DDE	7360	2480	68.5	3300
130125	A	2.8% Burt's Biochar	2,4 DDD	67.1	42.9	6.68	38.9

130125	A	2.8% Burt's Biochar	4,4 DDD	34.4	30.8	3.18	22.8
130125	A	2.8% Burt's Biochar	2,4 DDT	1050	467	44.7	522
130125	A	2.8% Burt's Biochar	4,4 DDT	1020	625	80.5	575
130125	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	***9610***	***3690***	***206***	**4500**
130125	B	2.8% Burt's Biochar	2,4 DDE	215	121	57.7	131
130125	B	2.8% Burt's Biochar	4,4 DDE	15700	6030	874	7530
130125	B	2.8% Burt's Biochar	2,4 DDD	169	126	83.2	126
130125	B	2.8% Burt's Biochar	4,4 DDD	119	103	129	117
130125	B	2.8% Burt's Biochar	2,4 DDT	2440	1380	1320	1710
130125	B	2.8% Burt's Biochar	4,4 DDT	3250	2250	2440	2650
130125	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	***21900***	***10020***	***4900***	**12300**
130125	C	2.8% Burt's Biochar	2,4 DDE	341	155	104	7.94
130125	C	2.8% Burt's Biochar	4,4 DDE	25200	8900	1950	149
130125	C	2.8% Burt's Biochar	2,4 DDD	238	156	100	23.8
130125	C	2.8% Burt's Biochar	4,4 DDD	129	115	150	9.18
130125	C	2.8% Burt's Biochar	2,4 DDT	3910	1540	1850	297
130125	C	2.8% Burt's Biochar	4,4 DDT	2860	1790	3040	665
130125	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	***32700***	***1200***	***7200***	**17500**

Table E-6 DDT Concentrations (ng/g) in Plant Root Tissue (2012 Study)

Root				
Field Study: Point Pelee National Park 2012				
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
130129	A	Control	2,4 DDE	128
130129	A	Control	4,4 DDE	14000
130129	A	Control	2,4 DDD	157
130129	A	Control	4,4 DDD	49.1
130129	A	Control	2,4 DDT	1840
130129	A	Control	4,4 DDT	1400
130129	***A Total***	***Control***	***∑DDT***	**17600**
130129	B	Control	2,4 DDE	315
130129	B	Control	4,4 DDE	35800
130129	B	Control	2,4 DDD	236
130129	B	Control	4,4 DDD	180
130129	B	Control	2,4 DDT	3960
130129	B	Control	4,4 DDT	3490
130129	***B Total***	***Control***	***∑DDT***	**44000**
130129	C	Control	2,4 DDE	325

130129	C	Control	4,4 DDE	29700
130129	C	Control	2,4 DDD	215
130129	C	Control	4,4 DDD	86.5
130129	C	Control	2,4 DDT	3950
130129	C	Control	4,4 DDT	2480
130129	***C Total***	***Control***	***∑DDT***	**36700**
130129	A	2.8% GAC	2,4 DDE	253
130129	A	2.8% GAC	4,4 DDE	17700
130129	A	2.8% GAC	2,4 DDD	292
130129	A	2.8% GAC	4,4 DDD	117
130129	A	2.8% GAC	2,4 DDT	2940
130129	A	2.8% GAC	4,4 DDT	4230
130129	***A Total***	***2.8% GAC***	***∑DDT***	**25500**
130129	B	2.8% GAC	2,4 DDE	445
130129	B	2.8% GAC	4,4 DDE	42000
130129	B	2.8% GAC	2,4 DDD	373
130129	B	2.8% GAC	4,4 DDD	207
130129	B	2.8% GAC	2,4 DDT	5060
130129	B	2.8% GAC	4,4 DDT	6620
130129	***B Total***	***2.8% GAC***	***∑DDT***	**54700**
130129	C	2.8% GAC	2,4 DDE	540
130129	C	2.8% GAC	4,4 DDE	45900
130129	C	2.8% GAC	2,4 DDD	487
130129	C	2.8% GAC	4,4 DDD	182
130129	C	2.8% GAC	2,4 DDT	5680
130129	C	2.8% GAC	4,4 DDT	4470
130129	***C Total***	***2.8% GAC***	***∑DDT***	**57200**
130129	A	2.8% BlueLeaf Biochar	2,4 DDE	367
130129	A	2.8% BlueLeaf Biochar	4,4 DDE	31200
130129	A	2.8% BlueLeaf Biochar	2,4 DDD	321
130129	A	2.8% BlueLeaf Biochar	4,4 DDD	175
130129	A	2.8% BlueLeaf Biochar	2,4 DDT	5750
130129	A	2.8% BlueLeaf Biochar	4,4 DDT	5780
130129	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**43500**
130129	B	2.8% BlueLeaf Biochar	2,4 DDE	279
130129	B	2.8% BlueLeaf Biochar	4,4 DDE	24800
130129	B	2.8% BlueLeaf Biochar	2,4 DDD	436
130129	B	2.8% BlueLeaf Biochar	4,4 DDD	226
130129	B	2.8% BlueLeaf Biochar	2,4 DDT	2990
130129	B	2.8% BlueLeaf Biochar	4,4 DDT	1800

130129	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**30500**
130129	C	2.8% BlueLeaf Biochar	2,4 DDE	334
130129	C	2.8% BlueLeaf Biochar	4,4 DDE	24100
130129	C	2.8% BlueLeaf Biochar	2,4 DDD	274
130129	C	2.8% BlueLeaf Biochar	4,4 DDD	212
130129	C	2.8% BlueLeaf Biochar	2,4 DDT	4270
130129	C	2.8% BlueLeaf Biochar	4,4 DDT	4760
130129	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**33900**
130129	A	2.8% Burt's Biochar	2,4 DDE	62.1
130129	A	2.8% Burt's Biochar	4,4 DDE	6830
130129	A	2.8% Burt's Biochar	2,4 DDD	71.6
130129	A	2.8% Burt's Biochar	4,4 DDD	34.4
130129	A	2.8% Burt's Biochar	2,4 DDT	927
130129	A	2.8% Burt's Biochar	4,4 DDT	693
130129	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**8620**
130129	B	2.8% Burt's Biochar	2,4 DDE	225
130129	B	2.8% Burt's Biochar	4,4 DDE	20085
130129	B	2.8% Burt's Biochar	2,4 DDD	251
130129	B	2.8% Burt's Biochar	4,4 DDD	131
130129	B	2.8% Burt's Biochar	2,4 DDT	2920
130129	B	2.8% Burt's Biochar	4,4 DDT	3010
130129	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**26600**
130129	C	2.8% Burt's Biochar	2,4 DDE	253
130129	C	2.8% Burt's Biochar	4,4 DDE	22500
130129	C	2.8% Burt's Biochar	2,4 DDD	253
130129	C	2.8% Burt's Biochar	4,4 DDD	73.8
130129	C	2.8% Burt's Biochar	2,4 DDT	3130
130129	C	2.8% Burt's Biochar	4,4 DDT	1710
130129	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**27900**

Table E-7 DDT Concentrations (ng/g) in Plant Shoots (Greenhouse Study)

Shoot				
	Greenhouse Study			
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
130129	A	Control	2,4 DDE	10.2
130129	A	Control	4,4 DDE	963
130129	A	Control	2,4 DDD	12.4
130129	A	Control	4,4 DDD	29.1
130129	A	Control	2,4 DDT	234
130129	A	Control	4,4 DDT	594
130129	***A Total***	***Control***	***∑DDT***	**1840**
130129	B	Control	2,4 DDE	18.6
130129	B	Control	4,4 DDE	1230
130129	B	Control	2,4 DDD	28.0
130129	B	Control	4,4 DDD	47.86
130129	B	Control	2,4 DDT	430
130129	B	Control	4,4 DDT	951
130129	***B Total***	***Control***	***∑DDT***	**2710**
130129	C	Control	2,4 DDE	25.8
130129	C	Control	4,4 DDE	1030
130129	C	Control	2,4 DDD	21.0
130129	C	Control	4,4 DDD	34.1
130129	C	Control	2,4 DDT	349
130129	C	Control	4,4 DDT	909
130129	***C Total***	***Control***	***∑DDT***	**2370**
130129	A	2.8% GAC	2,4 DDE	48.6
130129	A	2.8% GAC	4,4 DDE	1640
130129	A	2.8% GAC	2,4 DDD	81.4
130129	A	2.8% GAC	4,4 DDD	60.5
130129	A	2.8% GAC	2,4 DDT	1040
130129	A	2.8% GAC	4,4 DDT	3510
130129	***A Total***	***2.8% GAC***	***∑DDT***	**6380**
130129	B	2.8% GAC	2,4 DDE	17.7
130129	B	2.8% GAC	4,4 DDE	1340
130129	B	2.8% GAC	2,4 DDD	49.3
130129	B	2.8% GAC	4,4 DDD	112
130129	B	2.8% GAC	2,4 DDT	641
130129	B	2.8% GAC	4,4 DDT	2550
130129	***B Total***	***2.8% GAC***	***∑DDT***	**4710**
130129	C	2.8% GAC	2,4 DDE	30.8
130129	C	2.8% GAC	4,4 DDE	1450

130129	C	2.8% GAC	2,4 DDD	54.4
130129	C	2.8% GAC	4,4 DDD	12.6
130129	C	2.8% GAC	2,4 DDT	537
130129	C	2.8% GAC	4,4 DDT	1230
130129	***C Total***	***2.8% GAC***	***∑DDT***	**3310**
130129	A	2.8% BlueLeaf Biochar	2,4 DDE	11.4
130129	A	2.8% BlueLeaf Biochar	4,4 DDE	907
130129	A	2.8% BlueLeaf Biochar	2,4 DDD	26.0
130129	A	2.8% BlueLeaf Biochar	4,4 DDD	52.5
130129	A	2.8% BlueLeaf Biochar	2,4 DDT	359
130129	A	2.8% BlueLeaf Biochar	4,4 DDT	968
130129	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**2320**
130129	B	2.8% BlueLeaf Biochar	2,4 DDE	15.3
130129	B	2.8% BlueLeaf Biochar	4,4 DDE	1040
130129	B	2.8% BlueLeaf Biochar	2,4 DDD	38.4
130129	B	2.8% BlueLeaf Biochar	4,4 DDD	114
130129	B	2.8% BlueLeaf Biochar	2,4 DDT	469
130129	B	2.8% BlueLeaf Biochar	4,4 DDT	2070
130129	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**3750**
130129	C	2.8% BlueLeaf Biochar	2,4 DDE	10.9
130129	C	2.8% BlueLeaf Biochar	4,4 DDE	841
130129	C	2.8% BlueLeaf Biochar	2,4 DDD	30.2
130129	C	2.8% BlueLeaf Biochar	4,4 DDD	49.8
130129	C	2.8% BlueLeaf Biochar	2,4 DDT	477
130129	C	2.8% BlueLeaf Biochar	4,4 DDT	1220
130129	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**2630**
130129	A	2.8% Burt's Biochar	2,4 DDE	62.1
130129	A	2.8% Burt's Biochar	4,4 DDE	6830
130129	A	2.8% Burt's Biochar	2,4 DDD	71.6
130129	A	2.8% Burt's Biochar	4,4 DDD	34.4
130129	A	2.8% Burt's Biochar	2,4 DDT	927
130129	A	2.8% Burt's Biochar	4,4 DDT	693
130129	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**8620**
130129	B	2.8% Burt's Biochar	2,4 DDE	71.5
130129	B	2.8% Burt's Biochar	4,4 DDE	1630
130129	B	2.8% Burt's Biochar	2,4 DDD	49.3
130129	B	2.8% Burt's Biochar	4,4 DDD	102
130129	B	2.8% Burt's Biochar	2,4 DDT	808
130129	B	2.8% Burt's Biochar	4,4 DDT	3470
130129	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**6130**

130129	C	2.8% Burt's Biochar	2,4 DDE	60.4
130129	C	2.8% Burt's Biochar	4,4 DDE	691
130129	C	2.8% Burt's Biochar	2,4 DDD	25.0
130129	C	2.8% Burt's Biochar	4,4 DDD	<1.0
130129	C	2.8% Burt's Biochar	2,4 DDT	291
130129	C	2.8% Burt's Biochar	4,4 DDT	382
130129	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**1450**

Table E-8 DDT Concentrations (ng/g) in Plant Root Tissue (Greenhouse Study)

Root				
	Greenhouse Study			
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
130129	A	Control	2,4 DDE	223
130129	A	Control	4,4 DDE	55800
130129	A	Control	2,4 DDD	912
130129	A	Control	4,4 DDD	1350
130129	A	Control	2,4 DDT	3200
130129	A	Control	4,4 DDT	12000
130129	***A Total***	***Control***	***∑DDT***	**73500**
130129	B	Control	2,4 DDE	237
130129	B	Control	4,4 DDE	58400
130129	B	Control	2,4 DDD	2140
130129	B	Control	4,4 DDD	3930
130129	B	Control	2,4 DDT	2870
130129	B	Control	4,4 DDT	8870
130129	***B Total***	***Control***	***∑DDT***	**76500**
130129	C	Control	2,4 DDE	224
130129	C	Control	4,4 DDE	58500
130129	C	Control	2,4 DDD	1360
130129	C	Control	4,4 DDD	2110
130129	C	Control	2,4 DDT	3210
130129	C	Control	4,4 DDT	13600
130129	***C Total***	***Control***	***∑DDT***	**79000**
130129	A	2.8% GAC	2,4 DDE	397
130129	A	2.8% GAC	4,4 DDE	69400
130129	A	2.8% GAC	2,4 DDD	2860
130129	A	2.8% GAC	4,4 DDD	4710
130129	A	2.8% GAC	2,4 DDT	12400

130129	A	2.8% GAC	4,4 DDT	34800
130129	***A Total***	***2.8% GAC***	***∑DDT***	**125000**
130129	B	2.8% GAC	2,4 DDE	361
130129	B	2.8% GAC	4,4 DDE	76700
130129	B	2.8% GAC	2,4 DDD	1880
130129	B	2.8% GAC	4,4 DDD	3690
130129	B	2.8% GAC	2,4 DDT	9790
130129	B	2.8% GAC	4,4 DDT	37480
130129	***B Total***	***2.8% GAC***	***∑DDT***	**130000**
130129	C	2.8% GAC	2,4 DDE	358
130129	C	2.8% GAC	4,4 DDE	74200
130129	C	2.8% GAC	2,4 DDD	2960
130129	C	2.8% GAC	4,4 DDD	5740
130129	C	2.8% GAC	2,4 DDT	7840
130129	C	2.8% GAC	4,4 DDT	23600
130129	***C Total***	***2.8% GAC***	***∑DDT***	**115000**
130129	A	2.8% BlueLeaf Biochar	2,4 DDE	279
130129	A	2.8% BlueLeaf Biochar	4,4 DDE	61500
130129	A	2.8% BlueLeaf Biochar	2,4 DDD	3420
130129	A	2.8% BlueLeaf Biochar	4,4 DDD	5980
130129	A	2.8% BlueLeaf Biochar	2,4 DDT	4580
130129	A	2.8% BlueLeaf Biochar	4,4 DDT	10900
130129	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**86600**
130129	B	2.8% BlueLeaf Biochar	2,4 DDE	347
130129	B	2.8% BlueLeaf Biochar	4,4 DDE	57100
130129	B	2.8% BlueLeaf Biochar	2,4 DDD	2280
130129	B	2.8% BlueLeaf Biochar	4,4 DDD	4390
130129	B	2.8% BlueLeaf Biochar	2,4 DDT	7370
130129	B	2.8% BlueLeaf Biochar	4,4 DDT	24200
130129	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**95700**
130129	C	2.8% BlueLeaf Biochar	2,4 DDE	307
130129	C	2.8% BlueLeaf Biochar	4,4 DDE	55100
130129	C	2.8% BlueLeaf Biochar	2,4 DDD	2160
130129	C	2.8% BlueLeaf Biochar	4,4 DDD	3700
130129	C	2.8% BlueLeaf Biochar	2,4 DDT	5340
130129	C	2.8% BlueLeaf Biochar	4,4 DDT	15600
130129	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**82300**
130129	A	2.8% Burt's Biochar	2,4 DDE	284
130129	A	2.8% Burt's Biochar	4,4 DDE	62500
130129	A	2.8% Burt's Biochar	2,4 DDD	1410

130129	A	2.8% Burt's Biochar	4,4 DDD	3130
130129	A	2.8% Burt's Biochar	2,4 DDT	7290
130129	A	2.8% Burt's Biochar	4,4 DDT	29500
130129	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**104000**
130129	B	2.8% Burt's Biochar	2,4 DDE	293
130129	B	2.8% Burt's Biochar	4,4 DDE	63400
130129	B	2.8% Burt's Biochar	2,4 DDD	1990
130129	B	2.8% Burt's Biochar	4,4 DDD	3670
130129	B	2.8% Burt's Biochar	2,4 DDT	5450
130129	B	2.8% Burt's Biochar	4,4 DDT	16300
130129	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**91100**
130129	C	2.8% Burt's Biochar	2,4 DDE	298
130129	C	2.8% Burt's Biochar	4,4 DDE	69800
130129	C	2.8% Burt's Biochar	2,4 DDD	2170
130129	C	2.8% Burt's Biochar	4,4 DDD	3860
130129	C	2.8% Burt's Biochar	2,4 DDT	5550
130129	C	2.8% Burt's Biochar	4,4 DDT	15400
130129	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**97100**

Table E-9 DDT Concentrations (ng/g) in Worm Tissue

Worm				
Point Pelee National Park 2013				
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
14909	A	Control	2,4 DDE	959
14909	A	Control	4,4 DDE	39800
14909	A	Control	2,4 DDD	20200
14909	A	Control	4,4 DDD	25800
14909	A	Control	2,4 DDT	24600
14909	A	Control	4,4 DDT	149000
14909	***A Total***	***Control***	***∑DDT***	**619000**
14909	B	Control	2,4 DDE	755
14909	B	Control	4,4 DDE	284000
14909	B	Control	2,4 DDD	18500
14909	B	Control	4,4 DDD	18400
14909	B	Control	2,4 DDT	13400
14909	B	Control	4,4 DDT	76900
14909	***B Total***	***Control***	***∑DDT***	**41200**
14909	C	Control	2,4 DDE	1030
14909	C	Control	4,4 DDE	334000

14909	C	Control	2,4 DDD	20900
14909	C	Control	4,4 DDD	22900
14909	C	Control	2,4 DDT	20300
14909	C	Control	4,4 DDT	123000
14909	***C Total***	***Control***	***∑DDT***	**522000**
14909	A	2.8% GAC	2,4 DDE	909
14909	A	2.8% GAC	4,4 DDE	195000
14909	A	2.8% GAC	2,4 DDD	77100
14909	A	2.8% GAC	4,4 DDD	14700
14909	A	2.8% GAC	2,4 DDT	12300
14909	A	2.8% GAC	4,4 DDT	85800
14909	***A Total***	***2.8% GAC***	***∑DDT***	**38600**
14909	B	2.8% GAC	2,4 DDE	577
14909	B	2.8% GAC	4,4 DDE	214000
14909	B	2.8% GAC	2,4 DDD	15800
14909	B	2.8% GAC	4,4 DDD	14900
14909	B	2.8% GAC	2,4 DDT	11100
14909	B	2.8% GAC	4,4 DDT	65400
14909	***B Total***	***2.8% GAC***	***∑DDT***	**321000**
14909	C	2.8% GAC	2,4 DDE	666
14909	C	2.8% GAC	4,4 DDE	253000
14909	C	2.8% GAC	2,4 DDD	21600
14909	C	2.8% GAC	4,4 DDD	16100
14909	C	2.8% GAC	2,4 DDT	15600
14909	C	2.8% GAC	4,4 DDT	96200
14909	***C Total***	***2.8% GAC***	***∑DDT***	**40300**
14909	A	2.8% BlueLeaf Biochar	2,4 DDE	577
14909	A	2.8% BlueLeaf Biochar	4,4 DDE	214000
14909	A	2.8% BlueLeaf Biochar	2,4 DDD	16000
14909	A	2.8% BlueLeaf Biochar	4,4 DDD	18400
14909	A	2.8% BlueLeaf Biochar	2,4 DDT	12600
14909	A	2.8% BlueLeaf Biochar	4,4 DDT	73100
14909	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**335000**
14909	B	2.8% BlueLeaf Biochar	2,4 DDE	570
14909	B	2.8% BlueLeaf Biochar	4,4 DDE	211000
14909	B	2.8% BlueLeaf Biochar	2,4 DDD	14100
14909	B	2.8% BlueLeaf Biochar	4,4 DDD	16000
14909	B	2.8% BlueLeaf Biochar	2,4 DDT	15000
14909	B	2.8% BlueLeaf Biochar	4,4 DDT	86300
14909	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**34200**
14909	C	2.8% BlueLeaf Biochar	2,4 DDE	500
14909	C	2.8% BlueLeaf Biochar	4,4 DDE	192000

14909	C	2.8% BlueLeaf Biochar	2,4 DDD	13200
14909	C	2.8% BlueLeaf Biochar	4,4 DDD	16300
14909	C	2.8% BlueLeaf Biochar	2,4 DDT	14200
14909	C	2.8% BlueLeaf Biochar	4,4 DDT	82700
14909	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**319000**
14909	A	2.8% Burt's Biochar	2,4 DDE	709
14909	A	2.8% Burt's Biochar	4,4 DDE	203000
14909	A	2.8% Burt's Biochar	2,4 DDD	16800
14909	A	2.8% Burt's Biochar	4,4 DDD	16600
14909	A	2.8% Burt's Biochar	2,4 DDT	15600
14909	A	2.8% Burt's Biochar	4,4 DDT	92500
14909	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**34500**
14909	B	2.8% Burt's Biochar	2,4 DDE	368
14909	B	2.8% Burt's Biochar	4,4 DDE	125000
14909	B	2.8% Burt's Biochar	2,4 DDD	9910
14909	B	2.8% Burt's Biochar	4,4 DDD	9380
14909	B	2.8% Burt's Biochar	2,4 DDT	8360
14909	B	2.8% Burt's Biochar	4,4 DDT	49700
14909	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**20200**
14909	C	2.8% Burt's Biochar	2,4 DDE	508
14909	C	2.8% Burt's Biochar	4,4 DDE	149000
14909	C	2.8% Burt's Biochar	2,4 DDD	12900
14909	C	2.8% Burt's Biochar	4,4 DDD	10900
14909	C	2.8% Burt's Biochar	2,4 DDT	10100
14909	C	2.8% Burt's Biochar	4,4 DDT	58800
14909	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**24300**

Table E-10 DDT Concentrations (ng/g) in Polyoxymethylene

POM				
Point Pelee National Park 2013				
Data File (ASU)	Rep	Treatment	Compound	Concentration (ng/g)
120224	A	Control	2,4 DDE	379
120224	A	Control	4,4 DDE	52900
120224	A	Control	2,4 DDD	1290
120224	A	Control	4,4 DDD	1230
120224	A	Control	2,4 DDT	5020
120224	A	Control	4,4 DDT	25800
120224	***A Total***	***Control***	***∑DDT***	**86600**
120224	B	Control	2,4 DDE	390
120224	B	Control	4,4 DDE	55500
120224	B	Control	2,4 DDD	1480
120224	B	Control	4,4 DDD	1410
120224	B	Control	2,4 DDT	5680
120224	B	Control	4,4 DDT	26200
120224	***B Total***	***Control***	***∑DDT***	**90700**
120224	C	Control	2,4 DDE	381
120224	C	Control	4,4 DDE	43600
120224	C	Control	2,4 DDD	1290
120224	C	Control	4,4 DDD	1610
120224	C	Control	2,4 DDT	4200
120224	C	Control	4,4 DDT	22200
120224	***C Total***	***Control***	***∑DDT***	**73300**
120224	A	2.8% GAC	2,4 DDE	225
120224	A	2.8% GAC	4,4 DDE	34000
120224	A	2.8% GAC	2,4 DDD	1010
120224	A	2.8% GAC	4,4 DDD	1120
120224	A	2.8% GAC	2,4 DDT	3560
120224	A	2.8% GAC	4,4 DDT	21900
120224	***A Total***	***2.8% GAC***	***∑DDT***	**61800**
120224	B	2.8% GAC	2,4 DDE	279
120224	B	2.8% GAC	4,4 DDE	30500
120224	B	2.8% GAC	2,4 DDD	920
120224	B	2.8% GAC	4,4 DDD	850
120224	B	2.8% GAC	2,4 DDT	3350
120224	B	2.8% GAC	4,4 DDT	18000
120224	***B Total***	***2.8% GAC***	***∑DDT***	**53900**
120224	C	2.8% GAC	2,4 DDE	280
120224	C	2.8% GAC	4,4 DDE	37900
120224	C	2.8% GAC	2,4 DDD	1100
120224	C	2.8% GAC	4,4 DDD	1240

120224	C	2.8% GAC	2,4 DDT	4030
120224	C	2.8% GAC	4,4 DDT	22000
120224	***C Total***	***2.8% GAC***	***∑DDT***	**66600**
120224	A	2.8% BlueLeaf Biochar	2,4 DDE	246
120224	A	2.8% BlueLeaf Biochar	4,4 DDE	36600
120224	A	2.8% BlueLeaf Biochar	2,4 DDD	910
120224	A	2.8% BlueLeaf Biochar	4,4 DDD	1360
120224	A	2.8% BlueLeaf Biochar	2,4 DDT	3050
120224	A	2.8% BlueLeaf Biochar	4,4 DDT	17800
120224	***A Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**59900**
120224	B	2.8% BlueLeaf Biochar	2,4 DDE	248
120224	B	2.8% BlueLeaf Biochar	4,4 DDE	37200
120224	B	2.8% BlueLeaf Biochar	2,4 DDD	994
120224	B	2.8% BlueLeaf Biochar	4,4 DDD	1230
120224	B	2.8% BlueLeaf Biochar	2,4 DDT	3700
120224	B	2.8% BlueLeaf Biochar	4,4 DDT	20000
120224	***B Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**63300**
120224	C	2.8% BlueLeaf Biochar	2,4 DDE	215
120224	C	2.8% BlueLeaf Biochar	4,4 DDE	29500
120224	C	2.8% BlueLeaf Biochar	2,4 DDD	822
120224	C	2.8% BlueLeaf Biochar	4,4 DDD	1350
120224	C	2.8% BlueLeaf Biochar	2,4 DDT	2560
120224	C	2.8% BlueLeaf Biochar	4,4 DDT	15500
120224	***C Total***	***2.8% BlueLeaf Biochar***	***∑DDT***	**49900**
120224	A	2.8% Burt's Biochar	2,4 DDE	291
120224	A	2.8% Burt's Biochar	4,4 DDE	38600
120224	A	2.8% Burt's Biochar	2,4 DDD	1000
120224	A	2.8% Burt's Biochar	4,4 DDD	1300
120224	A	2.8% Burt's Biochar	2,4 DDT	3510
120224	A	2.8% Burt's Biochar	4,4 DDT	19100
120224	***A Total***	***2.8% Burt's Biochar***	***∑DDT***	**63800**
120224	B	2.8% Burt's Biochar	2,4 DDE	311
120224	B	2.8% Burt's Biochar	4,4 DDE	45200
120224	B	2.8% Burt's Biochar	2,4 DDD	1160
120224	B	2.8% Burt's Biochar	4,4 DDD	1630
120224	B	2.8% Burt's Biochar	2,4 DDT	3860
120224	B	2.8% Burt's Biochar	4,4 DDT	22700
120224	***B Total***	***2.8% Burt's Biochar***	***∑DDT***	**74800**
120224	C	2.8% Burt's Biochar	2,4 DDE	309
120224	C	2.8% Burt's Biochar	4,4 DDE	39700
120224	C	2.8% Burt's Biochar	2,4 DDD	1040
120224	C	2.8% Burt's Biochar	4,4 DDD	1100

120224	C	2.8% Burt's Biochar	2,4 DDT	3250
120224	C	2.8% Burt's Biochar	4,4 DDT	18400
120224	***C Total***	***2.8% Burt's Biochar***	***∑DDT***	**63900**

Table E-11 DDT Concentration (ng/g) in Soils

Soils							
Concentration (ng/g)							
	2012 (Anders Field)			2013 (Former Ag Site 6)			
Data File: 14522				*Data File: 14613*			
	AF-A	AF-B	AF-C	GAC-MD	Blueleaf-MD	Burts-MD	Control-MD
2,4 DDE	13.6	12.3	14.4	192	74.4	172	131
4,4 DDE	1760	1800	1800	30900	16200	26900	21500
2,4 DDD	34.6	29.6	34.1	496	122	400	145
4,4 DDD	43.3	41.3	43.5	575	121	364	127
2,4 DDT	94.9	97.6	94.4	3180	1040	2880	1920
4,4 DDT	498	498	509	20600	5750	13700	7110
Total	**2440**	**2470**	**2500**	**55900**	**23300**	**44400**	**30900**

Table E-12 QA/QC Chapter 7

Data File	[DDT] Spike (ng/g)	% of Target	[DDT] Blank (ng g^{-1})	RSD (%)	Average Efficiency (%)	Matrix
14522	96.25	96.25	1.00	21.69	104.41	Soils (2012)
14402	310.02	103.34	< 1.0	11.35	98.58	Soil (2013)
131022	67.57	112.62	< 1.0	5.14	92.55	Shoot (Harvest 1-2013)
131106	67.31	112.18	< 1.0	0.65	97.67	Shoot (Harvest 1-2013)
131028	56.76	94.60	< 1.0	3.44	96.70	Shoot (Harvest 1-2013)
131029	62.82	104.70	< 1.0	0.55	93.80	Shoot (Harvest 1-2013)
131107	60.36	100.60	< 1.0	10.04	94.92	Root (Harvest 1-2013)
131107	55.96	93.27	< 1.0	0.08	90.14	Root (Harvest 1-2013)
131217	64.21	107.02	< 1.0	10.91	92.07	Shoot (Harvest 2-2013)
131217	64.21	107.02	< 1.0	2.45	92.36	Shoot (Harvest 2-2013)
131224	61.79	102.98	< 1.0	4.51	102.01	Root (Harvest 2-2013)
131230	67.45	112.42	< 1.0	6.80	100.75	Root (Harvest 2-2013)
14909	67.00	111.67	< 1.0	20.20	75.50	Worm
14909	67.00	111.67	< 1.0	14.11	101.30	Worm
130117	61.32	102.20	< 1.0	2.02	88.21	Shoot (2012)
130121	63.49	105.82	< 1.0	8.15	99.70	Shoot (2012)
130121	62.05	103.42	< 1.0	4.62	89.97	Shoot (2012)
130125	62.49	104.15	< 1.0	1.87	96.34	Shoot (2012)
130129	63.63	106.05	< 1.0	0.03	94.02	Root (2012)
130125	64.15	106.92	< 1.0	1.36	94.93	Root (2012)
140220	65.55	109.25	< 1.0	-	78.06	POM
140224	62.68	104.47	< 1.0	-	96.10	POM
Average	*63.94*	*106.56*		*6.55*	*91.41*	

Table E-13 Plant Harvest Data (Field Study)

		Harvest 1			Harvest 2		
		Shoot		Root	Shoot		Root
Treatment	REP	Weight (g)	Length (cm)	Weight (g)	Weight (g)	Length (cm)	Weight (g)
Control	A	299.6	145	13.4	14.1	21	2.2
Control	B	388.1	151	14.8	41.6	34	14.5
Control	C	218.4	103	7.1	37.3	41	0.4
2.8% GAC	A	113.8	79	5.8	6.7	39	0.4
2.8% GAC	B	77.6	70	4.6	12.4	35	2.5
2.8% GAC	C	99.7	93	4	5.1	21	1.4
2.8% Burts	A	182.8	107	7.1	31.9	49	5.1
2.8% Burts	B	203.4	113	7.2	52.3	50	3.2
2.8% Burts	C	164.4	108	6.8	28.5	35	8.1
2.8% BlueLeaf	A	202.5	77	11.9	282.5	206	13.2
2.8% BlueLeaf	B	133.7	72	6.3	127.5	96	10.1
2.8% BlueLeaf	C	636	223	15.5	30.4	45	4.9

Table E-14 Plant Harvest Data (Greenhouse)

		Greenhouse		
		Shoot		Root
Treatment	REP	Length (cm)	Weight (g)	Weight (g)
Control	A	88	27.9	1.2
Control	B	73	22.3	0.8
Control	C	98	28.3	0.8
2.8% GAC	A	83	23.1	0.7
2.8% GAC	B	89	20.2	1.2
2.8% GAC	C	95	30.5	1
2.8% Burts	A	85	33.5	1.3
2.8% Burts	B	55	20.2	0.7
2.8% Burts	C	54	16.5	0.9
2.8% BlueLeaf	A	94	28.4	1.2
2.8% BlueLeaf	B	72	23.8	0.5
2.8% BlueLeaf	C	84	25.8	0.8

Table E-15 Worm Harvest Data

	Invertebrates					
	Worms Toxicity Study 1			Worms Toxicity Study 2		
Treatment	Weight Initial (g)	Weight Final (g)	% Survival	Weight Initial (g)	Weight Final (g)	% Survival
Control	5.3349	6.0788	96	3.13	3.1788	100
Control	5.3404	6.2012	100	2.75	2.8807	100
Control	6.5407	6.9299	104	2.97	2.6989	85
2.8 % GAC	5.9472	3.7601	96	2.96	2.4151	100
2.8 % GAC	6.2492	4.4809	112	3.54	2.21996	95
2.8 % GAC	5.7963	3.7467	96	3.19	1.8647	80
2.8% Burts	5.6823	6.8223	100	3.07	2.9607	100
2.8% Burts	6.677	5.1725	108	3.04	3.2832	100
2.8% Burts	4.2598	6.1343	100	2.58	2.6678	100
2.8% BlueLeaf	6.3918	6.5336	108	2.85	3.7664	100
2.8% BlueLeaf	5.7498	7.1771	100	2.82	2.2979	100
2.8% BlueLeaf	5.7991	6.0406	108	2.74	2.8419	100

Table E-16 Worm Avoidance Study

Invertebrate Avoidance Studies			
		Number of Worms	
Amendment	Wheel	Control	Amended
2.8% GAC	A	7	3
	B	10	0
	C	9	2
2/8% Burt's Biochar	D	3	7
	E	6	4
	F	3	7
2.8% BlueLeaf Biochar	A	7	3
	B	9	1
	C	8	2

Table E-17 Particle Size Distribution

Particle Size Distribution							
Treatment	**Sieve Number**	**Sieve Opening**	**Mass of Empty Sieve**	**Mass of Sieve + Soil**	**Sample Mass in Sieve**	**Retained**	**% Retained**
GAC A	4	4.7	536.00	536.01	0.01	0.00	0.02
	10	2	471.66	471.69	0.03	0.00	0.05
	18	1	411.95	442.58	30.63	0.51	50.66
	35	0.5	398.60	426.23	27.63	0.46	45.70
	60	0.25	364.97	366.78	1.81	0.03	2.99
	100	0.15	361.30	361.40	0.10	0.00	0.17
	200	0.0075	354.40	354.48	0.08	0.00	0.13
	pan		343.04	343.21	0.17	0.00	0.28
GAC B	4	4.7	535.99	536.00	0.01	0.00	0.02
	10	2	471.65	471.67	0.02	0.00	0.03
	18	1	411.95	443.66	31.71	0.53	53.00
	35	0.5	398.60	424.69	26.09	0.44	43.61
	60	0.25	364.97	366.55	1.58	0.03	2.64
	100	0.15	361.30	361.46	0.16	0.00	0.27
	200	0.0075	354.39	354.48	0.09	0.00	0.15
	pan		343.05	343.22	0.17	0.00	0.28
GAC C	4	4.7	535.98	535.99	0.01	0.00	0.02
	10	2	471.65	471.68	0.03	0.00	0.05
	18	1	411.93	444.65	32.72	0.54	54.15
	35	0.5	398.58	424.60	26.02	0.43	43.06
	60	0.25	364.96	366.40	1.44	0.02	2.38
	100	0.15	361.30	361.35	0.05	0.00	0.08
	200	0.0075	354.39	354.44	0.05	0.00	0.08
	pan		343.06	343.17	0.11	0.00	0.18
BlueLeaf Biochar A	4	4.70	536.02	545.27	9.25	0.15	15.39
	10	2.00	471.77	482.84	11.07	0.18	18.42
	18	1.00	412.04	421.79	9.75	0.16	16.22
	35	0.500	398.68	406.18	7.50	0.12	12.48
	60	0.250	364.99	369.34	4.35	0.07	7.24
	100	0.150	361.31	363.86	2.55	0.04	4.24
	200	0.0075	354.39	356.67	2.28	0.04	3.79
	pan		343.05	356.40	13.35	0.22	22.21
BlueLeaf Biochar B	4	4.7	536.03	545.58	9.55	0.16	15.88
	10	2	471.69	483.01	11.32	0.19	18.83
	18	1	411.96	421.58	9.62	0.16	16.00
	35	0.5	398.63	406.15	7.52	0.13	12.51
	60	0.25	364.97	369.22	4.25	0.07	7.07

	100	0.15	361.31	363.73	2.42	0.04	4.03
	200	0.0075	354.41	356.52	2.11	0.04	3.51
	pan		343.06	356.39	13.33	0.22	22.17
BlueLeaf Biochar C	4	4.7	536.01	543.63	7.62	0.13	12.73
	10	2	471.66	482.85	11.19	0.19	18.70
	18	1	411.98	421.85	9.87	0.16	16.49
	35	0.5	398.63	406.41	7.78	0.13	13.00
	60	0.25	364.99	369.31	4.32	0.07	7.22
	100	0.15	361.31	363.68	2.37	0.04	3.96
	200	0.0075	354.41	356.65	2.24	0.04	3.74
	pan		343.08	357.54	14.46	0.24	24.16
Burt's Biochar A	4	4.7	535.93	539.38	3.45	0.05	5.48
	10	2	471.67	486.84	15.17	0.24	24.11
	18	1	412.00	426.43	14.43	0.23	22.93
	35	0.5	398.74	412.80	14.06	0.22	22.34
	60	0.25	364.97	377.51	12.54	0.20	19.93
	100	0.15	361.27	364.45	3.18	0.05	5.05
	200	0.0075	354.36	354.41	0.05	0.00	0.08
	pan		343.02	343.07	0.05	0.00	0.08
Burt's Biochar B	4	4.7	536.03	540.69	4.66	0.08	7.76
	10	2	471.78	486.79	15.01	0.25	25.00
	18	1	412.08	426.56	14.48	0.24	24.11
	35	0.5	398.79	415.04	16.25	0.27	27.06
	60	0.25	364.99	374.55	9.56	0.16	15.92
	100	0.15	361.31	361.37	0.06	0.00	0.10
	200	0.0075	354.42	354.43	0.01	0.00	0.02
	pan		343.07	343.09	0.02	0.00	0.03
Burt's Biochar C	4	4.7	536.03	538.15	2.12	0.04	3.54
	10	2	471.78	486.02	14.24	0.24	23.80
	18	1	412.05	429.94	17.89	0.30	29.90
	35	0.5	398.70	414.29	15.59	0.26	26.05
	60	0.25	365.00	374.82	9.82	0.16	16.41
	100	0.15	361.31	361.47	0.16	0.00	0.27
	200	0.0075	354.41	354.42	0.01	0.00	0.02
	pan		343.07	343.08	0.01	0.00	0.02

APPENDIX E: SUPPORTING INFORMATION FOR CHAPTER 7

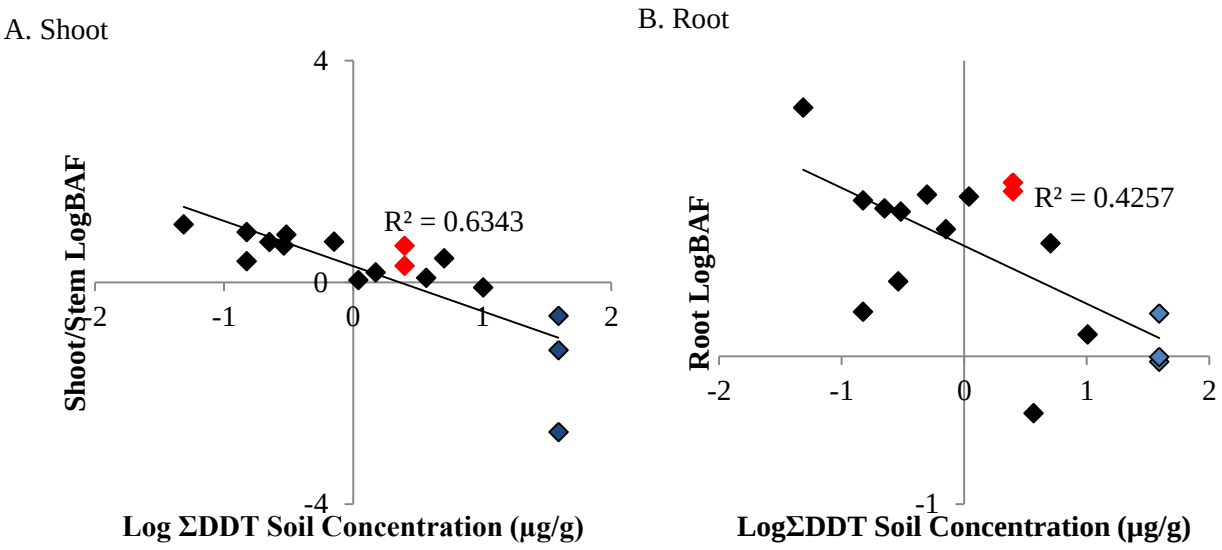

Figure E.10.1 Literature reported Log ΣDDT bioaccumulation factors (BAFs) and Log BAFs from the current study in *Cucurbita pepo* spp. *pepo* shoot/stem (a) and root tissues (b) soils with various concentrations of DDT (µg/g) and its metabolites (n=18) (7, 48, 151, 244, 246, 247, 250-252). The red and blue markers indicate the BAFs from the current study from soil contaminated with 2.5 µg/g and 39 µg/g ΣDDT, respectively.