REMEDIATION STRATEGIES FOR DICHLORODIPHENYLTRICHLOROETHANE AND DIELDRIN CONTAMINATED SOILS AT POINT PELEE NATIONAL PARK

STRATÉGIES DE REMÉDIATION DES SOLS CONTAMINÉS PAR LE DICHLORODIPHÉNYLTRICHLOROÉTHANE ET PAR LE DIELDRINE DANS LE PARC NATIONAL DE LA POINTE-PELÉE

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ABSTRACT

Organochlorine pesticides have been recognized as contaminants of concern due to their bioaccumulation, recalcitrance to degradation, and potential toxicity to humans and wildlife. Point Pelee National Park (PPNP), in Leamington, ON, is heavily contaminated with organochlorine pesticides (dichlorodiphenyltrichloroethane (DDT) and dieldrin) due to their substantial use during the 1950s and 1960s when the park was used as an orchard. Conventional treatments for organochlorine contaminated soils (e.g. excavation and incineration) are effective, but they can negatively affect sensitive ecosystems. Three different remediation strategies (phytoextraction, hydroxypropyl-β-cyclodextrin, and zero-valent iron) for dealing with organochlorine pesticide contamination at PPNP were investigated in this thesis. Two native grasses (*Panicum virgatum* and *Schizachyrium scoparium*), demonstrated great potential for phytoextraction, taking up significant amounts of DDT and dieldrin *in-situ* only five months after plot establishment. Hydroxypropyl-β-Cyclodextrin (HPβCD), a surfactant, forms water soluble complexes with low-polarity organic compounds, like DDT. Microbial studies using HPβCD showed a significant increase in DDT water solubility, a significant decrease in [ΣDDT] in soils, and no significant increase in overall microbial activity. Greenhouse experiments, using a combined approach of phytoextraction with HPβCD showed a significant increase in [ΣDDT] in *S. scoparium* shoots, however, they also suggested DDT mobilization, with higher [ΣDDT] found in the bottom section of unplanted soil treated with HPβCD. Hence, the *in-situ* application of HPβCD at PPNP is not recommended as an increase in DDT mobilization at the site is likely to cause groundwater contamination. Laboratory and field studies showed that the application of DARAMEND and EHC (zero-valent iron technology) to PPNP soils contaminated with organochlorine pesticides did not improve the degradation of DDT or dieldrin. The most suitable remediation strategy for PPNP was found to be phytoextraction by native grasses, as they will not negatively impact the sensitive ecosystem at PPNP or introduce invasive species to the park.

RÉSUMÉ

Les pesticides organochlorés ont été reconnus comme des contaminants préoccupants en raison de leur bioaccumulation, de leur récalcitabilité à la dégradation et de leur toxicité potentielle pour les humains et la faune. Le parc national de la Pointe-Pelée (PNPP), à Leamington, en Ontario, est fortement contaminé par les pesticides organochlorés (dichlorodiphényltrichloroéthane (DDT) et dieldrine) en raison de leur utilisation importante dans les années 1950 et 1960, lorsque le parc a été utilisé comme verger. Les traitements conventionnels pour les sols contaminés par des composés organochlorés (par exemple l'excavation et l'incinération) sont efficaces, mais ils peuvent affecter négativement les écosystèmes sensibles. Dans cette thèse, trois stratégies d'assainissement différentes (phytoextraction, hydroxypropyl-β-cyclodextrine, et fer zéro-valent) pour traiter la contamination par les pesticides organochlorés à PNPP ont été étudiées. Deux graminées indigènes (*Panicum virgatum* et *Schizachyrium scoparium*) ont démontré un grand potentiel de phytoextraction, prenant des quantités significatives de DDT et de dieldrine *in situ* seulement cinq mois après l'établissement de la parcelle. L'hydroxypropyl-β-cyclodextrine (HPβCD), un tensioactif, forme des complexes hydrosolubles avec des composés organiques à faible polarité, comme le DDT. Les études microbiennes avec la HPβCD ont montré une augmentation significative de la solubilité dans l'eau du DDT, une diminution significative de la [ΣDDT] dans les sols et aucune augmentation significative de l'activité microbienne globale. Les expériences en serre, en utilisant une approche combinée de la phytoextraction avec la HPβCD, ont montré une augmentation significative de la [ΣDDT] dans les pousses de *S. scoparium*, cependant elles ont également suggéré une mobilisation du DDT, avec une [ΣDDT] plus élevé dans la partie inférieure du sol non implanté traité par HPβCD. Par conséquent, l'application *in situ* de HPβCD au PNPP n'est pas recommandée car une augmentation de la mobilisation du DDT sur le site est susceptible de provoquer une contamination des eaux souterraines. Des études de laboratoire et sur le terrain ont montré que l'application de DARAMEND et EHC (technologie de fer à zéro-valeur) aux sols PNPP contaminés par des pesticides organochlorés n'a pas amélioré la dégradation du DDT ou de la dieldrine. La stratégie d'assainissement la plus appropriée pour le PNPP s'est révélée être la phytoextraction par des graminées indigènes, car elles n'auront ni d'impact négatif sur l'écosystème sensible du PNPP ni n'introduiront d'espèces envahissantes dans le parc.

CO-AUTORSHIP STATEMENT

The student's contribution to the thesis manuscript are as follow:

- Active participant in the initial development of research ideas and projects.
- Primary researcher responsible for the successful implementation and completion of laboratory and greenhouse experiments conduct at the Royal Military College of Canada (RMC, Kingston, ON), and field experiments conducted at Point Pelee National Park (PPNP, Leamington, ON), as well as analytical work completed at both the Analytical Service Unit at Queen's University (Kingston, ON) and at the RMC.
- Primary author on all three research papers.

Chapter 3: Dahmer CP, Rutter A, Zeeb B. *In-situ* Phytoextraction of DDT and Dieldrin by Native Grasses at Point Pelee National Park.

Chapter 4: Dahmer CP, Rutter A, Zeeb B. The Role of Hydroxypropyl-β-Cyclodextrin (HPβCD) in DDT Remediation at Point Pelee National Park. (In prep for submission to the International Journal of Phytoremediation).

Chapter 5: Dahmer CP, Rutter A, Zeeb B. The Use of Zero-Valent Iron (ZVI) Technology to Promote DDT and Dieldrin Degradation at Point Pelee National Park. (In prep for submission to the Remediation Journal).

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ANOVA	Analysis of Variance
ASU	Analytical Services Unit
BAF	Bioaccumulation Factor
βCD	β-cyclodextrin
CCME	Canadian Council of Ministers of Environment
CDs	Cyclodextrins
cm	centimeter
CMβCD	Carboxymethyl-β-Cyclodextrin
DAPI	4',6-diamidino-2-phenylindole
DCBP	Decachlorobiphenyl
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
GC/ECD	Gas Chromatography with Electron Capture Detection
HPβCD	Hydroxypropyl-β-Cyclodextrin
ISQG	Interim Sediment Quality Guidelines
K_{cw}	HPβCD-water partition coefficient
K_{ow}	Partition Coefficient
L	Liter
LD_{50}	Median Lethal Dose
m	meter
m^2	square meters (area)
mg/g	milligrams per gram
mg/kg	milligrams per kilogram
mg/L	micrograms per litre
mL	milliliter
ng	nanogram
ng/g	nanograms per gram
ng/L	nanograms per litre
nm^3	cubic nanometers (volume)
OMOE	Ontario Ministry of the Environment
PAHs	Polycyclic Aromatic Hydrocarbons
PEL	Probable Effect Levels
POPs	Persistent Organic Pollutants
ppm	Parts Per Million
PPNP	Point Pelee National Park

PV	Pore Volume
RMC	Royal Military College of Canada
TF	Translocation Factor
µg/g	micrograms per gram
µL	microliter
ΣDDT	DDT, DDE, and DDD

1. General Introduction

Dichlorodiphenyltrichloroethane (DDT) was the first synthetic insecticide developed in history, and it was marketed all over the world (Fiedler 2000; Baird & Cann 2012). Dieldrin is another organochlorine insecticide which is similar to DDT. Organochlorine pesticides, like DDT and dieldrin, have been recognized as potential health risks due to their bioaccumulation, recalcitrance to degradation, and potential toxicity to humans and wildlife (Corona-Cruz *et al.* 1999; Jorgenson 2001; Fiedler 2000; Baird & Cann 2012). Moreover, DDT and its metabolites, and dieldrin are known endocrine disruptors (Jorgenson 2001; Yang *et al.* 2010; Sudharshan *et al.* 2012). The use of DDT and dieldrin has been prohibited in most countries since the 1970s, and these two pesticides are amongst the 21 persistent organic pollutants (POPs) that require immediate phasing out, according to the 2010 Stockholm convention (Sudharshan *et al.* 2012).

Even after being banned or at least restricted in many countries, DDT and dieldrin are still present in the environment today. DDT is especially common in soils, in part because of its strong adsorption to solid particles (Corona-Cruz *et al.* 1999; Sudharshan *et al.* 2012). Across Canada, localized areas have been contaminated with organochlorine pesticides due to their historical use. For example, Point Pelee National Park (PPNP) in southwestern Ontario, is heavily contaminated with organochlorine pesticides (principally DDT and its metabolites, and dieldrin) due to their substantial use during the 1950s and 1960s (Smits *et al.* 2005). As a result of PPNP's former use as orchard land, legacy DDT and dieldrin contamination exists at levels that far exceed the park land guideline of 0.7 mg/kg for DDT set by the Canadian Council of Ministers of the Environment (CCME), and the 0.05 mg/kg guideline for dieldrin set by the Ontario Ministry of the Environment (OMOE).

Conventional treatments for organochlorine contaminated soils include excavation and incineration, thermal desorption, microwave-enhanced thermal treatment, soil washing with surfactants, and supercritical fluid extraction (Vidali 2001; Yang *et al.* 2010). Although effective, these methods can negatively affect sensitive ecosystems, like the ones at PPNP. Therefore, there is demand for environmentally friendly remediation technologies that maintain the park's integrity during the remediation process.

Phytoextraction is a green technology that uses vascular plants *in-situ* to extract soil contaminants into plant roots and translocate them to the shoots, which are then harvested and transported to a facility where they can be incinerated or composted (McCutcheon & Schnoor 2003; Suresh & Ravishankar 2004). Phytoextraction of DDT and dieldrin was considered problematic for many years due to the highly hydrophobic nature of these pesticides. However, in 1994, Hülster published a breakthrough study that opened the possibility for phytoextraction use in soils contaminated with all kinds of POPs. The potential of *Cucurbita pepo* species for phytoextraction of DDT and its metabolites has been shown in several subsequent studies (White & Hite 2001; White 2002; Lunney *et al.* 2004; Whitfield Åslund *et al.* 2010). Although there have been fewer studies on phytoextraction of dieldrin, cucurbits have also attracted attention because of their high-level accumulation ability (Matsumoto *et al.* 2009). At PPNP, Paul *et al.* (2015) evaluated the potential for weed species to phytoextract DDT. Results showed that some weed species (including native grasses) could obtain better phytoextraction results per unit area than *Cucurbita* species. However, more research was needed in this field to find the best approach to DDT and dieldrin remediation *in-situ* using the studied species, or to discover alternate green remediation techniques.

Hydroxypropyl-β-Cyclodextrin (HPβCD), it is a substituent from the naturally occurring β-Cyclodextrin, which is a product of starch's microbial degradation. In HPβCD, hydroxyl groups exist on the interior cavity, and hydroxypropyl groups remain on the exterior shell, resulting in a very unique structure that creates a hydrophilic shell, and a hydrophobic cavity (Wang & Brusseau 1993; Del Valle 2004). HPβCD is not commonly used as a remediation tool, but its ability to form complexes with low-polarity organic compounds, like DDT, make it a possible candidate for use in *in-situ* remediation (Brusseau *et al.* 1994). Between 2002 and 2007, four McMaster University theses (Marenco 2002; Badley 2003; Mironov 2004; Etherington 2007) reported on the possibility of using HPβCD to remediate soils contaminated with DDT at PPNP. These studies had positive results suggesting that the application of HPβCD to DDT contaminated soils enhanced *in-situ* microbial degradation of this pesticide. However, none at this research was published in the peer review literature. Further research is required to more clearly define the role of HPβCD in the remediation of organic contaminants, in particular DDT.

In recent years, another environmentally friendly bioremediation technique has emerged, zero-valent iron (ZVI) technology. ZVI has been established as an effective reductant and catalyst for a wide variety of common soil and water contaminants including, halogenated organic compounds, polycyclic aromatic hydrocarbons, chlorinated pesticides, and heavy metals (Zhang 2003; Grieger *et al.* 2010). *In-situ* application of ZVI products favours the growth of anaerobic microorganisms, which could be beneficial for accelerating biodegradation of organic compounds, like DDT and dieldrin (Zhang 2003). A few laboratory studies (Yang *et al.* 2010; El-Temsah & Joner 2013; El-Temsah *et al.* 2013) evaluated the potential for ZVI to promote DDT degradation in soil. Although, some reported complete degradation of DDT without significantly increased of its metabolites in the treated soil (El-Temsah & Joner 2013), others reported significant increase of DDT metabolites following the ZVI treatment (Yang *et al.* 2010; El-Temsah *et al.* 2013). Further research is required to ascertain the role of ZVI in the remediation of soils co-contaminated with DDT and dieldrin, and determine the true efficacy of this technology.

This M.Sc. thesis explores three different remediation strategies for dealing with DDT (and dieldrin) contamination at Point Pelee National Park, taking into account PPNP's distinctive features, including the need to preserve its ecologically unique flora and fauna. Following, this brief introduction, chapter two provides a literature review of DDT and dieldrin toxicity and their presence in the environment, and a review of the three remediation techniques explored here (phytoextraction, HPβCD, and ZVI). In chapter three, the feasibility of using phytoextraction with native grasses to remediate DDT and dieldrin contaminated soil *in-situ* at PPNP is evaluated. In chapter four, the potential of employing HPβCD as a remediation tool for soils contaminated with DDT is investigated. HPβCD was tested as tool to enhanced DDT microbial degradation and to enhance phytoextraction by native grasses. This chapter also discusses the role of HPβCD in DDT bioavailability to invertebrates. In chapter five, the role of ZVI in DDT and dieldrin remediation is explored using both laboratory and *in-situ* studies. Chapter six includes a discussion of the major findings and conclusions of this thesis as well as directions for future research. Raw data and quality control are included in Appendices A-C.

2. Literature Review

2.1 PERSISTENT ORGANIC POLLUTANTS

Persistent organic pollutants (POPs) are a group of organic substances that are toxic, persistent, bioaccumulative, and prone to long-range transport (Lohmann *et al.* 2007). These pollutants become concentrated and move through the food chain, affecting animal reproduction, development, and immunological functions (Wania & Mackay 1996). POPs persist in the environment, and have long half-lives in soils, sediments, air, or biota. Moreover, POPs are extremely difficult to break down to less hazardous substances due to the presence of a very stable carbon-halogen bond (Fiedler 2000). POPs' long-range transport potential and harmful effects on humans and wildlife led to several international agreements intended to reduce future environmental burdens. In 1998, the *Aarhus Protocol on Persistent Organic Pollutants* was signed. This protocol focused on a list of 16 substances and was ratified by seventeen countries. The *Stockholm Convention on Persistent Organic Pollutants*, signed by 191 governments between 2001 and 2003, seeks to eliminate or control the use of *the dirty dozen* (Breivik *et al.* 2004). *The dirty dozen* is a group of organic pollutants that includes nine agrochemicals (aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, mirex, heptachlor, hexachlorobenzene, toxaphene), and three industrial substances (polychlorinated biphenyls, dioxin, and furans) (Jorgenson 2001).

2.2 ORGANOCHLORINE PESTICIDES

2.2.1 Dichlorodiphenyltrichloroethane (DDT)

Othmar Zeidler first synthesized dichlorodiphenyltrichloroethane (DDT) in Germany in the year 1874. However, the insecticidal properties of DDT were only discovered in 1939 by a chemist named Paul Müller that worked at the time in the Swiss firm, Geigy. When the pesticidal properties of DDT were first discovered, this chemical seemed to be an ideal insecticide. It had very low toxicity to humans, was highly toxic to insects, and was persistent. DDT has an acute oral median lethal dose (LD_{50}) of 113-450 mg/kg and is considered to be only moderately toxic to vertebrates (Baird & Cann 2012). In 1941, products containing DDT entered the market and started to be used to combat disease-carrying insects (Fiedler 2000; Baird & Cann 2012). After the Second World War, DDT was applied to a variety of agricultural crops. The largest agricultural use of DDT has been on cotton (Fiedler 2000), although it was also used to control insect pests on fruit trees, and vegetable crops.

In 1948, Müller received the Nobel Prize in Medicine and Physiology in recognition of the many civilian lives saved by DDT (Baird & Cann 2012).

The scientific community had some reservations with respect to the use of DDT, due to its persistence in soil for several years, and its possible accumulation in the food chain. General public awareness began in 1962, with the publication of Rachel Carson's *Silent Spring*. In this book, Carson discusses the decline in the American robin population due to the massive amounts of DDT used in some regions of the United States (Baird & Cann 2012). In the early 1970s, there was a growing concern about the adverse environmental effects of DDT, which led to the implementation of restrictions and bans of DDT in many developed countries. Today, due to health and environmental concerns, all use of DDT is banned in most countries. However, its use as a disease control vector is still permissible in certain countries as a better alternative has not yet become available (Fiedler 2000; Miniero & L'lamiceli 2008).

2.2.2 Dieldrin

Dieldrin is also an organochlorine pesticide that is known to cause serious environmental problems. It has been prohibited over the past decades in most countries around the world, but is still routinely found in the environment, especially in agricultural soils (Matsumoto *et al.* 2009). Aldrin and dieldrin are similar compounds made by a chemical process known as the Diels-Alder reaction, hence their names, and dieldrin is also the main persistent degradation product of aldrin (CCME 1999a; Jorgenson 2001). Dieldrin was first commercially manufactured in 1950, and the combined production of aldrin and dieldrin peaked in the mid-1960s with about 20 million pounds produced per year (Jorgenson 2001; Murano *et al.* 2010). In the United States aldrin/dieldrin ranked second, after DDT, among agricultural chemicals most used in the 1960s (Jorgenson 2001). Due to its high toxicity and long persistence in the environment, dieldrin has been prohibited in many countries since the 1970s (Matsumoto *et al.* 2009). In Canada, the registration and use of dieldrin under the *Pest Control Products Act* were discontinued as of January 1st, 1991. Dieldrin has also been identified as a Track 1 substance by Environment Canada because it is persistent, bioaccumulative, released primarily as a result of human activities, and considered "CEPA-toxic" under the Canadian Environmental Protection Act (CCME 1999a).

2.2.3 DDT & Dieldrin Chemistry

A DDT molecule is a substituted ethane. At one carbon, chlorine atoms replace all three hydrogens, and at the other, two of the three hydrogens are replaced by a benzene-like ring (containing a chlorine atom) (Baird & Cann 2012). DDT has two main metabolites, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD), all three compounds are found in two isomeric forms, 2,4- and 4,4- (Aislabie *et al.* 1997). DDT, DDE, and DDD are commonly referred as ΣDDT.

The pathway of DDT degradation by dechlorination is usually DDT → DDE → DDD. DDT → DDE formation can occur through photochemical reactions in the presence of sunlight, and also through dehydrochlorination of DDT by bacteria and animals. DDT can also be degraded directly from DDT to DDD (Corona-Cruz *et al.* 1999). DDD is formed by reductive dechlorination that can be microbially mediated, or occur as a result of chemical reactions (Aislabie *et al.* 1997). DDE is generally the major degradation product of DDT, and is the primary breakdown product of DDT in aerobic environments, while DDD is the main product in anaerobic or aquatic environments (Gautam & Suresh 2006). The proposed complete pathway for the anaerobic transformation of DDT by bacteria and fungi suggests that DDD is reductively dechlorinated to 2,2-bis(4-chlorophenyl)ethylene (DDNU), which is further oxidized to 2,2-bis(4-chlorophenyl)ethanol (DDOH) and bis(4-chlorophenyl)-acetic acid (DDA), the latter of which in turn is decarboxylated to bis(4-chlorophenyl)methane (DDM). In addition, DDM is oxidized to 4,4-dichlorobenzophenone (DBP), which is not further metabolized under anaerobic conditions. Alternating anaerobic and aerobic incubation conditions can enhance DDX biodegradation by promoting reductive dechlorination of DDT-DPB with subsequent aerobic aromatic ring cleavage (Figure 2.1) (Yang *et al.* 2010).

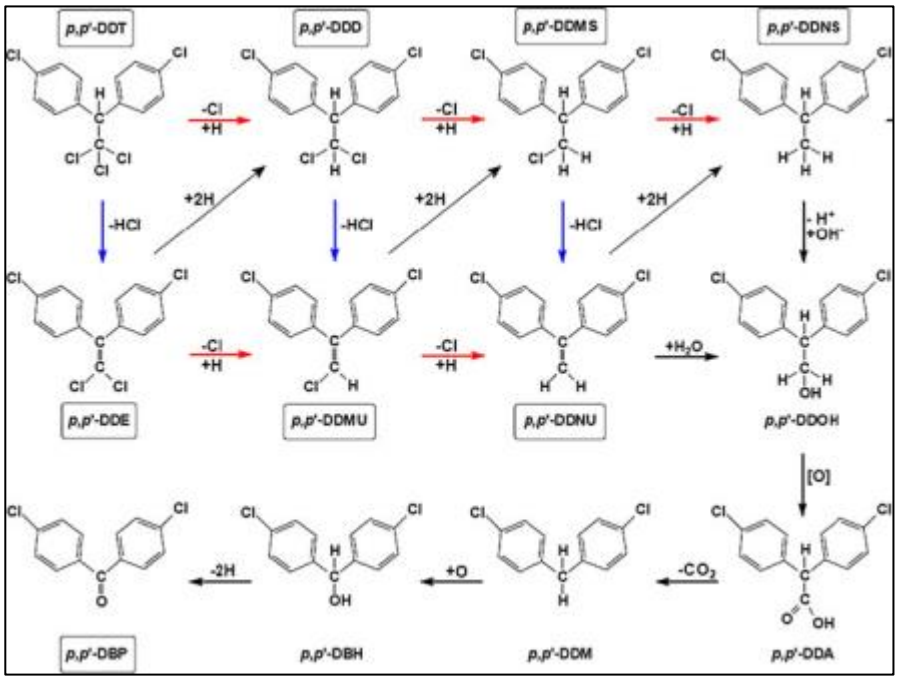

Figure 2.1. The potential pathways for microbial degradation of DDT. Blue arrows represent dehydrochlorination reactions, and red arrows represent reductive dechlorination reactions (from Sudharshan *et al.* 2012).

DDT, DDE, and DDD half-lives range from 2-15.6 years in soil, 17.7 hours to 7.4 days in air, 7 days to 1 year in surface waters, and 16 days to 31.3 years in groundwater (Howard *et al.* 1991). The half-life of DDT in soils can be affected by many factors including soil temperature, moisture content, and organic carbon content. There are reported DDT half-lives of up to 20 years in soils from the Netherlands, up to 30 years in Maine forest soils, up to 35 years in Maryland soils, and up to 40 years in Ontario soils (Crowe & Smith 2007; Clow *et al.* in press).

DDT has a high hydrophobicity with an octanol-water partition coefficient (K_{ow}) of 6.2. K_{ow} is defined by:

$$Kow = \frac{C\ octanol}{C\ water}$$

where $C_{octanol}$ represents the molar concentration of an organic compound in the octanol phase and C_{water} represents its molar concentration in the water phase at the

time of equilibrium. Because the K_{ow} value is often quite large it is usually written in base-10 logarithm (Hopkin *et al.* 2005; Baird & Cann 2012).

Dieldrin is a cyclodiene (Figure 2.2), and it was first formulated from a waste product of synthetic rubber, cyclopentadiene, by Julius Hyman in 1947 (Jorgenson 2001). This pesticide is also the main persistent degradation product of aldrin (CCME 1999a). Dieldrin (CAS number: 60-57-1) is a colourless crystalline compound, and technical grade dieldrin (95%) is a light-tan compound with a mild odor. This pesticide remains solid at ambient temperature with a melting point of 175-176ºC. Its vapor pressure is 0.4 mPa at 20ºC, and it has an identically high octanol-water partition coefficient (log K_{ow} = 6.2) as DDT (Matsumoto *et al.* 2009).

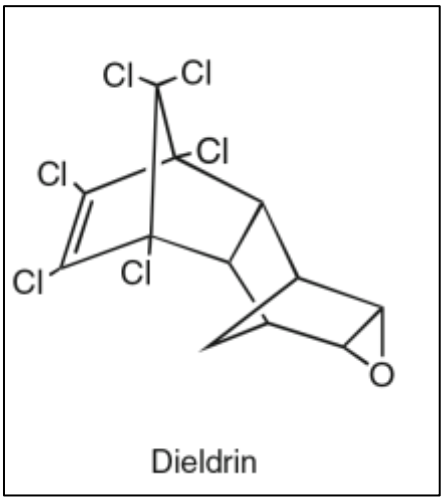

Figure 2.2. Chemical structure of dieldrin (Matsumoto *et al.* 2009).

The half-live of dieldrin in soil differ to some extent among reports (Donoso *et al.* 1979; Mcdougall & Wagga 1995; Meijer *et al.* 2001). Most have shown that this pesticide is highly persistent in soil, with a calculated half-life of about 25 years (Matsumoto *et al.* 2009). Moreover, dieldrin is hydrophobic, with an very low solubility in water (186 μg/L at 25-29ºC), and does not dissolve in the water passing through the soil, but is very soluble in fats, waxes, and oils (Jorgenson 2001).

2.2.4 DDT & Dieldrin in the Environment

DDT has been extensively used all over the world since World War II. Even after being banned or at least restricted in many countries, it is still universally found in the environment. DDT is especially common in soils, in part because of its strong adsorption to solid particles that result in great persistence (Corona-Cruz *et al.* 1999). DDT and/or its metabolites have been found in all birds and fish that have been analyzed, including ones living in deserts or at ocean depths (Baird & Cann 2012).

In the 1960s, dieldrin was recommended for use on approximately 90 crops, principally corn, hay, wheat, rye, barley and oats, and orchards and vegetables (Jorgenson 2001). In the early 2000s, high levels of dieldrin were detected in a variety of crops around the world (Matsumoto *et al.* 2009). The U.S. Food and Drug Administration (FDA) conducted a study in 1998 to evaluate pesticide residues in foods prepared as they would be consumed. Dieldrin was found in 9% of common foods, and had a 66.2% chance of exceeding safe limits for pesticides from food grown in the United States (Jorgenson 2001). Similar to DDT, dieldrin also binds strongly to soil particles and is resistant to leaching into groundwater (Fiedler 2000).

Due to their lipophilic characteristic, DDT and dieldrin tend to accumulate in the fatty tissues of ingesting organisms along the food chain (Aislabie *et al.* 1997; Jorgenson 2001). Moreover, DDT is prone to biomagnification, as its concentration increases along the food chain. Similarly, dieldrin is not easily metabolized in water and has limited capacity of being digested and excreted from the body. Dieldrin bioaccumulation is resistant to physical degradation and biologic metabolism (Jorgenson 2001).

There has been less use of both DDT and dieldrin in recent years, but their persistence has ensured that significant residues of both are still present in the environment today. Additionally, DDT and DDE can enter the environment as a result of long-range air transport from developing countries where DDT is still used to control mosquito vectors (Fiedler 2000; Matsumoto *et al.* 2009; Baird & Cann 2012).

2.2.5 DDT & Dieldrin Toxicity

Commercial DDT contains 70-80% 4,4-DDT, and this isomer can be further metabolized to 4,4-DDE. This form of DDT is more biodegradable, and has lower toxicity, but has been shown to have estrogenic activity (Hopkin *et al.* 2005), DDE has also been identified as an androgen receptor (Aislabie *et al.* 1997). The most common form of DDT stored in human fat is DDE from food sources (Baird & Cann 2012). North American, adults have ΣDDT stored in their body fat to the extent of 3 ppm. This has been associated with several health problems in humans, including increased risk of breast cancer in women (Corona-Cruz *et al.* 1999). Dieldrin has been linked to various health issues, including reproductive issues, as an estrogen disruptor. It is also known to cause liver damage, and neurological issues, and has been classified as a possible carcinogenic to humans by the U.S. EPA (Jorgenson 2001).

2.2.6 Canadian Soil & Sediment Quality Guidelines for DDT & Dieldrin

The Canadian Council of Ministers of the Environment (CCME) and the Ontario Ministry of the Environment (OMOE) have established protective guidelines for ΣDDT and dieldrin concentrations in soil and sediments (CCME 1999a; CCME 1999b; CCME 2001a; OMOE 2011). For the protection of human health and the environment in agricultural, residential and park land the ΣDDT concentration in soil should not exceed 0.7 mg/kg (CCME 1999b), and the dieldrin concentration in shallow soils should not exceed 0.05 mg/kg (OMOE 2011). The CCME guidelines for DDT, DDE, DDD, and dieldrin concentrations in sediments are summarized in table 2.1. The formal protocol used by CCME to derive sediment quality guidelines relies on both the National Status and Trend Program (NSTP) and the spiked-sediment toxicity test (SSTT) (CCME 2001b). Generally, the lower of the two values derived using either approach is recommended, however, if information is available to support only one approach, interim sediment quality guidelines (ISQG) are used, which is the case for both DDT and dieldrin. Additionally, guidelines for the probable effect level (PEL), which is the level above which adverse effects are expected to occur are frequently included in the sediment guidelines (CCME 2001b).

Table 2.1. Interim sediment quality guidelines (ISQG) and probable effect levels (PEL) for DDT, DDE, DDD, and dieldrin (μg/kg-dw) (CCME 1999a; CCME 2001a).

		Freshwater	Marine/Estuarine
DDT			
	ISQG	1.19	1.19
	PEL	4.77	4.77
DDE			
	ISQG	1.42	2.07
	PEL	6.75	3.74
DDD			
	ISQG	3.54	1.22
	PEL	8.51	7.81
Dieldrin			
	ISQG	2.85	0.71
	PEL	6.67	4.30

2.2.7 DDT & Dieldrin Contamination at Point Pelee National Park (PPNP)

Point Pelee National Park (PPNP) is the smallest national park in Canada (16 km^2), located south of the town of Leamington, Ontario. The park was established in 1918 with the intention of protecting and preserving its ecologically unique land and species. It consists of wetlands, Carolinian forest, beach habitats, and old fields (former farms and orchards) within a highly fragmented landscape. Point Pelee is internationally known as an important staging area for migratory birds during spring and fall migration and is a vital breeding area for many species of birds, especially passerines (Badley 2003; Smits *et al.* 2005; Denyes *et al.* 2016).

The park is contaminated from historical use of organochlorine pesticides (principally DDT and its degradation products and dieldrin) during the 1950s and 1960s (Smits *et al.* 2005). DDT was first used at PPNP in 1948, and was continuously and extensively used until 1967. It was mainly deployed in agricultural areas for pest control, and on roads, campgrounds, and picnic areas for mosquito control (Russell & Haffner 1997). DDT was for the most part applied in its granular form, as a particulate spray, but some trouble areas received a more intense treatment consisting of DDT *tossit bombs* (Badley, 2003). The *tossit bombs* refer to the directly application of DDT onto open waters in the marshes at PPNP (Russell & Haffner 1997). There are no records of aldrin or dieldrin use within the park, but they may have been used as soil insecticides until the mid-1960s when agriculture in the park was abandoned (Russell *et al.* 1995).

In the 1990s, studies performed on the park's wildlife found concerning high levels of DDT and dieldrin in tissue samples. In frogs (northern spring peppers) the reported ΣDDT concentration was over 1,100 μg/kg, while for dieldrin it was over 190 μg/kg (Russell *et al.* 1995). In 1997, the *Great Lakes Institute for Environmental Research* wrote a comprehensive report demonstrating that two of the 30 sites analyzed at PPNP had high DDT soil concentrations (~1,200-9,000 ng/g), and one had a very high concentration (~15,000 ng/g). Moreover, 200-800 ng/g of ΣDDT was present in frog tissue and snapping turtle egg samples found within the park (Russell & Haffner 1997).

The organochlorine pesticide contamination in PPNP is still a concern today. A recent study conducted by Clow *et al.* (in press) mapped the presence of DDT at the park, and found DDT concentrations above those recommended by CCME in samples of both sediment and soil. The average ΣDDT in sediment was 37.1 ng/g, far exceeding the CCME guideline of 1.19 ng/g. Soil samples were collected from 115 locations in the park, with 56.5% of them having concentrations above CCME guidelines. The soil samples averaged 23,000 ng/g DDT (Clow *et al.* in press). Concentrations of dieldrin exceeding CCME and OMOE guidelines have been also recently reported within the park boundaries (Smits *et al.* 2005).

Although the last known application of these pesticides at PPNP occurred almost 50 years ago, contamination persists, creating a need for finding efficient methods to remove DDT and dieldrin contamination while preserving the park's unique fauna and flora.

2.3 DDT & DIELDRIN REMEDIATION TECHNIQUES

2.3.1 Traditional Technologies

Conventional treatment for organochlorine contaminated soils includes excavation and incineration, thermal desorption, microwave-enhanced thermal treatment, soil washing with surfactants, and supercritical fluid extraction (Yang *et al.* 2010). These techniques usually involve digging up contaminated soil and removing it to a landfill, or capping and containing contaminated areas of a site (Vidali 2001; Yang *et al.* 2010). Digging up the contaminated soil moves the contamination to another site, usually a landfill, but does not deal directly with the contamination problem. This method is also expensive, and creates risks during the excavation, handling, and transportation processes. The capping method is only a temporary solution, as the contamination remains on site and any isolation barriers need to be monitored to maintain proper function (Vidali 2001).

An alternative remediation approach is the use of high-temperature incineration or various types of chemical decomposition. These methods completely destroy DDT or transform it to innocuous substances, but they also have drawbacks. First, these methods are generally not-publicly acceptable. Second, they tend to be costly, and third, they may expose workers and nearby residents to contaminants (Vidali 2001).

An increasingly popular alternative to these traditional remediation methods is bioremediation (Juwarkar *et al.* 2010). Bioremediation refers to the process that uses microorganisms, green plants or their enzymes to treat polluted sites in order to regain their original condition (Megharaj *et al.* 2011). It is also defined as the processes by which organic wastes are biologically degraded, under controlled conditions, to an innocuous state, or to levels below concentration limits established by regulatory authorities (Vidali 2001). In recent years, bioremediation techniques have been used for decontamination of surface and subsurface soils, freshwater and marine systems, groundwater, and land ecosystems (Juwarkar *et al.* 2010). These techniques are usually more economical than traditional methods, and can be used on site for many pollutants, which reduces exposure and transportation risks (Vidali 2001).

2.3.2 Phytotechnologies

Phytoremediation is a combination of the words *phyto* meaning plant and *remediation* meaning remedy. It is the use of a vascular plant's natural ability to contain, degrade or remove toxic chemicals and/or pollutants from soil or water (Fulekar 2010). Plants were first investigated in the treatment of metal contaminated wetland soils in the 1970s. In the early 1990s, the term phytoremediation appears in the technical literature for the first time (Russell 2005). The concept of using vascular plants to remediate soils contaminated with organic pollutants was based on observations. Organic chemicals' disappearance is accelerated in vegetated soils when compared to surrounding non-vegetated bulk soils (Alkorta & Garbisu 2001).

Phytoremediation can be an effective, non-intrusive, and inexpensive way to remediate soils (Alkorta & Garbisu 2001). It is more cost-effective than alternative mechanical or chemical methods of remediation. Estimates set the cost of phytoremediation at $10,000 to $30,000 per acre, which is 2-5 fold less expensive than traditional capping (Suresh & Ravishankar 2004). Phytoremediation is also an aesthetically pleasing and socially accepted technology (Alkorta & Garbisu 2001; Suresh & Ravishankar 2004). In addition, vegetation offers other benefits to contaminated sites. Plants increase the amount of organic carbon in the soil, which stimulates microbial activity. Deep-rooted vegetation helps to stabilize soil controlling windblown dust, and thereby reducing an important pathway for human exposure to contaminants via inhalation (Schnoor *et al.* 1995).

Phytoremediation does have some limitations, including the inability to extract contaminants located below a plant's rooting depth and the plants' inability to grow at the toxic levels of some contaminants. It is also a long-term process, often taking years to regulate the contamination levels. Moreover, in case of highly lipophilic persistent chemicals, such as DDT and dieldrin, bioavailability of contaminants is another limitation factor (Suresh & Ravishankar 2004).

In phytoextraction, a type of phytoremediation, contaminants are taken up by plants, and then translocated to above ground plant tissues. These tissues (shoots and sometimes roots) must then be harvested, and transported to a facility where they can be incinerated or composted. This technique has mainly been used in soils contaminated with metals, but can be used for decontamination of metalloids, radionuclides, perchlorates, benzene, toluene, ethylbenzene, and xylenes (BTEX), DDT, dieldrin, and pentachlorophenol (PCP) (McCutcheon & Schnoor 2003; Suresh & Ravishankar 2004) .

2.3.2.1 Phytoextraction of DDT & Dieldrin

Phytoextraction of organochlorine pesticides was considered problematic for many years due to the highly hydrophobic nature of this pesticide. In 1994, Hülster published a breakthrough study that opened the possibility of the use of phytoextraction in soils contaminated with POPs. In his study, zucchini plants bioaccumulated substantial quantities of polychlorinated dibenzo-p-dioxins and dibenzofurans in their roots. These plants were also able to translocate the contaminants into their aerial tissues without volatilization to the atmosphere (Hülster *et al.* 1994).

The potential of *Cucurbita pepo* species for phytoextraction of DDT and its metabolites has been shown in several subsequent studies (White & Hite 2001; White 2002; Lunney *et al.* 2004; Whitfield Åslund *et al.* 2010). Some studies suggested that cucurbit plants produce molecules in their roots exudates that help to desorb and solubilize hydrophobic compounds from soil particles, rendering them more available for uptake by the plant (Matsumoto *et al.* 2009; Lunney *et al.* 2004; Hülster *et al.* 1994). Their root exudates have high protein content, low total sugar content, and a high percentage of monosaccharides in sugar. In most other plant exudates, the proportions of proteins and sugars are reversed and monosaccharides are essentially absent (Matsumoto *et al.* 2009). Other plant species have also been tested for their DDT phytoextraction potential, including maize and forage (Mo *et al.* 2008), tomato, sunflower, soybean and alfalfa (Mitton *et al.* 2014). Maize, tomato, and alfalfa showed DDT accumulation potential in these studies.

Although there have been fewer studies on phytoextraction of dieldrin, cucurbits have attracted attention because of their high-level accumulation ability (Matsumoto *et al.* 2009). A study that compared shoot uptake of dieldrin of 32 plant species of arable crops growing in contaminated soil and demonstrated that the family *Cucurbitaceae* took up more dieldrin than the others, with zucchini having the highest uptake level (Otani *et al.* 2007). Jorgenson (2001), also reported that dieldrin in soil was readily absorbed into the pulp of vegetables, such squash, melons and cucumbers. Dannarumma (2009), found that in tomato, lettuce and celery there was no detectable translocation of dieldrin to the aerial parts. There is translocation to aerial parts of cucurbitaceous plants, and in zucchini it increased throughout the life cycle. In a different study, the ability of zucchini, white-flowered gourd, cucumber, tomato, komatsuna, and sunflower to uptake dieldrin hydroponically was evaluated. Dieldrin was detected in the roots of all plants grown in the hydroponic medium containing dieldrin, whereas it was not detected in the shoots of sorghum and sunflower. The values seen in the shoots of cucurbits were more than one order of magnitude higher than those in the shoots of non-cucurbits (Murano *et al.* 2010).

The goal of phytoextraction is to maximize the contaminant concentration in the harvestable tissue of the plant (shoot). The two qualities used to measure a plant's phytoextraction effectiveness are its bioaccumulation factor (BAF) and translocation factor (TF). BAF is defined by:

$$BAF = \frac{[plant\ tissue]}{[soil]}$$

and indicates a plant's ability to extract contaminants from soil. The TF is defined by:

$$TF = \frac{[shoots]}{[roots]}$$

and demonstrates the ability of a plant to translocate contaminants to the aboveground portion of its tissue. When shoot BAFs and TFs are greater than one, phytoextraction is likely to be a cost effective technique (Lunney *et al.* 2004; Whitfield Åslund *et al.* 2010).

For example, in the *Cucurbita* genera, the highest observed root BAFs are 16 in pumpkin, and 9.9 in squash for 4,4-DDE (White 2002). The relationship between DDT concentration in soil and BAF values was also reported. Soils contaminated with a high concentration of DDT (3,700 ng/g) had a smaller BAF (1.2) than soils contaminated with a low concentration of DDT (150 ng/g, BAF = 2.4) (Lunney *et al.* 2004).

Many studies have shown that DDT and dieldrin are strong candidates for phytoextraction. A variety of plant species have been tested for their ability to extract these pesticides with promising results. However, there is a need for more research in this field to find the best way to approach DDT and dieldrin remediation using the studied species, or even to discover better extractors.

2.3.2.2 Phytoextraction of DDT by Native Grasses Species

Native or indigenous plants are defined as those that naturally occur in the region, area or biome in which they originally evolved. These plants have coevolved with wildlife, fungi, and microbes to form mutually dependent relationships that are the foundation of a native ecosystem (Paul *et al.* 2015). The main advantages of using native species, like grasses and weeds, are that they are easy to cultivate and propagate, generally self-sustainable, relatively inexpensive, and are often hardier than many cultivated species (Ficko *et al.* 2010). Moreover, many native grasses are perennial species, which could be advantageous for phytoremediation by stabilizing, extracting, or degrading contaminants for longer time periods in a given year, and over several years (Ficko *et al.* 2010; Paul *et al.* 2015).

There are a limited number of studies available regarding the phytoextraction of persistent organic pollutants (POPs), like DDT or dieldrin, by native grasses or weed species. This is in part due to the fact that widespread utilization of phytoremediation can be limited by the small range or size of plants expressing remediation potential, and insufficient abilities of native plants to tolerate, detoxify, and accumulate contaminants (Arthur *et al.* 2005). Another barrier to the use of phytoextraction as a treatment for soils contaminated with POPs has been the inability of plants to accumulate sufficiently high concentration of these contaminants in their shoots (Whitfield Åslund *et al.* 2007).

Paul *et al.* (2015) evaluated the potential for weed species to phytoextract DDT from contaminated soils in PPNP. Results showed that some weed species (including native grasses) could obtain better phytoextraction results per square meter than *Cucurbita* species. For example, switchgrass (*Panicum virgatum*) extracted 2,100,000 ng of DDT/m^2, while *Curcubita pepo ssp pepo* cv. Howden extracted only 716,000 ng. Little bluestem (*Schizachyrium scoparium*) also showed potential for DDT extraction, and had 1,640,000 ng of DDT in the shoot tissue. Moreover, Paul *et al.* (2015) observed that the uptake of DDT by native grass species increased with increasing DDT concentration in soil. For example, in soil with high DDT contamination, *P. virgatum* and *S. scoparium* showed a better extraction potential than the known DDT extractor *C. pepo*.

There is great potential for phytoextraction with native grasses at PPNP, as two species effectively extracted DDT in an *in-situ* pilot scale plot. However, their ability to phytoextract dieldrin is yet to be confirmed. Moreover, there is a need to determine if the best solution for the DDT and dieldrin contamination at PPNP is phytoextraction and/or find a more efficient remediation technique.

2.4 HYDROXYPROPYL-β-CYCLODEXTRIN (HPβCD)

Cyclodextrins (CDs) are a family of cyclic oligosaccharides that are useful molecular chelating agents, and have a cage-like supramolecular structure. Because of their inclusion complex forming capability, CDs are widely used in many industrial products, technologies and analytical methods (Del Valle 2004). Cyclodextrins were first discovered in 1891 by Villiers (Szejtli 1998). A small amount of crystalline material was obtained from starch digested by *Bacilus amylobacter*, and this product was first named *cellulosine*. Villiers' bacterial culture was probably contaminated with heat-resistant spores of *Bacillus macerans*, which produced the cyclodextrin. In 1911, Schardinger described two crystalline products of starch that were produced by *Bacillus macerans* (Szejtli 1998; Del Valle 2004). He named these products *crystallised dextrin α* and *crystallised dextrin β*. In 1935, a new cyclodextrin was isolated and named *γ dextrin*. Structures of α and β-cyclodextrin were determined by X-ray crystallography in 1942. The fact that CDs can form inclusion complexes (discovered by Pringsheim), and the X-ray structure of γ-cyclodextrin were observed in 1948. In the 1950s, French and Cramer started to work intensively on the production of cyclodextrins, and characterized their true chemical and physical properties. Furthermore, in 1961 there was evidence of the existence of other natural cyclodextrins (δ-, ζ-, ξ-, and η-cyclodextrin) (Szejtli 1998; Del Valle 2004). Today, it is known that cyclodextrins are microbially produced as a result of the intramolecular transglycosylation reaction from the degradation of starch by cyclodextrin glucanotransferase (Brusseau *et al.* 1997; Del Valle 2004).

Three types of cyclodextrins are referred to as first generation or parent cyclodextrins: α-, β-, and γ-cyclodextrin. They are cyclic oligosaccharides formed by 6, 7, or 8 α—1,4-linked glucose units (Bardi *et al.* 2000; Del Valle 2004). These compounds have a hydrophilic shell with a relatively apolar cavity (Figure 2.3). The hydrophilic outside can be dissolved in water, and the apolar cavity provides a hydrophobic matrix (micro heterogeneous environment) that can form inclusion complexes with hydrophobic guest molecules (Brusseau *et al.*, 1994; Del Valle, 2004).

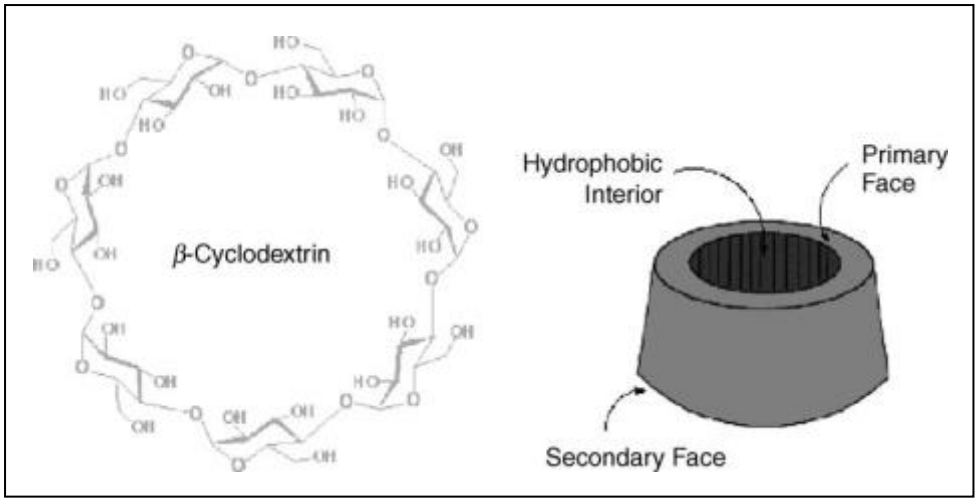

Figure 2.3. Chemical structure of β-cyclodextrin (from Del Valle 2004).

All CDs have the ability to form water-soluble inclusion complexes with non-polar, low solubility organic compounds. During this interaction, cyclodextrin is referred to as the *host* and the molecule contained within the cavity is the *guest*. The interaction with non-polar guests can be formed by hydrogen bonds or Van der Waal forces (Szejtli 1998). The following thermodynamic equilibrium equation describes the formation of inclusion complexes:

$$\mathbf{CD + S \leftrightarrow CD \bullet S}$$

where CD is the uncomplexed host cyclodextrin, S is the free guest compound, and CD•S is the concentration of complexed solutes. When dissolved, these compounds reach an equilibrium between the associated and disassociated compounds expressed by the complex stability constant K_a (Wang & Brusseau 1993; Szejtli 1998):

$$\mathbf{K_a = [CD \bullet S] / [CD]\,[S]}$$

The following equation represents the solubilisation of an organic compound by cyclodextrin:

$$\mathbf{S_t = S_0\,(1+K_aC_0)}$$

where S_t is the total aqueous-phase concentration of cyclodextrin solution in which both free and complexed species exist, S_0 is the aqueous solubility of the compound, K_a is the stability constant, and C_0 is the initial cyclodextrin concentration (Wang & Brusseau 1993).

The interactions between a low solubility guest and a CD in aqueous solutions have some consequences. The concentration of the guest in the dissolved phase increases, while the concentration of the dissolved cyclodextrin decreases. The diffusion and volatility of the included guest also decreases significantly. Moreover, the formerly hydrophobic guest becomes hydrophilic upon complexation (Szejtli 1998).

Many cyclodextrin derivatives were synthesized from the three naturally occurring ones. These derivatives have a changed hydrophobic cavity volume, and also modifications to improve solubility, stability, and the control of the guest molecule chemical activity. For example, up to 20 substituents have been linked to β-cyclodextrin in a regioselective manner (Del Valle 2004). These cyclodextrins were introduced in industry in the early 1980s in Japan. By the next decade, Japan became the largest cyclodextrin consumer in the world with an annual consumption of 1,800 tons, 80% of which went into the food industry where it is used as stabilizer for flouring agents, 10% into the cosmetic industry, and 5% were used in the pharmaceutical and agricultural industry. In the early 1990s, Procter & Gamble, an US based company, launched cyclodextrin-based fabric softener, becoming the largest single industrial user of cyclodextrins today (Loftsson & Duchêne 2007). Some derivatives, such as hydroxypropyl-β-cyclodextrin (HPβCD), are used in the pharmaceutical industry mostly as solubilizers, but also as stabilizers or to reduce local drug irritation. In HPβCD, hydroxyl groups exist on the interior cavity, and hydroxypropyl groups remain on the exterior shell (Figure 2.4), resulting in a very unique structure that creates a hydrophilic shell, and a hydrophobic slightly polar cavity (Wang & Brusseau 1993).

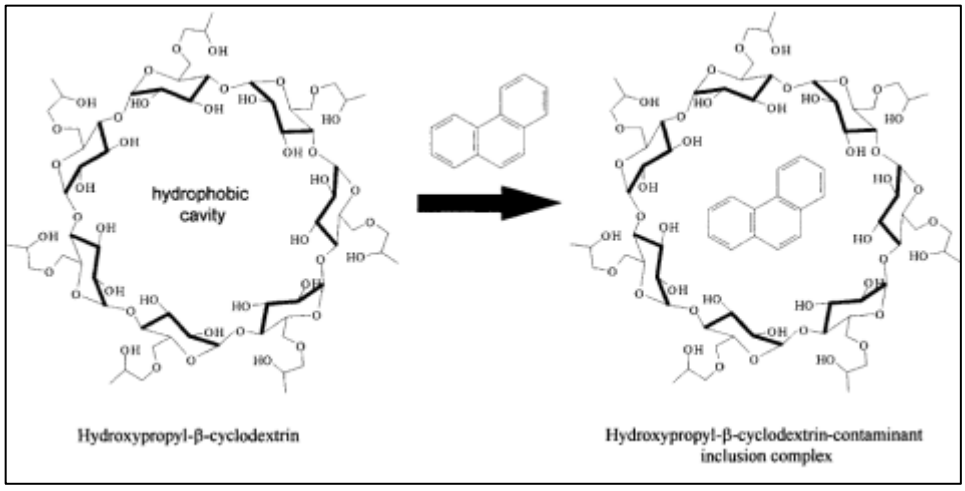

Figure 2.4. Chemical Structure of hydroxypropyl-β-cyclodextrin and a representation of an HPβCD-guest inclusion complex (Reid *et al.* 2004).

2.4.1 Uses of HPβCD in Remediation

In 1993, Wang and Brusseau were the first to investigate the possibility of cyclodextrin to form complexes with low-polarity organic compounds in order to increase these compounds' water solubility. Their results suggested that trichloroethene, chlorobenzene, naphthalene, anthracene, and 4,4-DDT solubilities were significantly increased by HPβCD (Table 2.2).

Table 2.2. Chemicals investigated for their ability to form complexes with cyclodextrin. Where K_{ow} is octanol-water partition coefficient and K_{cw} is the HPβCD-water partition coefficient (modified from Wang & Brusseau 1993).

Chemical	**log K_{ow}**	**log K_{cw}**	**Molecular Volume (nm^3)**
Trichloroethene	2.61	1.17	0.155
Chlorobenzene	2.84	1.92	0.180
Naphthalene	3.37	2.72	0.232
Anthracene	4.45	3.47	0.315
DDT	6.36	4.05	0.508

HPβCD molecules can enter and leave the entire water-saturated domain with no observed sorption, retardation, or pore exclusion. Due to these characteristics, this surfactant is being considered as an option to enhance desorption and removal of contaminants, thereby increasing the effectiveness of subsurface remediation. The nonreactive nature of cyclodextrin, combined with its large affinity for low-polarity organic compounds make it a possible candidate for use in *in-situ* remediation (Brusseau *et al.* 1994).

In 1997, Brusseau demonstrated that cyclodextrin could greatly enhance the simultaneous desorption and elution of phenanthrene and cadmium from three types of soil. The soils consisted of a sandy aquifer material containing minimal organic carbon and clay, a soil with relatively high clay content (10.2%), and a soil with a relatively high organic carbon content (1.4%). In this study, a mixture of 50:50 carboxymethyl-β-cyclodextrin (CMβCD) (0.5%) and HPβCD (0.5%) increased the removal of phenanthrene. The volume of cyclodextrin solution applied to the soil was calculated based on the soil's pore volume (PV):

$$\boldsymbol{PV = Area\ (m^2) \times Depth\ (m) \times Porosity \times 1000\ {}^{L}/_{m^3}}$$

The results showed that 86% of the initial mass was removed by the CMβCD-HPβCD solution after 20 pore volumes of flushing; and only 66% when CMβCD

was applied alone. The study results also indicated that aging (38 days old contaminants) appears to have a relatively small impact on the removal of cadmium and phenanthrene. These results suggested that cyclodextrin is a candidate for remediation of mixed wastes (Brusseau *et al.* 1997). However, in a previous study, they observed that DDT has a molecular volume of 0.508 nm^3, which is larger than the cavity volume of HPβCD (0.346 nm^3). Because of its molecular volume, DDT can achieve only a partial entry in the HPβCD cavity, leaving part of its volume in the solution phase. HPβCD-DDT has a partial nonpolar characteristic that could contribute to the relatively smaller solubility enhancement observed for DDT (Wang & Brusseau 1993).

Four McMaster University theses (Marenco 2002; Badley 2003; Mironov 2004; Etherington 2007) reported on the possibility of using HPβCD to remediate soils contaminated with DDT at PPNP. Marenco (2002) examined the relationship between soil conditions, former land-use, and the concentration of DDT in the soil. This study provided data characterizing different soil environments in term of their propensity for degrading DDT. According to Marenco (2002), flat, relatively lower-lying sites at PPNP are expected to degrade DDT faster than the relatively higher-lying areas, these latter sites should be target for future remediation projects.

Badley (2003) designed and implemented a remediation experiment using the applications of technical grade HPβCD (10% and 20% solutions) to remove DDT and its metabolites from surface soils within the former orchard area. The experiment consisted of a 3 m x 3 m square grid subdivided into nine treatment plots, with an initial DDT concentration of 33.64 µg/g. Three plots received the 10%-HPβCD solution, three plots received the 20%-HPβCD solution, and three did not receive any treatment (control). One pore volume (33L) of HPβCD solution was applied weekly for 13 (20% solution) and 19 (10% solution) weeks.

The rate at which water enters the soil to fill pores and move through the soil is defined as the rate of infiltration. The initial application of one pore volume of cyclodextrin was completed in approximately two hours without causing surface ponding. By the application of the sixth pore volume, the solution took three hours to infiltrate. By the 13^{th} week of application, the time required for the infiltration of one pore volume ranged between four and seven hours, and extensive ponding was observed. The 20% solution applications were discontinued at this point. The increase in both application time and ponding observed in the 20% plots was not experienced in the 10% application plots.

After approximately ten pore volumes of treatment, the initial DDT concentration of 33.64 µg/g was reduced to 3.4 µg/g in both treatments, and there was no appreciable change in the DDT concentration after this point. The 10%-HPβCD treatment resulted in a decrease of 90%, 77%, and 82% of the initial DDT, DDE, and DDD,

respectively present in the soil by week 19. The 20%-HPβCD treatment decreased the amount of DDT, DDE, and DDD by 90%, 73%, and 73%, respectively by week 13.

According to Badley (2003), the treatment results suggested that HPβCD increased the bioavailability of DDT, and enhanced its degradation by the microbial communities in the soil. Soil samples from the treatment plots showed an increase in bacterial cell numbers when analyzed by 4',6-diamidino-2-phenylindole (DAPI) fluorescent stain test. Hence, she suggested that the decrease in concentration might have been due to enhance *in-situ* biological degradation, and that vertical mobilization of the mass of DDT within the soil profile accounted for only a portion of the decrease seen in the surface concentration of the contaminants.

In a subsequent study, Smith (2004), obtained similar results in a column experiment using PPNP soils treated with one pore volume of 20%-HPβCD once a week. The abiotic columns received sodium azide (a biocide) and HPβCD solutions. The results showed no increase in moisture content or decreased infiltration rates. The biotic columns, treated only with HPβCD, had a significant moisture increase and decreased infiltration rates, suggesting that microbial activity plays a significant role in the soil profile treated with HPβCD (Smith 2004, in Etherington 2007).

Mironov (2004) collected two sets of samples of PPNP groundwater, the first in October 2003, and the second in March 2004. In the first set, a total of 14 sampling points was selected, and samples were collected at two depths (2.4 and 2.7 m below surface) for each point. The locations were determined based on groundwater flow expected from contaminants flushed from Badley's (2003) treatment plots. The second set of sampling points was based on the results from the first set.

DDT was found only at three locations: (1) one meter west of the west edge of the application grid at a concentration of 6.10 ng/L; (2) three meters east of the east edge of the application grid at a concentration of 1.49 ng/L; and (3) on the south edge of the application grid at a concentration of 9.27 ng/L. DDE was detected in eight of 14 samples at concentrations ranging from 1.64 to 17.0 ng/L. The observed DDT and DDE concentrations are 10-100 times above the DDT and DDE concentrations found in water in the area before the pilot-scale field experiment, and are attributed to the cyclodextrin applications to the remediation grid (Mironov 2004).

Mironov (2004) also used the unsaturated/saturated numerical model HYDRUS-2D to model the pilot-scale experiment conducted by Badley (2003) to assess the distribution of the mass of DDT and cyclodextrin in the groundwater system. The HYDRUS-2D model simulated solute transport without retardation or degradation, and generated a March 2004 DDT plume with a center of mass located 3-4 m east of the eastern edge of the remediation grid and approximately 3.5 m below the surface.

The maximum concentration of DDT in the simulated plume was 1.4 mg/L, which is not viable as DDT water solubility is < 1 mg/L. The DDT concentrations detected during the March 2004 groundwater sampling were 2 to 8 orders of magnitude below those simulated by HYDRUS-2D. Even when extreme possible values of groundwater velocity and net infiltration were input to the model, the simulated values of DDT concentration were 3 to 5 orders of magnitude above the observed values in groundwater samples. It was concluded that enhanced degradation of DDT must have been induced within the system by the presence of cyclodextrin solution (Mironov 2004).

All HPβCD treated plots had increased organic content after remediation, and no increase in DDT concentration was found at a depth 80 cm in November 2002. Moreover, less than 0.1% of the DDT mass from soil was found in groundwater in the vicinity of the remediation grid. It was concluded that co-metabolic degradation of DDT and its metabolites likely occurred within the A-horizon during the application of cyclodextrin solution (Badley 2003; Mironov 2004).

Etherington (2007) conducted a six column laboratory experiment. The columns were packed with PPNP DDT/DDE contaminated soil, and were divided in two groups of three each. The initial soil concentrations were 6.2 µg/g DDT and 3.9 µg/g DDE. The first three columns received one pore volume (120 ml) of 10%-HPβCD twice a day for five days, and the other three columns (control) received the same amount of deionized water. The columns' effluent from each treatment were collected and analyzed for DDT/DDE content. The average amount of DDT removed after each pore volume application was 0.3 µg/g and 0.2 µg/g DDE. The percentage of DDT and DDE mass removed after 10 pore volumes of 10%-HPβCD treatment was 19% and 21% respectively. The comparison between Badley 2003 field results and Etherington 2007 laboratory results led the author to conclude that the field application of HPβCD was approximately three times more effective in removal of DDT/DDE that the laboratory column study. According to Etherington (2007) this indicates that mobilization by 10%-HPβCD is not likely the sole mechanism for DDT and DDE removal from soil in the field remediation trials.

Contradictorily, Etherington (2007) asserted that the vast majority of cyclodextrin applied was not retained in the soil, and it was possible that a greater portion was drained from the soil profile into the groundwater. Moreover, she asserted that the microbial communities could not have been stimulated enough in the short period between treatment and sampling (one week) to degrade such a large amount of cyclodextrin. Taking this into consideration, it is possible that the microbial communities are not degrading any DDT or cyclodextrin-DDT complexes present in the soil. It should be noted that none of the thesis generated between 2002 and 2007 were published in the peer-reviewed literature in any form.

In an unrelated study, Wang (2005) demonstrated that HPβCD increases pyrene bioavailability to *Burkholderia* CRE 7 and enhanced its biodegradation. In this laboratory experiment, the bottoms of the vials were coated with a thin layer of pyrene. Over the 15 weeks of the experiment no measureable loss of pyrene occurred for the control (no HPβCD added), while in the vials containing HPβCD (10^4 mg/L), 14% pyrene biodegradation was observed. These results suggest that HPβCD may be useful for enhancing the bioavailability and biodegradation of pyrene (Wang *et al.* 2005). In a microcosm experiment, Stroud *et al.* (2009), tested the ability of *Pseudomonas sp.* and *Acinetobacter Iwoffi* to degrade phenanthrene and hexadecane respectively. After 100 days of incubation, the control soils had significantly higher levels of phenanthrene mineralisation than the HPβCD amended soils (10-40 mM). For hexadecane, significantly higher extents of mineralisation were observed in HPβCD treated soils by day 25, suggesting that microbial degradation enhancement is both contaminant and microorganism specific.

Recently, Romeh (2015) used HPβCD in combination with phytoremediation to remediate cyanophos (an organophosphorus insecticide) contaminated soils in Egypt. The application of 1%-HPβCD solution to soils planted with *Plantago major* L. resulted in the removal of 65% of this insecticide from spiked soils (20 μg/g), compared to 46% in the planted control in a nine day experiment. Furthermore, an increase in cyanophos concentration in shoots from 11.86 μg/g to 15.84 μg/g and in roots from 6.08 μg/g to 8.30 μg/g was observed in HPβCD treated plants.

Numerous studies (Reid *et al.* 2004; Stokes *et al.* 2005; Doick *et al.* 2006; Papadopoulos *et al.* 2007) have analyzed the ability of HPβCD to chemically predict bioavailability of polycyclic aromatic hydrocarbons (PAHs) by linking HPβCD chemical extractability to its microbial degradation. The cyclodextrin extraction technique conceptually mimics the interaction between organic contaminants and degrading microbial cells in soil. Papadopoulos (2007), concluded that correlations between the HPβCD extractable fraction and the microbially degradable fraction were very close in six soils studied. These correlations mean that the amounts of PAHs degraded by the catabolic activity of the indigenous microflora in each of the soils were correlated with the HPβCD-extractable PAH concentrations. But the ability of cyclodextrin extractions to actually predict full-scale bioremediation endpoints in the field is still uncertain. Moreover, Hartnik *et al.* (2008) successfully used the HPβCD extractability method to evaluate the bioavailability of two pesticides, chlorfenvinphos and α-cypermethrin, to earthworms.

In summary, there are several studies on the possible use of cyclodextrins as a tool to increase bioavailability and enhance biodegradative activity of microbes. Several of these studies were conducted within PPNP between 2002 and 2007. As a result of their apparent success, PPNP staff were keen to follow up with this work. Other researchers have analyzed cyclodextrin as a chemical method to predict the

bioavailability of a contaminant. There is a need for more research in both fields to more clearly define the role of cyclodextrin in the remediation of organic contaminants, in particular DDT.

2.5 ZERO-VALENT IRON

Metallic or zero-valent iron (ZVI) (Fe^0) is a moderate reducing reagent, which can react with dissolved oxygen (DO) and to some extent with water. These corrosion reactions can be accelerated or inhibited by manipulating the solution chemistry and/or solid (metal) composition (Zhang 2003). The surface of Fe^0 will quickly oxidize to either iron hydroxides or oxyhydroxide in the presence of oxygen since Fe^0 is only stable in a reducing environment. The formation of the iron oxide shell will subsequently decrease ZVI's reactivity (Grieger *et al*. 2010).

According to the equations below, iron-mediated reactions should produce a characteristic increase in pH and decline in solution redox potential (E_H). A highly reducing environment ($E_H < 0$) is created through the rapid consumption of oxygen and other potential oxidants and production of hydrogen (Zhang 2003).

$$2Fe^0_{(s)} + 4H^+_{(aq)} + O_{2(aq)} \rightarrow 2Fe^{2+}_{(aq)} + 2H_2O_{(l)}$$

$$Fe^0_{(s)} + 2H_2O_{(aq)} \rightarrow Fe^{2+}_{(aq)} + H_{2(g)} + 2OH^-_{(aq)}$$

Typically, in a close batch reactor, a pH increase of 2-3 units is observable while ORP reduction is in the range of 500-900 mV.

Nano zero valent iron (nZVI), are particles typically less than 100 nm in diameter (Figure 2.5). These particles can be synthesized by several methods, including sonochemical, electrochemical, gas phase reduction, and liquid phase reduction methods. In aqueous solution, all nZVI particles react with water and oxygen to form an outer iron (hydr)oxide layer, and as a result, nZVI particles have a core-shell structure (O'Carroll *et al.* 2013).

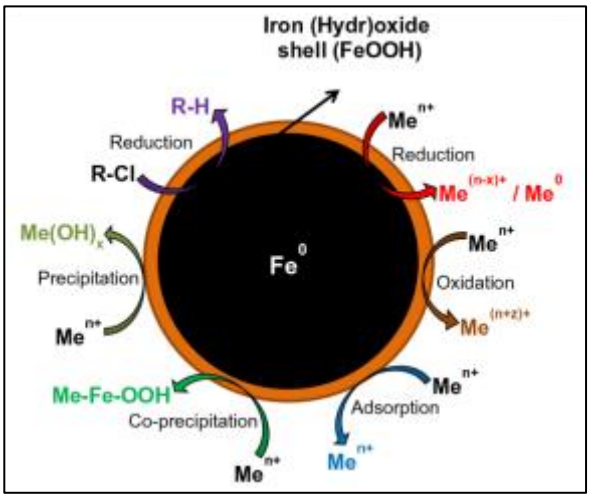

Figure 2.5. Core-shell structure of nZVI depicting various mechanisms for the removal of metals and chlorinated compounds (from O'Carroll *et al.* 2013).

Uncoated nZVI particles are prone to rapid aggregation and agglomeration often forming micro-sized fractal aggregates, which subsequently lead to a significant loss in reactivity and decreased environmental mobility. Therefore, there is an increasing interested in engineering nZVI with various surface coatings that reduce aggregation and maintain discrete particles ('stabilized' nAZVI) or control reactivity. Polymers, polyelectrolytes, and surfactants are among the main types of coatings used (Grieger *et al.* 2010; O'Carroll *et al.* 2013).

Due to its characteristics, ZVI has been largely established as an effective reductant and catalysts for a wide variety of common environmental contaminants including chlorinated organic compounds, and metal ions (Zhang 2003). Additionally, nZVI has been developed and used to degrade a wide range of organic and inorganic soil and water contaminants, including halogenated organic compounds, polycyclic aromatic hydrocarbons, pesticides, and heavy metals (Grieger *et al.* 2010)

2.5.1 Uses of ZVI & nZVI in Remediation

2.5.1.1 ZVI for Organochlorine Pesticide Remediation

The first use of zero valent metals for degradation of chlorinated compounds was studied by Sweeney and Fisher, who used metallic zinc for the degradation of halogenated organic compounds (O'Carroll *et al.* 2013). Subsequently, granular ZVI particles were used for environmental remediation since their introduction in 1994

by Gillham and O'Hannesin. The main application for ZVI has been their installation in permeable reactive barriers for the treatment of groundwater plumes. Although successful at many sites, ZVI applications have been limited for the most to shallow groundwater plumes in unconsolidated aquifers (Grieger *et al.* 2010).

Environmental applications of ZVI have been enthusiastically accepted by many users and regulatory agencies, especially in the USA, largely due to the low cost (Zhang 2003). However, there are a few challenges to the commercialization of ZVI, including the possibility of a public backlash, the fact that the technology is largely unknown to consultants, governments and site owners, as well as the lack of long-term studies (Mueller *et al.* 2012). Despite all these challenges, there are a few products in the market containing ZVI in their formulation, including DARAMEND and EHC. These two products contain a patented combination of organic carbon and ZVI, and are commercially available through a company called *PeroxyChem*. Unfortunately, there is minimal literature available related to the application of these two products to DDT and/or dieldrin contaminated soil and groundwater, all the existing information is in a single paper published by Seech *et al.* (2008).

DARAMEND is a soil amendment that provides a reduced environment that supports rapid and complete dechlorination of many chlorinated compounds (Seech *et al.* 2008). The combination of chemical and microbial oxygen consumption enables reliable generation of very low E_H conditions, which enhances both chemical and microbial dehalogenation processes. There are two key components of the DARAMEND bioremediation technique: (1) addition of the DARAMEND to the soil to be treated, and (2) regulation of oxygen availability and moisture content.

This soil amendment has been successfully applied to more than four million tons of soil, sediment, and other materials contaminated with various persistent organic compounds, including chlorinated herbicides and pesticides (like DDT and dieldrin), in many sites in the US, Canada, and Europe. During the application, soil and amendments are blended using a rotary tiller, driven by an agricultural tractor, with an effective penetration of two feet; the soil's water content is adjusted using agricultural irrigation equipment. In the Uniroyal Chemical site, in Ontario, Canada, the application of DARAMEND for nine months resulted in a reduction of 91.2% of the initial DDT contamination (53.5 mg/kg) (Seech *et al.* 2008).

On the other hand, EHC is specifically formulated for injection into the subsurface. The EHC slurry can be injected using a number of available technologies, including direct injection through GeoProbe rods and hydraulic or pneumatic fracturing. It can also be applied via direct placement into trenches or by using deep soil mixing equipment. Common applications include hot-spot treatment, plume treatment, and plume management suing a permeable reactive barrier (Seech *et al.* 2008).

The organic component of EHC is nutrient-rich, hydrophilic, and has high surface area, being ideal to support bacteria growth in groundwater. As the bacteria grow on EHC surfaces, indigenous heterotrophic bacteria consume dissolved oxygen and reduce the redox potential in groundwater. In addition, they ferment carbon and release a variety of volatile fatty acids, which diffuse from the site of fermentation into the groundwater plume and serve as electron donors for other bacteria, including dehalogenators and halorespiring species. Furthermore, the ZVI particles provide a reactive surface area that stimulates direct chemical dechlorination (Seech *et al.* 2008).

Although, most of the *in-situ* applications of ZVI technology uses commercially available products that contains the metallic form of ZVI (like DARAMEND and EHC), most of the academic research has moved towards ZVI nanoparticles.

2.5.1.2 Uses of nZVI Remediation Technology

Nano zero valent iron (nZVI) is the most commonly used nanomaterial for soil and groundwater remediation at the present time (Mueller *et al.* 2012). This technique is very effective in transforming a wide variety of common chlorinated contaminants. In a reductive environment, chlorinated compounds are partially or totally dechlorinated to ethane and chloride (Mueller *et al.* 2012). Shortly after the nZVI *in-situ* application, pH increase and ORP decrease at the site favoring the growth of anaerobic microorganisms, which could be beneficial for accelerated biodegradation. Production of hydrogen gas and also ferrous iron ions further promotes microbial growth (Zhang 2003). A laboratory microcosm study showed that the introduction of nZVI led to geochemical changes and shifts in microbial populations (Grieger *et al.* 2010).

Technical challenges of using nZVI in field applications include iron passivation through non-target reactions, the limited particle mobility, and the difficulties of scale-up from laboratory experiment to field tests (Mueller *et al.* 2012). Additionally, although nZVI particles are a powerful remediation tool, their colloidal chemistry is such that these particles tend to agglomerate and adhere to soil surfaces (Zhang 2003). Moreover, possible ecotoxicity effects of nZVI are largely unknown, and concerning effects in aquatic organisms have already been identified. For example, in *Oryzias latipes*, nZVI caused disturbance in the oxidative defense system in both, embryos and adults (Grieger *et al.* 2010).

So far, *in-situ* nZVI applications have mainly target contaminants in aqueous systems and groundwater. Usually, nZVI reactivity and degradation efficiency is less in soils than in aqueous solutions due to limited desorption or solubilisation of the contaminants in soil (El-Temsah & Joner 2013). A few laboratory studies (Yang *et al.* 2010; El-Temsah & Joner 2013; El-Temsah *et al.* 2013) evaluated the potential

for nZVI to promote DDT degradation in soil. Yang *et al.* (2010), reported that nZVI has a positive effect on the degradation of 4,4-DDT and 2,4-DDT, however there was an increase in DDD concentration in the nZVI treated soils. The authors concluded, that due to the increase in DDD concentration after transformation of DDT, the addition of nZVI did not significantly increased the degradation of ΣDDT.

El-Temsah & Joner (2013), reported that the addition of 1 and 10 g of nZVI per kg of soil to a historically DDT contaminated soil, resulted in a reduction of 24% and 28% of the contamination respectively after seven days of incubation. Moreover, no significant changes in DDD or DDE were found for any of the treatments. However, in the same study, concerning toxicity effects of nZVI on collembola (*Folsomnia candida*) and ostracods (*Heterocypris incongruens*) were found. For collembola, mortality reached 100% when adults were exposed to either nZVI concentration in soil for seven days, while after 30 days of incubation toxicity was reduced to about 60% and 80% mortality for 1 and 10g nZVI / kg of soil, respectively. For ostracods, toxicity effects of nZVI in soil and leachates decreased with increased incubation time.

El-Temsah *et al.* (2013), used DDT spiked soil in a column experiment. The addition of nZVI and subsequent leaching with water led to a reduction of almost 50% of the initial DDT concentration in the soil. DDT in leachates were below the detection limit, and DDT distribution within the different sections of the soil columns showed low DDT mobility. However, DDT degradation was followed by significant increase of DDD and DDE in soil treated with nZVI. The effects of nZVI in soil on germination of barley and flax were investigated as well. Strong negative effects of nZVI addition and leaching were observed in soil from all sections of the soil column with respect to root development of both species. Furthermore, attempts to germinate seed in soil freshly amended with nZVI (with no leaching) resulted in complete inhibition for two plant species.

Although nZVI particles have been used in several studies and applied in groundwater sites multiple times, to date there have been no quantitative estimates of nZVI particles in the environment. Monitoring nZVI at contaminated sites after *in-situ* application has usually focused on indirect geochemical parameters including pH, dissolved oxygen, and ORP (Grieger *et al.* 2010). Recent work has questioned the interpretation of ORP data as evidence of successful nZVI emplacement during field application, suggesting that the highly complex redox response indicates nZVI corrosion and transport of these corrosion products and not transport of the particles themselves (O'Carroll *et al.* 2013). Furthermore, studies also show that naturally occurring organic matter subsurface constituents may act in a similar fashion to surface modifications, potentially increasing the mobility of nZVI as with other colloids (O'Carroll *et al.* 2013). Additionally, the German Federal Institute for Geosciences and Natural Resources, has discontinued its activities in the field of

nZVI remediation since 2008. This institute has come to the conclusion that the technical difficulty of disseminating the iron below ground and the cost-benefit analysis indicate that nZVI technology is not yet ready for large-scale application (Mueller *et al.* 2012).

As nZVI still an emerging technology, the non-nanoscale form of ZVI is usually selected for large scale *in-situ* applications. This is particularly the case if ZVI products are being applied to soils, since most laboratory and pilot scale field studies done with nZVI focus on the use of this technology for groundwater treatment. Moreover, the toxicity of most nanoscale particles to many living organisms is still unknown, and their release into the environment needs to be closely monitored. The major contaminants at PPNP are organochlorine pesticides, specially DDT and dieldrin, making the park an ideal location to further test the efficiency of products containing ZVI.

3. *In-situ* Phytoextraction of DDT and Dieldrin by Native Grasses at Point Pelee National Park

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3.1. ABSTRACT

Point Pelee National Park (PPNP) is highly contaminated with organochlorine pesticides, due to the historical use of dichlorodiphenyltrichloroethane (DDT) and dieldrin. Phytoextraction, is a green technology, that has been successfully used in the past to remediate organochlorine contamination. In this study, the ability of two native grasses (*Schizachyrium scoparium* and *Panicum virgatum*) to phytoextract DDT and dieldrin *in-situ* in a pilot-scale plot was evaluated. A significant decrease in the concentration of both pesticides was demonstrated in only five months of phytoextraction, and hence this green technology is likely suitable for *in-situ* remediation at PPNP.

3.2. INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) and dieldrin are organochlorine pesticides that are persistent in the environment and can pose a threat to human health and wildlife (Matsumoto *et al.* 2009; Baird & Cann 2012). They are both included in the persistent organic pollutants (POPs) subgroup known as *the dirty dozen*. Between 2001 and 2003, 191 governments signed the *Stockholm Convention on POPs* that seeks to eliminate or control their use (Breivik *et al.* 2004). DDT and dieldrin have been banned in Canada since the 1970s, however they are still commonly found in

soils today, due in part to their strong adsorption to solid particles, resulting in great persistence (Corona-Cruz *et al.* 1999; Matsumoto *et al.* 2009; Sudharshan *et al.* 2012).

Point Pelee National Park (PPNP), in southwestern Ontario, is heavily contaminated with both DDT and dieldrin due to its historical use as orchard land. DDT was first applied at PPNP in 1948, and was continuously and extensively used until 1967 (Russell & Haffner 1997). The major contaminant at PPNP today is dichlorodiphenyldichloroethylene (DDE), which is the persistent by-product of DDT aerobic biotic degradation. Aldrin and dieldrin are similar compounds, and dieldrin is the main persistent degradation product of aldrin (CCME 1999a). There are no records of aldrin or dieldrin use within the park, but they may have been used as soil insecticides until the mid-1960s when agriculture at PPNP was abandoned (Russell *et al.* 1995). In the 1990s, studies performed on the park's wildlife found concerningly high levels of DDT and dieldrin in tissue samples of frogs and snapping turtles (Russell *et al.* 1995; Russell & Haffner 1997). Hundreds of soil samples have since been collected at PPNP to determine the levels of DDT contamination at the park. Many of these soil samples have DDT concentrations above the 700 ng/g recommended by the Canadian Council of Ministers of Environment (CCME) (Crowe & Smith 2007; Clow *et al.* in press), and dieldrin concentrations above the 50 ng/g recommended by the Ontario Ministry of the Environment (OMOE).

Point Pelee National Park is internationally known as an important staging area for migratory birds during spring and fall migration, and is a vital breeding area for many species of birds, especially passerines (Smits *et al.* 2005; Denyes *et al.* 2016). Conventional remediation strategies for organochlorine contaminated soils, such as excavation and incineration, can negatively affect sensitive ecosystems (Smits *et al.* 2005). Therefore, these methods are not suitable for PPNP, and there is a need for an environmentally friendly remediation technology that maintains the park's integrity during the clean-up process. Phytoextraction, is a type of phytoremediation, where contaminants are taken up by vascular plants, and then translocated to above ground plant tissues. These tissues must then be harvested, and transported to a facility where they can be incinerated, composted, or landfilled (McCutcheon & Schnoor 2003; Suresh & Ravishankar 2004). In recent years, many studies (White & Hite 2001; White 2002; Lunney *et al.* 2004; Otani *et al.* 2007; Donnarumma *et al.* 2009; Matsumoto *et al.* 2009; Whitfield Aslund *et al.* 2010) have shown that DDT and dieldrin are strong candidates for phytoextraction.

In 2015, Paul *et al.* demonstrated that two native grasses, switchgrass (*Panicum virgatum*) and little bluestem (*Schizachyrium scoparium*), have great potential to phytoextract DDT at PPNP. The current study, evaluated the potential of these two species to phytoextract both DDT and dieldrin *in-situ* at the park over a five month time period. Additionally, this study also cultivated the two grasses at their optimal

densities, establishing the maximum amount of DDT and dieldrin that can be extracted per unit area.

3.3. METHODS & MATERIALS

3.3.1. Site Description & Plot Selection

Point Pelee National Park (PPNP) located south of the town of Leamington, Ontario, consists of a peninsula of land (16 km^2) made up of marsh and woodland habitats. The soil at PPNP is classified as sandy and contains organochlorine pesticides contamination, composed predominantly of DDT (4,4-DDE and 4,4-DDT), and dieldrin which have weathered in place for over 40 years (Smits *et al.* 2005; Denyes *et al.* 2016).

A ten by three meter experimental plot was established at the *Delaurier* parking lot close to the trail entrance (41°56'56.14"N 82°31'6.32"W) of PPNP in June 2015. The plot location was selected by Parks Canada personnel, in an area open to the public, such that it could be used to educate the general population about the on-going pesticide remediation project at PPNP. The mean ΣDDT and dieldrin concentrations in the soil were 340 ± 85 ng/g and 5 ± 1 ng/g respectively.

3.3.2. Plant Selection

Panicum virgatum and *Schizachyrium scoparium* were selected for this study based on their potential to phytoextract DDT as reported by Paul *et al.* (2015), and the fact that they are both native to Ontario.

P. virgatum (switchgrass) it is a perennial sod-forming grass that grows 3 to 5 feet tall. The stem is round and usually has a reddish tint, and the seed head is an open, spreading panicle. *P. virgatum* grows well in moderately deep to deep, somewhat dry to poorly drained sandy to clay loam soils. Moreover, it grows at high density, produces a large amount of biomass, can be used as a bioenergy crop, and provides excellent nesting and fall and winter cover for pheasants, quail, and rabbits (USDA 2006).

S. scoparium (little bluestem) is a medium height (18 in to 3 ft) grass with a coarse stem and basal leaves. As a warm season grass, it begins growth in late spring and continues through the hot summer period until the first killing frost. Plants are green, but often purplish at the base of stem and the entire plant has a reddish cast after frost. *S. scoparium* is one of the most widely distributed native grasses in North America, growing on a wide variety of soils. This species has excellent drought and

fair shade tolerance, and fair to poor flood tolerance. *S. scoparium* is suitable for hay, and provides cover for ground birds and small mammals (USDA 2002).

3.3.3. Plot Implementation & Sample Collection

In June 2015, the 30 m^2 phytoremediation plot was thoroughly homogenized to a depth of 10 cm with a stiff rake, and divided into three sections. Following soil homogenization, four surface soil samples (0-10 cm) were collected, with one sample being collected from the center of each of the three sections, and a field duplicate collected from the second section. The first section measuring four by three meters, was planted with ~2,040 seedlings of *P. virgatum* (i.e. at its optimal density of 170 plants/m^2). The second section measuring one by one meter was established as a buffer zone, and the third section measuring five by three meters, and was planted with ~750 seedlings of *S. scoparium* (i.e. at its optimal density of 50 plants/m^2) (Figure 3.1). On October 2015, five months after the plot was established, six additional surface soil samples were collected at three random locations from each of the two planted plots. Additionally, three plants (shoots and roots) of each species were harvested. Soil and plant samples were placed into a labelled Whirl-Pak® bag and frozen at -20ºC until analysis.

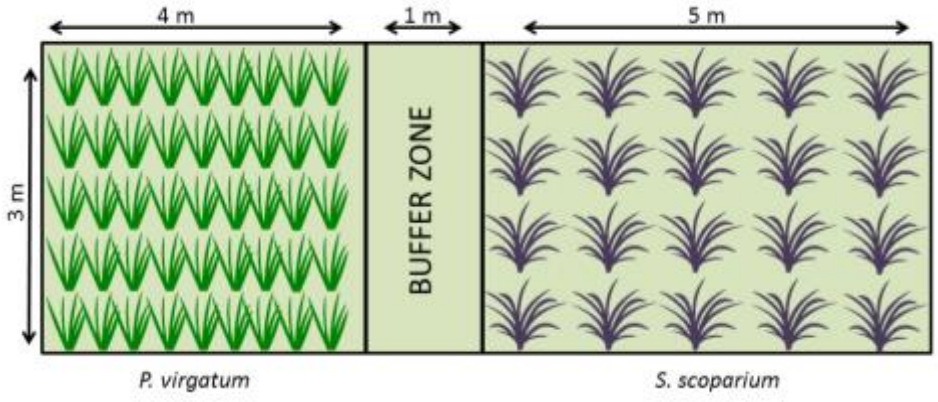

Figure 3.1. Representation of the phytoextraction plot established on June 2015, at the *Delaurier* parking lot at Point Pelee National Park.

3.3.5. Analytical Methods

3.3.5.1. Soil Samples

Soil samples (5 g wet weight) were air-dried overnight at room temperature. Approximately 1 g of sample was used for analysis. Soil samples were extracted using the accelerated solvent extraction (ASE) method with 30-40 mL 50:50 of hexane: acetone, 100µl of 1 ppm decachlorobiphenyl (DCBP) as a surrogate standard and ~15 g of Ottawa sand. The extract was concentrated by rotoevaporation and applied to a Florisil extraction column. The column was rinsed with hexane into a 10 mL volumetric flask (fraction 1, DDT). The same Florisil column was subsequently rinsed with dichloromethane (DCM) into 10 mL volumetric flask and placed into a 500 mL round bottom flask. The extract was concentrated by rotoevaporation and solvent exchanged to hexane. The extract was then placed in to 10 mL volumetric flask with hexane (fraction 2, dieldrin). Samples were transferred to gas chromatograph (GC) vials and analyzed by an HP 6890 GC equipped with a ^{63}Ni electron capture detector (GC/ECD), a SPB-1 fused silica capillary column. The carrier gas was helium at a flow rate of 2 mL/min. Nitrogen was used as a makeup gas for the ECD. The results were expressed as nanograms of pesticide per gram of dry weight soil.

3.3.5.2. Plant Samples

Plant samples (<10 g wet weight) were dried overnight in an oven at 25-30 ºC. Dried samples were finely grounded using an electric grinder (Thomas Scientific – model 3383-L10). Approximately 1 g of sample was used for analysis. Plant samples were extracted using the microwave extraction method with 15 mL 50:50 of hexane: acetone, and 100µl of 1 ppm DCBP as an internal surrogate standard. The extract was transferred to a glass syncore flask by pouring through a glass funnel with a filter paper (Fisher P8) filled with ~5 g of sodium sulphate. The extract was then concentrated by a Büchi syncore to approximately 2 mL, and applied to a Florisil extraction column (fraction 1, DDT). The same Florisil column was subsequently rinsed with dichloromethane (DCM) into 10 mL volumetric flask and placed into a 500 mL round bottom flask. The extract was concentrated by rotoevaporation and solvent exchanged to hexane. The extract was then placed in to 10 mL volumetric flask with hexane (fraction 2, dieldrin). Plant samples were analyzed by gas chromatography as described above for the soil samples.

3.3.6. Quality Assurance & Quality Control (QA/QC)

For every nine soil or plant samples extracted by either ASE or microwave, one analytical blank, and one control sample was included, as specified by the US-EPA method for organochlorine pesticides (US-EPA 2007). For soil samples, the analytical blank contained Ottawa sand, and the internal surrogate (DCBP), while for plant samples contained only the internal surrogate (DCBP). The control sample, in both cases, was spiked with 100 μL of 2 ppm organochlorine pesticide mixture (Appendix IX, from Supelco). Samples concentrations were corrected for surrogate recovery, and all analytical blanks were less than 1.0 ng/g (below detection limit). The mean difference between the control standard and the control standard target was less than 20%. All mean relative standard deviations between the analytical duplicates can be found in Appendix A.

3.3.7. Statistical Analysis

Statistical analyses were performed using R 3.2.2 (free software for statistical computing and graphics). All DDT and dieldrin concentrations are reported on a dry weight (ng/g) basis and recorded with the standard deviation of the mean. Data were tested for normality using the Shapiro-Wilk test. Data was analyzed by an one-way analysis of variance (ANOVA), followed by a post hoc Tukey comparison with significance level $p = 0.05$.

3.4. RESULTS & DISCUSSION

3.4.1. DDT and Dieldrin concentration in soil samples

Five months after the phytoextraction plot was implemented, reduction of both ΣDDT and dieldrin was found in soils collected from the root zone of both *S. scoparium* and *P. virgatum* plants (Figure 3.2). Soils from *P. virgatum* had a significant 52% decrease in ΣDDT concentration, while soils from *S. scoparium* had a not significant reduction of 33% (ANOVA, $F_{2,7} = 4.9$, $p < 0.05$, n=3,4 / Tukey-test, $p < 0.05$, $p > 0.05$). For dieldrin, soils from both species had a significant reduction in concentration (ANOVA, $F_{2,7} = 6.5$, $p < 0.05$, n=3,4 / Tukey-test, $p < 0.05$, $p < 0.05$).

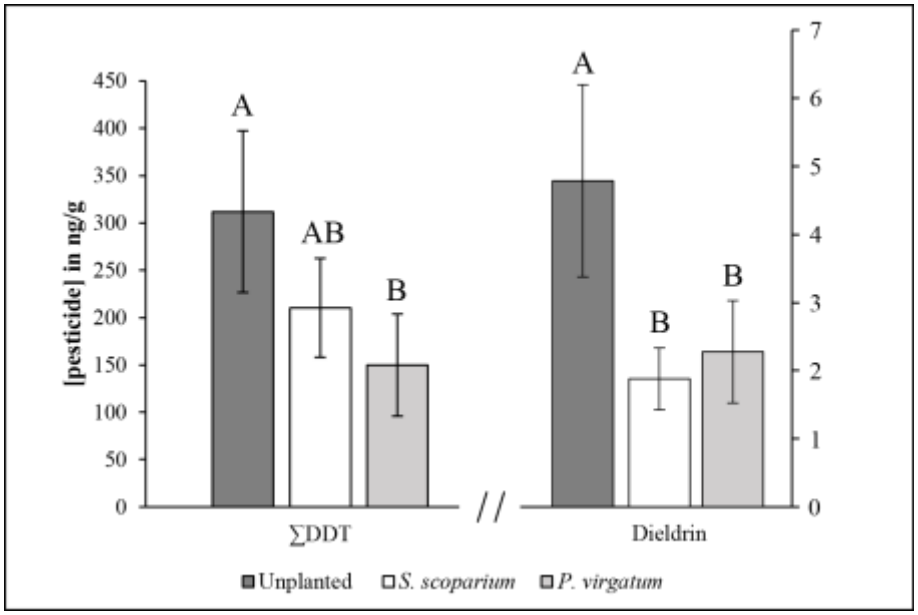

Figure 3.2. A comparison of the ∑DDT and dieldrin concentrations in soils collected on June, 2015 (unplanted) prior to plot establishment, and on October, 2015 (*S. scoparium* and *P. virgatum*) after five months of phytoextraction. Letters indicates where there is a significant difference.

3.4.2. DDT and Dieldrin concentration in plant tissue

At the time of harvesting (October 2015), plants from both species (*S. scoparium* and *P. virgatum*) had their health visually accessed, and were within the expected conditions for that time of the year (fall). After five months of phytoextraction, shoot ∑DDT and dieldrin concentrations ranged from 260 ± 90 ng/g (*P. virgatum*) to 190 ± 70 ng/g (*S. scoparium*), and 8 ± 4 ng/g (*P. virgatum*) to 5 ± 1 ng/g (*S. scoparium*), respectively. Moreover, there was no significant difference in [∑DDT] in shoots of *P. virgatum* and *S. scoparium* (ANOVA, $F_{1,4} = 1.07$, $p > 0.05$, n=3). Root ∑DDT and dieldrin concentrations ranged from 190 ± 50 ng/g (*P. virgatum*) to 310 ± 50 ng/g (*S. scoparium*), and 9 ± 3 ng/g (*P. virgatum*) to 5 ± 0.4 ng/g (*S. scoparium*), respectively. *P. virgatum* had significantly higher [∑DDT] in roots than *S. scoparium* (ANOVA, $F_{1,4} = 8.17$, $p < 0.05$, n=3). For dieldrin, there was no significant difference in the concentration in shoots and roots between the two grasses (ANOVA, $F_{1,4} = 1.59$, $p > 0.05$, n=3; ANOVA, $F_{1,4} = 6.76$, $p > 0.05$, n=3). These positive results were unpredicted, as Paul *et al.* (2015) previously reported shoot ∑DDT concentrations below detection limit at a low DDT contaminated site (~290 ng/g). However shoot ∑DDT concentrations in low DDT soil have been

reported for other species. Lunney *et al.* (2004) reported shoot ∑DDT concentrations of 375 ng/g, 98 ng/g, and 3.7 ng/g for pumpkin, zucchini and alfalfa respectively grown in low DDT contaminated soil (~150 ng/g).

Additionally, even though *P. virgatum* has a higher planting density (170/m^2), it did not extract significantly more ΣDDT or dieldrin per square meter than *S. scoparium* (ANOVA, $F_{1,4} = 2.05$, $p > 0.05$, n=3; ANOVA, $F_{1,4} = 4.75$, $p > 0.05$, n=3) (Table 3.1). These results showed that both native species have an equal ability to phytoextract organochlorine pesticides *in-situ* from PPNP soils.

Table 3.1. Comparison of shoot pesticide extraction per square meter for plants at the *Delaurier* phytoextraction plot. Although there are apparent differences, the total mean shoot pesticide extractions/m^2 are not significantly different between plant species.

Pesticide	Plant species	Mean shoot dry wt. (g)	Mean shoot [pesticide] (ng/g)	Plant density (plant/m^2)	Total mean shoot pesticide extraction/m^2 (ng)
DDT	*S. scoparium*	4.3	190	50	40,700
	P. virgatum	1.8	260	170	78,300
Dieldrin	*S. scoparium*	4.3	5	50	1,080
	P. virgatum	1.8	8	170	2,400

When the different DDT metabolites were taken into consideration, 4,4-DDE and 4,4-DDT were the main metabolites found in plant tissue, which is consistent with those present in the soil (Figure 3.3). However, the DDT metabolite ratios were not the same. The soil composition was 64% 4,4-DDE, 25% 4,4-DDT, 5% 2,4-DDT, 3% 2,4-DDD, 2% 4,4-DDD, and 1% 2,4-DDE, while the composition in *S. scoparium* tissues was 37% 4,4-DDE, 20% 4,4-DDT, 13% 2,4-DDD, 12% 4,4-DDD, 10% 2,4-DDT and 7% 2,4-DDE, and in *P. virgatum* tissues was 30% 4,4-DDE, 25% 4,4-DDT, 15% 2,4-DDT, 13% 2,4-DDD, 11% 4,4-DDD, and 6% 2,4-DDE. Whitfield Åslund *et al.* (2010) found that 57-63% of DDT metabolites consisted of 4,4-DDT in the shoot tissues of pumpkin, even though 4,4-DDT is less soluble in water than other metabolites. This study showed that *in-situ*, 37% and 30% of DDT metabolites consisted of 4,4-DDE in *S. scoparium* and *P. virgatum* respectively.

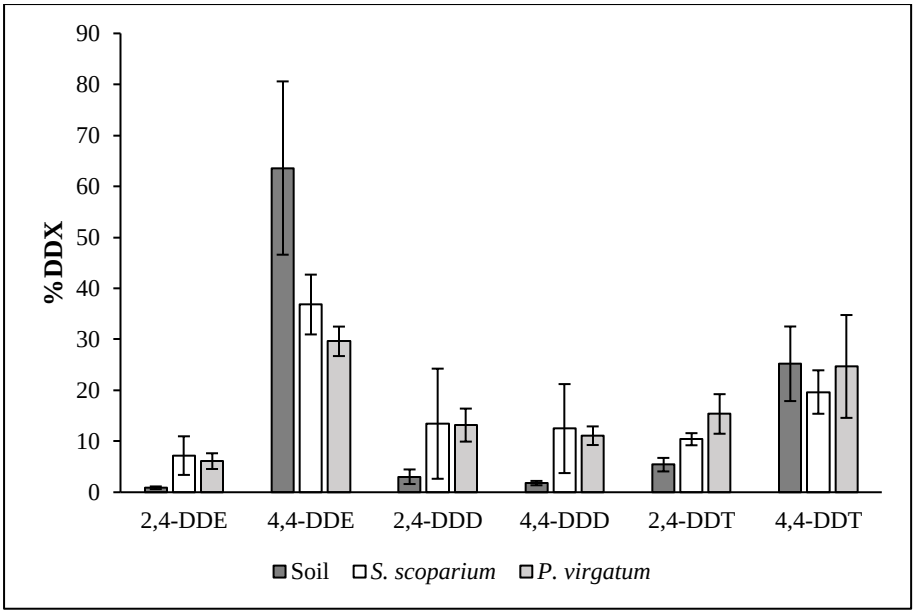

Figure 3.3. The percentage of each DDT metabolite in *S. scoparium* and *P. virgatum* tissue after five months of phytoextraction (October 2015), and in soil at plot implementation (June 2015). There are no significant differences between %DDX in the two species.

3.4.3. Bioaccumulation Factors (BAFs) & Translocation Factors (TFs)

The efficiency of phytoextraction can be calculated using bioaccumulation factors ($[\text{contaminant}]_{\text{plant tissue}}/[\text{contaminant}]_{\text{soil}}$). When shoot BAFs are greater than one, phytoextraction is likely to be a cost-effective technique (Lunney *et al.* 2004; Whitfield Åslund *et al.* 2010). The shoot BAFs of dieldrin, in *S. scoparium* and *P. virgatum*, were 2.2 and 4.3 respectively, suggesting that phytoextraction is an effective technique when the dieldrin concentration in soil is very low (~ 5 ng/g). For ΣDDT the shoot BAFs were 1.3 for *S. scoparium* and 1.2 for *P. virgatum*, again indicating that phytoextraction is a viable remediation strategy for these soils. Other than using BAFs, the efficiency of phytoextraction can also be calculated by translocation factors ($[\text{contaminant}]_{\text{shoot}}/[\text{contaminant}]_{\text{root}}$). Similar to BAFs, TFs greater than one are desirable. For ΣDDT, TFs were 1.0 for *S. scoparium* and 0.8 for *P. virgatum*; similarly, the TFs for dieldrin are 1.1 and 0.9 respectively. These findings are consistent with those described in Paul *et al.* (2015), and demonstrated that these two native grasses can phytoextract ΣDDT from soils where dieldrin co-contamination is present.

3.5. CONCLUSIONS

In-situ phytoextraction of ΣDDT and dieldrin was very successful at PPNP. In a short period of time (five months), both organochlorine pesticides in the soil were significantly reduced. Moreover, both *S. scoparium* and *P. virgatum* demonstrated an equal potential to extract large amounts of these two organochlorine pesticides when they occur as co-contaminants. Additionally, both species had shoot BAF>1 for both pesticides, indicating that phytoextraction by native grasses is a viable remediation technique for Point Pelee. These results are especially important as phytoextraction by native grasses has been identified as the best green technology to remediate the organochlorine pesticides contamination at PPNP, as it does not affect Point Pelee's sensitive ecosystem and does not introduce invasive species. In the future, it is essential to establish a phytoextraction plot in an area with higher ΣDDT and dieldrin co-contamination within the park to verify if the results presented here are replicable in those conditions.

4. The Role of Hydroxypropyl-β-Cyclodextrin (HPβCD) in DDT Remediation at Point Pelee National Park

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4.1 ABSTRACT

Point Pelee National Park (PPNP) is highly contaminated with dichlorodiphenyltrichloroethane (DDT) due to the historical use of this persistent organochlorine pesticide. Hydroxypropyl-β-Cyclodextrin (HPβCD) which has the ability to form water soluble complexes with low-polarity organic compounds, was previously used as a DDT remediation technology at PPNP in 2003. In the present study, HPβCD's ability to promote DDT microbial degradation, enhance DDT phytoextraction by two native grasses (*Schizachyrium scoparium* and *Panicum virgatum*), and increase DDT bioavailability to redworms (*Eisenia fetida*) was investigated. HPβCD was not able to promote DDT microbial degradation in PPNP soils, however it was able enhance the DDT phytoextraction ability of *S. scoparium* plants. Additionally, HPβCD application to PPNP soil increased 2,4-DDE and 2,4-DDD bioavailability to redworms. Unfortunately, as a result of the increased water solubility of DDT, this pesticide is able to move through the soil column, and groundwater contamination is a possibility. Due to this important issue, *in-situ* use of HPβCD to remediate DDT contamination is not recommended at PPNP.

4.2 INTRODUCTION

Hydroxypropyl-β-Cyclodextrin (HPβCD) is part of a family of cyclic oligosaccharides compounds that are produced by the bacterial degradation of starch. Due to its hydrophobic exterior and its relatively apolar cavity, HPβCD can increase the apparent aqueous solubility of low-polarity organic compounds as well as enhance their desorption and transport in soil. The hydrophilic exterior can be dissolved in water, while the apolar cavity provides a hydrophobic matrix (micro heterogeneous environment) that can form inclusion complexes with hydrophobic guest molecules, such as dichlorodiphenyltrichloroethane (DDT) (Brusseau *et al.* 1997; Del Valle 2004).

DDT is an organochlorine pesticide that was used worldwide from 1941 to the early 1970s, when it was banned in many countries due to human health and environmental concerns. Even though it has been banned for decades in North America, DDT is still universally found in the environment, especially in soils, due to its strong adsorption to solid particles. Furthermore, DDT tends to accumulate in the fatty tissues of ingesting organisms, and is able to bioaccumulate up the food chain (Corona-Cruz *et al.* 1999; Crowe & Smith 2007).

DDT was first applied at Point Pelee National Park (PPNP) in 1948, and was continuously and extensively used until 1967 (Russell & Haffner, 1997). In the 1990s and early 2000s, studies performed on the park's wildlife found concerningly high levels of DDT in tissue samples from frogs, snapping turtles, and birds (Russell *et al.* 1995; Russell & Haffner 1997; Smits *et al.* 2005). Hundreds of soil samples have since been collected at PPNP to determine the levels of DDT contamination at the park. Many of these soil samples have DDT concentrations above 700 ng/g recommended by the Canadian Council of Ministers of Environment (CCME) (Crowe & Smith 2007; Clow *et al.* in press).

Several studies (Schwartz & Bar 1995; Bardi *et al.* 2000; Zhou *et al.* 2007; Qiu *et al.* 2009) with the parent compound of HPβCD, β-cyclodextrin (βCD), showed that this specific cyclodextrin was able to increase bioavailability of organic pollutants and/or to decrease the toxicity of substrates towards bacterial cells. Between 2002 and 2007, several studies were conducted by McMaster researchers to investigate the possibility of remediating soils contaminated with DDT at PPNP using HPβCD. These studies are described in four MSc. Theses (Marenco 2002; Badley 2003; Mironov 2004; Etherington 2007), but were never published in the literature. McMaster researches suggested that HPβCD treatment increased the bioavailability of DDT, and enhanced its degradation via microbial communities present in PPNP's soil. However, Stroud *et al.* (2009) reported that the introduction of HPβCD into soils did not enhance the biodegradation phenanthrene. Although the reported results

at PPNP were promising, the mechanism of action of HPβCD was unclear, and mobilization of DDT into the groundwater during treatment was a distinct possibility. Moreover, this could cause a possible increase in DDT bioavailability to soil invertebrates, including worms.

More recently, phytoextraction of DDT has been studied at PPNP (Paul *et al.* 2015; Denyes *et al.* 2016). Phytoextraction is a green technology that uses vascular plants *in-situ* to extract soil contaminants into plant roots and translocate them to the shoots, which must then be harvested and transported to a facility for incineration or composting (McCutcheon & Schnoor 2003; Suresh & Ravishankar 2004). It is a non-intrusive, and inexpensive way to remediate soils (Alkorta & Garbisu 2001), being more cost-effective than alternative mechanical or chemical methods of remediation (Suresh & Ravishankar 2004). Paul *et al.* (2015) evaluated the potential for native weed species to phytoextract DDT from contaminated soils at PPNP. Their results showed that some native grass species, including *Panicum virgatum* (switchgrass) and *Schizachyrium scoparium* (little bluestem), have great potential to extract DDT from PPNP contaminated soils.

Additionally, combining plants with solubility enhancement agents such as surfactants (e.g. HPβCD), may improve phytoextraction of non-polar organic contaminants (like DDT). HPβCD has never been used to enhance organochlorine pesticides phytoextraction, however Romeh (2015), reported that the application of HPβCD to soils spiked with cyanophos (an organophosphorus insecticide) and planted with *Plantago major* resulted in a 65% removal of the initial concentration of that insecticide in the soil. Other cyclodextrins have also been tested with phytoextraction techniques. Wang *et al.* (2015), saw a significant increase in phenanthrene concentrations in the shoots of ryegrass (*Lolium perenne* L.) treated with another cyclodextrin derived from βCD, cysteine-β-cyclodextrin (CβC). Furthermore, Chen *et al.* (2010), reported a removal of 38.1% the initial concentration of PCB from soils treated by a phytoextraction (by *L. perenne L)* and βCD combined approach.

The current study revisits the McMaster studies in order to determine the mechanisms of HPβCD action when amended to DDT contaminated soils. A series of experiments were designed to determine if HPβCD in fact increases DDT microbial degradation in highly contaminated PPNP soils. Laboratory and greenhouse experiments investigate the mechanism behind significantly DDT reduction in PPNP soils following HPβCD application. The objectives were to determine if the application of HPβCD solution to PPNP soils enhanced microbial DDT degradation, and/or increased DDT phytoextraction by native grasses (*P. virgatum* and *S. scoparium*). DDT bioavailability to redworms (*Eisenia fetida*) following HPβCD application to soils was also evaluated.

4.3 METHODS & MATERIALS

4.3.1 Site Description & Soil Collection

Point Pelee National Park (PPNP) located south of the town of Leamington, Ontario, consists of a peninsula of land (16 km^2) made up of marsh and woodland habitats. PPNP is known as an important staging area for migratory birds and is also a vital breeding area for many species of birds (Smits *et al.* 2005; Crowe & Smith 2007).

For laboratory and greenhouse studies, surface soil (0-10 cm) was collected from two different areas of the park. Each collected soil was individually thoroughly homogenized using the process described in Low *et al.* (2008) and Ficko *et al.* (2011). Briefly, soil was sieved through a 1 cm^2 sieve and consolidated in one pile on a table. The original pile of soil was quartered by random scooping using a flat-bottom scoop. Each of the four piles was manually mixed and re-combined into a central pile by scooping from the four piles in an alternating manner. The procedure was repeated 30 times for complete homogenization. The first collection area is a former agricultural land area, and due to this historical use it has very high concentrations of DDT. Soils were collected at this location at three different times, once in 2014, when the mean ΣDDT concentration in the soil was 14,000 ± 1,100 ng/g (n = 3), and twice in 2015 (in June, mean ΣDDT concentration of 31,000 ± 6,600 ng/g (n = 3), and in October, mean ΣDDT concentration of 11,000 ± 300 ng/g (n = 3). The second collection area is known as *Sleepy Hollow*, where the pesticide contamination is lower, mean ΣDDT concentration of 1,300 ± 20 ng/g (n = 3).

4.3.2 Experimental Design

A series of seven experiments were designed to determine if the application of HPβCD solution to PPNP soils enhanced microbial DDT degradation, increased DDT phytoextraction by *Panicum virgatum* and *Schizachyrium scoparium*, and increased DDT bioavailability to *Eisenia fetida*.

4.3.2.1 DDT Solubility Experiment

The objective of this initial experiment was to confirm that an HPβCD solution is able to solubilize the DDT present in PPNP soils. Based on previous studies carried out by Badley (2003), a 10%-HPβCD solution was selected, and the volume applied was based on pore volume (PV), which is the measure of void space within a specific volume of soil. The PV was calculated by multiplying the soil volume (mL) to the average porosity of PPNP's soils (0.45) determined by Marenco (2002). The number of pore volumes applied (10) was also based on Badley's work which showed no

appreciable change in DDT concentrations after this point. Five grams of DDT contaminated PPNP soil (~14,000 ng/g; collected in 2014) was added to each of 6 x 100 mL Erlenmeyer flasks. The flasks were then divided into two groups of three with the first group treated with 1.6 mL of 10%-HPβCD solution a day, and the second group (control) with the same amount of deionized water. After 10 days, the liquid and soil layers were collected, and analyzed for DDT concentration.

4.3.2.2 Microbial Activity Experiment

Six microbial columns were designed to create a hospitable environment for the microbial community to develop. These columns were based on a previous study carried out by Etherington (2007) that showed a 19% reduction of DDT and a 21% reduction of DDE in soils treated with 10%-HPβCD in this environment. The glass columns measured 4.5 cm in diameter, 30 cm in length, and had a stopcock at the bottom that led directly into a 1L amber glass jar that collected any liquid runoff from the columns. On March 16, 2015, ~200 g of DDT contaminated PPNP soil (~14,000 ng/g; collected in 2014) was added to each of the six glass columns. The columns were then wrapped with aluminum foil to minimize light exposure, and set on the top of a laboratory bench where they were undisturbed during the duration of this experiment (six weeks). For the first week, all six columns were treated twice with 72 mL (equivalent of 1PV) of deionized water. As the soil was previously dried and homogenized, this was an important step to restore the soil's moisture content and promote microbial growth. From the week of March 23, 2015 to the week of April 23, 2015, the three columns in the treatment group received 72 mL of 10%-HPβCD solution twice a week, while the remaining three (control group) received the same amount of deionized water. The 10%-HPβCD solution was prepared by adding 200 g of HPβCD powder (Fisher, cat # FSSP9745080) to 2 L of deionized water, and stirring until the HPβCD was completely dissolved, forming a colourless solution. Over the course of the experiment, three soil samples (~12 g) were collected from each column to be analyzed for overall microbial activity using the fluorescein diacetate (FDA) method (Adam & Duncan 2001; Green *et al.* 2006) soils were removed from the columns, place into a labelled Whirl-Pak® bag and frozen at -20ºC until analysis. On the same day, the 1 L amber glass jars were sealed with parafilm, and store at 4ºC until analysis.

4.3.2.3 Optimal HPβCD Concentration Experiment

A previous study conducted by Badley (2003), showed no significant difference in the DDT removal from soils treated with 10% and 20% HPβCD solutions. As HPβCD is an expensive product (~$700/kg), it is important to test if lower concentrations have the same DDT removal efficiency, as this will prevent the use of unnecessary amounts of HPβCD and reduce the overall remediation cost. The

following experiment was designed to determine the optimal HPβCD concentration to be used in PPNP soils. Twelve microbial columns were created as described above. On June 23, 2015, ~200 g of DDT contaminated soil from PPNP (~14,000 ng/g; collected in 2014) was added to each of 12 glass columns. As before, the columns were wrapped in aluminium foil to minimize light exposure and placed on a laboratory bench top. For the first week of treatment, all 12 columns received 72 mL (1PV) of deionized water twice to restore the soils' moisture. From the week of June 29, 2015 to the week of July 27, 2015, the 12 columns were separated into four different treatment groups: (1) three were treated with 10%-HPβCD solution, (2) three were treated with 5%-HPβCD solution, (3) three were treated with 2.5%-HPβCD solution, and (4) three were treated with deionized water. HPβCD solutions were prepared as described above. Similarly, columns were treated twice a week with 72 mL of their respective HPβCD solution concentration or deionized water (control) for a total of 10 applications. Soil samples were collected from all the columns on weeks three and six to be analyzed for overall microbial activity. One week after the last HPβCD treatment (August 4, 2015), all soils were removed from the columns, placed into a labelled Whirl-Pak® bag and frozen at -20ºC until analysis. On the same day, the 1 L amber glass jars were sealed with parafilm, and store at 4ºC until analysis.

4.3.2.4 Greenhouse Experiment I

As HPβCD is known to have the ability to form water soluble complexes with DDT, it was hypothesized that this HPβCD-DDT complex will be more readily taken up by known DDT phytoextractors than DDT alone. In order to investigate this hypothesis, a greenhouse experiment was designed using two native grasses, *Panicum virgatum* (switchgrass) and *Schizachyrium scoparium* (little bluestem), previously shown by Paul *et al.* (2015) to phytoextract DDT from PPNP soils. Seedlings having the park's unique genetic background were obtained from PPNP personnel on June 16, 2015. On June 29, 2015, the seedlings were transferred from clean potting soil to DDT contaminated soils from PPNP. Two different soils from the park were used, one with a high DDT concentration (~31,000 ng/g, collected on June 2015) from the former agricultural land area, and another with a lower DDT concentration (~1,300 ng/g, collected on June 2015) from the *Sleepy Hollow* area. A total of 54 six inch pots were filled with ~800 g of soil, 27 with the high DDT soil and 27 with the low DDT soil. Thirty-six of the 54 pots then received two seedlings each; half of the 36 pots were planted with *P. virgatum* and the other half with *S. scoparium*. The pots were then divided into three different treatment groups: (1) 1PV (~200 mL) of 10%-HPβCD solution, (2) 50 mL of 10%-HPβCD, and (3) tap water (control). Hence, a total of 18 different combinations were created, and all treatments were completed in triplicate (Table 4.1). Finally, to minimize DDT lost due to

HPβCD solution runoff from the pots' bottom holes during the HPβCD application, all pot trays were covered in aluminium foil.

Table 4.1. Summary of all treatment conditions in the greenhouse experiment. All treatments were completed in triplicate, and two plants of the same species were planted per pot.

Treatment #	[DDT] in soil	Volume of HPβCD applied	Plant Species
1	High	1PV	*P. virgatum*
2	High	1PV	*S. scoparium*
3	High	50 mL	*P. virgatum*
4	High	50 mL	*S. scoparium*
5	High	Tap water	*P. virgatum*
6	High	Tap water	*S. scoparium*
7	High	1PV	No Plant
8	High	50 mL	No Plant
9	High	Tap water	No Plant
10	Low	1PV	*P. virgatum*
11	Low	1PV	*S. scoparium*
12	Low	50 mL	*P. virgatum*
13	Low	50 mL	*S. scoparium*
14	Low	Tap water	*P. virgatum*
15	Low	Tap water	*S. scoparium*
16	Low	1PV	No Plant
17	Low	50 mL	No Plant
18	Low	Tap water	No Plant

This experiment was carried out over 13 weeks, from June 29, 2015 to September 20, 2015. During this time, plants were measured bi-weekly to assess their growth and their visual health was noted. Plants were grown in the RMC greenhouse, at 23ºC ± 2ºC, and under natural sunlight. They were watered with tap water as needed and treated with the determined volume of a 10%-HPβCD solution once a week starting on July 16, 2015, for a total of 10 weeks. HPβCD solution was prepared as previously described. On September 20, 2015, plants were removed from the pots, washed using tap water, dried, separated into shoots and roots, and placed in a pre-labeled ziplock bag. Approximately 250-300 g of soil from each pot was also collected, placed into a labelled Whirl-Pak® bag and frozen at -20ºC until analysis. A total of 144 plants samples, and 54 soil samples were collected.

4.3.2.5 Greenhouse Experiment II

This experiment was designed in order to determine the fate of all DDT in soil amended with HPβCD. On May 25, 2016 nine pots (4" in size) were filled with ~280

g (dry weight) of DDT contaminated soil from PPNP (~11,000 ng/g, collected on October 2015) each. On the same day, six seedling of *S. scoparium* (grown from OSC seeds – lot # 14-8005) were transferred from clean potting soil to six of the nine pots containing PPNP soil. The pots were then divided into three different treatment groups: (1) 10%-HPβCD + *S. scoparium*, (2) tap water + *S. scoparium* (planted control), (3) 10%-HPβCD (unplanted control). HPβCD solution was prepared as previously described. All pots and trays were covered in aluminium foil to capture any DDT run-off that might occur. From June 10, 2016 to August 12, 2016, soils were treated once a week with 18 mL of HPβCD solution or tap water according to their treatment group. During the entire experiment, plants were measured weekly to assess their growth and their health was visually noted. They were also watered with tap water as needed. Plants were grown at the RMC greenhouse in the same conditions as previously described. On August 23, 2016, plants were removed from the pots, rinsed using tap water, dried using a paper towel, separated into shoots and roots, and placed in a pre-labeled ziplock bag. Soil samples (~3 g) from three different sections of each pot were collected, and classified as top (0-2 cm), middle (2-4 cm), and bottom (4-6 cm) sections. The remaining soil in each pot was also collected, and placed into a labelled Whirl-Pak® bag. All samples were frozen at -20ºC until analysis.

4.4.2.6 Bioavailability Experiment

This experiment was designed to investigate if the application of 10%-HPβCD solution to PPNP soils increases DDT bioavailability to redworms, a common soil invertebrate. The method used was modified from Morrison *et al.* (2000). Briefly, on July 08, 2016 15 x 500 mL mason jars were filled with potting soil or DDT contaminated soil from PPNP (~11,000 ng/g, collected on October 2015) to the mark of 350 mL. The jars were divided into three different treatment groups with five replicates in each group: (1) potting soil (clean control), (2) DDT-PPNP soil (DDT control), (3) DDT-PPNP soil + HPβCD. The soils from the first and second group were brought to ~90% of their moisture holding capacity using deionized water (~80-100 mL). The soils from the third group were brought to ~90% of their moisture holding capacity using a 10%-HPβCD solution. HPβCD solution was prepared as previously described. Five redworms (*Eisenia fetida*) purchased from *Vermi-Sprout* located in Dundas, ON, weighing ~0.3 g each were added to each jar. The top mouth of all jars was wrapped with plastic film (replacing the lid), and small holes were added to provide air circulation. All 15 jars were placed in a growth chamber (Conviron model ATC60) set to the follow conditions: temperature = 21ºC, humidity = 70%, and lights = 500 lux and 24 hrs on. After 8 days, worms were removed from the jars, rinsed with deionized water and placed in petri dishes (labeled as their respective jars) with filter paper on the bottom. The petri dishes were placed in the fridge at 4ºC for 72 hrs to allow the worms to depurate. After 72 hrs in the fridge,

worms were dried in an oven at 25ºC for 24 hrs, and finally they were placed in labelled Whirl-Pak® bags, and frozen at -20ºC until analysis. All the results presented here are in triplicates, as two out of the five replicates from each group had a poor extraction efficiency due to Soxhlet failure, and were excluded from the statistical analysis.

4.4.2.7 Avoidance Experiment

The objective of this experiment was to determine if invertebrates avoid soils treated with HPβCD. The method used is described in Denyes *et al.* (2016). Briefly, on July 15, 2016, ~120 g of DDT contaminated soil from PPNP (~11,000 ng/g, collected on October 2015) was added to each of six compartments of five avoidance wheels (Figure 4.1). The avoidance wheels are hexagonal in shape, made of steel, and have multiple holes between the compartments to allow worms to move freely (Environment Canada 2007). To bring moisture back to the dried soils, ~25 mL of deionized water or 10%-HPβCD solution was alternatingly added to each of the six compartments. HPβCD solution was prepared as previously described. Ten redworms (*E. fetida*), weighting ~0.2-0.3 g each were added to the center of each avoidance wheel. All wheels were covered with aluminium foil with small holes to optimize air circulation, and placed in a growth chamber (Conviron model ATC60). The growth chamber was set to the same conditions described above. After 48 hours, the five wheels were emptied, one compartment at a time. The number of worms in each compartment was recorded. Redworms were rinsed with deionized water, weighed, and discarded.

Figure 4.1. One of five avoidance wheels used in the invertebrate avoidance experiment.

4.3.3 Analytical Methods

4.3.3.1 Soil Samples

Soil samples (5 g wet weight) were air-dried overnight at room temperature. Approximately 1 g of sample was used for analysis. Soil samples were extracted using the accelerated solvent extraction (ASE) method with 30-40 mL 50:50 of hexane: acetone, 100 µl of 1 ppm decachlorobiphenyl (DCBP) as an internal surrogate standard and ~15 g of Ottawa sand. The extract was concentrated by rotoevaporation and applied to a Florisil extraction column. The column was rinsed with hexane into a 10 mL volumetric flask. Samples were transferred to gas chromatograph (GC) vials and analyzed by an HP 6890 GC equipped with a ^{63}Ni electron capture detector (GC/ECD), a SPB-1 fused silica capillary column. The carrier gas was helium at a flow rate of 2 mL/min. Nitrogen was used as a makeup gas for the ECD. The results were expressed as nanograms of DDT per gram of dry weight soil.

4.3.3.2 Plant Samples

Plant samples (<10 g wet weight) were dried overnight in an oven at 25-30 ºC. Dried samples were finely grounded using an electric grinder (Thomas Scientific – model 3383-L10). Approximately 1 g of sample was used for analysis. Plant samples were extracted using the microwave extraction method with 15 mL 50:50 of hexane: acetone, and 100 µl of 1 ppm DCBP as an internal surrogate standard. The extract was transferred to a glass syncore flask by pouring through a glass funnel with a filter paper (Fisher P8) filled with ~5 g of sodium sulphate. The extract was then concentrated by a Büchi syncore to approximately 2 mL, and applied to a Florisil extraction column. The column was rinsed with hexane into a 10 mL volumetric flask. Plant samples were analyzed by gas chromatography in the same way as described for the soil samples.

4.3.3.3 Water Samples

Water samples were extracted using the DCM liquid-liquid extraction method, with a separatory funnel. The original sample bottle contents (~700 mL) were poured into a separatory funnel, after 100 µl of 1 ppm DCBP was added to it. The remaining sample in the original bottle was extracted by adding 25 mL of DCM to the sample bottle, and then shaken, and poured directly to a round-bottom flask through a funnel with sodium sulphate. This procedure was repeated two more times. After that, 25 mL of DCM was added to the separatory funnel containing the original water sample. The separatory funnel was then carefully shaken for about 2 to 3 minutes. Once the two liquids physically separated, DCM was transferred to a round-bottom flask through a filter containing sodium sulphate. The extract was then concentrated by rotoevaporation and solvent exchanged to hexane, applied to a Florisil extraction column, and placed in to 10 mL volumetric flask. Liquid samples were analyzed by gas chromatography in the same way as described for the soil samples.

4.3.3.4 Redworm Samples

Redworm samples were extracted by Soxhlet using the method described in Denyes *et al.* (2016). Briefly, worm samples were dried at 25 ºC for 24 h immediately prior to analysis, then samples were finely chopped using metal scissors (rinsed with acetone between samples), and homogenized. Chopped worm samples were dried at room temperature for ~12-18 h, and then samples were ground with sodium sulphate and Ottawa sand. These samples were then extracted in a Soxhlet apparatus for 4 hrs at 4-6 cycles per hour in 250 mL of dichloromethane and 250 mL of a 1:1 hexane: acetone mixture. Redworm samples were analyzed by gas chromatography in the same way as described for the soil samples.

4.3.3.5 Microbial Activity Assay

Fluorescein diacetate (FDA) is a colourless compound that is hydrolysed by both free and membrane bound enzymes releasing a coloured end product (fluorescein). This end product absorbs strongly in the 490 nm wavelength, and can be measured by spectrophotometry. The ability to hydrolyse FDA is widespread among bacteria and fungi, therefore this assay provides a good estimate of total microbial activity in soils. The FDA method used was modified from Adam & Duncan (2001) and Green *et al.* (2006). Briefly, ~1 g of air dried soil was placed into a 125 mL Erlenmeyer flask. Subsequently, 50 mL of potassium phosphate buffer and 0.5 mL of FDA substrate were added to the flask. The flasks were incubated for three hours at 37ºC, and then samples were transfer to a centrifuge tube, and centrifuged for five minutes. The samples were then filtered using a Fisher Q5 paper filter, and the absorbance at 490 nm was measured using a spectrophotometer.

4.3.4 Quality Assurance & Quality Control (QA/QC)

For every nine soil or plant samples extracted by either ASE or microwave, one analytical blank, and one control sample was included, as specified by the US-EPA method for organochlorine pesticides (US-EPA 2007). For soil samples, the analytical blank contained Ottawa sand, and the internal surrogate (DCBP), while for plant samples contained the internal surrogate (DCBP) and 15 mL 50:50 of hexane: acetone. The control sample, in both cases, was spiked with 100 µL of 2 ppm organochlorine pesticide mixture (Appendix IX, from Supelco). Samples concentrations were corrected for surrogate recovery, and all analytical blanks were less than 1.0 ng/g (below detection limit). For water samples, the blank contained 500 mL of deionized water and 100 µL of DCBP, while the control was 500 mL of deionized water spiked with 100 µL of 2 ppm organochlorine pesticide mixture (Appendix IX, from Supelco), and 100 µL of DCBP. The mean difference between the control standard and the control standard target was less than 20%, unless otherwise specified in Appendix B. All the available mean relative standard deviations between the analytical duplicates can be found in Appendix B. For the microbial activity assay, the blank was treated exactly like all samples, except no soil was added to the flask, a slight colour development can occur spontaneously at 37 ºC and should be subtracted from the results obtained. For the assay negative control, 0.5 mL of acetone was added instead of the 0.5 mL of FDA substrate. Raw data for the microbial activity assay can be found in Appendix B.

4.3.5 Statistical Analysis

Statistical analyses were performed using R 3.2.2 (free software for statistical computing and graphics). All DDT concentrations are reported on a dry weight (ng/g) basis and recorded with the standard deviation of the mean. Data were tested for normality using the Shapiro-Wilk test. Data was analyzed by an one-way analysis of variance (ANOVA), followed by a post hoc Tukey comparison with significance level $p = 0.05$.

4.4 RESULTS & DISCUSSION

4.4.1 DDT Solubility Experiment

This simple experiment looked at the effects of 10%-HPβCD application to DDT contaminated soils in a closed system. The result of this experiment agrees with what was previously reported by Badley (2003). The 10%-HPβCD solution was able to significantly increase DDT solubility when compared to the deionized water control (ANOVA, $F_{1,3} = 290$, $p < 0.001$, $n = 3,2$). A significant amount of DDT was found in the liquid layer of the HPβCD treated flasks (Table 4.2), reinforcing the possibility of DDT mobilization into the park's groundwater during the *in-situ* trial performed by Badley (2003). Furthermore, once the ΣDDT extracted from the soil and liquid layers were added, there was no significant difference between the treatment and the control (ANOVA, $F_{2,6} = 247.6$, $p < 0.001$, $n = 3$ / Tukey-test, $p > 0.05$), demonstrating no significant microbial degradation.

Table 4.2. Location of ∑DDT following treatment with HPβCD (n = 3).

	Liquid Layer (%)	**Soil Layer (%)**
10%-HPβCD	33 ± 2	67 ± 9
Control	2 ± 2	98 ± 2

4.4.2 Microbial Activity Experiment

In this column experiment, soils treated with HPβCD had 44% lower ∑DDT concentration (ANOVA, $F_{1,4} = 247.6$, $p < 0.001$, $n=3$) than the control soils after five weeks of treatment (Figure 4.2). This significant reduction in [∑DDT] in treated soils is consistent with the ones in Badley (2003), that found a 40% reduction in [∑DDT] in a column experiment after the application of 18 PV of 10%-HPβCD to soils.

Additionally, Etherington (2007) showed a 19% reduction of DDT and a 21% reduction of DDE after 10 PV of 10%-HPβCD treatment.

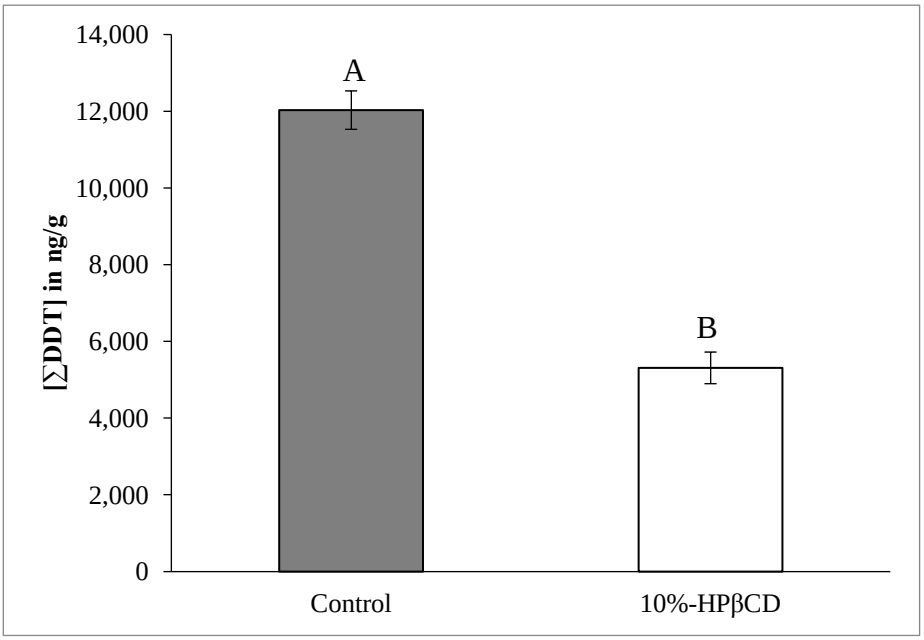

Figure 4.2. A comparison of the [∑DDT] in soils treated with 10%-HPβCD solution, and control soils treated with deionized water (n = 3), letters indicate where there is a significant difference.

Although these results seem promising, the ∑DDT concentration present in the runoff solution from the HPβCD treated columns was 495 μg/L, while the controls had only 0.12 μg/L. This result is supported by the results of the FDA assay, that showed no significant difference in the overall microbial activity in the columns treated with 10%-HPβCD when compared to the control columns treated with deionized water (ANOVA, $F_{1,4} = 1.88$, $p > 0.05$, $n = 3$). There was also no significant difference in the overall microbial activity between weeks of treatment (Figure 4.3).

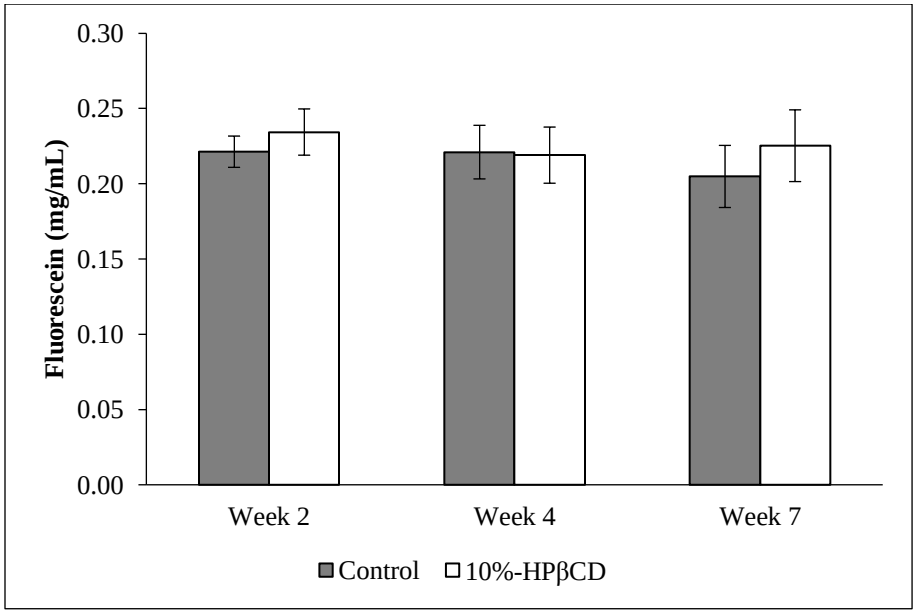

Figure 4.3. Microbial activity in the columns at weeks two, four, and seven (one week after the last treatment) measured by fluorescein diacetate assay, showing no significant difference between treatments or weeks. The 10%-HPβCD application in the columns had no significant effect on the measured microbial activity.

Successful increase in microbial degradation of organic contaminants following the application of other cyclodextrins have been reported before (Furuta *et al.* 2007; Zhou *et al.* 2007; Qiu *et al.* 2009), however they were all conducted in a closed flask using culture medium (Zhou *et al.* 2007; Qiu *et al.* 2009) or activated sludge (Furuta *et al.* 2007). Conversely, the results presented here are from a microbial column using soil collected from the field, and they suggest that even though the HPβCD treatment significantly reduced the ∑DDT concentration in the soils, the reduction was likely due to DDT mobilization into the runoff water, rather than DDT degradation. Moreover, no significant difference in overall microbial activity was detected by the FDA assay between treatments or between weeks.

4.4.3 Optimal HPβCD Concentration Experiment

In this column experiment, a reduction in ∑DDT concentration was observed in soils as the concentration of the HPβCD solution increased. Soils treated with 2.5%, 5% and 10% of HPβCD had a 4%, 19%, and 37% ∑DDT reduction respectively. However, [∑DDT] in soils treated with the two lower HPβCD concentrations (2.5%, and 5%,) were not significantly different from the control soils (ANOVA, $F_{3,8} = 2.48$, $p > 0.05$, $n = 3$) after five weeks of treatment; only soils treated with 10%-HPβCD

had a significant reduction in [∑DDT] (ANOVA, $F_{1,4} = 11$, $p < 0.05$, $n = 3$) (Figure 4.4). The results from the 5%-HPβCD solution here were lower than the significant 40% reduction of the initial DDT mass previously observed by Badley (2003).

The 10%-HPβCD results were consistent with the ones obtained in the previous microbial experiment, where a significant 44% reduction in soil ∑DDT concentration was observed. These results suggest that HPβCD concentrations lower than 10% are not efficient in increasing DDT degradation/removal from PPNP soils.

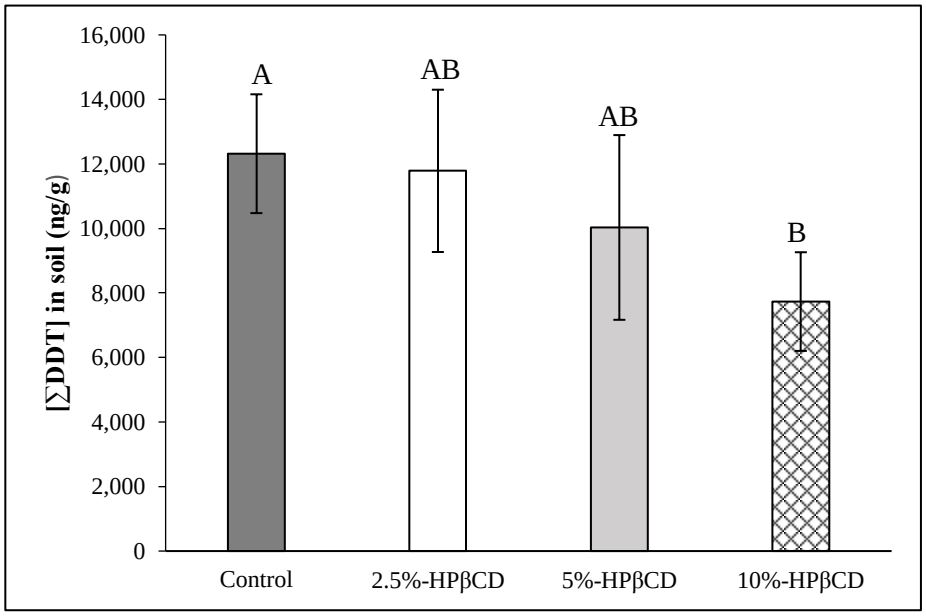

Figure 4.4. The ∑DDT concentrations in soils treated with three different HPβCD solutions (2.5%, 5%, and 10%), and control soils treated with deionized water (n = 3). Letters indicate where there is a significant difference.

In the liquid layer, all HPβCD had significant more ∑DDT than the control (ANOVA, $F_{3,8} = 240.6$, $p > 0.001$, $n = 3$). When the amount of ∑DDT found in the soil and liquid layers of the columns were added, there were no significant difference between all HPβCD and the control (ANOVA, $F_{3,8} = 0.75$, $p < 0.05$, $n = 3$). The results of this experiment showed that mobilization rather than microbial degradation was the main mechanism behind the reduction in [∑DDT] observed in soils treated with 10%-HPβCD (Figure 4.5).

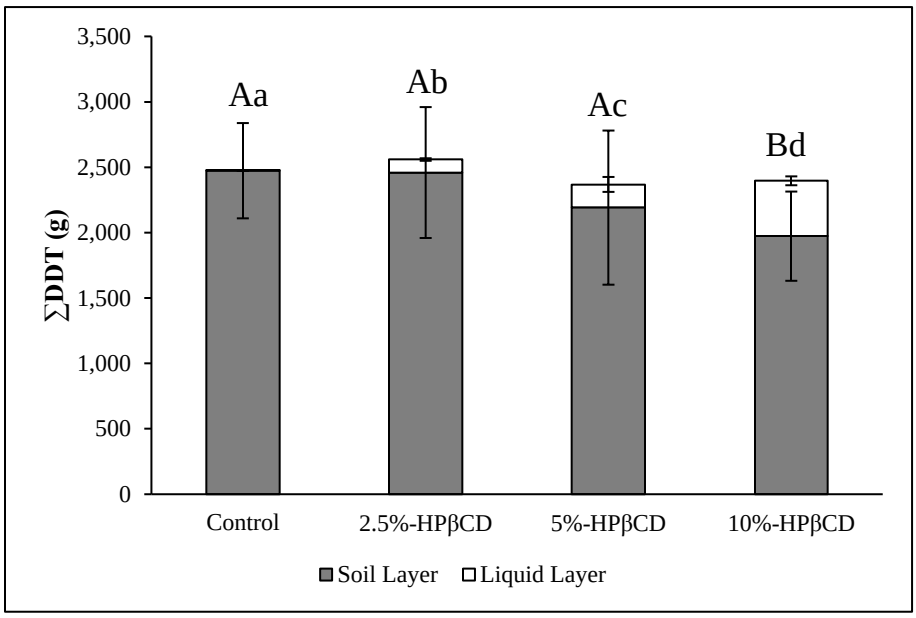

Figure 4.5. The ∑DDT in soil and in runoff water from samples treated with three different HPβCD solutions (2.5%, 5%, and 10%), and control samples treated with deionized water (n = 3). There is no significant difference in ∑DDT between all HPβCD treatments and the control when the two layers are added. Upper case letters indicate significant difference in soil samples, and lower case letters indicate significant difference in water samples.

The results of the FDA assay were consistent with the ones from the previous assay in that there were no significant difference in the overall microbial activity between the soils treated with different concentrations of HPβCD and the control soils (ANOVA, $F_{1,10} = 0.78$, $p > 0.05$, $n = 3$) (Figure 4.6).

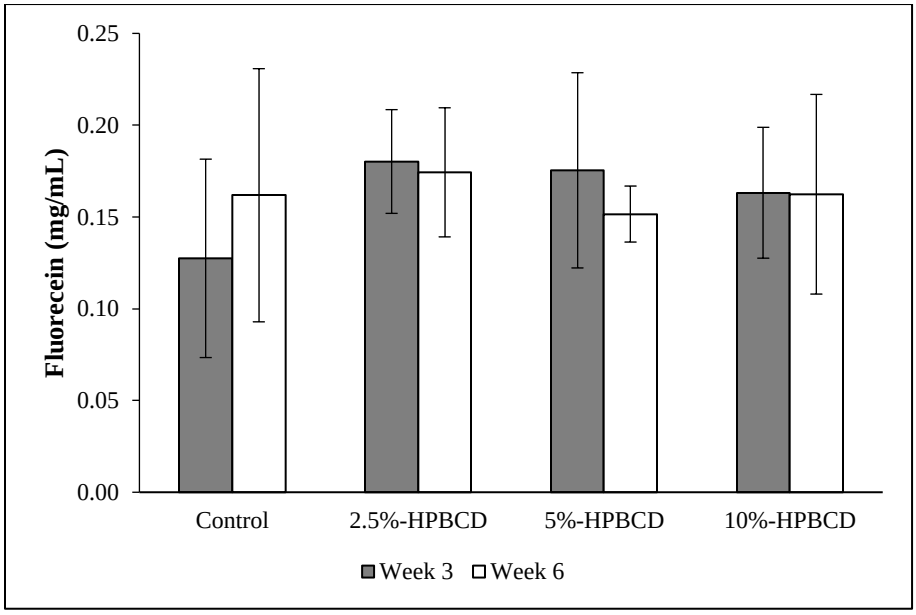

Figure 4.6. The overall microbial activity in the soils treated with different concentrations of HPβCD and the controls at weeks three and six of treatment. No significant difference in microbial activity between treatments or weeks was observed.

The results of both microbial column experiments suggest that HPβCD is not able to increase DDT microbial degradation. These results agree with the ones previously reported by Stroud *et al.* (2009) for phenanthrene. They found no significant difference in the mineralisation of this contaminant in soils amended with HPβCD in a microcosm experiment. Although, Badley (2003) observed a 1.5X increase in the number of bacteria in soil samples treated with 10%-HPβCD in the field, the microbial activity in the soil was never measured, and there was no clear link between the increase in bacterial cell number and the decrease in [ΣDDT] in the treated soils. As the FDA assay showed no significant difference in overall microbial activity between HPβCD treated soils and the control in both microbial column experiments presented here, the positive results observed earlier by Badley (2003) are likely due to DDT mobilization into PPNP's groundwater. Additionally, when the ΣDDT in the liquid layer was accounted for, there were no significant difference between samples treated with 10%-HPβCD and the control. **The use of HPβCD to promote DDT microbial degradation on site in PPNP is therefore not recommended**.

4.4.4 Greenhouse Experiment I

4.4.4.1 Plant Health

The overall health of *Schizachyrium scoparium* and *Panicum virgatum* plants (as determined visually) was not affected by HPβCD treatments, although the formation of a permanent dry HPβCD residue layer on top of the soils treated with the solution was observed in the last four weeks of treatment. Bi-weekly shoot measurements showed no significant differences between the heights of *S. scoparium* plants treated with the two different volumes of HPβCD and the control plants that received tap water growing in both low (~1,300 ng/g) and high (~31,000 ng/g) DDT contaminated soils (ANOVA, $F_{2,6} = 1.49$, $p > 0.05$, n=3; ANOVA, $F_{2,6} = 0.28$ $p > 0.05$, n=3). Moreover, there was no significant difference in the height of *S. scoparium* plants growing in the two different levels of soil contamination (ANOVA, $F_{2,1} = 0.13$, $p > 0.05$, n=3). However, *P. virgatum* plants, growing in low DDT soil, and receiving the 50 mL of 10%-HPβCD were significantly shorter than the ones in the control and 1PV of 10%-HPβCD groups by week eight (ANOVA, $F_{2,6} = 15.87$, $p < 0.01$, n=3 / Tukey-test, p = 0.004, 0.02). By week ten, the control plants were significantly taller than the ones in both HPβCD groups (ANOVA, $F_{2,6} = 11.31$, $p < 0.01$, n=3 / Tukey-test, p = 0.009, 0.03) (Figure 4.7). The same result was not observed with *P. virgatum* plants growing in high DDT soil. There was no significant difference between the height of these plants growing in the control soil or the ones treated with the two different volumes of 10%-HPβCD (ANOVA, $F_{2,6} = 4.82$, p = 0.06, n=3). However, even in high DDT soil, *P. virgatum* plants in the control group were observed to be visually taller (not significant) than the ones in the 1PV of 10%-HPβCD group (Tukey-test, p = 0.05, n=3).

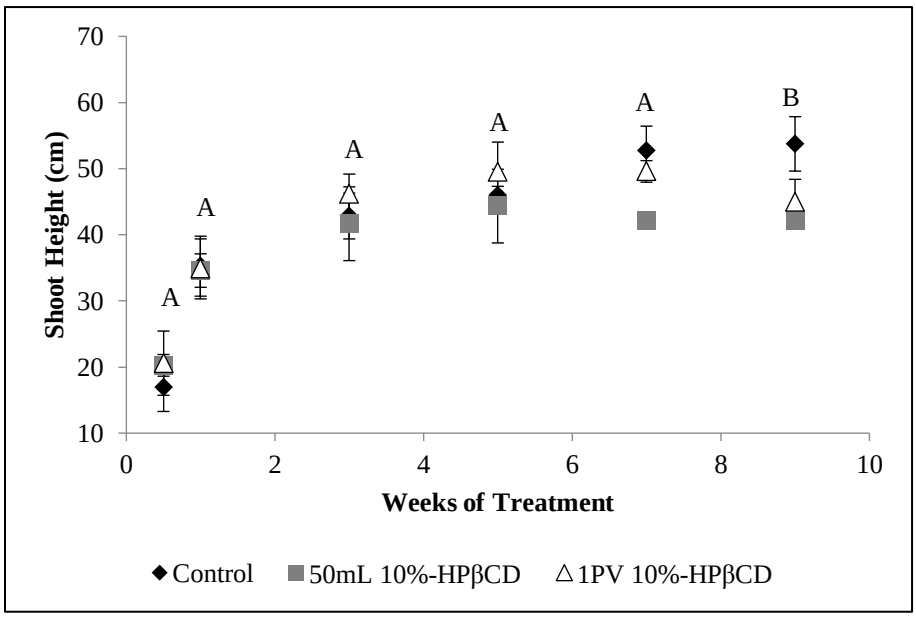

Figure 4.7. Shoot height of *P. virgatum* (measured in cm) grown in low DDT soil (1,300 ng/g) from the day of transplant (week zero) to the end of week ten of treatment. The *P. virgatum* plants treated with 10%-HPβCD weekly were significantly (B) shorter than the ones treated with tap water by week ten.

4.4.4.2 DDT concentration in plant tissues (low DDT soil)

In *P. virgatum* plants, [∑DDT] in shoots ranged from 170 ± 30 (n = 6) in the 1PV treatment group to 150 ± 40 ng/g (n = 6) in the control group. However, there was no significant difference in the concentration of ∑DDT in shoot tissues of *P. virgatum* plants treated with both volumes of 10%-HPβCD solution and the controls (ANOVA, $F_{2,15} = 0.95$, $p > 0.05$, n = 6) in low DDT soil (~1,300 ng/g). In roots, [∑DDT] ranged from 1,500 ± 290 ng/g (n = 6) in the control group to 700 ± 260 (n = 6) in the 1PV treatment group. The control group results were consistent with the 1,100 ng/g ∑DDT in root tissues observed by Paul *et al.* (2015) in *P. virgatum* plants grown at PPNP in a moderate ∑DDT-contaminated site (~5,000 ng/g). Moreover, control group roots extracted significantly more ∑DDT than both of HPβCD treatment groups (ANOVA, $F_{2,15} = 18.73$, $p < 0.01$, n = 6 / Tukey-test, $p < 0.001$, < 0.05) (Figure 4.8).

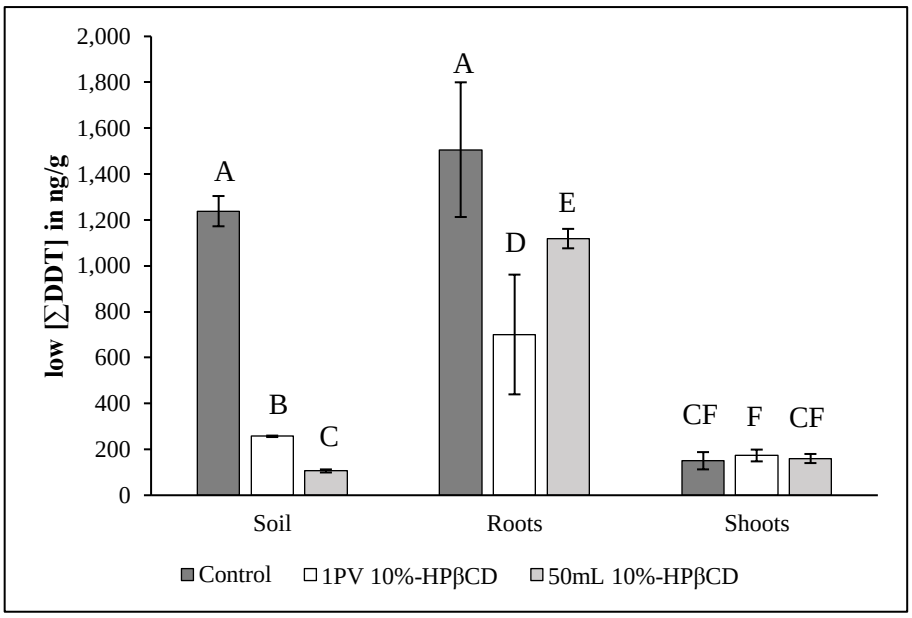

Figure 4.8. ΣDDT concentration found in low DDT soil (n = 3), roots (n = 6), and shoots (n = 6) of *P. virgatum* plants treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) and the control plants that received tap water (n = 6). Letters indicate where there is a significant difference.

In *S. scoparium*, [∑DDT] in shoots ranged from 470 ± 120 ng/g (n = 6) in the 50 mL treatment group to 190 ± 40 (n = 6) in the control group. Moreover, *S. scoparium* plants treated with both volumes of 10%-HPβCD solution had significantly higher concentration of ∑DDT in shoots than the ones in the control group (ANOVA, $F_{2,15}$ = 23.83, $p < 0.01$, n = 6 / Tukey-test, $p < 0.001$, < 0.001) in low DDT soil (~1,200 ng/g) (Figure 4.9). The [∑DDT] in *S. scoparium* shoots in all groups were lower than the 770 ng/g previously reported by Paul *et al.* (2015). In roots, [∑DDT] ranged from 1,500 ± 340 ng/g (n = 6) in the control group to 1,100 ± 140 (n = 6) in the 1PV treatment group. In *S. scopa*rium roots, the control group extracted significantly more ∑DDT than the ones in the 1PV HPβCD treatment group (ANOVA, $F_{2,15}$ = 3.95, $p < 0.05$, n=6 / Tukey-test, $p = 0.04$). There was no significant difference between the concentration of ∑DDT extracted from roots treated with 50 mL of 10%-HPβCD and the ones treated with tap water (control group) (Tukey-test, $p = 0.16$).

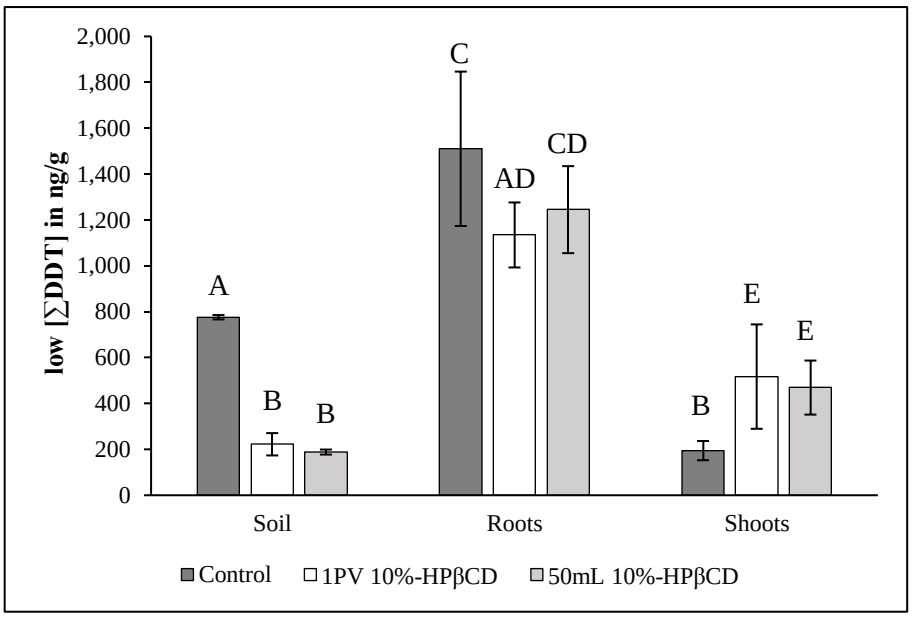

Figure 4.9. ΣDDT concentration in low DDT soil (n = 3), roots (n = 6), and shoots (n = 6) of *S. scoparium* plants treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) or tap water (control). Letters indicate where there is a significant difference.

These results indicate that *S. scoparium* is a better candidate than *P. virgatum* for a combined approach using phytoextraction and HPβCD in soils contaminated with lower concentrations of ∑DDT. The application of 50 mL of 10%-HPβCD significantly increased [∑DDT] in shoots, and did not affect the root's ability to extract DDT from the soil. Furthermore, these results agree with the ones previously reported by Romeh (2015) for cyanophos contaminated soils, where an increase of this insecticide in the leaves of *P. major* plants treated with 1%-HPβCD solution was observed.

When the different DDT metabolites are taken in consideration, there are also differences between the two grass species (Figure 4.10). The main metabolite present in *P. virgatum* shoots was 4,4-DDT with the highest percentage of 42% found in plants treated with 1PV of HPβCD. This result is consistent with Whitfield Åslund *et al.* (2010), that found that in the shoot tissue of *Curcubita pepo ssp pepo* 57-63% of DDT metabolites consisted of 4,4-DDT. However, for *S. scoparium* 2,4-DDE was the main metabolite present, with the highest percentage of 66% in plants treated with 50 mL of HPβCD. Additionally, 4,4-DDE, the main metabolite in the original soil (90%), represents only 20-10% of the metabolites present in *S. scoparium* and 23-10% in *P. virgatum*.

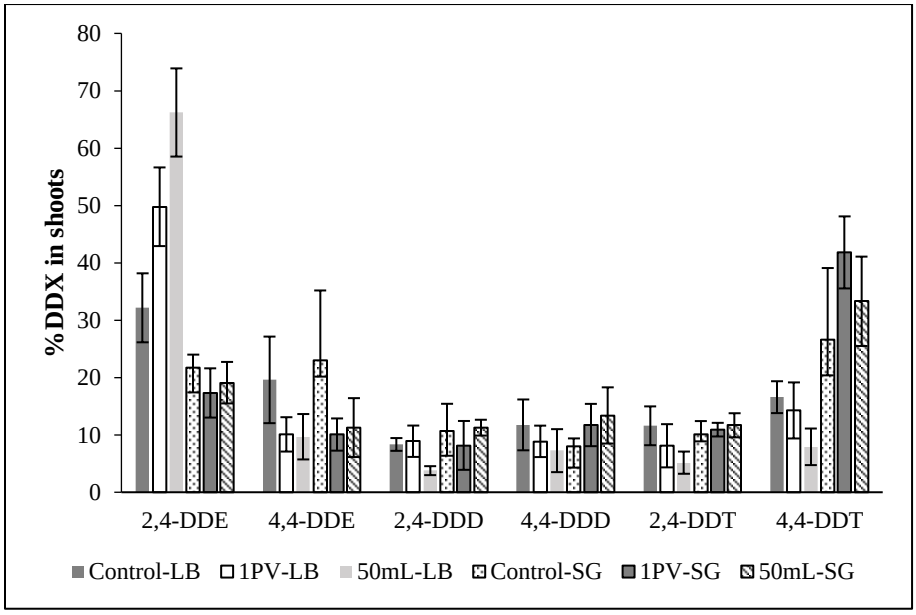

Figure 4.10. The percentage of each DDT metabolite in shoots of *S. scoparium* (LB) and *P. virgatum* (SG) plants grown in low DDT soil and treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) or tap water (control). *S. scoparium* plants extracted more 2,4-DDE, while *P. virgatum* plants extract more 4,4-DDT.

4.4.4.3 DDT concentration in soil samples (low)

In low DDT soil (~1,300 ng/g), soil samples from control pots planted with *P. virgatum* showed only a 3% not significant reduction in ΣDDT concentration when compared to the unplanted control (ANOVA, $F_{5,12} = 1538$, $p < 0.001$, n = 6 / Tukey-test, $p = 0.96$). However, soil samples from control pots planted with *S. scoparium* had a significant 39% reduction in [ΣDDT] (ANOVA, $F_{5,12} = 1192$, $p < 0.001$, n = 6 / Tukey-test, $p < 0.001$). Soils treated with 1PV of 10%-HPβCD solution, had a significant 85% (unplanted), 83% (*S. scoparium*), and 80% (*P. virgatum*) reductions in [ΣDDT] (ANOVA, $F_{8,18} = 681$, $p < 0.001$, n = 6 / Tukey-test, $p < 0.001$, 0.001, 0.001). These results are consistent with the ones obtained in the field by Badley (2003), when an 83% decrease in ΣDDT concentration was observed. In the 1PV 10%-HPβCD treatment, there was no significant difference between the unplanted soils and the ones planted with *S. scoparium* (Tukey-test = 0.52), while the soils planted with *P. virgatum* had significant higher [ΣDDT] than the unplanted ones (Tukey-test < 0.001). Soils treated with 50 mL of 10%-HPβCD solution, had a significant 91% (unplanted), 85% (*S. scoparium*), and 92% (*P. virgatum*) reduction in [ΣDDT] (ANOVA, $F_{8,18} = 681$, $p < 0.001$, n = 6 / Tukey- test, $p < 0.001$, 0.001,

0.001). Moreover, there was no significant difference between the unplanted soils and the ones planted with *P. virgatum* (Tukey-test = 0.65), while the soils planted with *S. scoparium* had significant higher [ΣDDT] than the unplanted ones (Tukey-test < 0.05). The significant reduction in [ΣDDT] in soils treated with both volumes of 10%-HPβCD cannot be explained by the significant increase of [ΣDDT] in plant tissue (Figure 4.11).

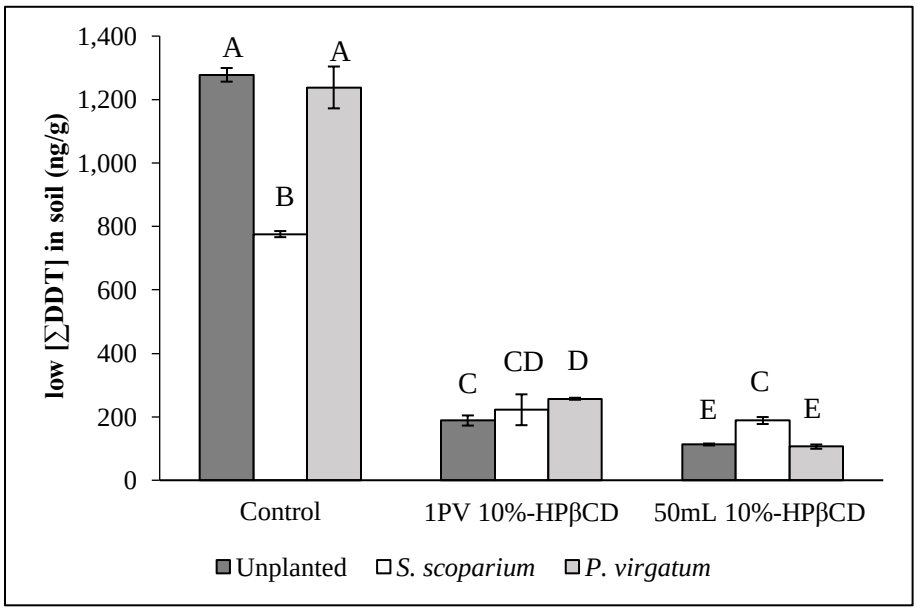

Figure 4.11. ΣDDT concentration in low DDT soil samples from unplanted or planted (with *S. scoparium* or *P. virgatum*) pots treated for 10 weeks with two different volumes of 10%-HPβCD (1PV or 50 mL) and the control samples treated with tap water (n = 3). Letters indicate where there is a significant difference.

The significant reductions of 85% and 91% in [∑DDT] in unplanted soil treated with 1PV and 50 mL of 10%-HPβCD respectively, were even greater than the ones observed in the microbial column experiments (44% and 37%). These reductions were also greater than the 34% reduction in organochlorine pesticides observed by Ye *et al.* (2014) in a soil washing experiment using carboxymethyl-β-cyclodextrin (CMβCD). Additionally, these significant reductions were consistent with the ones reported by Brusseau *et al.* (1997), where 86% of the initial phenanthrene was removed from the soil after a 20 PV application of mixture of CMβCD and HPβCD. These results suggest that phytoextraction is not the main mechanism behind the significant reduction in [∑DDT] in soils treated with both volumes of 10%-HPβCD, and DDT mobilization is likely occurring.

4.4.4.4 DDT concentration in plant tissues (high DDT soil)

In high DDT soil (~31,000 ng/g), [∑DDT] in shoots of *P. virgatum* plants ranged from 250 ± 20 (n = 6) in the 50 mL treatment group to 180 ± 40 ng/g (n = 6) in the control group. *P. virgatum* plants treated with 50 mL of 10%-HPβCD had significantly higher [∑DDT] in their shoots than ones in the control group (ANOVA, $F_{2,15} = 11.15$, $p < 0.05$, n=6 / Tukey-test < 0.001). However, ∑DDT concentration in shoots were lower than the 1,000 ng/g previously observed in the field ([∑DDT] ~ 10,200 ng/g) by Paul *et al.* (2015). In roots, [∑DDT] ranged from 25,100 ± 3,300 (n = 6) in the control group to 14,400 ± 2,200 ng/g (n = 6) in the 50 mL treatment group. Paul *et al.* (2015), reported a lower root [∑DDT] of 1,000 ng/g in the field ([∑DDT] ~ 10,200 ng/g). In this greenhouse trial, the control group had significantly higher [∑DDT] in roots than both of HPβCD treatment groups (ANOVA, $F_{2,15} = 18.73$, $p < 0.01$, n=6 / Tukey-test < 0.001, 0.05) (Figure 4.12).

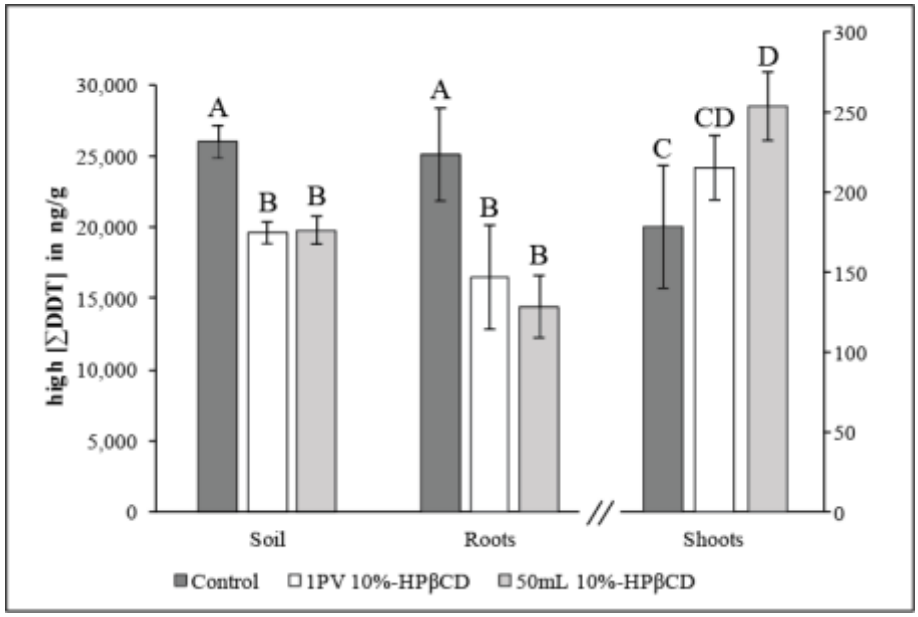

Figure 4.12. ΣDDT concentration in high DDT soil (n = 3), roots (n = 6), and shoots (n = 6) of *P. virgatum* plants treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) or tap water (control). Letters indicate where there is a significant difference.

In high DDT soil (~31,000 ng/g), [∑DDT] in *S. scoparium* shoots ranged from 810 ± 60 (n = 6) in the 50 mL treatment group to 170 ± 40 ng/g (n = 6) in the 1PV treatment group. Plants treated with 50 mL of 10%-HPβCD had significantly higher [∑DDT] in their shoots than ones in the control group (ANOVA, $F_{2,15} = 222.3$, $p < 0.01$, n=6 / Tukey-test, $p < 0.001$). The [∑DDT] in *S. scoparium* shoots in all groups were lower than the 3,600 ng/g previously reported by Paul *et al.* (2015). In roots, [∑DDT] ranged from 34,300 ± 3,400 (n = 6) in the control group to 25,700 ± 3,700 ng/g (n = 6) in the 1PV treatment group. As in low DDT soil, the roots of *S. scoparium* plants in the control group extracted significantly more ∑DDT than the plants in 1PV HPβCD treatment group (ANOVA, $F_{2,15} = 3.90$, $p < 0.05$, n = 6 / Tukey-test, $p < 0.04$). Additionally, there was no significant difference between the concentration of ∑DDT in roots treated with 50 mL of 10%-HPβCD and the ones treated with tap water (control group) (Tukey-test, p = 0.16; Tukey-test, p = 0.20) (Figure 4.13).

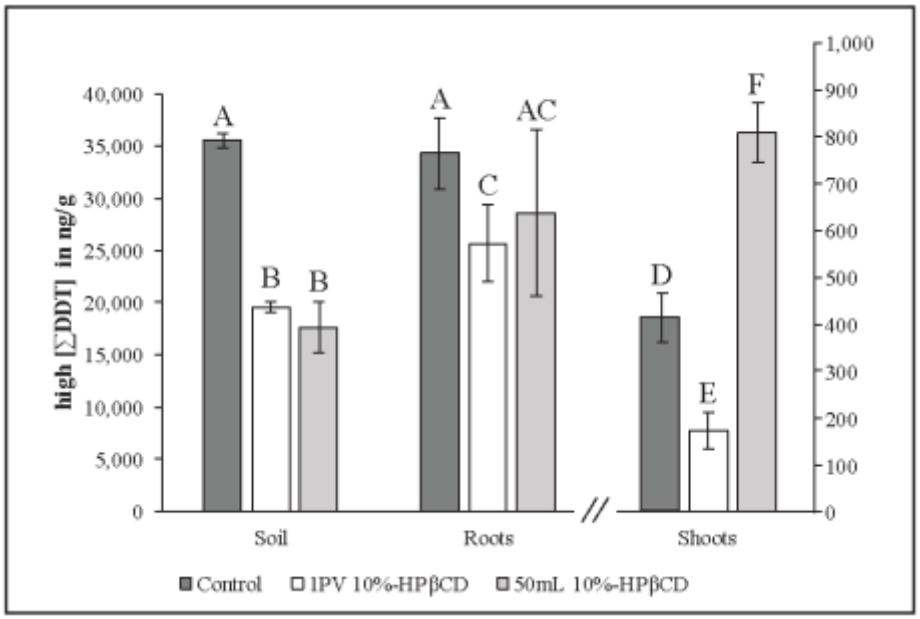

Figure 4.13. ΣDDT concentration found in high DDT soil (n = 3), roots (n = 6), and shoots (n = 6) of *S. scoparium* plants treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) or tap water (control). Letters indicate where there is a significant difference.

The amendment of 50 mL of 10%-HPβCD to soils increased in approximately 42% and 96% the ∑DDT concentration in *P. virgatum* and *S. scoparium* shoots, respectively. Not many soil amendments have successfully increased [∑DDT] in shoots before (White *et al.* 2003; Whitfield Åslund *et al.* 2010; Mitton *et al.* 2012). Other than HPβCD, organic acids amendment to soils have been able to significantly increase ∑DDT concentration in shoots of zucchini (White *et al.* 2003), and willow plants (Mitton *et al.* 2012). Whitfield Åslund *et al.* (2010) reported that the addition of 10%-Biosolve (another surfactant) significantly decrease the [∑DDT] in shoots of *Curcubita pepo ssp pepo* plants.

When the different DDT metabolites are taken into consideration, there were also differences between the two grass species (Figure 4.14), and between low and high DDT soils. In high DDT soil, the main metabolite present in *S. scoparium* shoots was 4,4-DDE, with the highest percentage of 53% in the control group, which is slightly less than its contribution in the original soil profile (76%), while in low DDT soil, the main metabolite was 2,4-DDE. For *P. virgatum*, the main metabolite present in shoot tissues varies between treatments, in the control group it was 2,4-DDE (34%), in the 1PV of HPβCD was 4,4-DDT (38%), and in the 50 mL of HPβCD was 4,4-DDE (42%). In low DDT soil, the main metabolite present in *P. virgatum* shoots was 4,4-DDT for all treatment groups. The low DDT soil and 1PV results in high DDT soil were consistent with Whitfield Åslund *et al.* (2010), that found that in the shoot tissue of *Curcubita pepo ssp pepo* 57-63% of DDT metabolites consisted of 4,4-DDT.

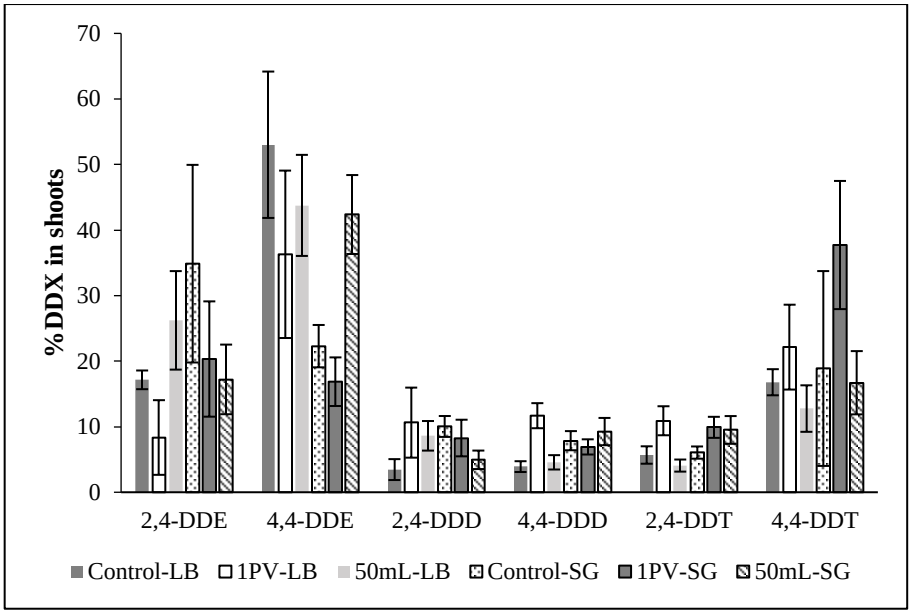

Figure 4.14. The percentage of each DDT metabolite in shoots of *S. scoparium* (LB) and *P. virgatum* (SG) plants grown in high DDT soil and treated for 10 weeks with two different volumes of 10%-HPβCD (1PV and 50 mL) or tap water (control). *S. scoparium* plants have a higher percentage of 4,4-DDE. For *P. virgatum* the percentage of DDT metabolites changed for each treatment.

The goal of phytoextraction is to maximize the contaminant concentration, in this case DDT, in the harvestable tissue of plant (shoots). This greenhouse experiment demonstraded that *S. scoparium* is a better candidate than *P. virgatum* to be used in a phytoextraction-HPβCD combined approach in both low and high DDT contaminated soils at PPNP. The application of 50 mL of 10%-HPβCD solution significantly increased [ΣDDT] in *S. scoparium* shoots without affecting the overall ability of this species to phytoextract DDT. Moreover, in high DDT soil, the concentration of all six DDT metabolites was significantly higher in the shoots of *S. scoparium* plants treated with 50 mL of 10%-HPβCD solution than the control plants (Figure 4.15).

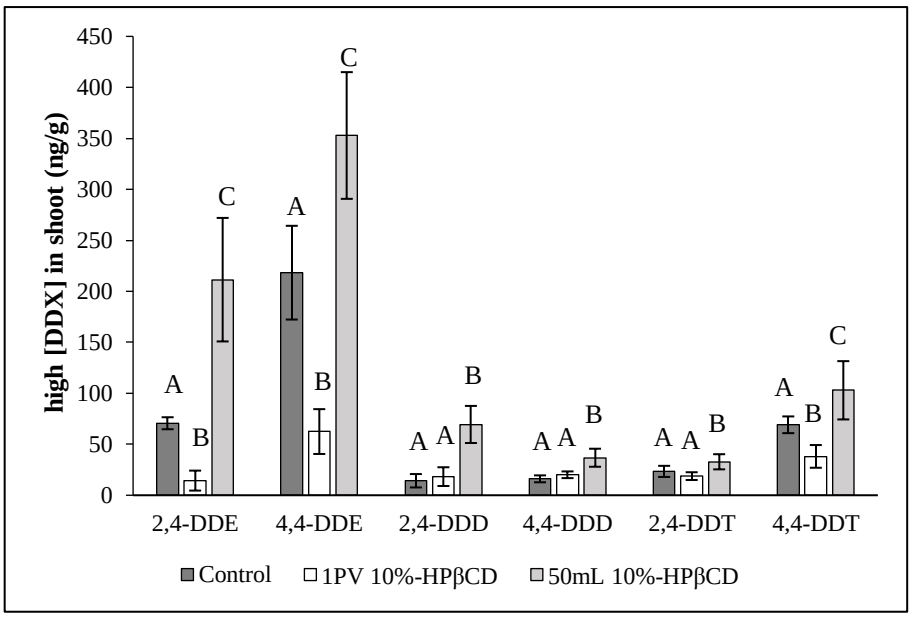

Figure 4.15. All DDT metabolites were found to be significantly higher in the shoots of *S. scoparium* plants treated with 50 mL of HPβCD. Letters indicates where there is a significant difference. The data from 2,4-DDE had to be log transformed to follow normal distribution (Shapiro-Wilk test, $p < 0.05$).

4.4.4. DDT concentration in soil samples (high)

In high DDT soil (~31,000 ng/g), similar to results obtained in the low DDT soil, there was no significant difference between those soils planted (with *S. scoparium* or *P. virgatum*) and those unplanted treated with both volumes of 10%-HPβCD (Figure 4.16). All soils treated with 1PV of 10%-HPβCD solution, had a significant 54% reduction in [ΣDDT] (ANOVA, $F_{8,18} = 127.5$, $p < 0.001$, $n = 6$ / Tukey-test, $p < 0.001$, 0.001, 0.001). Soils treated with 50 mL of 10%-HPβCD solution, had a significant 55% (unplanted), 59% (*S. scoparium*), and 54% (*P. virgatum*) reduction in [ΣDDT] (ANOVA, $F_{8,18} = 127.5$, $p < 0.001$, $n = 6$ / Tukey-test, $p < 0.001$, 0.001, 0.001). These reductions in ΣDDT concentration were consistent with the ones observed in the low DDT soil, in both microbial column experiments, and by Badley (2003) and Etherington (2007). Additionally, soil samples from control pots planted with *S. scoparium* and *P. virgatum* showed a significant 17% and 39% reduction in ΣDDT concentration when compared to the unplanted control (ANOVA, $F_{8,18} = 127.5$, $p < 0.001$, $n = 6$ / Tukey-test, $p < 0.001$, 0.001).

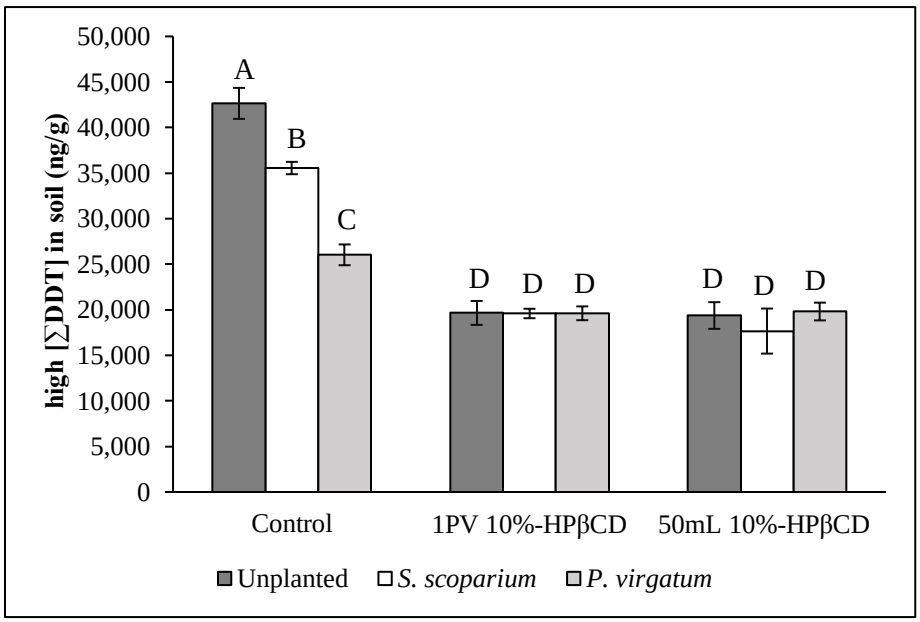

Figure 4.16. ΣDDT concentration in high DDT soil samples from unplanted and planted (with *S. scoparium* or *P. virgatum*) pots treated for 10 weeks with two different volumes of 10%-HPβCD (1PV or 50 mL) or tap water (control) (n=3). Letters indicate where there is a significant difference.

The results of both low and high DDT contaminated soils suggest that HPβCD is able to increase DDT phytoextraction, however, DDT mobilization is most likely the main mechanism behind the significant reduction in [ΣDDT] in HPβCD treated soils. **Hence, the use of HPβCD to enhance DDT phytoextraction on site in PPNP is not recommended.**

4.4.5. Bioaccumulation Factors (BAFs) & Translocation Factors (TFs)

The efficiency of phytoextraction can be calculated using bioaccumulation factors ($[contaminant]_{plant}/[contaminant]_{soil}$), and translocation factors ($[contaminant]_{shoot}/[contaminant]_{root}$). When shoot BAFs and TFs are greater than one, phytoextraction is likely to be a cost effective technique (Lunney *et al.* 2004; Whitfield Åslund *et al.* 2010). The highest shoot BAF (2.50) was calculated for *S. scoparium* plants grown in low DDT soil (~1,300 ng/g) and treated with 50 mL of 10%-HPBCD solution, and the lowest (BAF = 0.007) for *P. virgatum* plants in the control group grown in high DDT soil (~31,000 ng/g). Overall, *S. scoparium* plants had higher shoot BAFs than *P. virgatum* plants regarding the treatment type. Additionally, shoot BAFs were much higher in plants grown in low DDT soil, which is consistent with earlier work

performed by White *et al.* (2003), Lunney *et al.* (2004), and Paul *et al.* (2015). Moreover, *S. scoparium* and *P. virgatum* plants in the low DDT soil and treated with 50 mL of 10%-HPβCD had BAFs higher than the ones previously reported in the literature for these two species (Table 4.3).

Table 4.3. A comparison of shoot BAFs of *S. scoparium* and *P. virgatum* plants treated with 10%-HPβCD (*) and the ones previously reported for these two grasses, and other successful DDT phytoextractor species.

Species	**$[\Sigma DDT]_{soil}$ (ng/g)**	**BAF**	**Source**
*S. scoparium**	~1,300	2.50 ± 0.75	
S. scoparium	~290	< 0.50	Paul *et al.* (2015)
*P. virgatum**	~1,300	1.52 ± 0.25	
P. virgatum	~290	< 0.25	Paul *et al.* (2015)
Sporobolus cryptandrus	~290	< 0.25	Paul *et al.* (2015)
Cucurbita pepo cv. Howden	~150	2.4	Lunney *et al.* (2004)
Cucurbita pepo L. cv. Senator hybrid	~150	1.5	Lunney *et al.* (2004)

No TF greater than one was found in this experiment, the highest calculated TF was 0.48 for *S. scoparium* plants grown in low DDT soil and treated with 1PV of 10%-HPBCD solution, which is comparable to the TF of 0.42 previously obtained by Paul *et al.* (2015) in a field trial. Overall, *S. scoparium* plants had higher TFs than *P. virgatum* plants in all types of treatment and soil contamination level, except for the 1PV 10%-HPBCD treatment group in the high DDT soil.

4.4.5 Greenhouse Experiment II

This greenhouse experiment considered the fate of DDT in soils amended with HPβCD with and without the presence of a phytoextractor (*S. scoparium*).

4.4.5.1 Plant Health

Similar to the 2015 greenhouse experiment, the overall health of *S. scoparium* was not affected by HPβCD treatment (ANOVA, $F_{1,4} = 1.47$, $p > 0.05$, n=3).

4.4.5.2 DDT concentration in plant tissues

In shoots [ΣDDT] ranged from 490 ± 120 (n = 3) in the control group to 240 ± 120 in the 10%-HPβCD group. However, there was no significant difference in [ΣDDT] in shoots of *S. scoparium* plants treated with 10%-HPβCD and the ones in the control group (ANOVA, $F_{1,4} = 6.43$, $p > 0.05$, n=3). This result was inconsistent with the one obtained in the previous greenhouse experiment, where *S. scoparium* plants in the HPβCD group had a significantly higher [ΣDDT] in their shoots than the plants in the control group. Additionally, ∑DDT concentration in shoots were lower than the 810 ± 60 (n = 6) observed in the 2015 greenhouse experiment, and the 1,000 ng/g previously observed in the field ([∑DDT] ~ 10,200 ng/g) by Paul *et al.* (2015). It is important to note that *S. scoparium* plants in the 2015 greenhouse experiment were obtained from PPNP, while the plants in this experiment were grown from OSG seeds; this may partially explain the inconsistency between results. In roots, [ΣDDT] ranged from 21,700 ± 9,800 (n = 3) in the 10%-HPβCD group to 18,400 ± 7,500 in the control group. The root results were consistent with the ones previously reported, as there was no significant difference between the [ΣDDT] in plants treated with 10%-HPβCD and the ones treated with tap water (control) (ANOVA, $F_{1,4} = 0.21$, $p > 0.05$, n=3) (Figure 4.17). The 4,4-DDD was the only metabolite that had significantly higher concentration in the roots of *S. scoparium* plants treated with 10%-HPβCD than the ones in the control group (ANOVA, $F_{1,4} = 8.12$, $p < 0.05$, n=3).

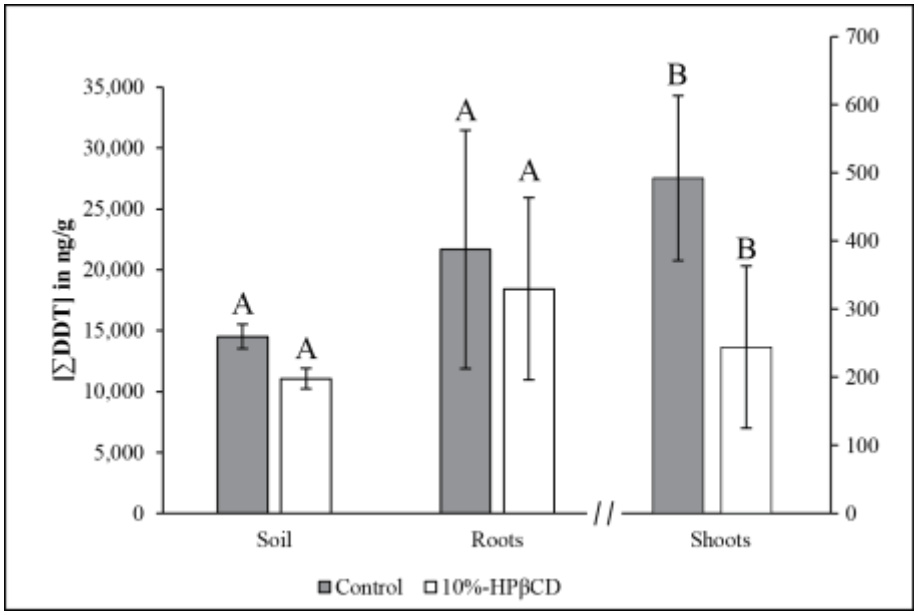

Figure 4.17. ΣDDT concentration in soil, roots, and shoots of *S. scoparium* plants treated for 10 weeks with 10%-HPβCD or tap water (control) (n=3). Letters indicate where there is a significant difference.

When the different DDT metabolites were taken into consideration, there were no differences between the two treatments (Figure 4.18). 4,4-DDE and 4,4-DDT were the main metabolites found in shoot tissue, which is consistent with the main metabolites present in the soil. These results were consistent with the ones observed in the 2015 greenhouse experiment, where 4,4-DDE was the main metabolite found in *S. scoparium* shoots.

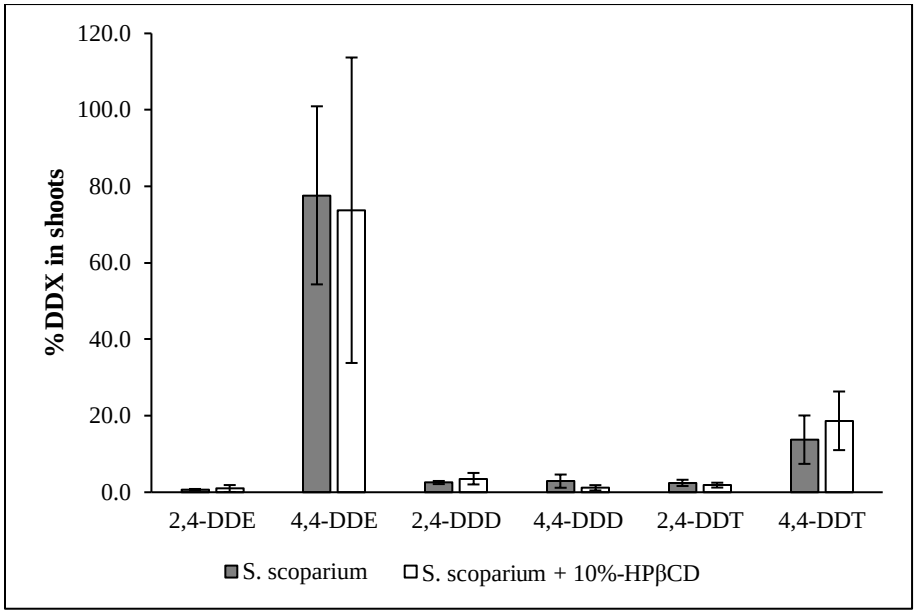

Figure 4.18. The percentage of each DDT metabolite in shoots of *S. scoparium* plants treated for 10 weeks with 10%-HPBCD or tap water. There were no differences between the two treatments.

4.4.5.3 DDT concentration in soil samples

There was no significant difference between those soils planted (with *S. scoparium*) and those unplanted treated with 10%-HPβCD (ANOVA, $F_{2,6} = 16.36$, $p < 0.01$, n = 3 / Tukey-test, $p > 0.05$). Soils treated with 10%-HPβCD solution, had a significant 23% (unplanted), and 28% (*S. scoparium*) reduction in [ΣDDT] (ANOVA, $F_{2,6} = 16.36$, $p < 0.01$, n = 3 / Tukey-test, $p < 0.05$, 0.01). These reductions in ΣDDT concentration were consistent with the ones observed in the previous greenhouse experiment, in both microbial column experiments, and by Badley (2003) and Etherington (2007). Additionally, unplanted soils treated with 10%-HPβCD had a higher ΣDDT concentration in the bottom section (4-6 cm), showing a pattern of HPβCD-DDT complex moving through the soil profile and being deposited further down in the soil (Figure 4.19).

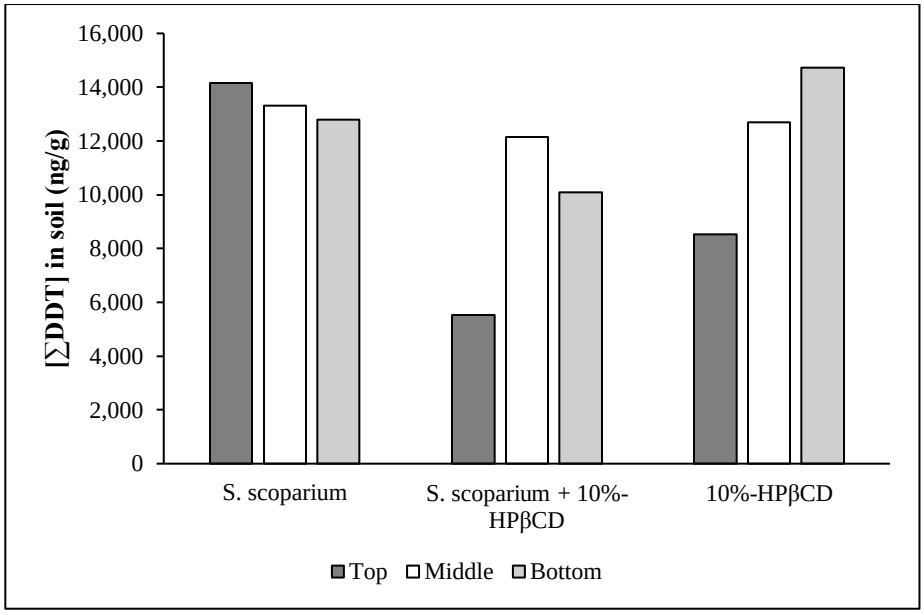

Figure 4.19. ΣDDT concentration in each of the three different sections of soil, that were classified as top (0-2 cm), middle (2-4 cm), and bottom (4-6 cm) sections (n = 1). In the unplanted soil treated with 10%-HPβCD, the [ΣDDT] decreased from the top to the bottom of the soil column.

4.4.6 Bioavailability Experiment

Redworms exposed to both DDT contaminated soil (~11,000 ng/g) and to DDT contaminated soil amended with 10%-HPβCD had significant higher ΣDDT concentration in their tissues than the ones exposed to potting soil (ANOVA, $F_{2,5}$ = 121.7, $p < 0.001$, n = 3 / Tukey-test, $p < 0.001$, 0.001). However, low concentrations of 4,4-DDE (90 ng/g) and 4,4-DDT (70 ng/g) were found in tissues of redworms exposed to potting soil. The addition of 10%-HPβCD to DDT contaminated soil did not significantly increase the [ΣDDT] in redworms (ANOVA, $F_{2,5}$ = 121.7, $p < 0.001$, n = 3 / Tukey-test, $p > 0.05$). When the different DDT metabolites were taken in consideration, the concentration of 2,4-DDE and 2,4-DDD was significantly higher in redworms exposed to soils treated with 10%-HPβCD (ANOVA, $F_{2,5}$ = 196.1, $p < 0.001$, n = 3 / Tukey-test, $p < 0.05$; ANOVA, $F_{2,5}$ = 73, $p < 0.001$, n = 3 / Tukey-test, $p = 0.01$), however, these two metabolites account for < 1% of the ΣDDT in PPNP soils. The main DDT metabolite in the soil is 4,4-DDE (73%), and this was also the main metabolite in redworm tissue (> 90%), in both HPβCD treated and untreated groups. The DDE uptake from the PPNP soil was consistent with the one previously reported by Morrison *et al.* (2000) for redworms in DDT aged soil (Table 4.4).

However, in Morrison *et al.* (2000) the highest metabolite assimilated from the soil was DDE, while in this study it was DDD.

Table 4.4. A comparison of the percentage DDX in soil that was assimilated by the redworms in this study (b) and in Morrison *et al.* (2000) (a). Aging periods are based on the last known application of DDT.

Compound	HPβCD	Soil Aging Period	Uptake (%)
2,4-DDT + 4,4-DDT	no	90 days	7.03[a]
	no	49 years	1.40[a]
	no	49 years	0.05[b]
	yes	49 years	0.05[b]
2,4-DDE + 4,4-DDE	no	90 days	9.23[a]
	no	49 years	1.75[a]
	no	49 years	1.15[b]
	yes	49 years	1.45[b]
2,4- DDD + 4,4-DDD	no	90 days	5.41[a]
	no	49 years	1.30[a]
	no	49 years	7.05[b]
	yes	49 years	10.93[b]
ΣDDT	no	90 days	7.32[a]
	no	49 years	1.64[a]
	no	49 years	0.53[b]
	yes	49 years	0.66[b]

The results of this experiment showed that the application of 10%-HPβCD to PPNP soils did not significantly increased ΣDDT bioavailability to redworms.

4.4.7 Avoidance Experiment

After 48 hours in the avoidance wheels, 82% of the redworms (*Eisenia fetida*) were found in compartments filled with PPNP DDT-contaminated soil treated with 10%-HPβCD solution. These results suggested that redworms not only do not avoid HPβCD treated soils, but they actually prefer it over control soils. This could be explained by the fact that redworms usually prefer moister soils, and it was observed in this experiment, and in previous ones, that HPβCD treated soils hold moisture for longer than control soils. Redworm weights were not affected by the application of 10%-HPβCD solution to soils, as out of the five wheel replicates, in three the weight of redworms significantly increased after the 48 hrs of incubation (ANOVA, $F_{1,18} = 30.34$, $p < 0.001$, n=10; ANOVA, $F_{1,18} = 20.15$, $p < 0.001$, n=10; ANOVA, $F_{1,18} = 18.95$, $p < 0.001$, n=10), and in the other two, there was no significant difference

between the weights before and after the incubation period (ANOVA, $F_{1,18} = 3.57$, $p > 0.05$, n=10; ANOVA, $F_{1,18} = 2.87$, $p > 0.05$, n=10).

4.6 CONCLUSIONS

The application of hydroxypropyl-β-cyclodextrin (HPβCD) to DDT contaminated soil from PPNP significantly increased DDT water solubility. Moreover, as no significant increase in overall microbial activity was found in both microbial experiments, it is likely that the application of HPβCD solution to soils led to groundwater contamination rather than microbial degradation in Badley's (2003) field trial. Additionally, soils treated with 10%-HPβCD solution were found to have significantly lower [ΣDDT] after 10 applications than control soils. These results show that the reduction of ΣDDT in HPβCD treated soils observed here and in previous work by Badley (2003), are due to an increase in DDT mobility, rather than an increase in DDT microbial degradation.

In the greenhouse experiments, the results indicate that *Schizachyrium scoparium* is a better candidate than *Panicum virgatum* to be used in a phytoextraction-HPβCD combined approach. The application of 50 mL of 10%-HPβCD solution significantly increased the ΣDDT concentration in *S. scoparium* shoots growing in PPNP soils with low and high DDT contamination. Moreover, *S. scoparium* plants grown in low DDT soil and treated with 50 mL of 10%-HPβCD had the highest shoot bioaccumulation factor (BAF = 2.50), and their height was not significantly affected by any treatment or soil type. Although, the *S. scoparium* results seem promising, phytoextraction is not the main mechanism behind the reduction in ΣDDT concentration in soils treated with HPβCD. Soil samples from unplanted controls treated with HPβCD also had a significant reduction in ΣDDT concentration when compared to untreated control soils. Additionally, there is no significant difference between ΣDDT reduction in planted (with *S. scoparium* or *P. virgatum*) and unplanted soils treated with HPβCD. Furthermore, unplanted soils treated with 10%-HPβCD had a higher ΣDDT concentration in the bottom section (4-6 cm), showing that DDT mobilization through the soil column is occurring in these soils.

Hydroxypropyl-β-cyclodextrin application to DDT contaminated soils from PPNP did increase the ability of *S. scoparium* plants to transfer this pesticide to their shoots, however DDT mobility also occurred. *In-situ* application of HPβCD at PPNP is not recommended, as the increase in DDT mobilization is likely to cause groundwater contamination. Additionally, the invertebrates studied (*Eisenia fetida*), did not avoid soils treated with 10%-HPβCD, and there was a significant increase in the bioavailability of 2,4-DDE and 2,4-DDD to redworms exposed to HPβCD treated soils, putting them at higher risk than the ones exposed to DDT contaminated soil

alone. Hence, we conclude that the use of phytoextraction alone is more beneficial, as it will not interfere with the park's unique ecosystem and will not spread the pesticide contamination into the park's groundwater system.

5. The Use of Zero-Valent Iron (ZVI) Technology to Promote DDT and Dieldrin Degradation at Point Pelee National Park

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5.1 ABSTRACT

Point Pelee National Park (PPNP) is highly contaminated with dichlorodiphenyltrichloroethane (DDT) and dieldrin, due to the historical use of these two persistent organochlorine pesticides. Zero valent iron (ZVI) technology has been successfully used in the past to promote organochlorine degradation in several locations in North America and Europe. In this study, the use of two commercially available ZVI products, DARAMEND and EHC, to promote DDT and dieldrin degradation in PPNP's soil and groundwater were investigated. DARAMEND was applied to PPNP's soil in a laboratory experiment and in an *in-situ* pilot-scale plot. In both cases, DARAMEND did not significantly increase DDT or dieldrin degradation in treated soils. The effectiveness of EHC was tested in a laboratory experiment that mimicked a groundwater environment using PPNP's pesticide contaminated soil. The result was consistent with the one reported for DARAMEND, in that there was no significant increase in DDT or dieldrin degradation in any of the samples treated with EHC. These results demonstrate that both of these ZVI commercially available products are not suitable for *in-situ* remediation at PPNP.

5.2 INTRODUCTION

DDT and dieldrin are organochlorine pesticides that are persistent in the environment and can pose a threat to human health and other living organisms. They are known to adsorb strongly to soils and sediments becoming less bioavailable overtime. DDT undergoes dechlorination under reducing conditions and forms dichlorodiphenyldichloroethane (DDD) which is able to degrade further to more polar products when subjected to aerobic treatment (Sudharshan *et al.* 2012). However, when subjected to aerobic biotic degradation, the decomposition of DDT results in the formation of dichlorodiphenyldichloroethylene (DDE), which is the major contaminant at Point Pelee National Park (PPNP) today. Recently, research and field projects have shown that the application of zero valent iron (ZVI) to soil and groundwater facilitates chemical and biological degradation of persistent organochlorine pesticides (Seech *et al.* 2008; Yang *et al.* 2010; El-Temsah & Joner 2013).

ZVI is a moderate reducing reagent, which can react with dissolved oxygen and to some extent with water (Zhang 2003), generating a reduced environment that may facilitate the degradation of organochlorine pesticides (Yang *et al.* 2010). Shortly after the ZVI application to soil or groundwater, the pH increases and the oxidation-reduction potential (ORP) decreases, favoring the growth of anaerobic microorganisms, which could be beneficial for accelerated biodegradation (Zhang 2003). Moreover, ZVI is very effective in transforming a wide variety of common chlorinated contaminants in a reductive environment. Chlorinated compounds, such as chlorinated methanes, brominated methanes, trihalomethanes, chlorinated ethenes, chlorinated benzenes, and other polychlorinated hydrocarbon pesticides, are partially or totally dechlorinated to ethane and chloride (Mueller *et al.* 2012).

DARAMEND and EHC are soil and groundwater amendments, respectively. They contain a patented combination of organic carbon and ZVI, and are commercially available through the company *PeroxyChem*. DARAMEND is a combination of tan/brown flakes that are composed 40-50% of iron and 50-60% of a patented organic amendment; it is solid at room temperature, and has a pH of 6.0 and a density of 0.97 kg/L (PeroxyChem 2010). Similarly, EHC is a light tan powder, made of potassium magnesium sulfate (25-35%), iron (25-35%), and patented organic amendment (25-35%) and viscosity modifier (0-10%). EHC has a pH of 5.6 as aqueous solution, and a 1.03 g/mL density(PeroxyChem 2016). A combination of chemical and microbial oxygen consumption enables reliable generation of very low ORP conditions, which enhances both the chemical and microbial dehalogenation processes. In addition, the organic carbon component supports microbial growth (Seech *et al.* 2008). DARAMEND has been successfully applied to more than four million tons of soil, sediment, and other materials contaminated with various

persistent organic compounds, including DDT, in many sites in the US, Canada, and Europe (Seech *et al.* 2008). On the other hand, EHC is specifically formulated for injection into the subsurface, and common applications include hot-spot treatment, plume treatment, and plume management using a permeable reactive barrier (Seech *et al.* 2008).

Although ZVI technologies have been largely used, especially in the US (Zhang 2003), two recent studies (Yang *et al.* 2010; El-Temsah *et al.* 2013) showed that while ZVI has a positive effect on the degradation of DDT, there was also an increase in DDD and DDE concentrations in the treated soils. This is concerning as the breakdown of parent compound can sometimes lead to the formation of more persistent recalcitrant degradation products. The formation of DDE from DDT breakdown is often considered a dead-end in the remediation process (Sudharshan *et al.* 2012).

At the request of Parks Canada personnel, who received free samples of DARAMEND and EHC from *PeroxyChem*, this study evaluates the potential of DARAMEND to promote DDT and dieldrin degradation in highly contaminated soil from PPNP in both laboratory and in *in-situ* experiments. Additionally, the ability of EHC to promote degradation of these pesticides in a laboratory environment that mimics groundwater was assessed.

5.3 METHODS & MATERIALS

5.3.1 Site Description & Soil Collection

Point Pelee National Park (PPNP), located south of the town of Leamington, Ontario, consists of a peninsula of land (16 km^2) made up of marsh and woodland habitats. PPNP is an important staging area for migratory birds and is also a vital breeding area for many species of birds (Smits *et al.* 2005; Crowe & Smith 2007). The soil at PPNP is classified as sandy and contains organochlorine pesticides contamination, composed predominantly of DDT (4,4-DDE and 4,4-DDT), and dieldrin which have weathered in place for over 40 years (Smits *et al.* 2005; Denyes *et al.* 2016).

For the laboratory studies, soil was collected from a site known to be former agricultural land with high residue soil pesticide concentrations. A total of ~50 kg of soil from the top 10 cm was collected in 2015. The collected soil was thoroughly homogenized using the process described in Low *et al.* (2008) and Ficko *et al.* (2011). Briefly, soil was sieved through a 1 cm^2 sieve and consolidated in one pile on a table. The original pile of soil was quartered by random scooping using a flat-bottom scoop. Each of the four piles was manually mixed and re-combined into a central pile by scooping from the four piles in an alternating manner. The procedure

was repeated 30 times. Following homogenization, mean ΣDDT and dieldrin concentrations in soil were 22,000 ± 3,400 ng/g (n=12), and 360 ± 50 ng/g (n=12), respectively.

A three by two meter pilot scale experimental plot was established in the *Delaurier Compound Site* (N 41º 57.653' W 082º 31.645) of PPNP on October 2015. Seven surface soil samples (0-10 cm) were collected from the plot area before the addition of DARAMEND. The average ΣDDT and dieldrin concentrations in the soil were 13,000 ± 6,900 ng/g and 440 ± 290 ng/g respectively.

5.3.2 Experimental Design

5.3.2.1 DARAMEND Laboratory Experiment

There are two key components of the DARAMEND bioremediation technique, the addition of the DARAMEND to the soil to be treated, and the regulation of oxygen availability and moisture content (Seech *et al.* 2008). In the laboratory, this was accomplished by adding 0.5-1% of DARAMEND to the targeted soil, and then bringing the soil to 90% of its water holding capacity. The DARAMEND used in this project (~2 kg) was provided by Parks Canada personnel, but this product is available for purchase from *PeroxyChem* at a cost of $0.50-0.80 /lb. A total of nine glass Mason jars, with sealed lids, were used in this experiment: 6x500 mL jars, and 3x250 mL jars. 250 g of PPNP soil (dry weight) were placed into each of the nine Mason jars, and they were divided into three groups: (1) PPNP soil + DARAMEND, (2) PPNP soil (control), (3) PPNP soil placed in the fridge (fridge control). In the treatment group, 1% DARAMEND was also added to the jars. In the first two groups, the soil was placed into 500 mL jars, and for the third group, 250 mL jars were used. All jars were wrapped in aluminium foil, and lids were tightly closed to create an anaerobic environment. The six 500 mL jars were placed in the dark for 5 days. The anaerobic cycle was followed by an aerobic cycle, where lids and foil were removed from the jars. During the aerobic cycle, soils were stirred daily and allowed to dry in a fume hood, this process took ~10 days. Anaerobic and aerobic cycles were repeated five times each. At the beginning of anaerobic cycles two through five, 0.5% of DARAMEND was added to each jar in the treatment group, and the water holding capacity was brought back to 90% in all six jars. Oxidation reduction potential (ORP) and pH measurements were taken at the end of each anaerobic cycle. Soil samples were taken at the beginning of the first, third, and fifth anaerobic cycles. The ~15 g soil samples were place into labelled Whirl-Pak® bags and frozen at -20ºC until analysis. The 250 mL untreated control jars were placed in the fridge (~4ºC) for the entire duration of the experiment, and were only sampled at the end of the experiment.

5.3.2.2 DARAMEND Field Trial

The DARAMEND field plot was divided into six one by one meter experimental plots, where the west three plots were treated with DARAMEND and the other three remained untreated (control group) (Figure 5.1). On the day of plot establishment (Oct 15, 2015), only 660 g of DARAMEND per treatment plot was available for application (whereas, the optimal amount per plot was calculated to be 1.96 kg). The remaining 1.3 kg of DARAMEND was applied to the plots almost a month later. Before the first DARAMEND application, surface soils (0-10 cm) in the plot were homogenized with a stiff rake as best as possible. The 660 g of DARAMEND was then carefully spread over each treatment plot, and thoroughly mixed with the top 10 cm of soil using a stiff rake. A total of ten surface soil samples (0-10 cm) were collected. Seven of these were collected before the addition of DARAMEND, with one sample being collected from the center of each of the six plots, and a field duplicate collected from the *Control A* plot. The final three samples were collected following DARAMEND application to the three experimental plots receiving treatment. After sampling, 45 gallons of water were evenly distributed amongst the six plots to bring their water holding capacity (WHC) to ~90%, and create an anaerobic environment. This watering process took about one hour. On November 12, 2015, the remaining 1.3 kg of DARAMEND was applied, as described above, to the three treatment plots by K. LeClair, a PPNP employee. From October 16, 2015 to December 15, 2015, all six plots were watered daily for about 30 minutes to maintain an anaerobic environment in the soil. After two months of treatment on November 15, 2015, seven additional surface soil samples were collected at random locations from each plot, and the field duplicate was collected from the *DARA A* plot. All soil samples collected on site were place into labelled Whirl-Pak® bags, and stored in a cooler during transport, and then frozen at -20ºC for three months until analysis.

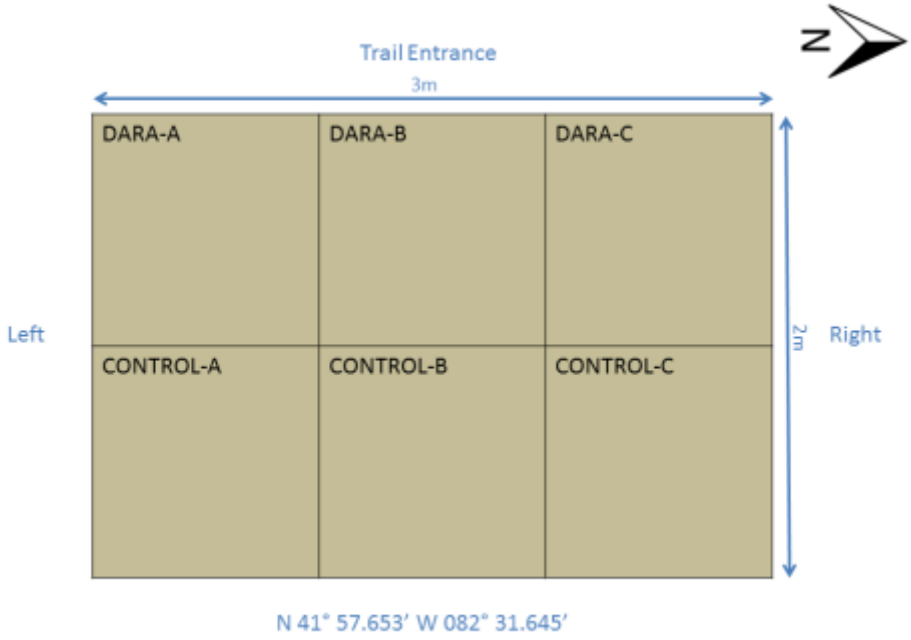

Figure 5.1. Representation of the three treatment plots (DARA) and the three control plots located in the *Delaurier Compound Site* at PPNP. GPS coordinates are included at the bottom of the figure.

5.3.2.3 EHC Laboratory Experiment

The EHC used in this project (~32 g) was provided by Parks Canada personnel, but this product is available for purchase from *PeroxyChem* at a cost of $1.50-2.50 /lb. To mimic a groundwater environment in the laboratory, 3.5 g or 2% of EHC was added to each of nine 1L Nalgene® bottles containing 175 g of PPNP soil. The bottles were then filled up with 800 mL of deionized water (no head space), and N_2 was bubbled into the bottles for two minutes before they were closed to ensure an anaerobic environment. Nine control bottles were treated identically except no EHC was added. All bottles were inverted and vented, by slightly unscrewing the lid, every day during the 45 days of treatment. ORP and pH measurements were taken on days 14, 28, and 45 as recommended by the PeroxyChem (2015a) protocol. At each sampling event, three EHC and three control bottles were sacrificed for the analysis. Soil samples were taken at the same time, placed into labelled Whirl-Pak® bags and frozen at -20ºC until analysis. As in the DARAMEND experiment, fridge controls were also used in this experiment. Three 250 mL Mason jars, with 175 g of

PPNP soil each, were placed in the fridge (~4ºC) for the entire duration of the experiment, and were only sampled at the end of the experiment.

5.3.3 Analytical Methods

Soil samples (5 g wet weight) were air-dried overnight at room temperature. Approximately 1 g of sample was used for analysis. Soil samples were extracted using the accelerated solvent extraction (ASE) method with 30-40 mL 50:50 of hexane: acetone, 100 µl of 1 ppm decachlorobiphenyl (DCBP) as surrogate standard and ~15 g of Ottawa sand. The extract was concentrated by rotoevaporation and applied to a Florisil extraction column. The column was rinsed with hexane into a 10 mL volumetric flask (fraction 1, DDT). The same Florisil column was subsequently rinsed with dichloromethane (DCM) into 10 mL volumetric flask and placed into a 500 mL round bottom flask. The extract was concentrated by rotoevaporation and solvent exchanged to hexane. The extract was then placed in to 10 mL volumetric flask with hexane (fraction 2, dieldrin). Samples were transferred to a gas chromatograph (GC) vials and analyzed by an HP 6890 GC equipped with a ^{63}Ni electron capture detector (GC/ECD), a SPB-1 fused silica capillary column. The carrier gas was helium at a flow rate of 2 mL/min. Nitrogen was used as a makeup gas for the ECD. The results were expressed as nanograms of pesticide per gram of dry weight soil.

5.3.4 Quality Assurance & Quality Control (QA/QC)

Nine soil samples were extracted and processed with one analytical blank, one control sample, and one analytical duplicate, as specified by the US-EPA method for organochlorine pesticides (US-EPA 2007). The control and blank were treated as described above. Samples concentrations were corrected for surrogate recovery, and all analytical blanks were less than 1.0 ng/g (below detection limit). The mean difference between the control standard and the control standard target was less than 20%; raw data can be found in Appendix C.

5.3.5 Statistical Analysis

Statistical analyses were performed using R 3.2.2 (free software for statistical computing and graphics). All DDT and dieldrin concentrations are reported on a dry weight (ng/g) basis and recorded with the standard deviation of the mean. Data were tested for normality using the Shapiro-Wilk test. Data was analyzed by an one-way analysis of variance (ANOVA), followed by a post hoc Tukey comparison with significance level $p = 0.05$.

5.4 RESULTS AND DISCUSSION

5.4.1 DARAMEND Laboratory Experiment

The pH and ORP readings recorded at the end of each of the five anaerobic cycles of this experiment are summarized in Table 5.1. All pH readings are in the range (between 5 and 8.5) specified by the *PeroxyChem* (2015b) protocol for proper application of DARAMEND. According to *PeroxyChem* this pH range guaranties maximum microbial activity in treated soils. Additionally, the protocol suggests that the ability of microorganisms to biodegrade many organic contaminants is controlled by oxygen availability, and the relationship between oxygen supply and redox potential is a direct and positive one. The soil's redox potential is measured by ORP in millivolts, and it is expected that the DARAMEND treated soils will have very low ORP readings. As anticipated, ORP readings decreased overtime in soils treated with DARAMEND, but stayed positive in control samples. Zhang (2003), observed a pH increase of 2-3 units, and ORP reduction of 500-900 mV in soils treated with ZVI, in a batch reactor experiment. In this experiment the pH and ORP changes observed were less notable. The pH was slightly reduced to 6.9 by the end of the first cycle in the DARAMEND samples, while remained at 7.6 in the control samples. For ORP, a reduction of 393 mV was observed by the end of cycle #5.

Table 5.1. Summary of pH and ORP readings recorded at the end of each anaerobic cycle.

	Cycle #1	**Cycle #2**	**Cycle #3**	**Cycle #4**	**Cycle #5**
			pH		
Control	7.6 ± 0.2	7.6 ± 0.02	7.8 ± 00.4	7.6 ± 0.02	7.6 ± 0.03
DARAMEND	6.9 ± 0.4	7.2 ± 0.1	7.2 ± 0.2	7.2 ± 0.03	7.5 ± 0.01
			ORP (mV)		
Control	264 ± 7	160 ± 11	125 ± 38	179 ± 12	251 ± 4
DARAMEND	64 ± 10	-97 ± 15	-102 ± 46	-113 ± 12	-142 ± 20

The experiment results showed no significant differences in ΣDDT (ANOVA, $F_{1,7} = 0.51$, $p > 0.05$, $n = 3$) and dieldrin (ANOVA, $F_{2,6} = 0.84$, $p = 0.48$, $n= 3$) concentrations between the soils treated with DARAMEND and the controls (Table 5.2). A closer look at the different DDT metabolites reveals a transformation of DDT to DDD (Figure 5.2). The concentrations of 2,4-DDT and 4,4-DDT were significantly reduced (ANOVA, $F_{2,6} = 15.83$, $p < 0.05$, $n =3$; ANOVA, $F_{2,6} = 11.66$, $p = 0.01$, $n =3$) in the soils treated with DARAMEND compared to the two controls, while the concentrations of 2,4-DDD and 4,4-DDD were significantly increased (ANOVA, $F_{2,6} = 14.35$, $p = 0.01$, $n =3$; ANOVA, $F_{2,6} = 81.88$, $p > 0.01$, $n =3$). These results are consistent with the expected degradation pathway of DDT in anaerobic environments. When DDT undergoes dechlorination under reducing conditions (e.g.

the anaerobic cycle of DARAMEND treatment), it is transformed to DDD, which is able to degrade further to more polar products such as 2,2-bis(4-chlorophenyl) (DDOH) ethanol when subjected to aerobic treatment. Technically a sequential reductive step followed by an oxidative process could mineralize DDT (Sudharshan *et al.* 2012).

Table 5.2. The concentrations of ΣDDT and dieldrin in the soils after five cycles of DARAMEND treatment. Soils in the control group (n=3) were exposed to the same anaerobic/aerobic cycles as the DARAMEND group (n=3), while soils in the control-fridge group (n=3) were kept in the refrigerator (4ºC) for the entire duration of the experiment.

	ΣDDT (ng/g)	**Dieldrin (ng/g)**
DARAMEND	25,300 ± 190	420 ± 30
Control	24,300 ± 1,500	380 ± 20
Control-Fridge	23,300 ± 2,400	410 ± 40

In this experiment, the DDD was not further transformed during the aerobic cycle, and accumulated in the soil (Figure 5.2). These findings are in agreement with two other studies (Yang *et al.* 2010; El-Temsah *et al.* 2013) that found that reduction the of DDT in soils treated with ZVI technologies (like DARAMEND) causes a subsequent increase of DDD in the same soils. It is possible that a longer treatment would lead to a reduction of the DDD in the soil. However, as 4,4-DDE is the major contaminant in the PPNP soil, it is important to note that this metabolite was not significantly decreased (ANOVA, $F_{2,6} = 4.08$, $p > 0.05$, $n = 3$) in the soils treated with DARAMEND.

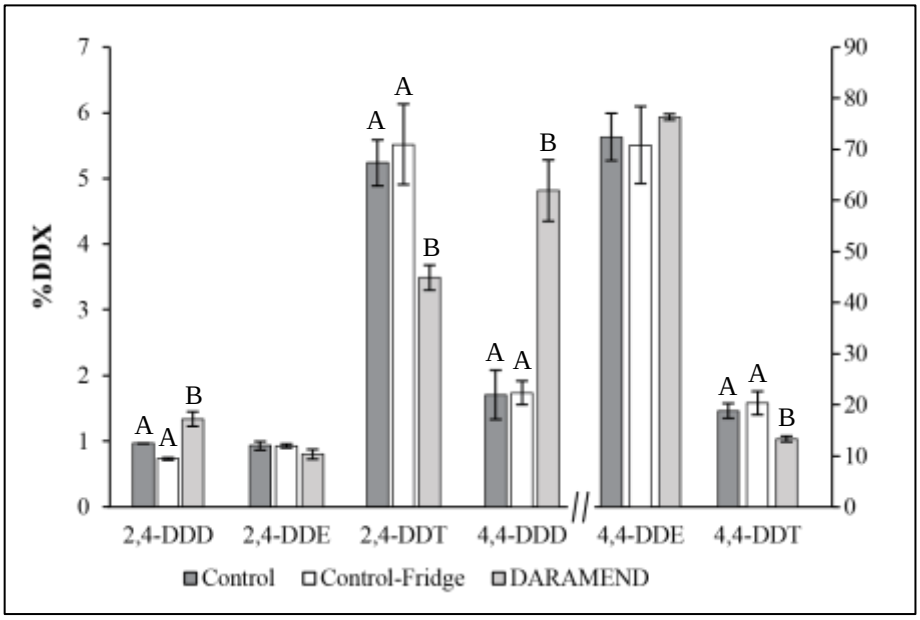

Figure 5.2. The percentage of each DDT metabolite found in the soil after five cycles of treatment (n=3). DARAMEND treated soils had a decrease in DDT and an increase in DDD, letters indicate where there is a significant difference.

5.4.2 DARAMEND Field Trial

There were no significant differences in ΣDDT (ANOVA, $F_{4,9} = 0.14$, $p = 0.96$, $n = 3$) and dieldrin (ANOVA, $F_{4,9} = 1.01$, $p = 0.45$, $n = 3$) concentrations between the soils treated with DARAMEND and the controls, or between the first and last day of treatment (Figure 5.3). Additionally, pesticides concentrations between all field samples varies greatly, due to the fact that it is harder to properly homogenize soils in a field plot to overcome the difference in amounts of DDT and dieldrin degradation naturally occurring in these soils.

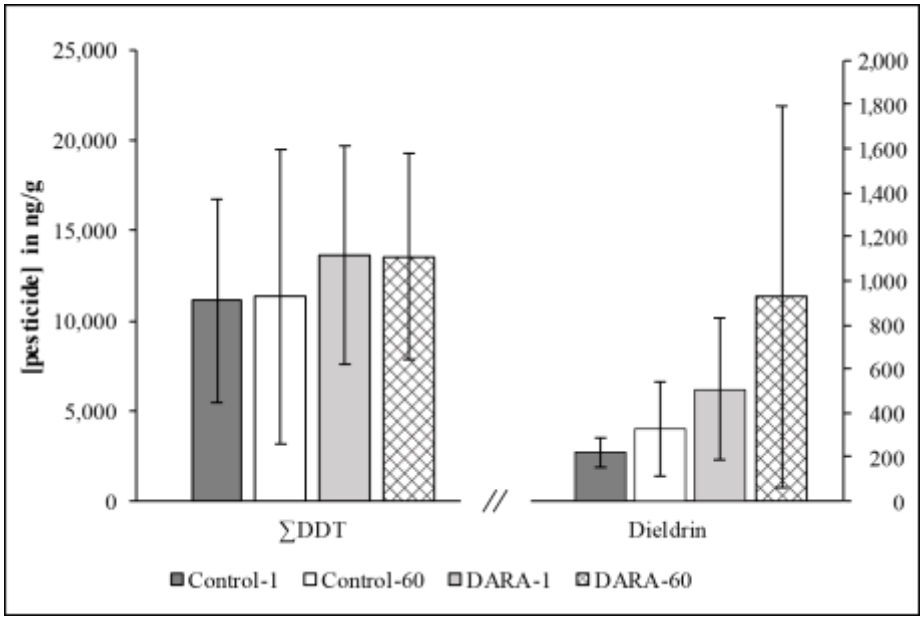

Figure 5.3. The concentrations of ΣDDT and dieldrin in the soils before (day 1) and after 60 days of the first DARAMEND application (n=3).

These results of one cycle of DARAMEND treatment on site at PPNP are comparable to the results of five cycles of the laboratory experiment completed earlier at RMC. In both cases, there were no significant differences in ΣDDT and dieldrin concentrations between the soils treated with DARAMEND and the controls. While the laboratory experiment resulted in a significant transformation of DDT to DDD in DARAMEND treated soils, this transformation was not observed in the field. Furthermore, 4,4-DDE, the major contaminant in the PPNP soil, was not significantly decreased in any of the soils treated with DARAMEND.

5.4.3 EHC Laboratory Experiment

EHC was applied to PPNP DDT contaminated soil in a laboratory simulated groundwater environment, as this product was specifically formulated for injection into the subsurface (Seech *et al.* 2008). ORP and pH were monitored during the treatment. The measurements were taken on days 14, 28, and 45 as recommended by the protocol. At each sampling event, three EHC and three control bottles were sacrificed for analyses. The soil's pH stayed in the recommended range (between 5 and 8.5) during the treatment (Table 5.3), creating an environment that promotes microbial activity. As expected, the ORP readings decreased overtime for the soils

treated with EHC, while the numbers stayed positive for the control samples (Table 5.3).

Table 5.3. Summary of pH and ORP (mV) readings at the beginning of the experiment (day 0) and on the three sampling dates.

	Day 0	**Day 14**	**Day 28**	**Day 45**
		pH		
Control	7.8 ± 0.04	7.2 ± 0.02	7.1 ± 0.06	6.8 ± 0.02
EHC	7.8 ± 0.06	6.4 ± 0.01	6.7 ± 0.02	6.7 ± 0.04
		ORP (mV)		
Control	264 ± 7	237 ± 3	175 ± 5	209 ± 1
EHC	262 ± 4	-49 ± 3	-93 ± 5	-83 ± 4

According to Seech *et al.* (2008), redox potentials as low as -550 mV are commonly observed in groundwater after EHC application. The lowest ORP reading recorded in this experiment for EHC treated bottles was -93 mV on day 28 of sampling, and the highest was -49 mV on the first sampling date (day 14) (Table 5.4).

Table 5.4. The concentrations of ΣDDT and dieldrin (ng/g) in the soils after 45 days of EHC treatment (n=3).

	ΣDDT (ng/g)	**Dieldrin (ng/g)**
EHC	22,000 ± 7,800	310 ± 160
Control	17,600 ± 2,300	290 ± 30
Control-Fridge	24,800 ± 150	360 ± 30

There were no significant differences in ΣDDT and dieldrin concentrations between the soils treated with EHC and the controls (ANOVA, $F_{2,6} = 1.90$, $p = 0.23$, $n = 3$). A closer look at the different DDT metabolites reveals no transformation of DDT to DDE, or DDD (Figure 5.4). The concentration of 4,4-DDT in the fridge control is significantly higher than both EHC and control soils (ANOVA, $F_{2,6} = 9.66$, $p = 0.01$, $n = 3$, Tukey-test, $p > 0.01$, 0.04). This is likely due to different amounts of degradation occurring naturally in the soils, and it is not a result of this specific experiment. Furthermore, 4,4-DDE is the major contaminant in the PPNP soil, and this metabolite was not significantly decreased in the soils treated with EHC.

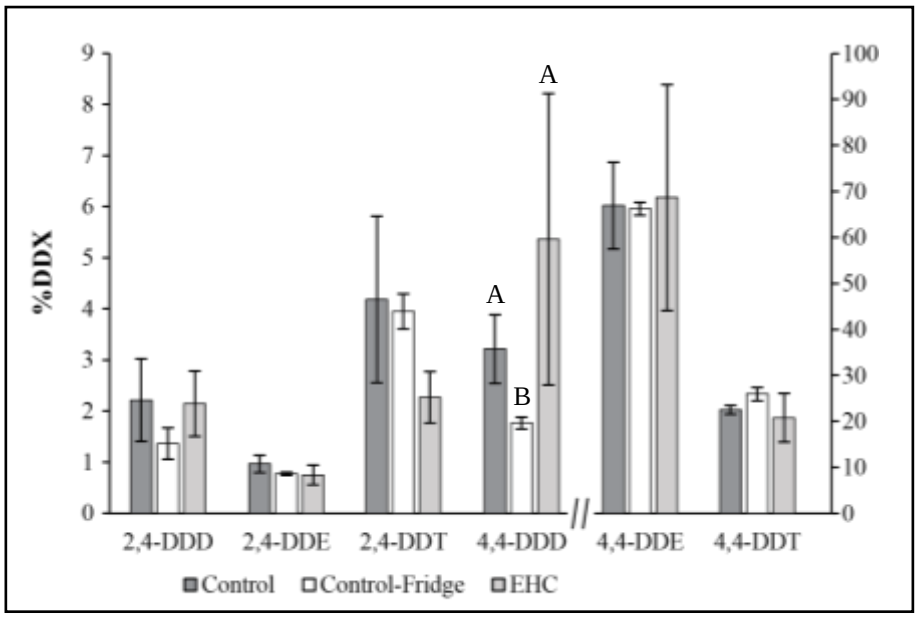

Figure 5.4. The percentage of each DDT metabolite found in the soil after 45 days of treatment (n = 3). For most metabolites, there is no significant difference between the soils treated with EHC and the controls, letters indicate where there is a significant difference.

6 CONCLUSIONS

DARAMEND application to DDT and dieldrin contaminated soils from Point Pelee National Park (PPNP) did not improve the degradation of these pesticides in either laboratory or field experiments. This soil amendment did not perform as previously reported by Seech *et al.* (2008) in other soils in Canada and North America. As bioremediation is known to be site specific, the results reported by this study suggests that DARAMEND is not a viable remediation option for PPNP's pesticide contamination.

There is currently no known DDT or dieldrin contamination of PPNP's groundwater. Further, due to the hydrophobic nature of these two pesticides it is very unlikely that there will be any contamination in the future. EHC is not recommended as a remediation tool, as it did not improve DDT or dieldrin degradation in a laboratory experiment that mimics the park's groundwater conditions.

Although, zero valent iron (ZVI) products have been successfully used to improve DDT and dieldrin degradation in the past, at PPNP the use of both DARAMEND and EHC did not result in any significant reduction of these two pesticides in soil or groundwater. Other remediation techniques might be more suitable to increase *in-situ* DDT and dieldrin degradation in PPNP.

6. Discussion and Conclusions

Dichlorodiphenyltrichloroethane (DDT) and dieldrin are anthropogenic organochlorine pesticides. Exposure to these compounds can cause adverse health effects to humans and wildlife. The use of DDT and dieldrin has been prohibited in most countries since the 1970s, however both pesticides are still present in the environment today. DDT is persistent in soils, in part because of its strong adsorption to solid particles (Corona-Cruz *et al.* 1999; Sudharshan *et al.* 2012). Soils contaminated with organochlorine pesticides are commonly remediated using conventional technologies such as excavation and incineration, thermal desorption, and soil washing with surfactants. These methods are expensive and can negatively affect sensitive ecosystems. Efforts are being made to develop environmentally friendly and cost-effective alternatives.

In this thesis, the feasibility of using phytoextraction with native grasses to remediate DDT and dieldrin contaminated soil *in-situ* at Point Pelee National Park (PPNP) was investigated. A field study showed high plant uptake into the species evaluated (*Panicum virgatum* and *Schizachyrium scoparium*), with each having a mean BAF>1 for both pesticides in only five months of phytoextraction (Chapter 3). These results demonstrated that phytoextraction by native grasses is a viable remediation technique to organochlorine pesticides contamination at PPNP. This is especially important as phytoextraction does not negatively impact PPNP's sensitive ecosystem and the use of native plants does not introduce invasive species.

Although the *in-situ* phytoextraction study was successful, the extent of DDT contamination at PPNP is vast and other alternatives are worth investigating. Therefore, the potential of using HPβCD as a remediation tool for soils contaminated with DDT was further explored. Microbial studies showed an increase in DDT water solubility, a significant decrease in ΣDDT concentration in soils, and no significant increase in overall microbial activity after the application of 10%-HPβCD solution to PPNP soils (Chapter 4). These results showed that the reduction of ΣDDT in HPβCD treated soils observed here and in previous work by Badley (2003), were due to an increase in DDT mobility, rather than an increase in DDT microbial degradation. Greenhouse studies showed that the application of 50 mL of 10%-HPβCD significantly increased ΣDDT concentration in *S. scoparium* shoots growing in PPNP soils with low (~1,300 ng/g) and high (~31,000 ng/g) DDT contamination. However, overall there was no significant difference between ΣDDT reduction in planted (with *S. scoparium* or *P. virgatum*) and unplanted soils treated with HPβCD. These results demonstrated that phytoextraction is not the main mechanism behind the reduction in ΣDDT concentration in soils treated with HPβCD. Additionally, higher ΣDDT concentration was found in the bottom section of unplanted soils

treated with 10%-HPβCD, reinforcing the idea that DDT mobilization rather than plant uptake is the main mechanism behind the reduction of ΣDDT concentration in these soils. Finally, growth chamber studies demonstrated that invertebrates (*Eisenia fetida*), did not avoid soils treated with 10%-HPβCD, and there was a significant increase in the bioavailability of 2,4-DDE and 2,4-DDD to redworms exposed to HPβCD treated soils. Together these studies indicated that the *in-situ* application of HPβCD at PPNP is not recommended, as an increase in DDT mobilization at the site is likely to cause groundwater contamination.

Another emerging and environmentally friendly bioremediation technique investigated in this thesis was zero-valent iron (ZVI) technology. Laboratory and field studies showed that the application of DARAMEND to DDT and dieldrin contaminated soils from PPNP did not improve the degradation of these pesticides (Chapter 5). Additionally, a laboratory study demonstrated that the application of EHC did not improve DDT or dieldrin degradation in an environment that mimics the park's groundwater conditions. Although these ZVI products have been reported to improve organochlorine pesticide degradation in other studies (Seech *et al.* 2008), at PPNP this remediation technique is not suitable.

This thesis investigated three different remediation strategies for the organochlorine pesticide (DDT and dieldrin) contamination at PPNP. I*n-situ* phytoextraction was very successful, and it is recommended that future work focus on establishing large phytoextraction plots in areas with higher DDT and dieldrin co-contamination within the park to verify phytoextraction feasibility in those conditions. Future work should further investigate the fate of DDT in HPβCD treated soils overtime, as this thesis demonstrates that in a short period HPβCD causes DDT mobilization. Future *in-situ* and laboratory studies with DARAMEND and EHC are not recommended, instead future work should focus on finding new soil amendments to work alone or in combination with phytoextraction.

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8. APPENDICES

Appendix A
Raw Data for Chapter 3: *In-situ* Phytoextraction of DDT and Dieldrin by Native Grasses at Point Pelee National Park.

Appendix B
Raw Data for Chapter 4: The Role of Hydroxypropyl-β-Cyclodextrin (HPβCD) in DDT Remediation at Point Pelee National Park.

Appendix C
Raw Data for Chapter 5: The Use of Zero-Valent Iron (ZVI) Technology to Promote DDT and Dieldrin Degradation at Point Pelee National Park.

Appendix D
Pictures from field and laboratory experiments in all data chapters.

APPENDIX A

Raw Data for Chapter 3

In-situ Phytoextraction of DDT and Dieldrin by Native Grasses at Point Pelee National Park

Table A-1. DDT and dieldrin concentration of soils of the 2015 *Delaurier* phytoextraction plot.

Table A-2. DDT and dieldrin concentration of plant tissues of the 2015 *Delaurier* phytoextraction plot.

Table A-1. DDT and dieldrin concentration of soils of the 2015 *Delaurier* phytoextraction plot. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)	Dieldrin (ng/g)
Section-SG-1[a]	2.70	197	6.14	6.10	18.2	85.3	315	5.19
Buffer Zone-1	2.29	158	8.65	4.66	12.8	57.7	244	3.40
Buffer Zone-1-Dup	2.07	165	7.17	4.96	14.5	63.6	258	3.98
Section-LB-1	3.90	274	16.0	7.75	22.1	108	432	6.58
Mean	2.74	198	9.49	5.87	16.9	78.6	312	4.79
SD	0.82	53.0	4.46	1.40	4.14	22.8	85.4	1.41
Section-SG-A-2[b]	3.73	125	1.30	16.2	32.7	80.8	260	1.43
Section-SG-B-2	5.01	121	3.28	11.3	17.4	57.3	215	1.87
Section-SG-C-2	1.13	83.0	1.18	9.60	16.7	44.4	156	2.34
Mean	3.29	110	1.92	12.4	22.3	60.8	210	1.88
SD	1.98	23.2	1.18	3.42	9.08	18.5	52.3	0.45
Section-LB-A-2	3.38	69.2	1.77	14.1	15.6	35.7	140	2.07
Section-LB-B-2	1.42	50.3	3.72	<1.0	15.8	30.4	102	3.11
Section-LB-C-2	4.99	103	4.27	<1.0	20.8	75.0	208	1.65
Mean	3.27	74.2	3.25	4.70	17.4	47.0	150	2.28
SD	1.79	26.7	1.31	8.14	2.97	24.3	53.9	0.75
Laboratory QA/QC								
Blank-1[c]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard-1	<1.0	20.0	<1.0	21.4	<1.0	22.4	-	23.7
Control Standard Target-1	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	100	-	107	-	112	-	118
Section-LB-1	3.58	273	19.8	8.20	22.2	114	-	6.90
Section-LB-1-Dup	4.23	274	12.2	7.30	22.0	101	-	6.26
RPD	16.7	0.34	47.9	11.7	0.98	12.0	-	9.8
Blank-2[d]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard-2	<1.0	22.2	<1.0	21.7	<1.0	22.2	-	23.5
Control Standard Target-2	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	111	-	109	-	111		117
Section-LB-C-2	5.23	94.1	4.48	<1.0	23.7	76.2	-	1.80
Section-LB-C-2-Dup	4.75	112	4.06	<1.0	17.9	73.7	-	1.49
RPD	9.65	-17.3	9.65	-	27.7	3.45	-	19.1

[a] Samples marked with the number one were collected on June 2015.
[b] Samples marked with the number two were collected on October 2015.

[c] QA/QC for samples marked with the numbers one.
[d] QA/QC for samples marked with the numbers two.

Table A-2. DDT and dieldrin concentration of plant tissues of the 2015 *Delaurier* phytoextraction plot. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)	Dieldrin (ng/g)
SG-SH-A[a]	8.35	69.6	26.2	18.8	17.3	32.1	172	4.32
SG-SH-B	20.3	104	34.3	61.0	32.4	97.7	350	12.3
SG-SH-C	23.0	71.3	19.7	21.9	26.1	88.4	250	7.93
Mean	17.2	81.6	26.7	33.9	25.3	72.8	257	8.17
SD	7.81	19.4	7.31	23.5	7.55	35.5	88.9	3.98
LB-SH-A	6.70	50.8	5.25	10.0	9.28	21.5	104	4.62
LB-SH-B	18.2	68.8	21.3	47.7	25.1	58.2	239	6.26
LB-SH-C	27.8	74.5	45.0	30.7	22.7	21.0	222	4.24
Mean	17.6	64.7	23.9	29.5	19.0	33.6	188	5.04
SD	10.6	12.4	20.0	18.8	8.54	21.3	73.8	1.07
SG-RT-A[b]	17.6	92.5	61.2	44.7	50.0	42.6	309	10.1
SG-RT-B	14.6	83.5	19.6	12.5	51.7	82.3	264	5.74
SG-RT-C	20.5	85.4	64.3	30.7	85.1	78.8	365	12.4
Mean	17.6	87.1	48.4	29.3	62.3	67.9	313	9.40
SD	2.92	4.72	25.0	16.1	19.8	22.0	50.4	3.37
LB-RT-A	6.87	67.7	16.2	10.3	28.5	35.2	165	4.74
LB-RT-B	8.30	72.3	13.0	37.3	11.5	23.4	166	4.29
LB-RT-C	14.7	88.9	53.7	7.33	22.3	66.4	253	5.06
Mean	9.95	76.3	27.6	18.3	20.8	41.7	195	4.70
SD	4.15	11.2	22.6	16.5	8.63	22.2	50.8	0.38
Laboratory QA/QC								
Blank-1[c]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard-1	<1.0	20.9	<1.0	20.4	<1.0	19.4	-	23.7
Control Standard Target-1	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	105	-	102	-	97.0		119
SG-SH-C	25.2	70.0	19.8	18.9	29.1	80.7	-	8.69
SG-SH-C-Dup	20.8	72.6	19.6	25.0	23.1	96.1	-	7.18
RPD	19.0	3.67	0.78	27.8	23.0	17.4	-	19.0
Blank-2[d]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard-2	<1.0	22.2	<1.0	21.7	<1.0	22.2	-	23.5
Control Standard Target-2	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	112	-	108	-	11	-	117
SG-RT-B	12.1	82.2	18.9	12.1	55.6	77.3	-	5.54
SG-RT-B-Dup	17.2	84.9	20.3	12.9	47.8	87.3	-	5.95

RPD	35.4	3.28	7.05	7.05	15.2	-12.2	-	-7.05

[a] Samples named 'SH' are shoot tissues.
[b] Samples named 'RT' are root tissues.
[c] QA/QC for shoot samples.
[d] QA/QC for root samples.

APPENDIX B

Raw Data for Chapter 4

The Role of Hydroxypropyl-β-Cyclodextrin (HPβCD) in DDT Remediation at Point Pelee National Park

Table B-1. DDT concentrations of soils of the DDT solubility experiment.

Table B-2. DDT concentrations of waters of the DDT solubility experiment.

Table B-3. DDT concentrations of soils of the microbial activity experiment.

Table B-4. DDT concentrations of waters of the microbial activity experiment.

Table B-5. Recorded absorbance and calculated concentrations for the microbial activity experiment.

Table B-6. DDT concentrations of soils of the optimal HPβCD concentration experiment.

Table B-7. DDT concentrations of waters of the optimal HPβCD concentration experiment.

Table B-8. Recorded absorbance and calculated concentrations for the microbial activity experiment.

Table B-9. DDT concentrations of soils of the greenhouse experiment I.

Table B-10. DDT concentrations of shoot tissues of the greenhouse experiment I.

Table B-11. DDT concentrations of root tissues of the greenhouse experiment I..

Table B-12. DDT concentrations of soils of the greenhouse experiment II. Quality assurance and quality control data are included at the bottom of the table.

Table B-13. DDT concentrations of shoot and root tissues of the greenhouse experiment II.

Table B-13. DDT concentrations of soil and redworms of the bioavailability experiment.

Table B-14. Recorded redworms weight for the bioavailability experiment.

Table B-15. Recorded redworms weight for the avoidance experiment.

Table B-1. DDT concentrations of soils of the DDT solubility experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)
Untreated-A	159	10,900	63.6	66.4	622	3,140	15,000
Untreated-B	161	10,400	65.0	62.1	649	2,930	14,300
Untreated-C	149	9,670	55.0	56.8	576	2,250	12,700
Mean	157	10,300	61.2	61.8	616	2,770	14,000
SD	6.28	627	5.41	4.4	37.1	467	1,100
Control-A	102	8,000	48.9	45.6	448	2,060	10,700
Control-B	110	7,340	46.9	46.6	383	2,100	10,000
Control-C	109	8,010	51.3	53.8	480	2,210	10,900
Mean	107	7,890	49.0	48.7	437	2,130	10,600
SD	5	387	2.24	4.45	49.1	94.8	479
10%-HPβCD-A	103	5,980	36.4	35.3	296	1,300	7,750
10%-HPβCD-B	158	7,270	50.3	52.6	403	1,930	9,870
10%-HPβCD-C	100	6,000	37.2	39.5	323	1,700	8,200
Mean	120	6,420	42.4	42.4	341	1,640	8,600
SD	32.7	740	7.80	9.02	56	322	1,120
Laboratory QA/QC							
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard	<1.0	19.1	<1.0	17.4	<1.0	17.3	-
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	95.5	-	87.0	-	86.5	-

Table B-2. DDT concentrations of waters of the DDT solubility experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	**2,4-DDE (μg/L)**	**4,4-DDE (μg/L)**	**2,4-DDD (μg/L)**	**4,4-DDD (μg/L)**	**2,4-DDT (μg/L)**	**4,4-DDT (μg/L)**	**ΣDDT (μg/L)**
Control-A	3.50	237	3.50	1.31	23.6	92.8	362
Control-C	0.82	41.8	1.33	0.18	4.25	16.2	64.6
Mean	2.16	139	2.42	0.75	13.9	54.5	213
SD	1.90	138	1.54	0.79	13.7	54.2	210
10%-HPβCD-A	42.4	1,900	26.1	20.9	522	1,680	4,190
10%-HPβCD-B	38.9	2,000	22.3	17.3	479	1,520	4,080
10%-HPβCD-C	44.7	2,050	25.0	18.2	586	1,890	4,620
Mean	42.0	1,990	24.5	18.8	529	1,700	4,300
SD	2.91	77.7	1.95	1.90	53.9	189	285
Laboratory QA/QC							
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	1.63	-
Control Standard	<1.0	19.09	<1.0	18.69	<1.0	19.42	-
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	95.5	-	93.5	-	97.1	-

Table B-3. DDT concentrations of soils of the microbial activity experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	**2,4-DDE (ng/g)**	**4,4-DDE (ng/g)**	**2,4-DDD (ng/g)**	**4,4-DDD (ng/g)**	**2,4-DDT (ng/g)**	**4,4-DDT (ng/g)**	**ΣDDT (ng/g)**
Control-A	142	8,120	72.5	195	842	2,920	12,300
Control-B	186	8,080	84.5	168	888	2,950	12,300
Control-C	155	7,570	73.0	168	834	2,650	11,400
Mean	161	7,90	76.7	177	854	2,840	12,000
SD	22.9	305	6.77	15.6	29.5	161	500
10%-HPβCD-A	77.5	3,770	85.6	195	324	1,110	5,560
10%-HPβCD-B	89.0	4,000	87.0	192	280	885	5,540
10%-HPβCD-C	77.7	3,310	97.3	174	292	886	4,830
Mean	81.4	3,690	90.0	187	299	961	5,310
SD	6.57	355	6.40	11.1	22.9	131	413
Laboratory QA/QC							
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard	<1.0	23.4	<1.0	20.8	<1.0	21.0	-
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	117	-	104	-	105	-

Table B-4. DDT concentrations of waters of the microbial activity experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (μg/L)	4,4-DDE (μg/L)	2,4-DDD (μg/L)	4,4-DDD (μg/L)	2,4-DDT (μg/L)	4,4-DDT (μg/L)	ΣDDT (μg/L)
Control-A	<0.02	0.07	0.03	0.02	<0.02	0.04	0.18
Control-B	<0.02	0.07	<0.02	0.02	<0.02	0.04	0.16
Control-C	<0.02	0.05	<0.02	<0.02	<0.02	0.06	0.13
Mean	<0.02	0.07	<0.02	<0.02	<0.02	0.05	0.16
SD	-	0.01	-	-	-	0.01	0.03
10%-HPβCD-A[a]	-	-	-	-	-	-	-
10%-HPβCD-B	5.94	111	22.0	118	84.7	221	563
10%-HPβCD-C	5.28	126	14.3	74.7	70.8	229	521
Mean	5.61	118	18.2	96.5	77.7	225	542
SD	0.47	11.0	5.47	30.9	9.84	5.60	30.0
Laboratory QA/QC							
Blank	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-
Control Standard	<0.02	0.28	<0.02	0.33	<0.02	0.35	-
Control Standard Target	<0.02	0.40	<0.02	0.40	<0.02	0.40	-
%QC recovery	-	70.0[b]	-	82.5	-	87.5	-

[a] Sample excluded due to low extraction efficiency.
[b] Mean difference between the control standard and the control target was more than 20%.

Table B-5. Recorded absorbance and calculated concentrations for the microbial activity experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Absorbance (490 nm)	Concentration (mg/mL)
Control-A2	1.39	0.23
Control-B2	1.35	0.22
Control-C2	1.27	0.21
Mean	1.34	0.22
SD	0.06	0.01
10%-HPβCD-A2	1.42	0.24
10%-HPβCD-B2	1.50	0.25
10%-HPβCD-C2	1.32	0.22
Mean	1.41	0.23
SD	0.09	0.02
Control-A4	1.22	0.20
Control-B4	1.30	0.22
Control-C4	1.47	0.24
Mean	1.33	0.22
SD	0.13	0.02
10%-HPβCD-A4	1.45	0.24
10%-HPβCD-B4	1.26	0.21
10%-HPβCD-C4	1.26	0.21
Mean	1.41	0.23
SD	0.09	0.02
Control-A7	1.35	0.22
Control-B7	1.26	0.21
Control-C7	1.11	0.18
Mean	1.41	0.23
SD	0.09	0.02
10%-HPβCD-A7	1.52	0.25
10%-HPβCD-B7	1.26	0.21
10%-HPβCD-C7	1.30	0.22
Mean	1.41	0.23
SD	0.09	0.02
Laboratory QA/QC		
Control-B4	1.30	0.22
Control-B4-Dup	1.35	0.22
RPD [b]	3.77	3.88
Blank	0.05	-
Negative Control	-0.40	-

[a] Numbers after the letter ID represent the week of the sample collection.
[b] Relative percent difference of the analytical duplicates.

Table B-6. DDT concentrations of soils of the optimal HPβCD concentration experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)
Control-A	159	10,300	285	107	751	2,370	13,900
Control-B	159	6,330	304	177	688	2,670	10,300
Control-C	183	8,180	301	82.7	1,070	2,870	12,700
Mean	167	8,260	297	122	837	2,630	12,300
SD	13.4	1,980	10.3	48.9	205	250	1,840
2.5%-HPβCD-A	127	6,420	98.4	134	508	1,590	8,880
2.5%-HPβCD-B	171	10,100	300	220	708	1,840	13,360
2.5%-HPβCD-C	177	9,500	354	222	693	2,160	13,110
Mean	158	8,680	251	192	636	1,860	11,790
SD	27.4	1,980	135	50.2	111	286	2,510
5%-HPβCD-A	160	8,400	359	176	603	1,770	11,500
5%-HPβCD-B	193	8,890	240	185	602	1,780	11,900
5%-HPβCD-C	88.6	4,690	42.9	166	405	1,340	6,730
Mean	147	7,330	214	176	537	1,630	10,000
SD	53.5	2,290	160	10	114	252	2,860
10%-HPβCD-A	94.1	5,590	208	76.4	412	1,310	7,690
10%-HPβCD-B	81.9	4,340	210	90.3	353	1,150	6,220
10%-HPβCD-C	120	6,680	225	106	443	1,700	9,280
Mean	98.7	5,540	214	91.1	403	1,390	7,730
SD	19.6	1,170	9.57	15.0	45.6	284	1,530
Laboratory QA/QC							
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard	<1.0	20.5	<1.0	21.3	<1.0	22.5	-
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	102	-	106	-	112	-
Control-C	182	8,112	282	88	996	2,761	-
Control-C-Dup	183	8,232	320	77	1,146	2,974	-
RPD [a]	0.63	1.47	12.8	12.9	14.0	-7.43	-

[a] Relative percent difference of the analytical duplicates.

Table B-7. DDT concentrations of waters of the optimal HPβCD concentration experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (μg/L)	4,4-DDE (μg/L)	2,4-DDD (μg/L)	4,4-DDD (μg/L)	2,4-DDT (μg/L)	4,4-DDT (μg/L)	ΣDDT (μg/L)
Control-A	<0.02	0.03	<0.02	<0.02	<0.02	<0.02	0.03
Control-B	<0.02	0.02	<0.02	<0.02	<0.02	<0.02	0.02
Control-C	<0.02	0.03	<0.02	<0.02	<0.02	<0.02	0.03
Mean	<0.02	0.03	<0.02	<0.02	<0.02	<0.02	0.03
SD	-	0.003	-	-	-	-	0.003
2.5%-HPβCD-A	1.52	103	11.02	16.4	8.63	1.48	142
2.5%-HPβCD-B	1.40	90.6	9.79	15.9	9.27	19.3	146
2.5%-HPβCD-C	1.02	76.9	8.93	10.1	7.56	17.3	122
Mean	1.31	90.3	9.91	14.1	8.49	12.7	137
SD	0.26	13.2	1.05	3.52	0.86	9.75	13.1
5%-HPβCD-A	2.24	157	13.1	24.9	19.9	52.0	269
5%-HPβCD-B	2.25	163	17.3	36.9	8.98	16.1	245
5%-HPβCD-C	2.05	148	14.1	21.1	12.0	26.3	223
Mean	2.18	156	14.8	27.6	13.6	31.5	246
SD	0.11	7.83	2.17	8.23	5.61	18.5	22.9
10%-HPβCD-A	5.40	358	30.1	62.2	35.2	83.0	574
10%-HPβCD-B	5.15	367	43.0	75.8	18.3	33.1	543
10%-HPβCD-C	4.80	337	17.8	29.4	57.4	192	639
Mean	5.11	354	30.3	55.8	37.0	103	585
SD	0.30	15.4	12.6	23.8	19.6	81.5	49.2
Laboratory QA/QC							
Blank	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-
Control Standard	<0.02	0.44	<0.02	0.36	<0.02	0.36	-
Control Standard Target	<0.02	0.40	<0.02	0.40	<0.02	0.40	-
%QC recovery	-	110	-	90.0	-	90.0	-

Table B-8. Recorded absorbance and calculated concentrations for the microbial activity experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Absorbance (490 nm)	Concentration (mg/mL)
Control-A3	0.62	0.07
Control-B3	1.25	0.18
Control-C3	0.92	0.13
Mean	0.93	0.13
SD	0.32	0.06
2.5%-HPβCD-A3	1.43	0.21
2.5%-HPβCD-B3	1.15	0.16
2.5%-HPβCD-C3	1.14	0.16
Mean	1.24	0.18
SD	0.16	0.03
5%-HPβCD-A3	1.52	0.23
5%-HPβCD-B3	0.90	0.12
5%-HPβCD-C3	1.22	0.18
Mean	1.21	0.18
SD	0.31	0.06
10%-HPβCD-A3	0.91	0.12
10%-HPβCD-B3	1.31	0.19
10%-HPβCD-C3	1.20	0.17
Mean	1.14	0.16
SD	0.21	0.04
Control-A6	0.52	0.08
Control-B6	1.19	0.20
Control-C6	1.25	0.21
Mean	0.99	0.16
SD	0.41	0.07
2.5%-HPβCD-A6	1.12	0.18
2.5%-HPβCD-B6	1.23	0.20
2.5%-HPβCD-C6	0.83	0.14
Mean	1.06	0.17
SD	0.21	0.03
5%-HPβCD-A6	0.87	0.14
5%-HPβCD-B6	1.03	0.17
5%-HPβCD-C6	0.88	0.14
Mean	0.93	0.15
SD	0.09	0.02
10%-HPβCD-A6	0.88	0.14

10%-HPβCD-B6	0.74	0.12
10%-HPβCD-C6	1.35	0.22
Mean	0.99	0.16
SD	0.32	0.05
Laboratory QA/QC		
5%-HPβCD-A3	1.52	0.23
5%-HPβCD-A3-Dup	1.44	0.22
RPD [b]	5.41	4.44
Blank	0.04	-
Negative Control	-0.06	-

[a] Numbers after the letter ID represent the week of the sample collection.
[b] Relative percent difference of the analytical duplicates.

Table B-9. DDT concentrations of soils of the greenhouse experiment I. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Treatment[b]	[∑DDT] soil	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	∑DDT (ng/g)
Control-A-3	Tap Water	Low	3.50	1,160	5.50	4.48	31.2	91.8	1,290
Control-B-3	Tap Water	Low	3.69	1,130	5.61	4.32	30.1	80.7	1,250
Control-C-3	Tap Water	Low	3.49	1,150	5.35	4.28	31.5	92.0	1,290
Mean	-	-	3.56	1,140	5.49	4.36	31.0	88.2	1,280
SD	-	-	0.12	14.6	0.13	0.10	0.73	6.47	21.5
Control-LB-A-3	LB + Tap Water	Low	1.94	700	5.11	2.29	17.8	59.3	787
Control-LB-B-3	LB + Tap Water	Low	1.81	682	5.15	2.05	17.5	60.1	769
Control-LB-C-3	LB + Tap Water	Low	1.83	688	4.87	2.98	16.5	58.0	772
Mean	-	-	1.86	690	5.04	2.44	17.3	59.2	776
SD	-	-	0.07	9.32	0.15	0.49	0.66	1.07	9.61
Control-SG-A-3	SG + Tap Water	Low	2.98	1,180	4.67	2.26	30.1	96.8	1,310
Control-SG-B-3	SG + Tap Water	Low	2.19	1,100	3.62	1.51	23.5	71.3	1,200
Control-SG-C-3	SG + Tap Water	Low	2.64	1,070	4.29	1.98	28.3	85.1	1,110
Mean	-	-	2.60	1,120	4.19	1.91	27.3	84.4	1,240
SD	-	-	0.39	53.3	0.53	0.38	3.44	12.7	65.9
50ML-A-2	50 mL-10%-HPβCD	Low	<1.0	92.3	2.17	<1.0	3.13	15.9	115
50ML-B-2	50 mL-10%-HPβCD	Low	<1.0	90.1	2.37	1.28	3.27	16.0	114
50ML-C-2	50 mL-10%-HPβCD	Low	<1.0	86.6	2.21	1.55	3.24	15.6	110
Mean	-	-	-	89.7	2.25	1.19	3.21	15.9	113
SD	-	-	-	2.89	0.10	0.42	0.07	0.22	2.60
50ML-LB-A-2	LB + 50 mL-10%-HPβCD	Low	<1.0	163	2.33	1.49	5.90	27.2	201
50ML-LB-B-2	LB + 50 mL-10%-HPβCD	Low	<1.0	150	2.93	1.39	5.59	20.7	181
50ML-LB-C-2	LB + 50 mL-10%-HPβCD	Low	<1.0	151	2.88	1.71	5.06	21.3	183
Mean	-	-	-	155	2.71	1.53	5.51	23.1	188
SD	-	-	-	7.38	0.33	0.16	0.42	3.62	11.0
50ML-SG-A-2	SG + 50 mL-10%-HPβCD	Low	<1.0	81.0	2.78	1.43	2.16	10.8	99
50ML-SG-B-2	SG + 50 mL-10%-HPβCD	Low	<1.0	92.4	3.64	0.94	2.45	11.8	112
50ML-SG-C-2	SG + 50 mL-10%-HPβCD	Low	<1.0	87.9	3.21	1.48	2.89	11.8	108
Mean	-	-	-	87.1	3.21	1.28	2.50	11.5	106
SD	-	-	-	5.72	0.43	0.30	0.37	0.58	6.73

1PV-A-1	1PV-10%-HPβCD	Low	<1.0	170	1.94	1.50	6.14	27.1	207
1PV-B-1	1PV-10%-HPβCD	Low	<1.0	145	1.76	1.22	5.36	22.0	176
1PV-C-1	1PV-10%-HPβCD	Low	<1.0	149	2.13	1.32	5.44	24.2	183
Mean	-	-	-	154	1.94	1.35	5.64	24.4	188
SD	-	-	-	13.2	0.18	0.14	0.43	2.58	16.3
1PV-LB-A-1	LB + 1PV-10%-HPβCD	Low	<1.0	152	2.25	1.04	4.68	23.7	184
1PV-LB-B-1	LB + 1PV-10%-HPβCD	Low	<1.0	172	1.19	1.42	5.21	24.7	206
1PV-LB-C-1	LB + 1PV-10%-HPβCD	Low	<1.0	238	2.39	1.82	7.31	27.2	277
Mean	-	-	-	187	1.94	1.43	5.73	25.2	222
SD	-	-	-	44.8	0.66	0.39	1.39	1.78	48.6
1PV-SG-A-1	SG + 1PV-10%-HPβCD	Low	<1.0	221	2.06	1.40	6.89	27.9	260
1PV-SG-B-1	SG + 1PV-10%-HPβCD	Low	<1.0	216	1.77	1.22	6.64	26.8	254
1PV-SG-C-1	SG + 1PV-10%-HPβCD	Low	<1.0	219	1.87	1.41	6.87	27.9	257
Mean	-	-	-	218	1.90	1.35	6.80	27.5	257
SD	-	-	-	2.18	0.15	0.11	0.14	0.62	3.11
Control-A-6	Tap Water	High	427	30,300	881	224	3,370	5,470	40,700
Control-B-6	Tap Water	High	449	33,600	843	213	3,240	5,610	43,900
Control-C-6	Tap Water	High	455	31,800	920	246	3,470	5,420	42,300
Mean	-	-	444	31,900	882	228	3,360	5,500	42,300
SD	-	-	14.7	1,620	38.3	16.8	117	96.8	1,610
Control-LB-A-6	LB+ Tap Water	High	210	26,100	572	202	2,530	5,290	34,900
Control-LB-B-6	LB+ Tap Water	High	208	27,200	537	260	2,660	5,410	36,200
Control-LB-C-6	LB+ Tap Water	High	202	26,700	579	246	2,620	5,160	35,500
Mean	-	-	207	26,700	563	236	2,600	5,280	35,500
SD	-	-	4.28	550	22.8	30.4	66.9	125	677
Control-SG-A-6	SG + Tap Water	High	219	20,500	604	257	1,190	3,360	26,100
Control-SG-B-6	SG + Tap Water	High	193	19,000	618	227	1,170	3,600	24,900
Control-SG-C-6	SG + Tap Water	High	202	21,100	507	172	1,140	3,980	27,100
Mean	-	-	204	20,200	576	219	1,170	3,650	26,000
SD	-	-	13.1	1,060	60.8	42.8	25.4	314	1,140
50ML-A-5	50 mL-10%-HPβCD	High	120	16,400	466	110	1,020	2,660	20,700
50ML-B-5	50 mL-10%-HPβCD	High	97.7	15,900	362	109	719	2,370	19,500
50ML-C-5	50 mL-10%-HPβCD	High	96.5	14,600	356	88.1	750	2,260	18,200
Mean	-	-	105	15,600	395	102	830	2,430	19,500

SD	-	-	13.2	883	62.1	12.4	165	207	1,270
50ML-LB-A-5	LB + 50 mL-10%-HPβCD	High	104	16,500	387	110	907	2,500	20,500
50ML-LB-B-5	LB + 50 mL-10%-HPβCD	High	79.6	12,700	338	84.2	691	2,140	16,000
50ML-LB-C-5	LB + 50 mL-10%-HPβCD	High	81.3	13,100	372	76.0	708	2,070	16,400
Mean	-	-	88.3	14,100	366	90.1	769	2,230	17,700
SD	-	-	13.5	2,090	25.4	17.7	120	217	2,470
50ML-SG-A-5	SG + 50 mL-10%-HPβCD	High	89.3	14,100	437	91.0	756	2,340	18,700
50ML-SG-B-5	SG + 50 mL-10%-HPβCD	High	110	16,300	491	125	927	2,600	20,570
50ML-SG-C-5	SG + 50 mL-10%-HPβCD	High	108	16,100	421	110	882	2,530	20,130
Mean	-	-	103	15,800	450	109	855	2,490	19,800
SD	-	-	11.6	708	36.5	17	88.7	133	975
1PV-A-4	1PV-10%-HPβCD	High	98.4	15,500	378	112	808	2,540	19,400
1PV-B-4	1PV-10%-HPβCD	High	101	16,700	452	130	918	2,790	21,000
1PV-C-4	1P-10%-HPβCD	High	92.6	14,800	412	91.7	811	2,290	18,500
Mean	-	-	97.4	15,600	414	111	846	2,540	19,600
SD	-	-	4.36	959	36.8	18.9	63.0	250	1,310
1PV-LB-A-4	LB + 1PV-10%-HPβCD	High	93.7	15,300	419	104	838	2,440	19,200
1PV-LB-B-4	LB + 1PV-10%-HPβCD	High	109	15,900	453	127	899	2,700	20,200
1PV-LB-C-4	LB + 1PV-10%-HPβCD	High	102	15,200	460	111	897	2,640	19,400
Mean	-	-	102	15,500	444	114	878	2,590	19,600
SD	-	-	7.83	362	21.9	11.7	34.6	138	518
1PV-SG-A-4	SG + 1PV-10%-HPβCD	High	103	16,500	457	111	908	2,660	20,700
1PV-SG-B-4	SG + 1PV-10%-HPβCD	High	108	16,300	412	79.2	904	2,610	20,400
1PV-SG-C-4	SG + 1PV-10%-HPβCD	High	93.2	14,700	474	116	903	2,580	18,900
Mean	-	-	101	15,800	448	102	905	2,620	20,000
SD	-	-	7.35	973	32.0	20.0	2.65	42.6	988

Laboratory QA/QC									
Blank-1	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-1	-	-	<1.0	23.8	<1.0	22.7	<1.0	25.0	-
Control Standard Target-1	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery		-	-	119	-	113	-	125[c]	
1PV-C-1	1PV-10%-HPβCD	Low	<1.0	149	2.13	1.32	5.44	24.2	183
1PV-C-Dup-1	1PV-10%-HPβCD	Low	<1.0	147	2.01	1.33	5.57	23.8	181
RPD [d]	-	-	-	1.02	6.01	0.85	2.32	1.66	
Blank-2	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-2	-	-	<1.0	19.8	<1.0	22.1	<1.0	20.1	-
Control Standard Target-2	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	99.1	-	110	-	101	-
50ML-C-2	50 mL-10%-HPβCD	Low	<1.0	86.6	2.21	1.55	3.24	15.6	110
50ML-C-Dup-2	50 mL-10%-HPβCD	Low	<1.0	84.0	1.91	1.43	3.15	15.1	106
RPD	-	-	-	3.03	14.4	7.90	2.83	3.24	-
Blank-3	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-3	-	-	<1.0	22.0	<1.0	20.6	<1.0	22.2	-
Control Standard Target-3	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	110	-	103	-	111	-
Control-C-3	Tap Water	Low	3.44	1,160	5.26	4.16	32.3	93.3	1,300
Control-C-Dup-3	Tap Water	Low	3.49	1,150	5.35	4.28	31.5	92.0	1,290
RPD	-	-	1.47	1.16	1.69	2.93	2.41	1.34	-
Blank-4	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-4	-	-	<1.0	19.9	<1.0	19.9	<1.0	20.7	-
Control Standard Target-4	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	99.4	-	99.5	-	103	-
1PV-SG-A-4	SG + 1PV-10%-HPβCD	High	103	16,500	457	111	908	2,660	20,700
1PV-SG-A-Dup-4	SG + 1PV-10%-HPβCD	High	100	14,500	394	104	821	2,440	18,300
RPD	-	-	2.81	13.2	14.7	6.58	10.0	8.81	-
Blank-5	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-5	-	-	<1.0	21.3	<1.0	21.5	<1.0	21.3	-
Control Standard Target-5	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-

%QC recovery	-	-	-	106		107	-	106	-
50ML-C-5	50 mL-10%-HPβCD	High	97.0	14,600	356	88.0	750	2,260	18,200
50ML-C-Dup-5	50 mL-10%-HPβCD	High	90.5	14,000	350	101	699	2,210	17,500
RPD	-	-	6.91	4.43	1.62	13.5	7.03	2.33	-
Blank-6	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-6	-	-	<1.0	20.9	<1.0	21.58	<1.0	21.20	-
Control Standard Target-6	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	105	-	108	-	106	-
Control-C-6	Tap Water	High	455	31,800	920	246	3,470	5,420	42,300
Control-C-Dup-6	Tap Water	High	471	33,700	899	267	3,750	5,130	44,200
RPD	-	-	3.38	5.78	2.30	8.30	7.71	5.53	-

[a] Numbers after the dash represent the extraction batch number of the sample.
[b] LB represent soil from pots planted with *S. scoparium*, and SG soil from pots planted with *P. virgatum*.
[c] Mean difference between the control standard and the control target was more than 20%.
[d] Relative percent difference of the analytical duplicates.

Table B-10. DDT concentrations of shoot tissues of the greenhouse experiment I. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Species	Tissue	Treatment	[∑DDT] soil	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	∑DDT (ng/g)
Control-LB-A-P1-7	*S. scoparium*	Shoot	Tap Water	Low	66.2	55.7	19.4	15.8	29.0	34.5	221
Control-LB-A-P2-7	*S. scoparium*	Shoot	Tap Water	Low	62.9	47.4	15.4	18.2	9.7	28.3	182
Control-LB-B-P1-7	*S. scoparium*	Shoot	Tap Water	Low	46.2	20.3	13.1	15.0	17.5	25.4	137
Control-LB-B-P2-7	*S. scoparium*	Shoot	Tap Water	Low	57.2	27.3	14.2	40.3	30.2	39.1	208
Control-LB-C-P1-7	*S. scoparium*	Shoot	Tap Water	Low	64.2	70.5	18.3	36.4	34.7	30.3	255
Control-LB-C-P2-7	*S. scoparium*	Shoot	Tap Water	Low	69.3	17.6	15.2	14.4	16.8	31.5	165
Mean	-	-	-	-	61.0	39.8	15.9	23.4	23.0	31.5	195
SD	-	-	-	-	8.31	21.4	2.43	11.8	9.70	4.81	41.9
Control-SG-A-P1-1	*P. virgatum*	Shoot	Tap Water	Low	44.7	67.6	8.9	12.7	18.6	19.1	172
Control-SG-A-P2-1	*P. virgatum*	Shoot	Tap Water	Low	41.6	61.6	13.4	13.5	22.4	46.6	199
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	Low	20.5	20.3	14.7	11.3	5.9	32.3	105
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	Low	23.9	31.5	17.6	8.2	11.1	15.9	108
Control-SG-B-P1-2	*P. virgatum*	Shoot	Tap Water	Low	36.2	15.5	24.7	12.5	16.9	64.9	171
Control-SG-B-P1-2	*P. virgatum*	Shoot	Tap Water	Low	30.3	14.8	10.5	11.9	18.5	62.0	148
Mean	-	-	-	-	32.9	35.2	15.0	11.7	15.6	40.1	150
SD	-	-	-	-	9.67	23.6	5.69	1.87	5.98	21.1	37.6
50ML-LB-A-P1-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	394	45.0	16.6	19.7	13.2	57.5	546
50ML-LB-A-P2-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	207	38.6	14.7	12.6	16.8	40.7	330
50ML-LB-B-P1-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	381	97.6	17.2	77.7	27.3	59.5	661
50ML-LB-B-P2-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	324	20.8	18.9	19.2	20.5	18.7	422

50ML-LB-C-P1-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	271	58.2	19.6	51.7	37.1	23.2	460
50ML-LB-C-P2-8	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	Low	274	23.0	14.9	33.9	25.5	24.7	396
Mean	-	-	-	-	309	47.2	17.0	35.8	23.4	37.4	469
SD	-	-	-	-	71.7	28.3	1.99	24.8	8.53	18.0	118
50ML-SG-A-P1-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	38.6	19.0	19.7	21.3	14.9	70.3	184
50ML-SG-A-P2-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	25.8	21.8	16.6	33.5	17.0	46.0	161
50ML-SG-B-P1-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	29.5	7.2	17.9	23.2	18.0	32.0	128
50ML-SG-B-P2-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	30.9	21.1	18.8	16.2	21.2	59.5	168
50ML-SG-C-P1-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	38.3	33.5	18.1	15.7	20.5	46.7	173
50ML-SG-C-P2-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	Low	20.6	9.1	16.1	16.2	18.9	67.0	148
Mean	-	-	-	-	30.6	18.6	17.9	21.0	18.4	53.6	160
SD	-	-	-	-	7.04	9.59	1.34	6.85	2.33	14.6	19.9
1PV-LB-A-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	202	25.2	28.9	55.7	34.7	73.0	419
1PV-LB-A-P2-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	220	71.5	50.1	50.6	73.5	37.1	503
1PV-LB-B-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	224	44.1	36.6	35.6	46.0	72.6	459
1PV-LB-B-P2-6	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	229	44.4	46.6	33.4	22.1	36.7	412
1PV-LB-C-P1-6	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	245	31.3	20.6	20.5	21.7	68.9	408
1PV-LB-C-P2-6	*S. scoparium*	Shoot	1PV of 10%-HPβCD	Low	216	62.5	62.1	45.9	25.7	97.8	510
Mean	-	-	-	-	223	46.5	40.8	40.3	37.3	64.3	452
SD	-	-	-	-	14.2	17.8	15.1	13.0	20.0	23.6	46.1
1PV-SG-A-P1-3	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	24.6	20.4	9.7	15.5	15.4	53.6	139
1PV-SG-A-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	33.8	15.1	5.4	24.4	18.9	52.1	150
1PV-SG-B-P1-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	26.6	10.6	27.0	20.4	16.6	82.4	184

1PV-SG-B-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	37.6	16.3	7.9	12.0	18.6	76.9	169
1PV-SG-C-P1-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	29.7	21.3	24.4	32.8	23.8	75.1	207
1PV-SG-C-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	23.8	18.9	14.0	17.0	19.7	97.3	191
Mean	-	-	-	-	29.4	17.1	14.7	20.3	18.8	72.9	173
SD	-	-	-	-	5.45	3.96	8.97	7.43	2.92	17.4	25.7
Control-LB-A-P1-8	*S. scoparium*	Shoot	Tap Water	High	72.1	261	13.9	11.9	18.4	72.9	451
Control-LB-A-P2-8	*S. scoparium*	Shoot	Tap Water	High	66.5	189	16.7	15.9	26.4	65.4	380
Control-LB-B-P1-8	*S. scoparium*	Shoot	Tap Water	High	70.1	257	13.1	18.7	22.4	79.8	462
Control-LB-B-P2-7	*S. scoparium*	Shoot	Tap Water	High	62.1	242	8.03	19.1	22.9	70.3	424
Control-LB-C-P1-7	*S. scoparium*	Shoot	Tap Water	High	74.1	218	25.7	12.2	32.5	70.9	433
Control-LB-C-P2-7	*S. scoparium*	Shoot	Tap Water	High	78.8	142	8.02	19.0	17.8	55.4	321
Mean	-	-	-	-	70.6	218	14.2	16.1	23.4	69.1	412
SD	-	-	-	-	5.88	45.97	6.56	3.39	5.46	8.20	52.5
Control-SG-A-P1-1	*P. virgatum*	Shoot	Tap Water	High	47.8	42.2	17.4	16.4	12.1	43.2	179
Control-SG-A-P2-1	*P. virgatum*	Shoot	Tap Water	High	92.9	45.9	14.4	11.8	12.9	44.6	222
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	High	22.2	45.0	15.4	12.7	9.0	12.3	117
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	High	83.2	32.7	18.8	15.5	11.8	12.9	175
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	High	77.6	33.2	19.0	10.7	9.2	11.4	161
Control-SG-B-P1-2	*P. virgatum*	Shoot	Tap Water	High	48.9	39.2	22.3	16.9	9.8	77.4	214
Mean	-	-	-	-	62.1	39.7	17.9	14.0	10.8	33.6	178
SD	-	-	-	-	26.8	5.76	2.84	2.60	1.66	26.5	38.4
50ML-LB-A-P1-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	149	426	88.2	44.6	45.6	72.2	826
50ML-LB-A-P2-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	277	295	48.0	25.1	35.5	99.2	779

50ML-LB-B-P1-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	182	305	59.7	41.2	29.8	119	736
50ML-LB-B-P2-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	159	357	78.2	40.3	27.5	144	806
50ML-LB-C-P1-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	209	430	89.5	43.5	34.0	113	919
50ML-LB-C-P2-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	292	305	53.1	26.1	24.7	70.1	771
Mean	-	-	-	-	211	353	69.4	36.8	32.8	103	806
SD	-	-	-	-	60.6	62.1	18.2	8.83	7.43	28.6	63.4
50ML-SG-A-P1-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	35.9	120	8.07	31.0	21.3	32.9	249
50ML-SG-A-P2-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	28.3	116	14.3	21.7	30.2	51.7	262
50ML-SG-B-P1-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	31.4	93.7	13.0	27.6	25.2	39.7	231
50ML-SG-B-P2-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	56.1	125	18.4	15.9	16.2	58.2	290
50ML-SG-C-P1-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	59.7	87.5	10.4	23.5	29.6	25.2	236
50ML-SG-C-P2-3	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	50.5	102	11.2	21.1	22.1	46.5	253
Mean	-	-	-	-	43.6	107	12.5	23.5	24.1	42.4	253
SD	-	-	-	-	13.5	15.3	3.57	5.28	5.37	12.2	21.4
1PV-LB-A-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	13.0	76.6	22.1	18.9	17.0	40.4	188
1PV-LB-A-P2-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	6.33	39.0	14.4	17.5	20.3	44.0	142
1PV-LB-B-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	8.98	82.9	9.8	24.8	14.4	31.8	173
1PV-LB-B-P2-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	29.9	84.6	15.5	23.5	16.0	54.0	223
1PV-LB-C-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	5.57	35.7	12.7	19.2	20.1	21.1	114
1PV-LB-C-P2-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	22.6	56.1	35.1	16.8	24.9	37.3	193
Mean	-	-	-	-	14.4	62.5	18.3	20.1	18.8	38.1	172
SD	-	-	-	-	9.80	22.0	9.20	3.29	3.81	11.1	38.9
1PV-SG-A-P1-3	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	64.5	46.1	25.6	11.6	27.0	74.5	249

1PV-SG-A-P2-3	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	53.6	40.7	13.6	16.2	24.2	64.2	213
1PV-SG-B-P1-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	18.2	40.5	19.4	16.8	19.5	111	226
1PV-SG-B-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	41.7	30.8	21.3	13.8	18.7	76.0	202
1PV-SG-C-P1-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	59.4	35.7	18.5	18.0	18.6	58.7	209
1PV-SG-C-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	24.8	23.8	8.47	12.8	19.9	102	192
Mean	-	-	-	-	43.7	36.3	17.8	14.9	21.3	81.1	215
SD	-	-	-	-	18.9	7.98	6.00	2.50	3.46	21.0	20.2
Laboratory QA/QC											
Blank-1	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-1	-	-	-	-	<1.0	17.3	<1.0	17.5	<1.0	14.9	-
Control Standard Target-1	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC	-	-	-	-	-	86.5	-	87.5	-	74.7[b]	-
Control-SG-B-P1-1	*P. virgatum*	Shoot	Tap Water	High	77.6	33.2	19.0	10.7	9.2	11.4	161
Control-SG-B-P1-Dup-1	*P. virgatum*	Shoot	Tap Water	High	88.9	35.3	15.8	6.2	9.8	12.4	169
RPD [c]	-	-	-	-	13.5	6.25	18.2	53.3	6.55	9.15	-
Blank-2	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-2	-	-	-	-	<1.0	18.9	<1.0	19.4	<1.0	20.0	-
Control Standard Target-2	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	94.5	-	97.0	-	100	-
50ML-SG-B-P2-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	56.1	125	18.4	15.9	16.1	58.2	290
50ML-SG-B-P2-Dup-2	*P. virgatum*	Shoot	50 mL of 10%-HPβCD	High	22.8	167	17.2	15.0	17.0	57.6	296
RPD	-	-	-	-	84.5	28.2	6.29	5.83	5.41	1.05	-
Blank-3	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-3	-	-	-	-	<1.0	17.8	<1.0	18.5	<1.0	19.3	-

Control Standard Target-3	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-89.1	-	92.4	-	96.6	-	-
1PV-SG-A-P1-3	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	64.5	46.0	25.6	11.6	27.0	74.5	249
1PV-SG-A-P1-Dup-3	*P. virgatum*	Shoot	1PV of 10%-HPβCD	High	59.3	128	28.3	10.2	22.7	82.2	330
RPD	-	-	-	-	8.37	93.9	10.3	12.6	17.4	9.81	-
Blank-4	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control Standard-4	-	-	-	-	<1.0	17.2	<1.0	18.8	<1.0	19.48	
Control Standard Target-4	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.00	
%QC recovery	-	-	-	-	-	86.1	-	93.9	-	97.4	-
1PV-SG-C-P2-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	23.8	18.93	14.0	17.0	19.7	97.3	191
1PV-SG-C-P2-Dup-4	*P. virgatum*	Shoot	1PV of 10%-HPβCD	Low	22.5	18.5	13.7	16.6	19.3	91.3	182
RPD	-	-	-	-	5.64	2.27	2.27	2.25	2.30	6.45	-
Blank-5	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-5	-	-	-	-	<1.0	18.2	<1.0	18.1	<1.0	19.0	-
Control Standard Target-5	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	91.1	-	90.4	-	95.1	-
1PV-LB-A-P1-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	13.0	76.6	22.1	18.9	17.0	40.4	188
1PV-LB-A-P1-Dup-5	*S. scoparium*	Shoot	1PV of 10%-HPβCD	High	11.87	73.0	24.3	20.5	21.4	41.3	192
RPD	-	-	-	-	8.82	4.41	9.51	8.24	23.2	2.18	-
Blank-6	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control Standard-6	-	-	-	-	<1.0	21.1	<1.0	20.1	<1.0	25.0	
Control Standard Target-6	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	
%QC recovery	-	-	-	-	-	105	-	100	-	125[b]	-
50ML-LB-B-P2-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	159	356	78.2	40.3	27.5	144	806
50ML-LB-B-P2-Dup-6	*S. scoparium*	Shoot	50 mL of 10%-HPβCD	High	137	354	117	38.4	39.2	142	827

RPD	-	-	-	-	15.2	0.72	39.7	4.96	36.0	1.70	-
Blank-7	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-7	-	-	-	-	<1.0	20.7	<1.0	18.0	<1.0	19.0	-
Control Standard Target-7	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	104	-	90.3	-	95.2	-
Control-LB-C-P1-7	*S. scoparium*	Shoot	Tap Water	Low	64.2	70.5	18.3	36.4	34.7	30.3	254
Control-LB-C-P1-Dup-7	*S. scoparium*	Shoot	Tap Water	Low	74.0	51.5	23.8	20.3	33.9	39.5	243
RPD	-	-	-	-	14.2	31.2	26.3	56.7	2.28	26.3	-
Blank-8	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-8	-	-	-	-	<1.0	2126	<1.0	21.7	<1.0	21.9	-
Control Standard Target-8	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	106	-	108	-	110	-
Control-LB-A-P2-8	*S. scoparium*	Shoot	Tap Water	High	66.5	189	16.7	15.8	26.4	65.4	380
Control-LB-A-P2-Dup-8	*S. scoparium*	Shoot	Tap Water	High	341	167	16.4	16.0	29.0	65.3	-
RPD	-	-	-	-	135	12.2	1.93	1.19	9.33	0.19	-

[a] Numbers after the dash represent the extraction batch number of the sample.
[b] Mean difference between the control standard and the control target was more than 20%.
[c] Relative percent difference of the analytical duplicates.

Table B-11. DDT concentrations of root tissues of the greenhouse experiment I. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	Species	Tissue	Treatment	[∑DDT] soil	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	∑DDT (ng/g)
Control-LB-A-P1-5	*S. scoparium*	Root	Tap Water	Low	359	1,200	74.2	28.8	86.0	311	2,060
Control-LB-A-P2-5	*S. scoparium*	Root	Tap Water	Low	267	1,150	39.2	35.5	50.6	121	1,670
.0Control-LB-B-P1-6	*S. scoparium*	Root	Tap Water	Low	39.0	897	65.7	39.6	129	118	1,290
Control-LB-B-P2-6	*S. scoparium*	Root	Tap Water	Low	39.4	722	75.9	51.5	49.0	161	1,100
Control-LB-C-P1-8	*S. scoparium*	Root	Tap Water	Low	185	915	41.7	28.3	53.8	145	1,370
Control-LB-C-P2-8	*S. scoparium*	Root	Tap Water	Low	92.1	1,000	51.8	57.3	89.0	288	1,580
Mean	-	-	-	-	164	981	58.1	40.2	76.2	191	1,510
SD	-	-	-	-	131	176	16.1	12.0	31.5	86.1	336
Control-SG-A-P1-4	*P. virgatum*	Root	Tap Water	Low	16.5	872	55.5	33.5	116	207	1,300
Control-SG-A-P2-4	*P. virgatum*	Root	Tap Water	Low	43.3	1,210	87.7	26.2	43.1	139	1,550
Control-SG-B-P1-5	*P. virgatum*	Root	Tap Water	Low	493	715	326	38.1	147	162	1,880
Control-SG-B-P1-5	*P. virgatum*	Root	Tap Water	Low	236	1,010	214	37.0	181	134	1,810
Control-SG-B-P1-8	*P. virgatum*	Root	Tap Water	Low	159	563	168	40.5	82.5	147	1,160
Control-SG-B-P1-8	*P. virgatum*	Root	Tap Water	Low	35.4	782	119	32.4	132	230	1,330
Mean	-	-	-	-	164	859	162	34.6	117	170	1,500
SD	-	-	-	-	183	229	98.4	5.08	48.7	39.5	294
50ML-LB-A-P1-3	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	40.3	695	45.2	30.7	66.7	148	1,030
50ML-LB-A-P2-3	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	42.0	684	59.0	53.4	104	146	1,090
50ML-LB-B-P1-4	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	131	884	66.8	45.6	66.8	233	1,430

50ML-LB-B-P2-4	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	143	795	52.2	18.9	38.5	81	1,130
50ML-LB-C-P1-7	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	132	879	54.3	34.4	52.4	164	1,320
50ML-LB-C-P2-8	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	208	857	53.0	48.0	86.0	229	1,481
Mean	-	-	-	-	116	799	55.1	38.5	69.2	167	1,240
SD	-	-	-	-	64.5	90.5	7.25	12.8	23.5	57.4	190
50ML-SG-A-P1-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	77.5	548	174	59.1	112	159	1,130
50ML-SG-A-P2-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	45.1	578	163	58.9	128	176	1,150
50ML-SG-B-P1-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	35.9	591	165	64.4	126	149	1,130
50ML-SG-B-P2-4	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	33.9	548	171	41.3	75.7	167	1,040
50ML-SG-C-P1-7	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	105	533	166	70.3	115	163	1,150
50ML-SG-C-P2-7	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	154	548	166	47.0	53.7	144	1,110
Mean	-	-	-	-	75.3	558	168	56.8	102	160	1,120
SD	-	-	-	-	47.6	22.2	4.19	10.8	30.2	11.6	42.4
1PV-LB-A-P1-1	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	183	516	159	38.5	54.8	131	1,080
1PV-LB-A-P2-1	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	125	692	253	61.1	56.0	91.1	1,280
1PV-LB-B-P1-2	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	32.2	846	54.3	29.5	32.0	109	1,100
1PV-LB-B-P2-2	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	43.9	830	61.6	33.5	45.4	105	1,120
1PV-LB-C-P1-7	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	168	738	137	47.5	93.0	122	1,300
1PV-LB-C-P2-7	*S. scoparium*	Root	1PV of 10%-HPβCD	Low	94.1	528	79.3	44.8	73.1	99.3	918
Mean	-	-	-	-	108	692	124	42.5	59.1	109	1,130
SD	-	-	-	-	62.6	143	76.0	11.3	21.4	14.7	142
1PV-SG-A-P1-1	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	71.1	259	111	33.4	47.6	71.1	593
1PV-SG-A-P2-1	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	78.1	169	61.4	47.6	51.7	60.9	469

1PV-SG-B-P1-2	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	120	528	55.2	33.3	67.9	121	926
1PV-SG-B-P2-2	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	41.3	523	197	42.0	137	125	1,070
1PV-SG-C-P1-6	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	17.4	218	29.3	26.5	28.8	82.9	403
1PV-SG-C-P2-6	*P. virgatum*	Root	1PV of 10%-HPβCD	Low	17.3	426	48.6	43.9	129	82.4	748
Mean	-	-	-	-	57.6	354	83.7	37.8	77.0	90.5	701
SD	-	-	-	-	40.1	159	61.9	7.98	45.1	26.5	261
Control-LB-A-P1-5	*S. scoparium*	Root	Tap Water	High	390	18,800	750	280	1,810	7,500	29,500
Control-LB-A-P2-5	*S. scoparium*	Root	Tap Water	High	493	22,800	767	212	1,750	7,660	33,700
Control-LB-B-P1-5	*S. scoparium*	Root	Tap Water	High	476	21,600	924	162	1,830	6,300	31,300
Control-LB-B-P2-6	*S. scoparium*	Root	Tap Water	High	234	27,400	889	258	1,740	6,450	37,000
Control-LB-C-P1-8	*S. scoparium*	Root	Tap Water	High	156	25,600	628	269	1,840	7,310	35,800
Control-LB-C-P2-8	*S. scoparium*	Root	Tap Water	High	395	28,200	588	261	1,720	7,300	38,500
Mean	-	-	-	-	357	24,100	758	240	1,780	7,080	34,300
SD	-	-	-	-	135	3,640	135	44.8	51.1	573	3,440
Control-SG-A-P1-4	*P. virgatum*	Root	Tap Water	High	308	12,900	696	79.8	2,560	6,890	23,500
Control-SG-A-P2-4	*P. virgatum*	Root	Tap Water	High	267	17,500	716	111	1,650	5,720	26,000
Control-SG-B-P1-5	*P. virgatum*	Root	Tap Water	High	342	17,300	693	116	1,140	7,300	26,900
Control-SG-B-P1-5	*P. virgatum*	Root	Tap Water	High	265	15,300	694	86.1	1,070	4,700	22,100
Control-SG-B-P1-8	*P. virgatum*	Root	Tap Water	High	278	14,900	630	202	1,210	4,660	21,900
Control-SG-B-P1-8	*P. virgatum*	Root	Tap Water	High	260	20,400	640	213	1,720	7,340	30,600
Mean	-	-	-	-	287	16,400	678	135	1,560	6,100	25,200
SD	-	-	-	-	32.1	2,590	34.6	58.4	560	1,240	3,350
50ML-LB-A-P1-3	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	78.3	12,700	660	538	1,790	4,820	20,600

50ML-LB-A-P2-3	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	178	16,000	774	327	1,110	5,980	24,400
50ML-LB-B-P1-4	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	183	26,200	560	67.5	1,840	6,310	35,100
50ML-LB-B-P2-4	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	221	28,000	699	138	1,100	7,370	37,500
50ML-LB-C-P1-7	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	352	12,600	773	132	1,140	4,810	19,800
50ML-LB-C-P2-7	*S. scoparium*	Root	50 mL of 10%-HPβCD	High	261	22,500	659	229	1,940	8,720	34,300
Mean	-	-	-	-	212	19,700	687	238	1,490	6,330	28,600
SD	-	-	-	-	91.7	6,810	81.0	172	408	1,520	7,940
50ML-SG-A-P1-2	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	117	7,740	601	102	845	3,090	12,500
50ML-SG-A-P2-2	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	135	10,600	577	232	920	4,150	16,600
50ML-SG-B-P1-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	93.7	9,600	653	314	869	3,670	15,200
50ML-SG-B-P2-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	133	10,200	618	357	864	3,200	15,300
50ML-SG-C-P1-7	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	64.4	6,690	564	29.4	847	2,800	10,100
50ML-SG-C-P2-7	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	104	9,680	508	70.7	886	4,670	15,900
Mean	-	-	-	-	108	9,070	587	184	872	3,600	14,400
SD	-	-	-	-	26.6	1,520	50	136	27.8	708	2,180
1PV-LB-A-P1-1	*S. scoparium*	Root	1PV of 10%-HPβCD	High	171	23,200	539	390	1,230	5,450	31,000
1PV-LB-A-P2-1	*S. scoparium*	Root	1PV of 10%-HPβCD	High	114	16,300	535	669	884	4,660	23,200
1PV-LB-B-P1-2	*S. scoparium*	Root	1PV of 10%-HPβCD	High	128	15,800	646	837	976	4,820	23,200
1PV-LB-B-P2-2	*S. scoparium*	Root	1PV of 10%-HPβCD	High	152	13,400	577	1,060	736	5,540	21,400
1PV-LB-C-P1-6	*S. scoparium*	Root	1PV of 10%-HPβCD	High	153	20,100	1,200	466	1,310	5,440	28,600
1PV-LB-C-P2-6	*S. scoparium*	Root	1PV of 10%-HPβCD	High	137	18,800	898	736	1,160	4,880	26,700
Mean	-	-	-	-	142	17,900	732	693	1,050	5,130	25,700
SD	-	-	-	-	20.2	3,510	265	246	222	389	3,700

1PV-SG-A-P1-1	*P. virgatum*	Root	1PV of 10%-HPβCD	High	89.7	8,470	198	205	675	3,230	12,900
1PV-SG-A-P2-1	*P. virgatum*	Root	1PV of 10%-HPβCD	High	88.5	12,700	348	234	1,030	4,770	19,200
1PV-SG-B-P1-1	*P. virgatum*	Root	1PV of 10%-HPβCD	High	119	13,900	662	235	908	4,020	19,800
1PV-SG-B-P2-2	*P. virgatum*	Root	1PV of 10%-HPβCD	High	134	10,500	597	255	839	3,740	16,100
1PV-SG-C-P1-6	*P. virgatum*	Root	1PV of 10%-HPβCD	High	86.0	13,900	539	150	1,070	3,820	19,500
1PV-SG-C-P2-6	*P. virgatum*	Root	1PV of 10%-HPβCD	High	82.5	7,520	513	174	583	2,540	11,400
Mean	-	-	-	-	100	11,200	476	209	850	3,690	16,500
SD	-	-	-	-	21.2	2,770	172	40.3	192	751	3,660
Laboratory QA/QC											
Blank-1	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-1	-	-	-	-	<1.0	21.2	<1.0	19.7	<1.0	19.4	-
Control Standard Target-1	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	106	-	98.7	-	96.9	-
1PV-SG-B-P1-1	*P. virgatum*	Root	1PV of 10%-HPβCD	High	119	13,900	662	235	908	4,020	19,800
1PV-SG-B-P1-Dup-1	*P. virgatum*	Root	1PV of 10%-HPβCD	High	127	12,500	662	238	848	4,010	18,400
RPD [b]	-	-	-	-	6.65	10.4	0.06	1.27	6.88	0.14	-
Blank-2	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-2	-	-	-	-	<1.0	21.5	<1.0	21.7	<1.0	20.1	-
Control Standard Target-2	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	108	-	108	-	100	-
50ML-SG-A-P2-2	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	135	10,600	577	232	920	4,150	16,600
50ML-SG-A-P2-Dup-2	*P. virgatum*	Root	50 mL of 10%-HPβCD	High	145	11,300	432	264	999	4,010	17,200

RPD	-	-	-	-	7.49	6.87	28.8	12.9	8.19	3.26	-
Blank-3	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-3	-	-	-	-	<1.0	20.2	<1.0	21.8	<1.0	21.1	-
Control Standard Target-3	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	101	-	109	-	105	-
50ML-SG-B-P1-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	36.0	591	165	64.0	126	149	1,130
50ML-SG-B-P1-Dup-3	*P. virgatum*	Root	50 mL of 10%-HPβCD	Low	33.5	522	158	51.1	142	157	1,060
RPD	-	-	-	-	7.18	12.3	4.29	22.4	11.6	5.05	-
Blank-4	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-4	-	-	-	-	<1.0	19.8	<1.0	20.9	<1.0	21.9	-
Control Standard Target-4	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC	-	-	-	-	-	99.2	-	104	-	109	-
Control-SG-A-P2-4	*P. virgatum*	Root	Tap Water	Low	43.0	1,210	88.0	26.0	43.0	139	1,550
Control-SG-A-P2-Dup-4	*P. virgatum*	Root	Tap Water	Low	44.9	1,110	85.1	34.2	41.8	131	1,450
RPD	-	-	-	-	4.33	8.51	3.33	27.3	2.73	5.72	-
Blank-5	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-5	-	-	-	-	<1.0	19.8	<1.0	20.4	<1.0	21.74	-
Control Standard Target-5	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.00	-
%QC recovery	-	-	-	-	-	98.9	-	102	-	109	-
Control-LB-B-P1-5	*S. scoparium*	Root	Tap Water	High	476	21,600	924	162	1,830	6,290	31,300
Control-LB-B-P1-Dup-5	*S. scoparium*	Root	Tap Water	High	457	20,900	1,040	248	1,910	6,160	30,700
RPD	-	-	-	-	4.02	3.28	12.2	41.8	4.46	2.11	-
Blank-6	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-6	-	-	-	-	<1.0	20.6	<1.0	19.6	<1.0	19.3	-

Control Standard Target-6	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	103	-	97.9	-	96.3	-
1PV-LB-C-P2-6	*S. scoparium*	Root	1PV of 10%-HPβCD	High	137	18,800	898	736	1,160	4,880	26,700
1PV-LB-C-P2-Dup-6	*S. scoparium*	Root	1PV of 10%-HPβCD	High	138	18,800	827	694	1,180	4,880	26,500
RPD	-	-	-	-	0.53	0.35	8.19	5.89	1.87	-0.03	-
Blank-7	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-7	-	-	-	-	<1.0	20.4	<1.0	20.0	<1.0	20.9	-
Control Standard Target-7	-	-	-	-	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	-	-	-	-	102	-	99.9	-	105	-
5OML-LB-C-P1-7	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	132	879	53.9	34.5	51.8	164	1,320
50ML-LB-C-P1-Dup-7	*S. scoparium*	Root	50 mL of 10%-HPβCD	Low	153	951	64.6	39.0	39.7	149	1,400
RPD	-	-	-	-	14.9	7.92	18.0	12.1	26.5	9.34	-
Blank-8	-	-	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-8	-	-	-	-	<1.0	20.12	<1.0	21.62	<1.0	21.14	-
Control Standard Target-8	-	-	-	-	<1.0	20.00	<1.0	20.00	<1.0	20.00	-
%QC recovery	-	-	-	-	-	101	-	108	-	106	-
Control-LB-C-P2-8	*S. scoparium*	Root	Tap Water	Low	92	1,003	52	57	89	288	1,581
Control-LB-C-P2-Dup-8	*S. scoparium*	Root	Tap Water	Low	102	1,058	46	52	93	300	1,650
RPD	-	-	-	-	10.1	5.32	13.4	9.33	4.01	4.01	-

[a] Numbers after the dash represent the extraction batch number of the sample.
[b] Relative percent difference of the analytical duplicates.

Table B-12. DDT concentrations of soils of the greenhouse experiment II. Quality assurance and quality control data are included at the bottom of the table.

Sample Name[a]	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)
Control-LB-A-1	226	11,000	268	85.8	1,340	2,650	15,600
Control-LB-B-1	212	10,700	295	62.8	1,310	1,860	14,400
Control-LB-C-1	208	10,200	192	82.7	1,090	1,800	13,600
Mean	215	10,600	252	77.1	1,250	2,100	14,500
SD	9.7	404	53.2	12.5	137	474	1,000
10%-HPβCD-LB-A-1	120	8,970	106	73.7	931	1,740	11,900
10%-HPβCD-LB-B-1	114	7,830	232	52.4	806	1,230	10,300
10%-HPβCD-LB-C-1	115	8,550	162	57.6	773	1,360	11,000
Mean	116	8,450	167	61.2	837	1,440	11,100
SD	3.50	577	63.2	11.1	83.2	265	840
10%-HPβCD-A-1	140	8,750	140	77.4	967	1,490	11,600
10%-HPβCD-B-1	138	9,400	314	60.0	1,050	1,230	12,100
10%-HPβCD-C-1	137	8,970	262	64.4	927	1,390	11,700
Mean	138	9,040	239	67.3	981	1,370	11,800
SD	1.46	331	89.6	9.08	62.7	131	322
Untreated-A-2	122	11,500	316	16.5	815	2,820	15,600
Untreated-B-2	133	11,000	300	18.7	753	2,590	14,800
Untreated-C-2	163	11,400	168	17.4	944	3,170	15,900
Mean	140	11,300	261	17.5	837	2,860	15,400
SD	21.3	265	81.2	1.09	97.6	292	555
Control-LB-A-Top-2	137	10,200	396	24.7	747	2,650	14,100
Control-LB-B-Middle-2	119	9,970	390	14.3	651	2,170	13,300
Control-LB-C-Bottom-2	100	9,360	287	18.3	716	2,300	12,800
10%-HPβCD-LB-A-Top-2	28.3	4,120	177	3.5	253	949	5,530
10%-HPβCD-LB-B-Middle-2	90.4	9,190	189	15.6	582	2,070	12,100
10%-HPβCD-LB-C-Bottom-2	77.9	7,520	202	12.3	498	1,790	10,100
10%-HPβCD-A-Top-2	66.8	6,520	239	12.2	351	1,330	8,520
10%-HPβCD-B-Middle-2	74.7	9,460	320	18.0	593	2,220	12,690
10%-HPβCD-C-Bottom-2	105	10,900	350	16.1	735	2,630	14,740
Laboratory QA/QC							
Blank-1[b]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-1	<1.0	23.8	<1.0	19.8	<1.0	16.8	-
Control Standard Target-1	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	119	-	98.8	-	83.8	

10%-HPβCD-LB-C-1	139	8,480	256	55.6	915	1,170	-
10%-HPβCD-LB-C-Dup-1	134	9,460	267	73.1	939	1,610	-
RPD [e]	3.98	10.9	4.40	27.2	2.68	31.7	-
Blank-2[c]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-2	<1.0	17.4	<1.0	13.9[d]	<1.0	16.4	-
Control Standard Target-2	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	87.0	-	69.5	-	82.0	-
10%-HPβCD-C-Bottom-2	108	10,300	363	16.3	711	2,630	-
10%-HPβCD-C-Bottom-Dup-2	102	11,500	336	15.9	759	2,620	-
RPD	5.54	11.0	7.60	2.80	6.46	0.38	-

[a] Numbers after the dash represent the extraction batch number of the sample.
[b] QA/QC for samples marked with the numbers one.
[c] QA/QC for samples marked with the numbers two.
[d] Mean difference between the control standard and the control target was more than 20%.
[e] Relative percent difference of the analytical duplicates.

Table B-13. DDT concentrations of soil and redworms of the bioavailability experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name [a]	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)
Untreated-Soil-A	21.5	8,400	71.0	34.9	322	2,560	11,400
Untreated-Soil-B	19.8	8,500	73.4	35.0	328	2,690	11,600
Untreated-Soil-C	20.3	8,170	69.3	33.5	328	2,530	11,100
Mean	20.5	8,350	71.2	34.5	326	2,590	11,400
SD	0.89	169	2.08	0.82	3.31	85.6	248
Potting-Soil-W-A	<1.0	26.4	<1.0	<1.0	<1.0	71.0	97.4
Potting-Soil-W-B	<1.0	116	<1.0	<1.0	<1.0	61.0	177
Potting-Soil-W-C	<1.0	128	<1.0	<1.0	<1.0	85.1	213
Mean	<1.0	90.3	<1.0	<1.0	<1.0	72.3	163
SD	<1.0	55.6	<1.0	<1.0	<1.0	12.1	59.3
PPNP- W-A	124	69,000	443	1,930	17.1	151	71,700
PPNP -W-B	233	127,000	731	5,960	360	3,400	137,700
PPNP -W-C	120	79,800	489	3,920	197	1,650	86,200
Mean	159	91,900	555	3,940	192	1,730	98,500
SD	63.9	30,800	154	2,010	172	1,630	34,700
PPNP+HPβCD-W-A	177	92,400	2,610	3,630	33.8	195	99,000
PPNP+HPβCD-W-B	171	97,700	1,930	3,820	237	2,210	106,100
PPNP+HPβCD-W-C	146	79,400	2,040	4,010	12.7	189	85,800
Mean	165	89,800	2,190	3,820	94.6	865	97,000
SD	16.0	9,420	365	190	124	1,160	10,300
Laboratory QA/QC							
Blank-S[b]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard-S	<1.0	19.5	<1.0	22.4	<1.0	21.5	-
Control Standard Target-S	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	97.5	-	112	-	107	-
Blank-W[c]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Control Standard -W	<1.0	17.3	<1.0	17.7	<1.0	23.2	-
Control Standard Target-W	<1.0	20.0	<1.0	20.0	<1.0	20.0	-
%QC recovery	-	86.5	-	88.5	-	116	-

[a] Letter 'W' are redworms samples.
[b] QA/QC for soil samples.
[c] QA/QC for redworms samples.

Table B-14. Recorded redworms (*Eisenia fetida*) weight for the bioavailability experiment.

Worm #	Before Weight (g)	After Weight (g)
Potting Soil		
A1	0.50	0.25
A2	0.27	0.26
A3	0.29	0.27
A4	0.28	0.30
A5	0.26	0.25
Mean	0.32	0.27
SD	0.10	0.02
B1	0.37	0.25
B2	0.26	0.38
B3	0.36	0.44
B4	0.27	0.32
B5	0.36	-
Mean	0.32	0.35
SD	0.05	0.08
C1	0.31	0.33
C2	0.27	0.39
C3	0.31	0.22
C4	0.30	0.29
C5	0.25	0.25
Mean	0.29	0.30
SD	0.03	0.07
D1	0.25	0.26
D2	0.29	0.34
D3	0.34	0.29
D4	0.25	0.34
D5	0.29	0.25
Mean	0.28	0.30
SD	0.04	0.04
E1	0.25	0.42
E2	0.33	0.28
E3	0.37	0.36
E4	0.25	0.30
E5	0.26	0.25
Mean	0.29	0.32
SD	0.05	0.07
PPNP Soil		
A1	0.25	0.32

A2	0.38	0.47
A3	0.30	0.35
A4	0.26	0.40
A5	0.25	0.40
Mean	0.29	0.39
SD	0.06	0.06
B1	0.26	0.39
B2	0.25	0.33
B3	0.30	0.33
B4	0.25	0.37
B5	0.29	0.39
Mean	0.27	0.36
SD	0.02	0.03
C1	0.32	0.33
C2	0.31	0.33
C3	0.27	0.46
C4	0.25	0.39
C5	0.25	0.31
Mean	0.28	0.36
SD	0.03	0.06
D1	0.32	0.37
D2	0.30	0.33
D3	0.28	0.35
D4	0.25	0.36
D5	0.26	0.43
Mean	0.28	0.37
SD	0.03	0.04
E1	0.26	0.37
E2	0.33	0.42
E3	0.35	0.56
E4	0.27	0.32
E5	0.31	0.32
Mean	0.30	0.40
SD	0.04	0.10
PPNP Soil + HPβCD		
A1	0.29	0.23
A2	0.25	0.32
A3	0.25	0.28
A4	0.30	0.57
A5	0.25	0.32
Mean	0.27	0.34
SD	0.02	0.13

B1	0.28	0.33
B2	0.25	0.36
B3	0.33	0.27
B4	0.26	0.39
B5	0.28	-
Mean	0.28	0.34
SD	0.03	0.05
C1	0.27	0.35
C2	0.33	0.26
C3	0.26	0.32
C4	0.35	0.47
C5	0.25	0.42
Mean	0.29	0.36
SD	0.04	0.08
D1	0.26	0.33
D2	0.25	0.36
D3	0.25	0.32
D4	0.31	0.36
D5	0.25	0.36
Mean	0.26	0.35
SD	0.03	0.02
E1	0.26	0.33
E2	0.26	0.34
E3	0.25	0.29
E4	0.30	0.40
E5	0.30	0.38
Mean	0.27	0.35
SD	0.02	0.04

Table B-16. Recorded redworms (*Eisenia fetida*) weight for the avoidance experiment.

Wheel ID	Worm Number	Before Weight (g)	After Weight (g)
A	1	0.25	0.33
	2	0.25	0.34
	3	0.27	0.30
	4	0.25	0.28
	5	0.30	0.30
	6	0.26	0.31
	7	0.25	0.30
	8	0.25	0.30
	9	0.25	0.38
	10	0.25	0.31
	Mean	0.25	0.33
	SD	0.25	0.34
B	1	0.26	0.31
	2	0.26	0.28
	3	0.28	0.32
	4	0.20	0.36
	5	0.29	0.45
	6	0.24	0.29
	7	0.23	0.39
	8	0.22	0.30
	9	0.24	0.28
	10	0.23	0.34
	Mean	0.25	0.33
	SD	0.03	0.05
C	1	0.29	0.27
	2	0.20	0.26
	3	0.24	0.23
	4	0.22	0.25
	5	0.21	0.21
	6	0.21	0.35
	7	0.23	0.31
	8	0.20	0.24
	9	0.29	0.28
	10	0.20	0.22
	Mean	0.23	0.26
	SD	0.03	0.04
D	1	0.20	0.20
	2	0.20	0.31

	3	0.20	0.30
	4	0.20	0.43
	5	0.24	0.28
	6	0.24	0.16
	7	0.20	0.31
	8	0.20	0.25
	9	0.20	0.23
	10	0.34	0.22
	Mean	0.22	0.27
	SD	0.04	0.08
E	1	0.22	0.25
	2	0.22	0.25
	3	0.24	0.31
	4	0.20	0.23
	5	0.20	0.25
	6	0.20	0.23
	7	0.20	0.25
	8	0.20	0.22
	9	0.20	0.24
	10	0.22	0.27
	Mean	0.21	0.25
	SD	0.01	0.03

APPENDIX C

Raw Data for Chapter 5

The Use of Zero-Valent Iron (ZVI) Technology to Promote DDT and Dieldrin Degradation at Point Pelee National Park

Table C-1. Calculated moisture content (MC) and measured pH for PPNP soils used in the DARAMEND and EHC laboratory experiments.

Table C-2. Recorded pH and OPR values for DARAMEND laboratory experiment.

Table C-3. DDT and dieldrin concentrations of soils of the DARAMEND laboratory experiment.

Table C-4. Recorded ORP values for DARAMEND field experiment.

Table C-5. DDT and dieldrin concentrations of soils of the DARAMEND field plot.

Table C-6. Recorded pH and OPR values for EHC experiment.

Table C-7. DDT and dieldrin concentrations of soils of the EHC experiment.

Table C-1. Calculated moisture content (MC) and measured pH for PPNP soils used in the DARAMEND and EHC laboratory experiments.

Sample Name	MC (%)
1	7.23
2	7.17
3	7.18
4	7.01
5	6.34
Mean	6.98
SD	0.37
Sample Name	pH
1	7.77
2	7.82
3	7.84
4	7.87
5	7.76
Mean	7.82
SD	0.05

Table C-2. Recorded pH and OPR values for DARAMEND laboratory experiment.

Sample Name	pH	ORP (mV)
Cycle #1		
Control-A	7.71	269
Control-B	7.35	267
Control-C	7.66	256
Mean	7.57	264
SD	0.20	7.00
DARAMEND-A	7.37	63
DARAMEND-B	6.72	74
DARAMEND-C	6.75	54
Mean	6.95	63.7
SD	0.37	10.0
Cycle #2		
Control-A	7.61	148
Control-B	7.58	163
Control-C	7.58	170
Mean	7.59	160
SD	0.02	11.2
DARAMEND-A	7.11	-82
DARAMEND-B	7.23	-96
DARAMEND-C	7.12	-112
Mean	7.15	-96.7
SD	0.07	15.0
Cycle #3		
Control-A	7.85	183
Control-B	7.87	196
Control-C	7.94	222
Mean	7.89	200
SD	0.05	19.9
DARAMEND-A	7.56	-115
DARAMEND-B	7.68	-116
DARAMEND-C	7.72	-119
Mean	7.65	-117
SD	0.08	2.08
Cycle #4		
Control-A	7.78	134
Control-B	7.80	83
Control-C	7.85	157
Mean	7.81	125
SD	0.04	37.9

DARAMEND-A	7.33	-136
DARAMEND-B	7.23	-119
DARAMEND-C	6.99	-50
Mean	7.18	-102
SD	0.17	45.5
Cycle #5		
Control-A	7.59	178
Control-B	7.59	191
Control-C	7.63	167
Mean	7.60	179
SD	0.02	12.0
DARAMEND-A	7.19	-124
DARAMEND-B	7.24	-100
DARAMEND-C	7.25	-116
Mean	7.23	-113
SD	0.03	12.2

Table C-3. DDT and dieldrin concentrations of soils of the DARAMEND laboratory experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)	Dieldrin (ng/g)
Control-A-5[a]	243	17,900	297	483	1,360	4,500	24,800	404
Control-B-5	214	18,600	227	452	1,190	4,960	25,600	360
Control-C-5	220	16,400	175	312	1,270	4,260	22,600	392
Mean	226	17,600	233	416	1,270	4,570	24,300	385
SD	15.7	1,120	60.9	91.2	85.0	356	1,550	22.5
DARAMEND-A-5	222	19,200	313	1,120	938	3,390	25,200	427
DARAMEND-B-5	185	19,500	330	1,190	856	3,490	25,500	385
DARAMEND-C-5	199	19,300	368	1,350	853	3,210	25,300	446
Mean	202	19,300	337	1,220	882	419	25,300	419
SD	18.6	153	28.0	118	48.2	142	191	31.3
Control-Fridge-A	208	16,300	164	397	1,320	4,460	22,800	392
Control-Fridge-B	216	18,400	173	451	1,410	5,350	26,000	454
Control-Fridge-C	222	14,900	170	368	1,130	4,390	21,200	377
Mean	216	16,500	169	405	1,290	4,730	23,300	407
SD	7.17	1,760	4.26	42.3	143	535	2,450	41.0
Laboratory QA/QC								
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard	<1.0	22.8	<1.0	21.2	<1.0	22.1	-	23.4
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	114	-	106	-	110	-	-

[a] Numbers after the dash represent the anaerobic cycle of the sample.

Table C-4. Recorded ORP values for DARAMEND field experiment. All recorded values are from March 2016 (measured from previously frozen soil samples).

Sample Name	ORP (mV)
Control-A1[a]	194
Control-B1	189
Control-C1	192
Mean	191
SD	2.08
DARAMEND-A1	187
DARAMEND-B1	175
DARAMEND-C1	176
Mean	179
SD	6.66
DARAMEND-A2[b]	170
DARAMEND-B2	181
DARAMEND-C2	196
Mean	182
SD	13.0
Control-A3	195
Control-B3	205
Control-C3	206
Mean	202
SD	6.08
DARAMEND-A3[c]	228
DARAMEND-B3	224
DARAMEND-C3	225
Mean	226
SD	2.08

[a] Samples marked with the number one (A1) were collected on October 2015, before the application of DARAMEND to the soil.
[b] Samples marked with the number two (A2) were collected on October 2015, after the application of DARAMEND to the soil.
[c] Samples marked with the number three (A3) where collected on December 2015.

Table C-5. DDT and dieldrin concentrations of soils of the DARAMEND field plot. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)	Dieldrin (ng/g)
Control-A1[a]	61.5	6,620	251	50.3	662	2,200	9,840	289
Control-A1-Dup	175	18,400	560	98.4	1,660	4,440	25,300	919
Control-B1	94.2	6,730	185	19.7	422	870	8,320	215
Control-C1	58.3	5,390	196	30.7	447	1,260	7,380	155
Mean	97.3	9,290	298	49.8	798	2,190	12,700	395
SD	54.5	6,110	177	34.8	585	1,600	8,470	354
DARAMEND-A1	89.1	6,910	205	49.7	755	2,780	10,800	432
DARAMEND-A2[b]	86.2	7,940	252	49.9	756	2,120	11,200	427
DARAMEND-B1	152	11,900	501	124	1,330	3,250	17,300	626
DARAMEND-B2	138	11,900	596	309	1,570	6,000	20,500	875
DARAMEND-C1[c]	-	-	-	-	-	-	-	-
DARAMEND-C2	76.3	6,360	201	45.3	635	1,870	9,190	227
Mean	108	9,000	351	116	1,010	3,210	13,800	517
SD	34.1	2,710	185	113	414	1,660	4,860	245
Control-A3[d]	33.1	2,380	157	24.3	258	585	3,440	96.6
Control-B3	96.3	7,700	628	71.1	763	1,550	10,800	366
Control-C3	137	11,200	756	292	1,590	5,760	19,700	520
Mean	88.6	7,090	514	129	870	2,630	11,300	327
SD	52.2	4,440	315	143	672	2,750	8,200	214
DARAMEND-A3	106	8,780	653	107	1,160	2,550	13,400	832
DARAMEND-B3	154	11,800	900	229	1,900	4,400	19,400	1,730
DARAMEND-C3	72.9	5,600	350	60.5	596	1,340	8,020	241
Mean	100	7,940	574	115	1,050	2,390	12,170	750
SD	39.9	2,980	256	79.2	632	1,470	5,440	714
Laboratory QA/QC								
Blank[e]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard	<1.0	20.0	<1.0	21.4	<1.0	22.4	-	23.7
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	100	-	107	-	112	-	118
Blank[f]	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0

Control Standard	<1.0	24.1	<1.0	22.8	<1.0	22.3	-	23.5
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	120	-	114	-	111	-	117

[a] Samples marked with the number one (A1) were collected on October 2015, before the application of DARAMEND to the soil.
[b] Samples marked with the number two (A2) were collected on October 2015, after the application of DARAMEND to the soil.
[c] Unsuccessful extraction.
[d] Samples marked with the number three (A3) where collected on December 2015.
[e] QA/QC for samples marked with the numbers one and two.
[f] QA/QC for samples marked with the number three.

Table C-6. Recorded pH and OPR values for EHC experiment.

Sample Name	pH	ORP (mV)
Day 0		
Control-A	7.77	269
Control-D	7.82	267
Control-G	7.84	256
Mean	7.81	264
SD	0.04	7.00
EHC-A	7.87	258
EHC-D	7.76	266
EHC-G	7.81	262
Mean	7.81	262
SD	0.06	4.00
Day 14		
Control-A	7.18	233
Control-B	7.16	237
Control-C	7.15	240
Mean	7.16	237
SD	0.02	3.51
EHC-A	6.36	-45
EHC-B	6.35	-51
EHC-C	6.37	-51
Mean	6.36	-49.0
SD	0.01	3.46
Day 28		
Control-D	7.04	179
Control-E	7.15	170
Control-F	7.07	177
Mean	7.09	175
SD	0.06	4.73
EHC-D	6.66	-89
EHC-E	6.70	-91
EHC-F	6.69	-99
Mean	6.68	-93.0
SD	0.02	5.29
Day 45		
Control-G	6.86	210
Control-H	6.85	207
Control-I	6.82	209
Mean	6.84	209
SD	0.02	1.53
EHC-G	6.69	-85
EHC-H	6.70	-78
EHC-I	6.62	-86
Mean	6.67	-83.0
SD	0.04	4.36

Table C-7. DDT and dieldrin concentrations of soils of the EHC experiment. Quality assurance and quality control data are included at the bottom of the table.

Sample Name	2,4-DDE (ng/g)	4,4-DDE (ng/g)	2,4-DDD (ng/g)	4,4-DDD (ng/g)	2,4-DDT (ng/g)	4,4-DDT (ng/g)	ΣDDT (ng/g)	Dieldrin (ng/g)
Control-G-45[a]	202	13,200	556	703	1,070	4,090	19,800	319
Control-H-45	170	12,300	302	520	613	4,060	18,00	293
Control-I-45	141	9,970	317	480	537	3,780	15,200	265
Mean	171	11,800	392	568	740	3,980	17,700	292
SD	30.5	1,670	143	119	288	171	2,310	26.7
EHC-G-45	214	21,400	637	1,910	624	5,880	30,700	491
EHC-H-45	151	12,600	385	875	473	4,300	18,800	223
EHC-I-45	133	11,500	400	770	407	3,600	16,800	211
Mean	166	15,200	474	1,180	502	4,590	22,100	309
SD	42.9	5,430	141	630	111	1,170	7,490	158
Control-Fridge-A	184	16,400	280	405	900	6,650	24,800	327
Control-Fridge-B	198	16,100	310	456	973	6,630	24,700	390
Control-Fridge-C	194	16,800	425	455	1,070	6,020	25,000	372
Mean	192	16,400	339	438	981	6,430	24,800	363
SD	7.27	351	76.4	29.2	85.5	358	148	32.3
Laboratory QA/QC								
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0
Control Standard	<1.0	22.2	<1.0	21.4	<1.0	20.8	-	22.1
Control Standard Target	<1.0	20.0	<1.0	20.0	<1.0	20.0	-	20.0
%QC recovery	-	111	-	107	-	104	-	110

[a] Numbers after the dash represent the number of incubation days.

APPENDIX D

Pictures from field and laboratory experiments in all data chapters.

Figure D-1. *P. virgatum* plot at the Delaurier parking lot of Point Pelee National Park in October 2015.

Figure D-2. *S. scoparium* plot at the Delaurier parking lot of Point Pelee National Park in October 2015.

Figure D-3. The six columns in the microbial activity experiment at the phytoremediation laboratory at RMC.

Figure D-4. The columns in the optimal HPβCD concentration experiment at RMC.

Figure D-5. *P. virgatum* plants from the greenhouse experiment I at the RMC greenhouse on August 2015.

Figure D-6. A representation of the three soil sections collected from each pot in the greenhouse experiment II.

Figure D-7. A representation of one of the five avoidance wheels used in the invertebrates avoidance experiment.

Figure D-8. The three treatment plots (DARA) and the three control plots located in the *Delaurier Compound Site* at PPNP.

Figure D-9. A representation of the EHC experiment that simulated a groundwater environment at the RMC laboratory.

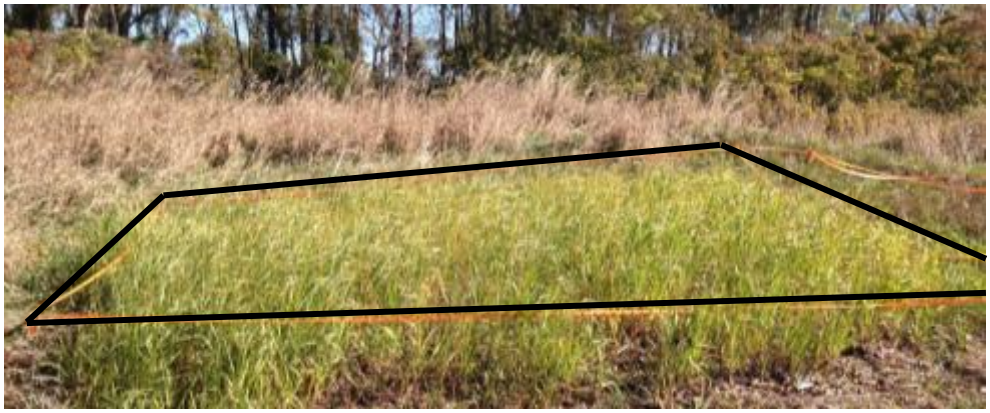

Figure D-1. *P. virgatum* plot at the *Delaurier* parking lot of Point Pelee National Park in October 2015.

Figure D-2. *S. scoparium* plot at the *Delaurier* parking lot of Point Pelee National Park in October 2015.

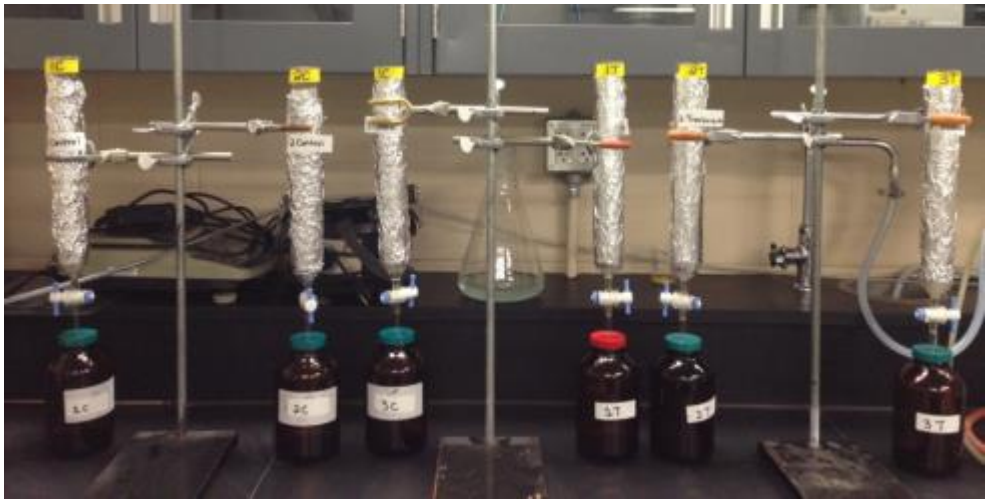

Figure D-3. The six columns in the microbial activity experiment at the phytoremediation laboratory at RMC.

Figure D-4. The columns in the optimal HPβCD concentration experiment at RMC.

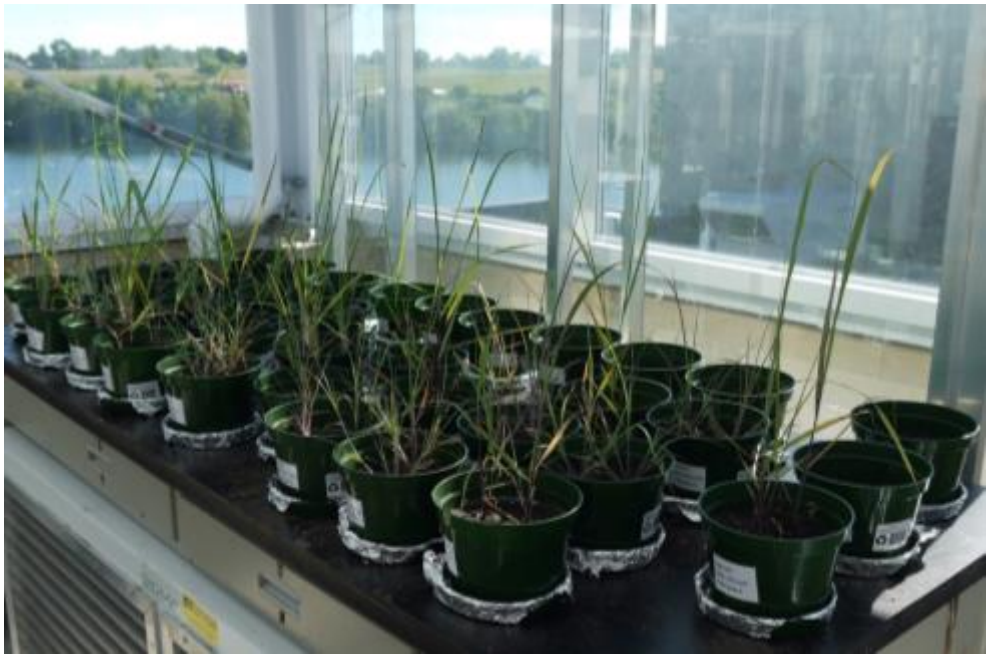

Figure D-5. *P. virgatum* plants from the greenhouse experiment I at the RMC greenhouse on August 2015.

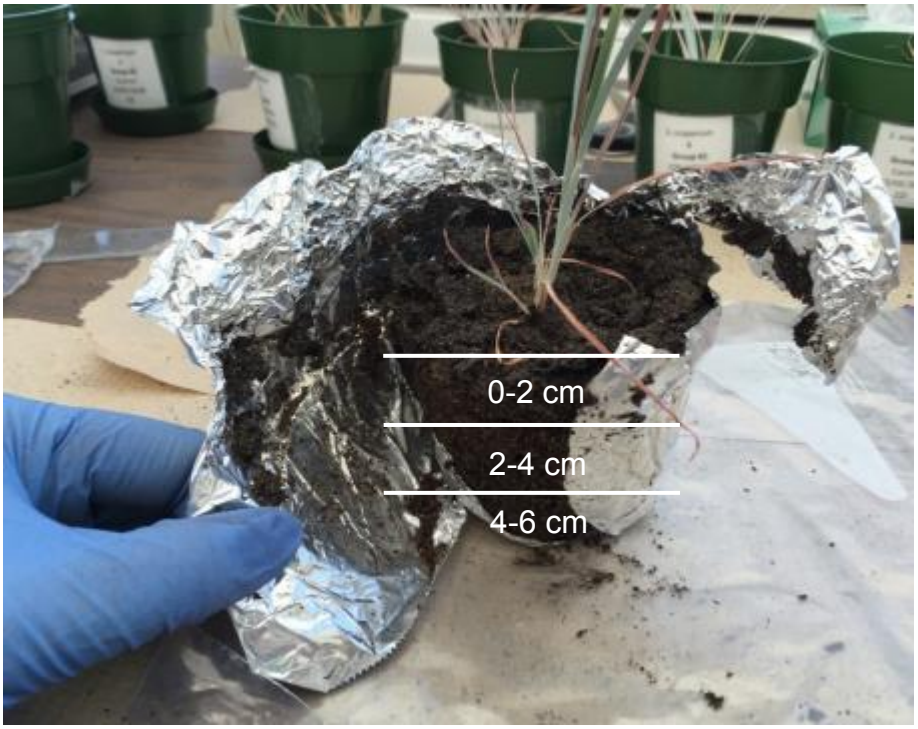

Figure D-6. A representation of the three soil sections collected from each pot in the greenhouse experiment II.

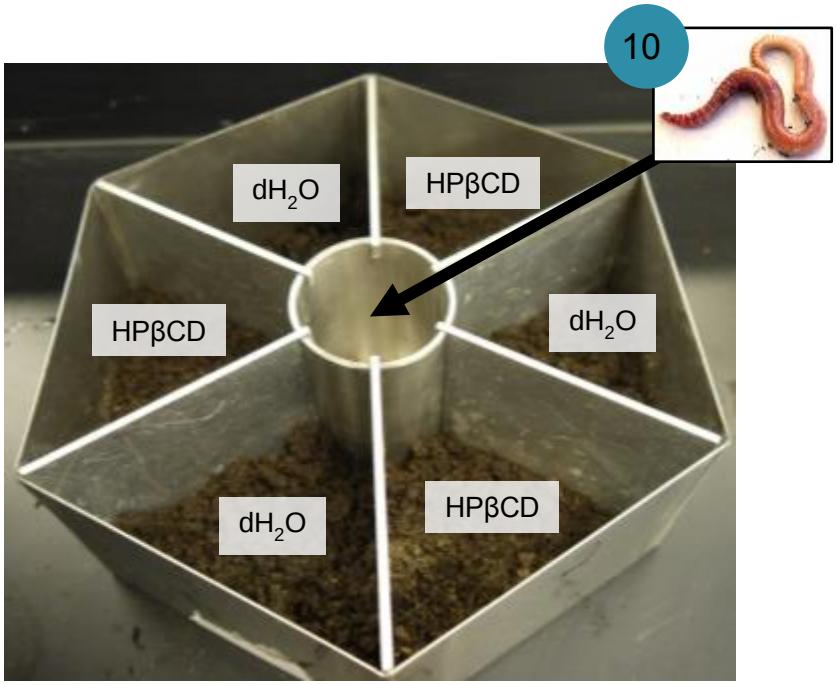

Figure D-7. A representation of one of the five avoidance wheels used in the invertebrates avoidance experiment.

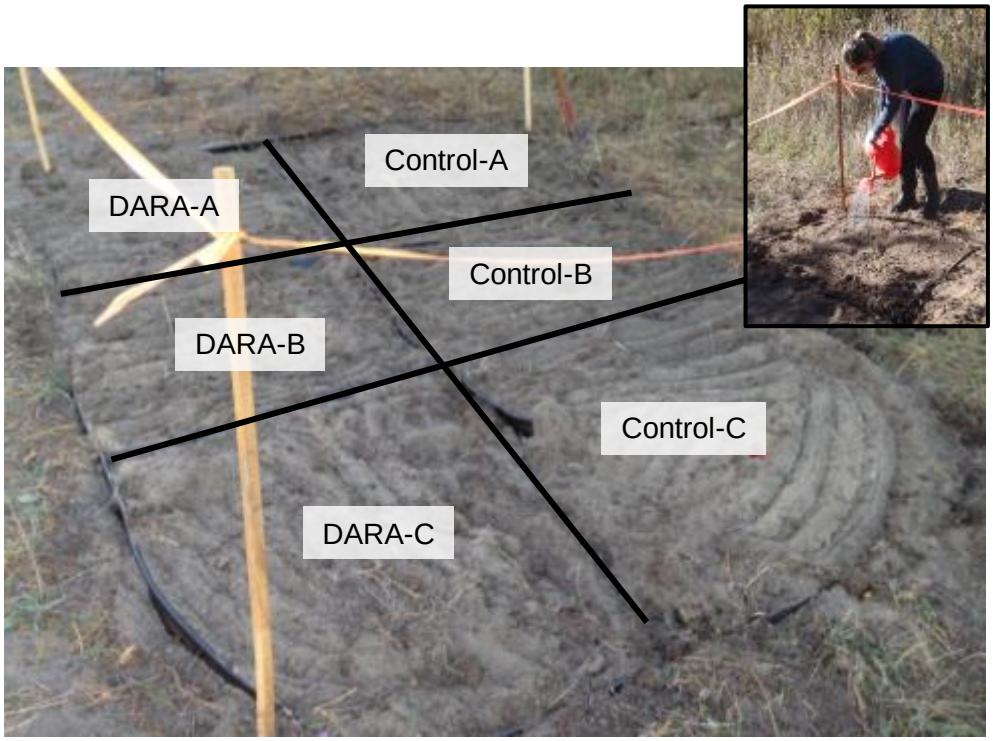

Figure D-8. The three treatment plots (DARA) and the three control plots located in the *Delaurier Compound Site* at PPNP.

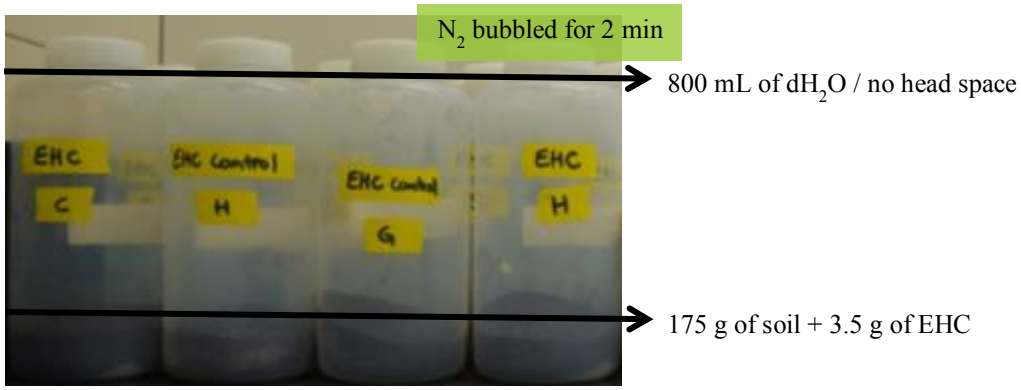

Figure D-9. A representation of the EHC experiment that simulated a groundwater environment at the RMC laboratory.