PHYTOEXTRACTION OF WEATHERED DDT-CONTAMINATED SOIL AT POINT PELEE NATIONAL PARK USING NATIVE AND NATURALIZED WEED SPECIES

PHYTOEXTRACTION DE SOL MÉTÉORISÉ CONTAMINÉ PAR LE DDT AU PARC NATIONAL POINT PELEE EN UTILISENT DES ESPÈCES MAUVAISES HERBES INDIGÈNES ET NATURALISÉES

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by

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Dedicated

To

My Parents

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Abstract

Phytoextraction is a potential remediation technique for low to moderate levels of dichlorodiphenyltrichloroethane (DDT)-contaminated soil. Identification of new species as phytoextractors of DDT is a vital step in establishing phytoextraction as a commercially-viable and environmental-friendly remediation technique. Thirteen of native and naturalized weed species were investigated both in field and greenhouse studies using DDT-contaminated soil from Point Pelee National Park (PPNP), ON, Canada. A screening study established the extraction capabilities of nine wild growing species and determined that four of these *Trifolium pratense*, *Symphyotrichum novae-angliae*, *Solanum ptycanthum Dun*. *Verbascum thapsus* have a higher DDT extraction potential than the known DDT phytoextractor *Cucurbita. pepo* ssp. *pepo* cv. Howden assuming plants are grown at optimal densities. A subsequent field trial was conducted using three native weed species and *C. pepo* at PPNP in low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. This study determined that two native species i.e. *Schizachyrium scoparium* and *Panicum virgatum* had higher shoot DDT extractions than *C. pepo* at the high DDT-contaminated site. An interesting and unique finding was that, DDT uptake of *C. pepo* is concentration dependent with a maximum uptake observed at a threshold soil DDT ~5000 ng/g. In contrast, such threshold soil DDT concentration was absent for weed species where uptake increases linearly with increasing soil DDT concentration. A final greenhouse study using four perennial native and naturalized weed species in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soils collected from PPNP, further demonstrated that weed species are capable of significant DDT phytoextraction *Trifolium pratense* in both low and high DDT-contaminated soil regardless of growing conditions exceeded *C. pepo* extraction by considering optimum planting density. Hence, it can be summarized that native (or naturalized) weed species show significant potential/prospect as phytoextractors of DDT, especially at ecologically sensitive sites where it is important to minimize habitat disturbance. It is noted that this is the first comprehensive study of DDT phytoextraction using of native weed species.

Résumé

La phytoextraction est une technique pouvant remédier les sols contaminés avec des niveaux faibles à modérés de dichlorodiphenyltrichloroéthane (DDT). L'identification de nouvelles espèces ayant la capacité de phytoextraire le DDT est une étape essentielle dans l'établissement de la phytoextraction comme technique de remédiation commercialement viable et respectueuse de l'environnement. Treize espèces d'herbes indigènes et naturalisées ont été étudiées dans des essais *in-situ* et dans une serre avec des sols contaminés au DDT provenant du parc national de la Pointe-Pelée (PNPP), ON, Canada. Une évaluation préalable a permis de déterminer les capacités d'extraction de neuf espèces sauvages et a démontré que quatre de ces espèces, *Trifolium pratense*, *Symphyotrichum novae-angliae*, *Solanum ptycanthum Dun.* et *Verbascum thapsus* ont un potentiel d'extraction de DDT plus élevé que le phytoextracteur de DDT connu, *Cucurbita pepo* ssp *pepo* cv. Howden, lorsqu'elles sont cultivées à des densités optimales. Par la suite, une étude *in-situ* au PNPP a été réalisée avec trois espèces d'herbes indigènes ainsi que *C. pepo* dans des sols contaminés avec des concentrations : faible (291 ng/g), modérée (5083 ng/g) et haute (10,192 ng/g) de DDT. À la lumière de cette étude, les pousses de deux espèces indigènes, *Schizachyrium scoparium* et *Panicum virgatum*, ont extrait plus de DDT que *C. pepo* au site ayant la plus haute concentration de contaminant. Il est intéressant de noter que l'absorption de DDT par *C. pepo* dépend de la concentration de DDT dans le sol et que l'absorption maximale a été observée à un seuil de ~5 000 ng/g DDT. En revanche, un tel seuil n'a pas été observé avec les espèces d'herbes indigènes, l'absorption a augmenté de façon linéaire avec la concentration de DDT dans le sol. Une dernière étude conduite avec quatre plantes vivaces indigènes et naturalisées cultivées en serre dans des sols du PNPP contaminés avec des concentrations de DDT faible (2300 ng/g) et haute (17,500 ng/g) a démontré que les espèces d'herbes ont été en mesure de phytoextraire le DDT de façon significative. *Trifolium pratense* cultivé dans les sols contaminés avec de faibles et hauts niveaux de DDT, peu importe les conditions de croissance, a dépassé la capacité de phytoextraction de *C. pepo* en tenant compte de la densité de plantation optimale. Par conséquent, il a été conclu que les espèces d'herbes indigènes (ou naturalisés) montrent un potentiel significatif pour phytoextraire le DDT, en particulier sur les sites écologiquement sensibles où il est important de réduire au minimum la perturbation de l'habitat. Il est à noter que ceci est la première étude complète traitant de la phytoextraction du DDT par des espèces d'herbes indigènes.

Co-Authorship Statement

This thesis contains the following manuscripts where the writer of this thesis is the principle author. The co-authors – Prof. Barabara A. Zeeb and Dr. Allison Rutter are the thesis supervisor and co-supervisor, respectively.

Chapter 3: Paul, S. ; Rutter, A., Zeeb, B. A. 2014. An Evaluation of Native and Naturalized weed species for the Phytoextraction of DDT at Point Pelee National Park, Leamington, ON. [Submitted to International Journal of Environmental Science and Technology, November 2014]

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Table of Contents

Acknowledgements iii
Abstract iv
Co-Authorship Statement vi
List of Tables x
List of Figures xi
List of Abbreviations xiii
Chapter 1 General Introduction 1
Chapter 2 Literature Review 5
2.1 Persistent organic pollutants (POPs) 5
2.2 DDT 5
2.2.1 DDT in the environment 5
2.2.2 DDT Chemistry 6
2.2.3 Physical properties of DDT 9
2.2.4 DDT in the ecosystem 9
2.2.5 DDT toxicity in the environment 10
2.2.6 Canadian soil quality guidelines of DDT 10
2.3 Levels of DDT in Point Pelee National Park (PPNP) 11
2.4 DDT remediation techniques 12
2.4.1 Traditional technologies (excavation/digging) 12
2.5 Phytotechnologies 12
2.5.1 Background 12
2.5.2 Phytoextraction of organic contaminants by *Cucurbita pepo* 13
2.5.3 Mechanism of phytoextraction by *C. pepo* 15
2.6 Native weed species for phytoextraction 16
Chapter 3 19
An Evaluation of Native and Naturalized weed species for the Phytoextraction of DDT at Point Pelee National Park, Leamington, ON 19
3.1 Introduction 21
3.2 Methodology 23
3.2.1 Site description 23
3.2.2 Sample collection 23
3.2.3 Analytical procedures 24
3.2.4 Quality assurance and control 24
3.2.5 Statistical analysis 25
3.3 Results 25
3.3.1 Soil concentration of DDT 25
3.3.2 Bioaccumulation Factors (BAFs) and Translocation Factors (TLFs) 25

3.3.3 DDT root and shoot extraction 27
3.4 Discussion 29
3.4.1 Bioaccumulation factors and Translocation factors 29
3.4.2 Shoot extraction 29
3.5 Conclusion 32
Chapter 4 34
Phytoextraction of DDT-contaminated soil at Point Pelee National Park, Leamington, ON, using *Cucurbita pepo* cv. Howden and native grass species 34
4.1 Introduction 36
4.2 Materials and Methods 37
4.2.1 Site description and selection 37
4.2.2 Plant selection 38
4.2.3 Field plot preparation and cultivation scheme 39
4.2.4 Soil and plant sample collection 40
4.2.5 Selection of harvested plants for analysis 41
4.2.6 Analytical procedures for soil and plant samples 41
4.2.7 Quality assurance and control 42
4.2.8 Statistical analysis 42
4.3 Result and Discussion 42
4.3.1 Plant growth and biomass of plant tissues 42
4.3.2 DDT concentration in *C. pepo* plant tissues. 43
4.3.3 Bioaccumulation Factors (BAFs) and Translocation Factors (TLFs) in *C. pepo* 46
4.3.4 DDT concentration in root and shoot tissues of native grass species 47
4.3.5 BAFs in native grass species 50
4.3.6 Phytoextraction potential based on optimal plant density 51
4.4 Conclusions 54
Chapter 5 55
Phytoextraction of weathered DDT by perennial Native and Naturalized weed species under greenhouse conditions 55
5.1 Introduction 57
5.2 Methodologies 58
5.2.1 Soil selection and processing 58
5.2.2 Species selection, seed collection, and germination 59
5.2.3 Experimental design 59
5.2.4 Harvesting 60
5.2.5 Analytical procedure 61
5.2.6 Quality assurance and control 62
5.2.7 Statistical analysis 62

5.3 Results and Discussion 62
5.3.1 Plant growth characteristics 62
5.3.2 DDT concentrations in root and shoot tissues 63
5.3.3 Bioaccumulation factors (BAFs) 66
5.3.4 Root and shoot DDT extraction 68
5.3.5 Phytoextraction efficiency 71
5.3.6 DDT metabolite accumulation and translocation pattern in root and shoot tissues 73
5.4 Conclusions 74
Chapter 6 76
Final Discussion and Summary 76
7. References 79
8. Appendices 92
Appendix A 92
Appendix B 104
Appendix C 124

List of Tables

.
Table 2.1: Soil quality guidelines for DDT (total) ng/g. (CCME, 1999)................11
Table 3.1: Optimal planting density per square metre, mean shoot dry weight and calculated extraction per square metre for nine plant species of PPNP…................31
Table 4.1: Comparison of shoot DDT extraction per square metre for plants at the moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites in Point Pelee National Park...53
Table 5.1: A comparison of mean shoot [DDT], and shoot DDT extraction between greenhouse and field studies using weed species and *C. pepo* shoot extractions those exceeded *C. pepo* were shown in bold. 72

List of Figures

Figure 2.1: Commercial grade ∑DDT is the combination of the above six metabolites: 77.1% *p,p'*-DDT, 14.9% *o,p'*- DDT, 4.0% *p,p'*-DDE, 0.1% *o,p'*-DDE, 0.3 % *p,p'*-DDD, 0.1 % *o,p'*-DDD and 3.5% undefined compounds 7
Figure 2.2: Conversion of DDT into its metabolites DDD and DDE 8
Figure 2.3: An overview of different phytoremediation techniques. 13
Figure 3.1: Comparison of a) root and b) shoot BAFs between plant species at DDT-contaminated sites in PPNP. 26
Figure 3.2: DDT extractions in a) root and b) shoot tissues for plant species at DDT-contaminated sites in Point Pelee National Park. Error bars represent the standard deviation of the mean.. 28
Figure 4.1: Schematic diagram of *C. pepo* and native grass species plots at each of the three DDT-contaminated sites in Point Pelee National Park. 40
Figure 4.2: Mean [DDT] for *C. pepo* plants grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites in PPNP. 43
Figure 4.3: *C. pepo* shoot and root DDT concentrations in relation to DDT concentrations in soil.. 44
Figure 4.4: DDT concentrations in shoot segments of *C. pepo* at the low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. 46
Figure 4.5: Comparison of shoot and root BAFs and TLFs of *C. pepo* grown at the low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites.. 47
Figure 4.6: Comparison of DDT concentrations between the native grass species at the low (291 ng/g), moderate (5083 ng/g) and high DDT (10192 ng/g)-contaminated sites in a) root and b) shoot tissues.. 49
Figure 4.7: Comparison of root a) and b) shoot BAFs between the native weed species at low (291 ng/g), moderate (5083 ng/g) and high DDT (10192 ng/g)-contaminated sites.. 51

Figure 5.1: Schematic representation of the placement of weed seedlings (n = 4) in containers of low and high DDT-contaminated soil, respectively.. 60
Figure 5.2: Mean DDT concentrations in a) root tissue and b) shoot tissue of four plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soils. 65
Figure 5.3: Comparison of BAFs in a) roots and b) shoots of four plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soils. 68
Figure 5.4: Total mean extractions in a) roots and b) shoots for each plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soil... 70
Figure 5.5: a) Percentages of DDT metabolites in low and high DDT-contaminated soil. Percentages of DDT metabolites in b) root and c) shoot tissue in high DDT-contaminated soil. 74

List of Abbreviations

Acronym/Abbreviation	Defininition
µg	microgram
ANOVA	analysis of variance
ATC	atomatic temperature control
ATSDR	Agency for Toxic Substance and Disease Registry
BAF	bioaccumulation factor
CCME	Canadian Council of Ministers of the Environment
cf	correction factor
Dw	dry weight
DCBP	decachlorobiphenyl
DDT	dichlorodiphenyltrichloroethane
DDE	dichlrodiphenyldichloroethylene
DDD	dichlorodiphenyldichloroethane
DCM	
ECD	electron capture detector
g	gram
GC	gas chromatography
HCB	hexachlorobenzene
HMWOA	high molecular weight organic acids
IGECE	Institute for Green Energy and Clean Environment
LMWOA	low molecular weight organic acids
ng	nanogram
PAHs	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxins
PCDF	dichlorodibenzofuran
POP	persistant organic pollutant
PPNP	Point Pelee National Park
QA	quality assurance
QC	quality control
RPM	revolutions per minute
RSD	relative standard deviation
SQG_{HH}	soil quality guideline for human health
SQG_{E}	soil quality guideline for environment health
ssp	subspecies
TLF	translocation factor
US EPA	US environmental protection agency
ww	wet weight

Chapter 1

General Introduction

Dichlorodiphenyltrichloroethane (DDT) is a persistent organochlorine pesticide that was used extensively in North America and worldwide for controlling pests in agriculture (US EPA, 1980) from 1940 to 1960. DDT was also used to combat insect vectors of malaria, typhus and other insect-borne diseases (Agency for Toxic Substance and Disease Registry, 2002; US EPA, 2012) from 1940 to 1971. Although DDT was an overwhelming success for controlling pests and malaria, it was eventually determined to be toxic to many biological organisms. In 1962, Rachel Carson raised valid concerns regarding DDT in her book, 'Silent Spring'. She revealed that DDT adversely affected wildlife and the environment as well as threatening human health.The persistence of DDT in the environment, and its bioaccumulation in animal fatty tissue through the food chain, may lead to biomagnification of DDT in living cells (CCME, 1999; US EPA, 1980). In the 1960s it was determined that DDT was responsible for adverse health impacts in animals (reproductive and growth problems), birds (eggshell thinning) and humans (nervous system disorders, reproductive, and developmental problems, immune response suppression, cancer, and endocrine disruption) (US EPA, 2012; Buccini *et al*., 2004; Agency for Toxic Substance and Disease Registry, 2002).

In North America, an enormous amount of DDT was manufactured between the years 1945 to 1983; production was ceased in 1985 (ATSDR, 2002; CCME, 1999). Although the use of DDT in Canada was restricted in 1969 and ended by the mid-1980s, it remains a concern as DDT is still being detected in Canadian soil (Webber and Wang, 1995; CCME, 1999; Bailey *et al*., 2005; Crowe and Smith, 2007; Evans *et al*., 2007). One example of ongoing DDT contamination in Canada is at Point Pelee National Park (PPNP), where a significant amount of DDT can still be detected in the soil.

PPNP was Canada's first national Park, and was established in 1918 near Leamington in southern Ontario. The Park is naturally significant because it is one of the premier places in North America for watching bird migrations (spring and autumn migration) (Park Canada, 2014). DDT was historically used for controlling pests in agricultural areas of the Park, and for controlling mosquitoes in the camp and picnic areas. The highest concentration of DDT recorded by Crowe and Smith in 2007 (316,000 ng/g) was measured in an area referred to as 'Former Agricultural Land'. Due to the slow degradation of DDT in soil, it is still found in many other areas of the park at concentrations exceeding the CCME guidelines of 700 ng/g

(Russell and Haffner, 1997; Crowe and Smith, 2007). A growing concern is that significant levels of DDT are being found in PPNP reptiles, amphibians, and even birds and their dietary compounds (Russell and Haffner, 1997 and Smits *et al*., 2004).

Approximately 700 plant species are found in PPNP. This includes an inventory of native weed species, such as *Solidago canadensis* (Canada goldenrod), *Solanum ptycanthum Dun* (eastern black nightshade), *Silene vulgaris* (bladder campion), *Asclepias syriaca* (milkweed), *Leonurus cardiac* (motherwort), and *Symphyotrichum novae-angliae* (New England aster) and naturalized species, such as *Cornus sanguinea* (dogwood), *Verbascum thapsus* (mullein), and *Trifolium pratense* (red clover) etc. In recent years, PPNP authorities have been working towards removing non-native/invasive species under Point Pelee's Habitat Restoration Project, and re-vegetating restoration sites with native weed species with particular emphasis on native grass species. The main aim of this project is to create a savannah habitat to protect the native plants and animals and to provide an environment for their survival (Park Canada, 2014).

A human risk assessment for DDT, DDD, DDE, and dieldrin in the soil at the camp Henry site in PPNP was conducted by Water Technology International Corp in1998. Based on the extent of DDT, different types of remedial options were proposed, however, these conventional remedial options are not suitable for the Park as they will destroy the ecologyical integrity and archeological significance of the Park. Therefore, Park personnel are interested in investigating environmentally viable, ecologically suitable, and inexpensive remedial options. *In-situ* DDT phytoextraction may provide a potential option. It costs 2-10 times less than conventional techniques as it is possible to use existing agricultural techniques and equipment (Cunningham and Ow, 1996; Pilon-Smits Freeman, 2006).

Phytoextraction is a sub-category of phytoremediation in which plants accumulate significant amounts of contaminants (inorganic/organic) from soil via root tissue and translocate those contaminants to the shoot tissue of the plant (Abhilash *et al*., 2009; Pilon-Smits Freeman, 2006). The harvested shoot biomass volume is reduced by composting and then disposing of this smaller amount of biomass by traditional techniques, i.e., landfilling or incineration (Sas-Nowosielska *et al*., 2004; Lazzari *et al*., 1999). To date, phytoextraction has been adopted as a successful technology for metal contaminant remediation (Baker *et al*., 1994, Cunningham *et al*., 1995; Koopmans *et al*., 2007; McGrath and Zhao, 2003; Zhao *et al*., 2003). After a breakthrough study of the accumulation of greater amounts of polychlorinated dibenzodioxins (PCDDs) and dichlorodibezofuran (PCDFs) in shoot tissue by Hüstler (1994), researchers began to investigate whether plants could accumulate hydrophobic organic contaminants such as DDT or PCBs (White, 2000, 2001; White *et al*., 2006; Zeeb *et al*., 2006; Whitfield Åslund *et al*.,

2008). Some plants of the *Cucurbita* genera show outstanding performance in phytoextraction of DDT and its metabolites (White, 2000, 2001, 2002; White *et al*., 2003; Lunney *et al*., 2004).

Phytoextraction of organic contaminants using native (or naturalized) weed species is less well documented, although some significant success has been achieved using weed species for metal remediation (Aboulroos *et al.,* 2006; Porebska and Ostrowska, 1999; Lasat *et al*., 1998). Native plants are those that have originated in a region naturally without intentional or unintentional human involvement (Morse *et al*., 2004), whereas naturalized plants are non-native species that are able to reproduce and sustain populations through several life cycles (Richardson *et al*., 2000), while remaining in balance with the ecological community to which they belong (Richardson *et al*., 2004). Recently, native weed species were used for PCB remediation (Ficko *et al*., 2010, 2011a, 2011b). No study has been conducted in which native (or naturalized) weed species have been used to phytoextract DDT. Native and naturalized weed species were chosen for phytoextraction DDT at PPNP as they can grow without any assistance and are widely available. Advantages of using weed species rather than crop species are that they have high tolerance for growing in unfertile soil conditions, are easy to propagate, are cost effective and are resilient species.

The overall goal of this thesis is to remediate DDT-contaminated sites in PPNP, using the green technology of phytoextoextraction. The key goals are: (i) to identify potential phytoextractors of DDT amongst PPNP native and naturalized species, (ii) to quantify their phytoextraction capabilities and compare them with the known phytoextractor *C. pepo* cv. Howden (*C. pepo*) under field conditions, and (iii) to investigate phytoextraction capability of four perennial species under greenhouse conditions.

In this thesis, chapter 2 provides a literature review describing DDT and its physiochemical properties, toxicity, and concentrations in Canadian soil. It also reviews phytoextraction as a green remediation technology and describes the uses of native (or naturalized) species in phytoextraction studies to date. Chapter 3 describes a screening investigation of nine wild grown native and naturalized species in PPNP. The main focus of this chapter is to identify potential phytoextractors of DDT and compare similar species with the PCB phytoextractors in Ficko *et al*. (2010). Chapter 4 describes a comparative phytoextraction study between *C. pepo* cv. Howden and three PPNP native grass species under field condition. This chapter also describes the soil concentration effects on the known phytoextractor *C. pepo*. Chapter 5 describes a greenhouse study of four native and naturalized weed species in the low and high DDT-contaminated soil. This study focuses on the phytoextraction of DDT on a per square metre basis (theoretical density value) and compares DDT metabolite accumulation. Finally, chapter 6

summarizes the overall findings and implications of this work in the context of phytoremediation of organic contaminants. Supplementary information and raw data are provided in Appendices A to C.

Chapter 2

Literature Review

2.1 Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are organic compounds, such as organochlorine insecticides, industrial chemical products or by-products, and industrial and household chemicals (Ritter *et al.,* 1995; Government of Canada, 2006). The Stockholm Convention on POPs (an international legally binding agreement) was ratified in May 2001 by over 100 countries including the United States, Canada and all members of the European Union (Porta *et al*., 2002). The aim of this agreement is to eliminate twelve particular POPs referred to as the dirty dozen which include eight pesticides (aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, mirex, and toxaphene), two industrial chemicals (hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs)), and two by-products of POPs (dichlorodibezodioxins and dichlorodibenzofurans (PCDDs and PCDFs). The main goal of the Convention was to stop the production, use and trade of POPs due to their negative impact on humans and animals (Porta *et al*., 2002). POPs are stable and persistent in the environment and they resist photolytic, biological, and chemical degradation (Ritter *et al.,* 1995).

2.2 DDT

2.2.1 DDT in the environment

In 1874, DDT was first synthesized in a laboratory by Othmar Zeidler in Germany (Matolcsy *et al*., 1988; Turusov *et al*., 2002). It was not until 1939 that the Swiss chemist Paul Hermann Muller discovered the insecticidal properties of DDT in controlling clothes moths. During the Second World War, DDT was extensively used among both military and civilian populations for combating malaria, typhus, and other insect-borne diseases (Lawless, 1977). In 1948, Muller was awarded the Nobel Prize in the field of Physiology and Medicine for bringing an 'outbreak of typhus' under control by using large quantities of DDT for the first time (Nobel Foundation, 2009; Bate, 2007).

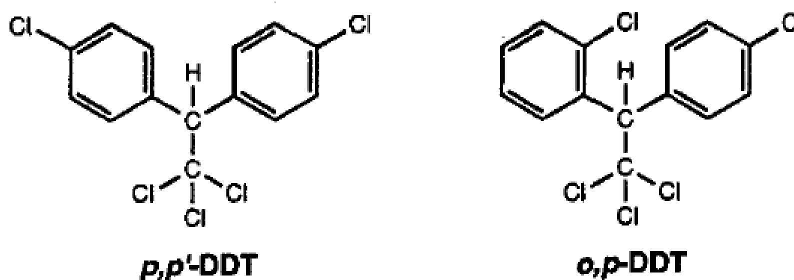
In the USA and Canada, DDT was first made commercially available in 1945 for domestic and industrial purposes due to its effectiveness as a pesticide in

controlling insect pests in crops. Low manufacturing cost and low price ($1 US per pound in 1945 and $0.25 US per pound in the mid-1950s) resulted in DDT's widespread use (CCME, 1999). At one time, DDT was registered as an agricultural pesticide on 334 crops against 240 pest species (McEwen and Stephenson, 1979). As a result of its low price, DDT usage was expanded from agricultural to household purposes in the form of granules, aerosols, smoke candles, lotions, and charges for vaporizers (Smith, 1991; CCME, 1999). DDT was never manufactured in Canada, however annual production in the USA reached its peak in 1963 when approximately 82,000 metric tons were produced (Geneva, 1979). In total, approximately 1.5 million tons of DDT was manufactured in the USA between 1945 and 1983 (CCME, 1999).

In her groundbreaking 1962 book, *Silent Spring*, Rachel Carson demonstrated public concern over the dangers of the extensive uses of pesticides, and reported the need for better pesticide controls. *Silent Spring* illustrated the worst impacts of synthetic chemicals on the environment and catalogued the environmental impacts of the indiscriminate spraying of DDT in the US. It went on to question the logic of releasing large amounts of chemicals into the environment without fully understanding their effects on ecology or human health. Based on the detrimental environmental impacts on wildlife, as well as the probable impact on human health, registrations for DDT were cancelled by the U.S. Environmental Protection Agency in 1972. Most uses of DDT came to an end in Canada by the mid-1980s due to environmental and human health concerns (CCME, 1999).

2.2.2 DDT Chemistry

DDT refers specifically to 1,1- trichloro-2,2-bis (*p*-chlorophenyl) ethane which is comprised of two isomeric forms, *o,p'*-DDT and *p,p'*-DDT (CCME, 1999). DDT has a number of metabolites with similar chemical properties and structures to the parent product (Figure 2.1). *o,p'*-DDE and *p,p'*-DDE (1,1-dichloro-2,2-bis *p*-chlorophenyl ethylene) and *o,p'*-DDD and *p,p'*–DDD (1,1-dichlro-2,2- bis *p*-chlorophenyl ethane) (Aislabie *et al*., 1997) form due to microbiological and abiotic transformations in the environment.



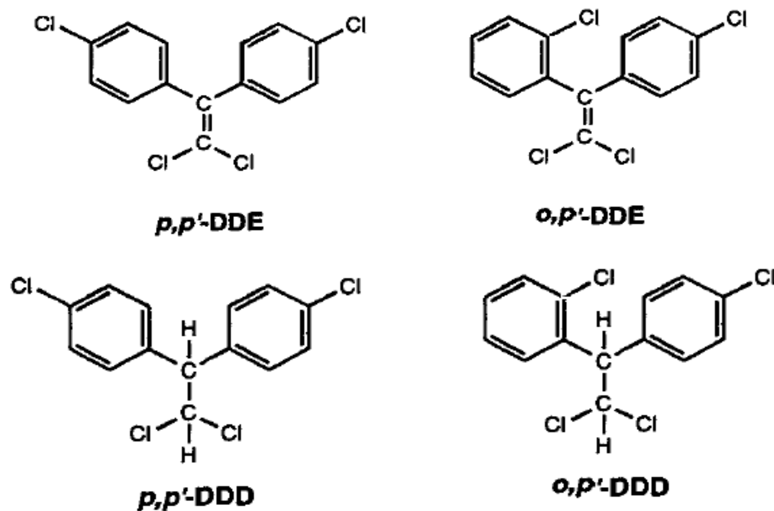


Figure 2.1: Commercial grade ∑DDT is the combination of the above six metabolites: 77.1% *p,p'*-DDT, 14.9% *o,p'*- DDT, 4.0% *p,p'*-DDE, 0.1% *o,p'*-DDE, 0.3 % *p,p'*-DDD, 0.1 % *o,p'*-DDD and 3.5% undefined compounds (Aislabie *et al.,* 1997).

In the environment, DDT may be exposed to processes, such as biodegradation, photodegradation, and photooxidation (Corona-Cruz *et al*., 1999). DDT can be degraded both aerobically and anaerobically (Corona-Cruz *et al*., 1999) as shown in the Figure 2.2. In an aerobic pathway, DDT may be metabolized to DDE by a photochemical dechlorination reaction (Maugh, 1973). DDE is the most persistent and abundant metabolite of DDT in the environment due to the occurrence of a chlorine substituent (Crowe and Smith, 2007; Aislabie *et al.,* 1997). Spencer and Cliath (1972) documented that *p,p'*-DDE has a higher vapor pressure than *p,p'*-DDT. Most *p,p'*-DDT is found in the atmosphere or in well-aerated soil as a volatized form of *p,p'*-DDE. Factors such as soil temperature, moisture content, and carbon content control the conversion of DDT to DDE in the presence of aerobic conditions and temperate soils (Crowe and Smith, 2007; Spencer *et al.,* 1996).

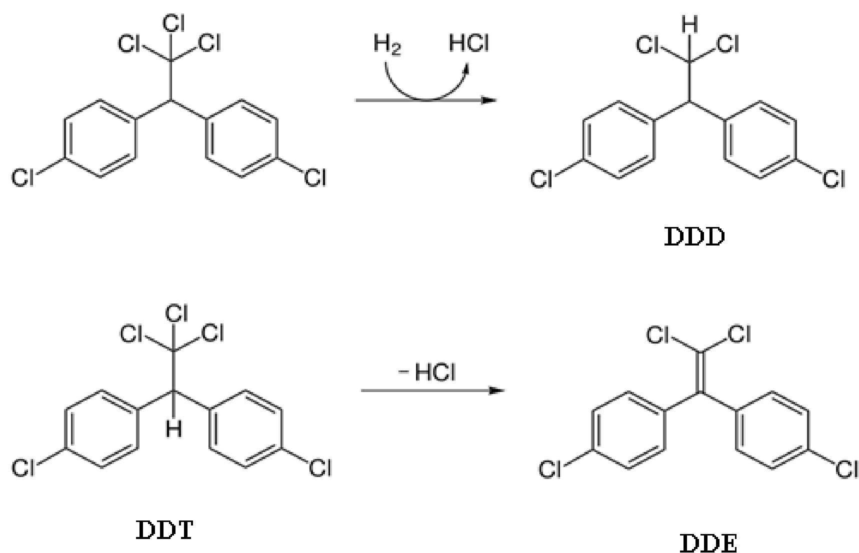


Figure 2.2: Conversion of DDT into its metabolites DDD and DDE

In an anaerobic pathway, DDT may be converted into DDD via a reductive dechlorination reaction. In flooded anaerobic soils, the metabolic conversion of DDT to DDD is much faster than the degradation of DDD. Different types of bacteria (e.g., *Escherichia coli, Enterobacter aerogenes, Pseudomonas putida*) and fungi (e.g., *Saccharomyces cerevisiae*) play a significant role in the conversion of DDT to DDD. The conversion, or biotransformation, of DDT to DDD is a co-metabolic process (Aislabie *et al.,* 1997) where there is simultaneous degradation of two compounds and the degradation of the second compound depends on the existence of the first compound. The degradation rate of DDE is also quite slow in both the aerobic and anaerobic environments (Crowe and Smith, 2007; ATSDR 2002). At the vapor stage, these three compounds (DDT, DDD, and DDE) can be degraded in the environment by reaction with the sun (ATSDR, 2002).

DDT can readily be adsorbed to soil organic carbon due to its high organic carbon partition coefficient (K_{oc} = 1.5 x 10^5) and this may limit its degradation in soil (Kaufman, 1974). The half-life of DDT varies according to different environmental factors, such as temperature, soil type, moisture, and pH (Biljana and Durisic-Mladenovic, 2007). The half-life ranges from 2-15 years in aerobic temperate soil and from 16-100 days in anaerobic soil (CCME, 1999).

DDT can occur in the atmosphere either in a gaseous form, attached to particles, or evaporated from the soil of warmer regions. In this latter state, DDT moves towards colder regions and condense when the temperature falls. This continuous cycle of volatilization and condensation is referred to as the 'Grasshopper' or 'Global Distillation' effect.

2.2.3 Physical properties of DDT

DDT is a white crystalline or waxy solid at room temperature, and it is tasteless, nonflammable, and odorless (CCME, 1999). The melting points of *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD are 109 ^{o}C, 89 ^{o}C and 109-110 ^{o}C, respectively. There is no particular boiling point of *p,p'*-DDT as it decomposes at a very high temperature, whereas *p,p'*-DDE and *p,p'*-DDD have boiling points of 336 ^{o}C and 350 ^{o}C, respectively (Howard and Meylan, 1997).

Due to their hydrophobicity, isomers of DDT are highly soluble in non-polar organic compounds such as ethanol (20 g/L) or acetone (580 g/L), but are very sparingly soluble in water (CCME, 1999). K_{ow} is the octanol-water partition co-efficient, which is simply defined by: $K_{ow} = C_{octanol}/C_{water}$ 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. K_{ow} is generally expressed as a unit-less ratio on a logarithmic scale. Generally, POPs have high log K_{ow} values ranging from 4 to 7. DDT metabolites, such as DDD and DDT have lower K_{ow} values and slightly higher solubilities in water compared to total DDT which has a log K_{ow} of 6.91 (ATSDR, 2002).

2.2.4 DDT in the ecosystem

DDT and its metabolites exist in the soil and enter easily into the food chain through the ingestion of contaminated soil and sediment by vertebrates and invertebrates. DDT can also migrate through plants to different levels of consumers in the food web. When DDT enters into the food web, it has a tendency to bioaccumulate in the lipid cells of animals (ATSDR, 2002; Streit, 1992). As a result, animals at the top of the food chain, including fish, predatory birds, mammals, and humans tend to have the greatest concentrations of DDT. Hence, biomagnification of DDT generates the highest risk of acute and chronic toxic effects at these higher levels (Jongbloed *et al*., 1995).

2.2.5 DDT toxicity in the environment

The National Toxicology Program (NTP) has termed DDT as 'moderately toxic' and the World Health Organization (WHO) has defined DDT as a 'moderately hazardous' compound based on rat oral LD_{50} of 113 mg/kg (WHO, 2005; Kegley *et al*., 2010). Humans are at higher risk of being affected by DDT residues through food, because of their position at the top of the food chain. Several ecological studies have proven an association between liver cancer and DDT exposure in humans (Beard *et al.,* 2006). Moreover, DDT also may cause pancreatic cancer, liver and biliarytract cancer, multiple myeloma, cardiovascular disease, and possibly diabetes (Beard *et al.,* 2006). DDT and its metabolites cause several types of mutagenic and carcinogenic effects and adverse effects on mammals, in terms of reproduction, growth, and immunocompetence (CCME, 1999; ATSDR, 2002). It has been found that liver hematomas in mice were increased following dietary exposure of 42,800 ng *p,p'*-DDT/kg/day for 15 to 30 weeks (ATSDR, 2002).

The correlation between DDT exposure and eggshell thinning was established by different studies (ATSDR, 2002; Peakall *et al*., 1973). Eggshell thinning is one of the major concerns that gained public awareness during the 1960s and 1970s from field observation and experimental studies of raptor populations, including the bald eagle, peregrine falcon, and osprey. Eggshell thinning is a kind of reproductive failure and exotoxicologic effect in avian species (Holm *et al*., 2006). Moreover, DDT is capable of creating phytotoxicity in a number of agricultural crops (CCME, 1999). It was reported that the Blakemore strawberry plant (*Fragaria* x*ananassa*), showed significant sensitivity to DDT-contaminated soil. The growth of the root of this plant was inhibited in 3000 ng/g DDT-contaminated soil and the number of plants produced decreased by approximately by 21% in 6000 ng/g DDT-contaminated soil (CCME, 1999).

2.2.6 Canadian soil quality guidelines of DDT

DDT guidelines for soil have been developed by Canadian Council of Ministers of Environment (CCME, 1999) to protect invertebrates, amphibians, reptiles, birds, mammals, and aquatic life. According to the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, 700 ng/g of DDT is recommended for residential/parkland and agricultural land uses, whereas 1200 ng/g of DDT is recommended for industrial and commercial land (Table 2.1) (CCME, 1999). In the Interim soil quality criteria, CCME also modeled another guideline for the different levels of consumers in the food chain to limit biomagnification of DDT and to protect against resulting ecological impacts. The guideline values are 1500 ng/g for primary consumers, 2000 ng/g for secondary

consumers and 700 ng/g for tertiary consumers (CCME, 1991). According to the Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota, 14 ng/g of DDT and less in tissue is recommended (CCME, 1998).

Table 2.1: Soil quality guidelines for DDT (total) ng/g. (CCME, 1999)

Guideline	Land use			
	Agricultural	Residential/Parkland	Commercial	industrial
SQG_{HH} = soil quality guideline for human health	700	700	1200	1200
SQG_{E} = soil quality guideline for environmental health	700	700	1200	1200

2.3 Levels of DDT in Point Pelee National Park (PPNP)

Point Pelee National Park (PPNP), situated in southern Ontario was established in 1918. Migratory routes and mass breeding of birds have made the Park biologically significant. However, some areas in the Park are contaminated with DDT due to the historical uses of DDT for controlling pests in agricultural areas and mosquitoes in roadways, campgrounds and picnic areas during 1950s and 1960s (Crowe and Smith, 2007; Smits *et al.*, 2004). A significant amount of DDT (15,000 ng/g in soil) was reported in the agricultural area of the Park in 1997 (Russell and Haffner). In the same study, it was documented that significant amounts of DDT were migrating to PPNP amphibians and reptiles. Later, Smits *et al*. (2004) detected DDT in the tissues of birds and the dietary compounds of birds. In 2007, Crowe and Smith completed a comprehensive study of PPNP soil, sediment and water and reported a maximum concentrations of DDT (316,000 ng/g) in the former agricultural area of the Park. DDT concentrations reported in the Park generally far exceed the CCME guideline for Residential/Parkland uses of 700 ng/g.

2.4 DDT remediation techniques

2.4.1 Traditional technologies (excavation/digging)

A common way of remediating DDT-contaminated soil is via excavation and landfilling. Following excavation, DDT-contaminated soil can also be incinerated, a process in which it is degraded into non-toxic substances. These techniques are expensive and cause destruction of soil as well as ecological disturbance to the surrounding areas, and hence may not be feasible in all situations. For example, DDT clean up in residences along the west side of Kenwood Avenue in Los Angeles was carried by the US EPA who adopting an excavation and incineration technique. The total cost of this project was $10.1 million (US EPA, 2002). In Canada, incineration of organically contaminated soil is restricted to two permanent high temperature thermal destruction units in Alberta and Quebec (CCME, 1999). In some situations, an *ex-situ* soil washing method can be adopted. More than 50 percent of soil DDT can be washed out using 1% anionic and 1% nonionic surfactants (Ghazali, 2010), but may not be cost effective due to high levels of DDT in the wash water. Hence, a sequential clean-up is required (Citizen Guide to Soil Washing, EPA, 1996). For these reasons, researchers are actively developing environmentally viable and more cost-effective techniques such as phytotechnologies.

2.5 Phytotechnologies

2.5.1 Background

Phytoremediation (sometimes termed phytotechnology) is a process that uses vascular plants to remove, transfer, sequester, stabilize, destroy or degrade toxic organic or inorganic contaminants in soil, sediments or water (Russell, 2005; UNEP, 2011). The ultimate objective of phytoremediation is to remediate contaminated areas of land or water.

Phytoremediation has found acceptance on a broad scale for controlling runoff and minimizing soil erosion, and enhancing the aesthetic beauty of sites, while not disturbing the environment. It is also cost effective with low labor, equipment and operational expenses (Russell, 2005). It has been estimated that phytotechnologies are 40% less expensive than other *in-situ* techniques and 90% less expensive than *ex-situ* techniques (ITRC, 2001). However, phytotechnologies have some limitations in terms of phytotoxicity (plants need to be able to survive in contaminated soil), seasonal characteristics (growing season may be too short), and are much slower compared to conventional clean-up techniques.

Phytoextraction is a subtype of phytoremediation in which plants take up inorganic or organic contaminants through their roots and transfer them to the above ground portion of the plant as shown in Figure 2.3. It appears that POPs such as DDT or other chlorinated substances cannot be extensively metabolized in the interior portion of the plant tissues (Fundersberg and Neish, 1968). However, these chlorinated substances can be accumulated in lignin molecules inside of the plant by covalent bonding and that plant tissue can easily be harvested (Khan, 1980).

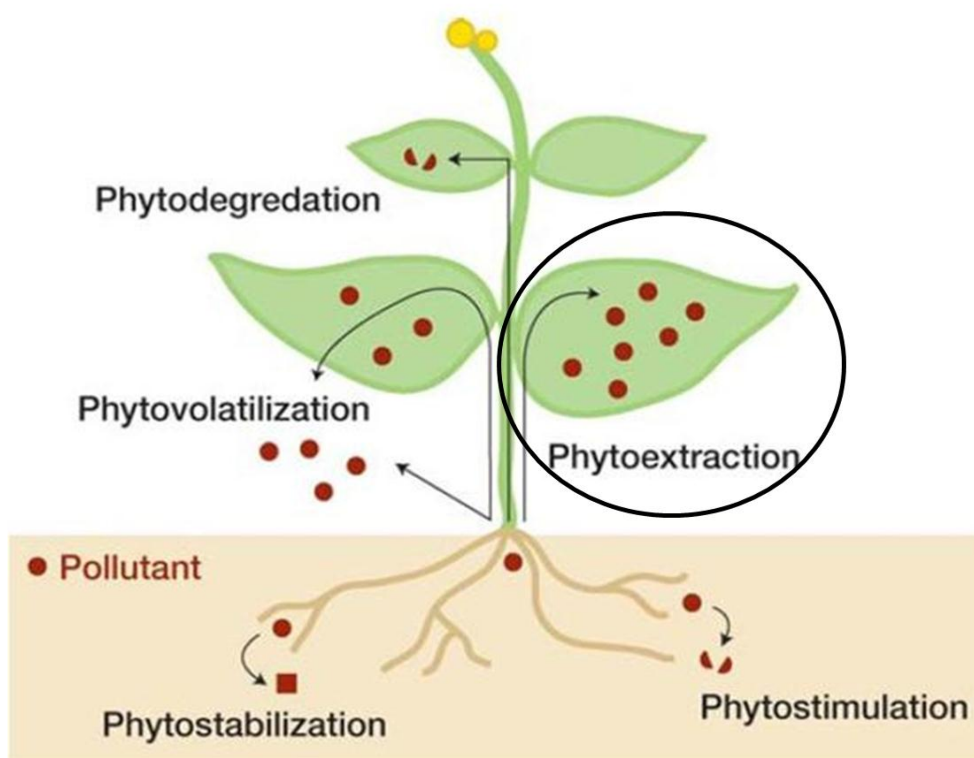


Figure 2.3: An overview of different phytoremediation techniques. (Adapted from Instute for Green Energy and Clean Environment (IGECE), 2010).

2.5.2 Phytoextraction of organic contaminants by *Cucurbita pepo*

It was anticipated that hydrophobic contaminants (including POPs) would not easily be desorbed from soil organic matter and made available for the plants to take up (Hülster *et al*., 1994; Zeeb *et al*., 2006; Ye *et al*., 1992) as POPs have a high K_{OW} hindering effective transportation into vegetative tissues (Blaylock *et al*., 1997). However in 1994, Hülster made a breakthrough in the phytoextraction of dioxins (PCDDs) and furans (PCDFs). Zucchini plants (belonging to the *Cucurbita*

genus) bioaccumulated substantial quantities of PCDDs/PCDFs in roots and translocated these contaminants into the aerial tissues of the plants rather than releasing the contaminants to the atmosphere by volatilization (Hüstler *et al*., 1994).

Using the basic principles of the Hülster *et al.*(1994) study, White found that plants of the *Cucurbita* genera (squash and pumpkin) extracted 0.40 to 2.4 % *p,p'*-DDE from the soil. This high *p,p'*-DDE extraction rate from the soil correlates with the phytoextraction of heavy metals by hyperaccumulating species (White, 2002). Furthermore, it was observed that *Cucurbita pepo* (zucchini) could phytoextract 1.3% of hydrophobic weathered *p,p'*-DDE and translocate it into the shoot tissue (Wang *et al*., 2004). Research was also conducted at the subspecies level for *C. pepo*. The study revealed a variation of phytoextraction capability in *C. pepo* ssp *pepo* versus *C. pepo* ssp *texana* where *C. pepo* ssp *pepo* had higher *p,p'*-DDE extraction (0.301 %) than *C. pepo* ssp *texana*, (0.065%) (White *et al*., 2003). *C. pepo* has now been successfully used for DDT extraction in numerous studies (Lunney *et al.*, 2004, 2010; Whitfield Åslund *et al*., 2010). *C. pepo* was also used for PCB (Zeeb *et al*., 2006; Whitfield Åslund *et al*., 2007, 2008) and chlordane (Mattina *et al.,* 2004) phytoextraction.

The efficiency of phytoextraction can be calculated using a bioaccumulation factor (BAF = $[DDT]_{plant\ tissue}$ / $[DDT]_{soil}$). Similarly, a translocation factor (TLFs = $[DDT]_{shoot\ tissue}$ / $[DDT]_{root\ tissue}$) can be used to determine how efficiently DDT is transferred from the plant root to the plant shoot. The goal of phytoextraction is to maximize the contaminant concentration in the harvestable tissue of the plant. Ideally, the shoot BAF and TLF will be greater than one and phytoextraction is then likely to be a cost effective technique (Ficko *et al.,* 2010; Whitfield Åslund *et al.,* 2010; Lunney *et al.,* 2004).

High BAFs are observed in some species for organic contaminants, but BAFs are generally not as high as those observed for metal contaminants. The highest root BAFs of 16 and 9.9 were found in the *Cucurbita* genera (pumpkin/squash) for *p,p*'-DDE (White, 2002). In another study, the root and shoot BAFs of *C. pepo* ssp *pepo* were observed to be 7.2 and 5.4, respectively in *p,p*'-DDE-contaminated soil (White *et al*., 2003). Moreover, Lunney *et al*. (2004) reported the effect of soil concentration on BAF and TLF where the shoot BAF were 1.2 and 2.4, respectively in high (3700 ng/g) and low (150 ng/g) DDT-contaminated soil.

2.5.3 Mechanism of phytoextraction by *C. pepo*

Plants release exudates, which are classified as low molecular weight organic acids (LMWOA) and high molecular weight organic acids (HMWOA), sugars (as organic compounds), amino acids and carbon dioxide (as volatiles). LMWOA such as di- and tri-carboxylic organic acids may make POPs like DDT more bioavailable for plant. These LMWOA compounds, including citric acid, malic acid, and oxalic acid assist in nutrient acquisition by chelating inorganic micronutrients, such as Fe, Zn, Cu, and Mn from the soil structure (Singer *et al*., 2003). It has been hypothesized that root exudates may destroy the local inorganic soil matrix and assist in the partial or complete dissolution of soil organic matters, which may lead to the organic contaminant being more accessible for uptake (White, 2002). Researchers have tried to understand the mechanism of phytoextraction of DDT by *Cucurbita pepo* species as this species has been found to be a particularly good accumulator of POPs (White, 2001; White *et al*., 2003, 2005). It was determined that *C. pepo* may accumulate significant quantities of POPs because of the different patterns of LMWOA that exude from the roots into the rhizosphere zone in comparison to other non-accumulating plants. These specific root exudates may play a significant role in increasing the bioavailability of POPs like DDT for the plant. However, the release and movement of contaminants through different plants are related to species characteristic such as plant physiology and plant metabolism (White, 2001; Inui *et al*., 2008).

It appears that hydrophobic contaminants such as DDT or PCBs can selectively sorb to lipid cells in roots (Collins *et al*., 2006; Trapp and Matthies, 1995). These contaminants can then move through the xylem sap (Greenwood *et al*., 2011). A xylem sap study of various *C. pepo* subspecies suggested that both the concentrations and the patterns of chlordane components were different among these species (Mattina *et al.,* 2004).

A hydroponic study hypothesized that during the accumulation of hydrophobic contaminants, accumulation of *p,p*-DDE followed an order i.e., root>>stem>leaf blade of *C. pepo* ssp *pepo* (zucchini) (Gent *et al*., 2007). It was further hypothesized by Gent *et al*. (2007) that contaminants might travel in the plant tissue from areas of higher gradient to lower gradient until they achieve equilibrium. In the same year, Whitfield Åslund observed the PCB accumulation along *C. pepo* shoot and noted that total PCB concentration decreased exponentially with increasing distance from the root. It was also documented that less chlorinated PCB congeners moved further along the shoot than higher chlorinated congeners (Whitfield Åslund *et al*., 2007, 2008). Similarly for DDT in *C. pepo,* the shoot followed the same exponential decreases of DDT concentration with increased distance from the root (Whitfield Åslund *et al*., 2010). In addition, higher proportions of less water soluble DDT isomers were found in the *C. pepo*

shoot tissue, which was the opposite effect to that of PCBs (Whitfield Åslund*et al*., 2010).

2.6 Native weed species for phytoextraction

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 our native ecosystems (Castillo and Elkins 2009). Naturalized plants are those that have been introduced from another area and then become established. The naturalization of a plant in an area can be a good thing or a bad thing, depending on the characteristics of that particular plant (Richardson *et al*., 2000). A plant may be termed 'invasive' if it spreads without human assistance, and has negative effects on the environment (or economy) (Pattison *et al*., 1998). Here, we refer to naturalized plants as those that have adapted to the general climate, microclimate, altitude, soil, and rainfall of an area without being detrimental to the ecosystem.

Some weed species have been determined to be efficient phytoextractors of metals (Cunningham *et al*., 1995; Raskin *et al*., 1997; Lasat *et al*., 1998; Zhao *et al*., 2003; Lone *et al*., 2008). For example, zinc and cadmium were successfully extracted by species in the *Brassicaceae* family. *Brassica juncea* (Indian mustard) is capable of accumulating several metals, including Se, Pb, Cr, Ni, Zn, and Cu (Aboulroos *et al.,* 2006). Evidences suggest that *Amaranthus retroflexus* (redroot pigweed) can remove radioactive cesium 137 (^{137}Cs) up to 40 times higher in shoot tissues than *Brassica juncea* and *Phaseolus acutifolius* (tepary bean) (Lasat *et al*., 1998). Similarly, *Pteris vittata* (Chinese brake fern) can extract 20 times more As into shoot tissues than is found in soil (Salido *et al*., 2003). In 1999, Porebska and Ostrowska, found some promising species (out of 40 wild-grown weed species surveyed) which could remove a significant quantity of Zn, Cu, Pb, and Cd. This study also revealed that metal uptake by the plants was dependent on the amount of metal in the soil in a form which was more bioavailable for the particular plant species. In the 1990's researchers began to investigate phytoextraction of non-metal contaminants such as radioactive and organic contaminants (Baker *et al*., 1994; Ghosh and Singh, 2005; Kopf Johnson., 2006).

There are several advantages to using native (or naturalized) weed species over crops species. These include the fact that they are: i) sustaining, ii) easy to propagate, iii) cost effective, and iv) have little chance of being consumed by herbivores resulting in a lower possibility of contaminants being transmitted through the food chain (Ficko *et al.*, 2010). There are also some disadvantages to using weed species as phytoextractors. Some of these species are an important part of the food chain, some are very noxious and may cause harm to other living plants

and organisms, and most produce a low amount of biomass compared to crop species.

Extraction and translocation of weathered *p,p'*-DDE has been been observed in the species *Lolium multiflorum* (rye), *Brassica juncea* (mustard), *Brassica napus* (canola), *Vicia villosa* (vetch), *Cajanus cajan* (pigeonpea), *Trifolium incarnatum* (clover), *Arachis hypogaea* (peanut), and three cultivars of *Lupinus albus* (white lupin) (White *et al*., 2005). Vetch showed phytoextraction percentages ranging from 0.06% to 0.22%. This study indicated that bioavailability of *p,p'*-DDE to these species is comparatively lower than other species like *C. pepo* (White *et al*., 2005). Likewise, Lunney *et al*. (2004) conducted a comparative study between *C. pepo* and three weed species (*Festuca arundinacea* (tallfescue), *Lolium multiflorum* (rye grass), and *Medicago sativa* (alfalfa)) and observed lower DDT extractions in weed species than *C. pepo*.

Kopf Johnson *et al.* (2006) determined that naturally grown species, including *Amaranthus retroflexus* (redroot pigweed), *Ambrosia artemisiifolia* (ragweed), *Cirsium arvense* (Canada thistle), *Echinochlola crus-gali* (barnyard grass), and *Polygonum pericardia* (ladies thumb) at a PCB-contaminated site were able to accumulate of 700 to 13,700 ng/g PCBs into their shoots. In 2007, a comparative field trial was conducted between *C. pepo* and two native species *Carex normalis* (sedge) and *Festuca arundica* (tallfesque) where *C. normalis* appeared to accumulate higher root and shoot PCB concentration than the known phytoextractor *C. pepo* (Whitfield Åslund *et al*., 2007). Based on the above study, Ficko *et al*. (2010) conducted a comprehensive investigation in weathered PCB-contaminated soil using 27 weed species. This study showed that many of the weed species had the capability to extract PCBs from the soil and accumulate significant concentrations in both root and shoot tissues. The mean shoot concentration ranged from 80 ng/g for *Cirsium vulgare to* 35,000 ng/g for *Vicia craca*.

Extraction of contaminants per unit area

It is important to compare the phytoextraction capabilities of different plants on a per unit area basis. The density at which plants grow optimally is a key consideration to maximizing contaminant phytoextraction. The optimal planting density of *C. pepo* cv. Howden is one per square metre (OMAFRA, 2000). In contrast, weed species grow at much higher densities (Ficko *et al.*, 2010). For example, *Chrysanthemum leucanthemum* (ox-eye daisy) grows at 20 plants per square metre (Pill *et al*., 1994). Likewise, *Symphyotrichum novae-angiae* (New England aster) grows at 144 plants per square metre (USDA, 2003) and up to 8000 *Trifolium pratense* (red clover) plants grow per square metre (Black, 1960).

Plant shoot biomass is another important consideration in phytoextraction as higher shoot biomass will correspond to a higher contaminant extraction. In one study, the mean dry shoot biomass of *C. pepo* was 313 g and the shoot PCB concentration was 6700 ng/g giving a maximum shoot PCB extraction of 2,100,000 ng (Whitfield Åslund *et al*., 2007). In contrast, *S. canadensis* had a dry shoot biomass of ~62 g and PCB concentration of 6774 ng/g with maximum shoot PCB extraction 420,000 ng (Ficko *et al.,* 2010) which was several times lower than *C. pepo*. Interestingly, *S. canadensis* could extract a greater quantity of PCBs when optimal planting density was considered. Similarly, 18 other native species took up equal or greater quantities of PCBs than *C. pepo* on a per metre square basis (Ficko *et al*., 2010). A field trial was conducted using three promising PCB phytoextracting native species (*Chrysanthemum lecunthemum*, *Rumex crispus* and *Solidago canadensis*) in monoculture plots at a PCB-contaminated site. It was again found that these three species extracted similar or greater amounts of PCB on a per unit area basis than known phytoextractor *C. pepo* (Ficko *et al.,* 2011b).

Chapter 3

An Evaluation of Native and Naturalized weed species for the Phytoextraction of DDT at Point Pelee National Park, Leamington, ON

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Abstract

The phytoextraction capacities of nine wild grown native and naturalized weed species, including *Solidago canadensis* (Canada goldenrod), *Solanum ptycanthum Dun* (eastern black nightshade), *Cornus sanguinea* (dogwood), *Silene vulgaris* (bladder campion), *Asclepias syriaca* (milkweed), *Leonurus cardiac* (motherwort), *Verbascum thapsus* (mullein), *Symphyotrichum novae-angliae* (new England aster), and *Trifollium pratense* (red clover) were evaluated from dichlorodiphenyltrichloroethane (DDT)-contaminated sites in Point Pelee National Park (PPNP), southern Ontario, Canada. These species were chosen as they are native (or naturalized) and abundant in DDT-contaminated areas of the park. Maximum root and shoot extractions of 284,000 and 12,000 ng, were measured in *S. novae-angliae* and *V. thapsus*, respectively. The highest DDT shoot extraction potential per square metre (96,000,000 ng) was calculated for *T. pratense*. Interestingly, nearly half (44%) have shown higher shoot extraction capabilities than that of the known phytoextractor *Cucurbita pepo* cv. Howden (pumpkin). In addition, shoot DDT extractions per square metre for *V. thapsus* and *T. pratense* were similar or identical to those previously recorded by these species for polychlorinated biphenyls. This study shows potential for the use of native weed species to both remediate and restore the ecological integrity at DDT-contaminated sites in Point Pelee National Park.

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3.1 Introduction

Dichlorodiphenyltrichloroethane (DDT) contamination is extensive in North America and other parts of the world due to its widespread use beginning in 1939 for controlling agricultural pests and disease vectors. Based on its detrimental environmental impacts on wildlife, as well as likely impacts on human health, the use of DDT has been restricted in Canada since 1969. However, as it is not easily degraded, large amounts of DDT and its metabolites are still being detected in Canadian soils (CCME, 1999). The Canadian environmental quality guidelines for maximum DDT soil concentration in parks or residential lands is 700 ng/g (CCME, 1999). Above this level, it is recommended that soils be remediated to protect the environment and human health. Between 1947 and 1967, DDT was applied to several areas within Point Pelee National Park (PPNP), Ontario, Canada to control mosquitoes and plant pests. Elevated levels of DDT (up to 316,000 ng/g) are still found in PPNP soil due to its high persistence and long half-life (Crowe and Smith, 2007). Traditional remedial technologies, including excavation and incineration of DDT-contaminated soil, are not suitable within PPNP because of the destructive nature of these methods on the surrounding ecosystem.

Various phytotechnologies have emerged as promising, cost-effective, environmentally friendly, and non-destructive remediation techniques for both inorganic and organic contaminants (USEPA, 2012; Russell, 2005). Phytoextraction is a form of phytoremediation, where plants take up contaminants through their roots and transfer them to the harvestable plant shoots. The shoots are then harvested and composted to reduce their volume, and the composted biomass is transferred to a waste incineration or hazardous waste landfill facility (Macek *et al*., 2000; Baylock and Huang, 2000; Michel *et al*., 2001; Sas-Nowosielska *et al*., 2004).

To date, plants of the *Cucurbita* genera and other food crops have often been used for organic contaminant phytoextraction (Hülster *et al*., 1994; Mattina *et al*., 2000; Lunney *et al*., 2004; Whitfield Åslund *et al*., 2010, White, 2002, White *et al*., 2003; Mattina *et al*., 2006; Zeeb *et al*., 2006, Whitfield Åslund *et al*., 2007, 2008), based on their ability to produce large amounts of biomass.There are however, several advantages to using native (or naturalized) weed species as phytoextractors. Weed species are often easier to propagate at contaminated sites, are self-sustaining, have lower associated cost, and have little chance of being consumed by humans (Ficko *et al*., 2010). Furthermore, thousands of weed species exist with different physiologies, distinct root systems, root exudates, and growth patterns. Some species are perennial, a factor which may help to extract, degrade or stabilize contaminants over longer periods of time. To date, numerous weed

species have been used as efficient phytoextractors of metals (Cunningham *et al*., 1995; Raskin *et al*., 1997; Lasat *et al*., 1998; Zhao *et al*., 2003; Lone *et al*., 2008).

Some researchers have begun looking at the potential of weed species for phytoextracting persistent organic pollutants (POPs). Bush *et al*. (1986) found that *Lythrum salicaria* (purple loosestrife) could accumulate PCBs in root tissues, but translocate only a small amount (0.5%) into the readily harvestable aerial tissues. A study by Whitfield Åslund *et al*. (2007) showed that *Carex normalis* (sedge) could accumulate 13000 ng/g PCB into its shoot tissue, which was half of that taken up by the known phytoextractor, *C. pepo*. Recently, Ficko *et al*. (2010) completed a comprehensive investigation of twenty-seven weed species grown on high (31000 ng/g) and low (4700 ng/g) PCB-contaminated soil. Maximum shoot extractions of 420,000 ng for *S.canadensis* (Canada goldenrod) and 120,000 ng for *C. leucanthemum* (ox-eye daisy) were observed. Moreover, 18 of 27 species were determined to extract higher amounts of PCBs per unit area than that of the known phytoextractor *C. pepo* when optimal plant density was taken into account.

The efficiency of phytoextraction can be measured using bioaccumulation factors (BAFs) ($[DDT]_{plant\ tissue}$ / $[DDT]_{soil}$) and translocation factors (TLFs) ($[DDT]_{shoot}$ / $[DDT]_{root}$). Ideally, phytoextraction will maximize the contaminant concentration in the harvestable portion of the plant, and hence the higher the BAFs and TLFs, the better. To date, several weed species have been demonstrated to have root BAFs >1, but most shoot BAFs and TLFs were <1 (Lunney *et al*., 2004; White *et al*., 2005). However, in a recent study, Ficko *et al.* (2010) found maximum root BAFs of 10 and 9.9 in *Solanum nigrum* and *Brassica nigra*, respectively for PCBs. In the same study, shoot BAFs > 1 in *Viccicia cracca* and *Polygonum persicaria* were demonstrated and these were higher than the shoot BAFs of *C. pepo* cv. Howden, a known PCB phytoextractor (Low *et al*., 2010; Zeeb *et al*., 2006; Whitfield Åslund *et al*., 2007). For DDT, shoot BAFs of *C. pepo* ranged from 1.1 (Lunney *et al*., 2010) to 1.2 to 2.4 (Lunney *et al*., 2004). Comparatively higher root and shoot BAFs were documented in different cultivars of *C. pepo* ssp *pepo* at *p,p'*-DDE contaminated soil (White *et al.*, 2003, 2010).

The current study surveys a number of common native (or naturalized) weed species in PPNP to determine which have the potential to efficiently extract DDT. The results are compared to a similar survey of native weed species found growing in PCB-contaminated soil (Ficko *et al*., 2010). This work has a practical significance as PPNP authorities have adopted a policy under Point Pelee's Habitat Restoration Project to remove all non-native/invasive species from the park and re-vegetate with species native to the area. Ideally, species used for re-vegetation will improve conditions for breeding butterflies and birds. However, if these species efficiently phytoextract DDT, they might prove to be harmful to some species. PPNP staff would like to identify the phytoextraction capabilities of native (or

naturalized) weed species in order to put into place a realistic plan for remediation at PPNP while minimizing risk to sensitive organisms. This may entail strategic planting of successful phytoextractors in some areas, and phytoexluders (i.e. plants that do not readily take up DDT) in others.

3.2 Methodology

3.2.1 Site description

Point Pelee National Park (PPNP) is situated in southern Ontario, Canada and extends towards the western basin of Lake Erie. The soil in several areas of the park are highly contaminated with DDT and its metabolites (∑DDT = mixture of *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*–DDD). Park soil is heterogeneously contaminated with DDT, depending on the historical application of this pesticide (ranging from 225 ng/g to 108,000 ng/g). The organic carbon content in the soil is 3.2% and the soil is classified as predominantly sand and silt.

3.2.2 Sample collection

In the summer of 2011, five native weed species (*S. canadensis* (Canada goldenrod), *S. vulgaris* (bladder campion), *A. syriaca* (milkweed), *L. cardiac* (motherwort), *S. ptycanthum Dun* (eastern black nightshade)) and two naturalized species (*C. sanguinea* (dogwood) and *V. thapsus* (mullein)) were collected from DDT-contaminated sites in PPNP where they grew naturally. In the summer of 2012, two additional species, one naturalized (*Trifolium pratense* (red clover) and another native (*S.novae-angliae* (new England aster)) species were harvested from these sites. Seven species included in this study are common, perennials and two species (*S. ptycanthum Dun* is annual but occasionally perennial and *V. thapsus* is biennial) found growing wild throughout PPNP and each were represented by a minimum of three sample individual plants (n = 2-4).

Plants were harvested using a trowel to loosen the soil around the root and excess soil was removed by shaking. Plants were then separated into root and shoot portions with scissors, which were rinsed with methanol between cuts. Plant sections were washed with clean running water, blotted dry with paper towel, weighed to a hundredth of a gram, and stored separately in labeled Whirlpak® bags. Soil samples (0-10 cm depth) associated with each individual plant's root system were collected in labeled Whirlpak® bags using a trowel. All samples were frozen until analysis.

3.2.3 Analytical procedures

Soil samples (20 g wet weight) were air-dried overnight at room temperature for 24 h. Approximately 2 g of sample were used for analysis. 10 to 15 g of representative plant sample were chosen for each plant tissue analysis. Plant samples were chopped and thoroughly homogenized prior to drying in an oven at 25-28 °C. The dried plant mass was then ground with mortar and pestle.

All of the prepared soil and plant samples were extracted using a soxhlet extractor with 100 µl of 1 ppm decachlorobiphenyl (DCBP) as an internal surrogate standard. Approximately 10 g of Ottawa sand, 10 g of sodium sulphate were mixed with the sample and 250 ml of dichloromethane (DCM) were used in a round bottom flask with the sample for an extraction period of 4 to 5 h (4-6 cycles/h). The extraction involved solvent vaporization and condensation. A Buchi syncore was used to concentrate the extract to approximately 2 ml. The concentrated extract was then solvent exchanged by adding 5 ml aliquots of hexane at least three times. Finally, the extract was filtered through a Florisil column and made up to 10 ml in a volumetric flask.

An Agilent 6890 Plus gas chromatograph was used to analyze the extracted plant and soil samples. The chromatograph was equipped with a ^{63}Ni electron capture detector (GC/ECD), and a SPB-1 methyl silixonae capillary column (30 m, 0.25 mm ID x 0.25 µm film thickness). The initial temperature was set at 100 ºC for 1 minute, with ramping at 8 ºC/min to 180 ºC. This was followed by 3 ºC/min to 220 ºC, remaining for 3 min at 220 ºC. Finally, the chromatograph was ramped at 20 ºC/min to 300 ºC where it was held at 300 ºC for 5 minutes. A constant flow was maintained during the procedure. The total estimated run time was 36.3 minutes. Temperature was maintained at 250 ºC and nitrogen was applied as a make-up gas for the ECD detector. Helium was used as a carrier gas.

3.2.4 Quality assurance and control

For every nine samples processed by Soxhlet, one analytical blank (Ottawa sand and anhydrous sodium sulfate), and one control sample (a blank sample spiked with 100 µl of 2 ppm organochlorine pesticide mixture (Appendix IX, supelco)) were included. Sample concentrations were corrected for surrogate recovery. No blanks contained any of the DDT isomer, which was below the detection limit. All of the control sample recoveries were between 85% and 106% of the projected value. The mean recovery for the internal surrogate was 93% for both plant and soil samples. The mean relative standard deviation between plant samples and their analytical duplicate was 22%, and 9.8% for soil samples and their analytical duplicate.

3.2.5 Statistical analysis

A one way Analysis of Variance (ANOVA) was used to compare the data following testing for normality (Kolmogorov-Smirnov test). A one way ANOVA was employed to compare shoot and root DDT uptake and extraction as well as shoot and root BAFs of native weed species. All data were analyzed using the statistical software TIBCO Spotfire S^+ with a significance level at $p = 0.05$.

As the wild-grown plant species were grown in heterogeneous DDT-contaminated soil, it is difficult to make a direct comparison of DDT uptake in plant tissues between species. Therefore, DDT plant extractions were normalized to the mean soil concentration (21,000 ng/g). A correction factor (cf) was calculated by dividing the mean soil [DDT] by the soil [DDT] associated with each individual plant root system. Each plant's DDT uptake was then multiplied by the correction factor. A similar normalization technique was adopted by White (2002).

3.3 Results

3.3.1 Soil concentration of DDT

DDT soil concentration associated with the 30 plant samples ranged from 225 to 108,000 ng/g with a mean soil [DDT] of 21000 ng/g.

3.3.2 Bioaccumulation Factors (BAFs) and Translocation Factors (TLFs)

The mean root BAF of *S. novae-angliae* (2.8) was significantly higher than that of the other species which ranged from 0.2 (*S. ptycanthum Dun*) to 0.62 (*T. pratense*) ($p < 0.05$) (Figure 3.1a). Mean shoot BAFs ranged from 0.01 (*S. canadensis* and *L. cardiac*) to 0.12 (*S. novae-angliae*) (Figure 3.1b), but no significant differences were observed ($p = 0.05$). TLFs ranged from 0.04 (*S. canadensis*) to 0.29 (*V. thapsus*) but no significant difference was found between the species (Table A 6, Appendix A).

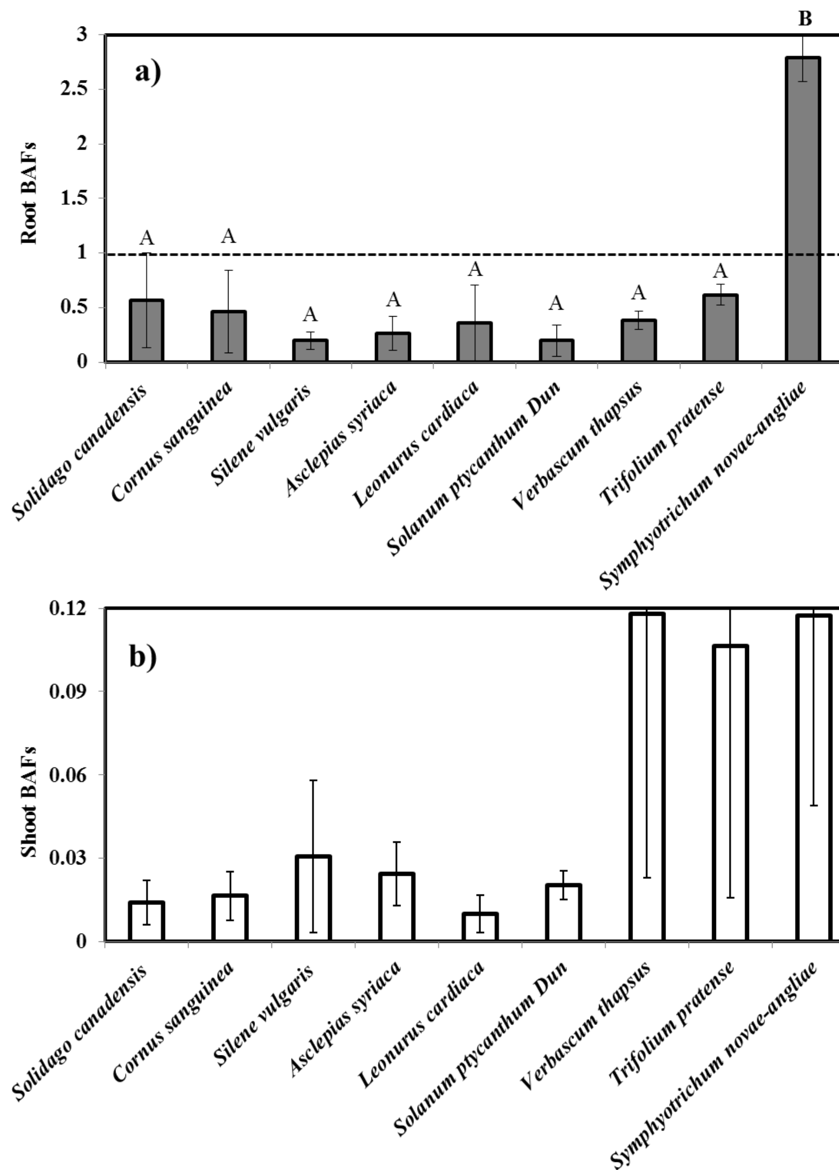

Figure 3.1: Comparison of a) root and b) shoot BAFs between plant species at DDT-contaminated sites in PPNP. Error bars show the standard deviation of the mean and the 1:1 line is indicated by a dotted line. Root BAFs followed by different letters indicate a significant difference between the species ($p < 0.05$). There were no significant differences in shoot BAFs.

3.3.3 DDT root and shoot extraction

Normalized root DDT extractions ranged from 2050 ng in *S. ptycanthum Dun* to 284,000 ng in *S. novae-angliae* (Figure 3.2a) with the latter species being significantly different from all of the others (p = 0.001). Although, mean shoot extractions ranged from 700 ng in *S. ptycanthum Dun* to 12000 ng in *S. novae-angliae* (Figure 3.2b), there was no significant difference between any of these species. Raw data for root and shoot DDT concentrations are provided in (Table A 1, Appendix A)

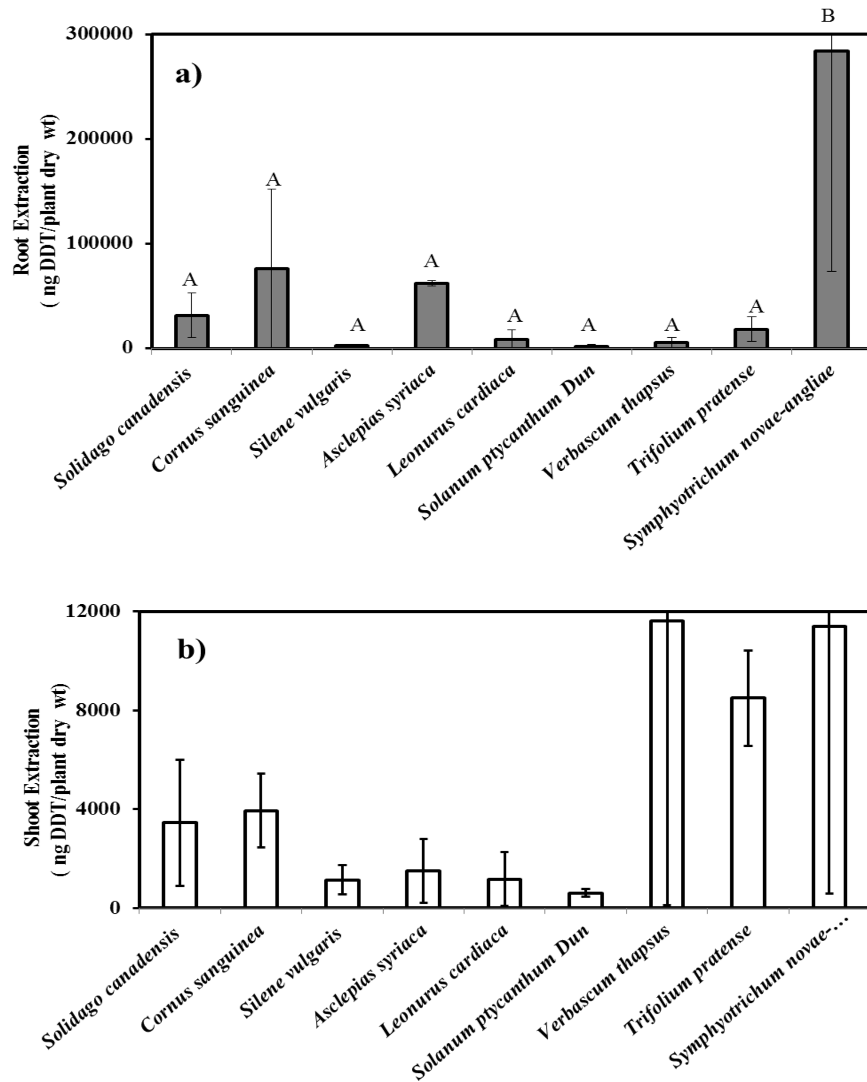

Figure 3.2: DDT extractions in a) root and b) shoot tissues for plant species at DDT-contaminated sites in Point Pelee National Park. Error bars represent the standard deviation of the mean. A one-way ANOVA was used to compare the root and shoot DDT extractions between species. Letters indicate a significant differences in root DDT extractions between the species (*p* = 0.001). There were no significant differences in shoot DDT extractions (*p* = 0.05).

3.4 Discussion

3.4.1 Bioaccumulation factors and Translocation factors

No species in the current study exceeded a shoot BAF of 1. The root BAF of *T. pratense* (red clover) (BAF = 0.62) in the present study is lower than that of *T. incarnatum* (crimson clover) (BAF = 6) reported by White *et al*. in 2005, however in this latter study, besides the difference in plant species, the BAF is presented in terms of *p,p*-DDE only, whereas the present study takes into account all of the metabolites of DDT.

Five species in the present study are common to a study carried out by Ficko *et al*. (2010) in which PCB phytoextraction was studied. In each case, the same trends were observed in shoot BAFs and TLFs for *S. novae- angliae, V. thapsus,* and *T. pratense*. The root BAF of 2.8 in *S. novae-angliae* in the present study was higher than that of *S. novae- angliae* (1.1) when used as a PCB extractor (Ficko *et al*., 2010). Shoot DDT BAF (0.01) in *S. canadensis* is comparatively lower than that of PCB BAF (0.27) (Ficko *et al*., 2010). A maximum TLF of 0.29 was recorded in *V. thapsus* and this is higher than the PCB TLF (0.16) calculated for the same species by Ficko *et al*. (2010).

The overall trend of BAFs and TLFs in common species between the two studies indicates that successful PCB extractors appear to also be good DDT extractors. Differences in DDT uptake, bioaccumulation, and translocation in the surveyed species may be due to the variations in growth pattern of plants, age and time of harvest at the species level, as variations have also been noted in *p,p'*-DDE uptake, bioaccumulation, and translocation of *C. pepo* at the subspecies level (White *et al*., 2003).

3.4.2 Shoot extraction

Shoot extraction takes into account DDT concentration in the shoot as well as the shoot biomass. It is essential to calculate total shoot extraction for a true comparison of phytoextraction capability among species (Whitfield Åslund *et al*., 2007; Ficko *et al*., 2010) as plants may accumulate lower levels of DDT, yet attain higher extractions if they are high in biomass.

A maximum dry shoot biomass of 62 g was calculated in *S. canadensis* by Ficko *et al*. (2010), whereas a dry biomass of only 11.4 g was calculated in *S. canadensis* in this study. The dry shoot biomass of *T. pratense* in this study was also eight fold lower than that of *T. incarnatum* (crimson clover) in White *et al*. (2005). However, the root and shoot extractions of *T. pratense* in this study were

19000 and 8500 ng, respectively, which were higher than that of the *T. incarnatum* in White's (2005) study using *p,p'*-DDE contaminated soil.

True shoot extraction can be underrepresented if the biomass of the plant species on a per area basis is not considered. In order to accurately compare phytoextraction capabilities among plants, a standard planting density (or optimal density) for each plant species was determined from the literature (Table 3.1). Taking into account, this optimal planting density, *T. pratense* (with an optimal density of 8000/m^2; Black, 1960) had the highest DDT extraction of 96,000,000 ng among the investigated species. Four species in this study (i.e., *S. novae-angliae*, *T. pratense, S. ptycanthum dun* and *V. thapsus*) achieved higher shoot DDT extractions than the known phytoextractor *C. pepo* (e.g. Lunney *et al*., 2004) on a per square metre basis. An onsite study at DDT-contaminated sites in Point Pelee National Park is required to directly compare the extraction efficiency of *C. pepo* to the wild grown native and naturalized weed species.

Table 3.1: Optimal planting density per square metre, mean shoot dry weight and calculated extraction per square metre for nine plant species of PPNP. The mean DDT shoot extraction of *C. pepo* ssp. *pepo* cv. Howden grown in DDT-contaminated soil from contaminated site (Lunney *et al*., 2004) is included for comparison. DDT extractions exceeding *C. pepo* are presented in bold.

Present Study (DDT)					Ficko et al., 2010 (PCB)	
Plant Species	**n**	**Optimal Density/ m^2**	**Mean Dry wt/plant (g)**	**DDT Extraction (ng)/m^2**	**Mean Dry wt/plant (g)**	**PCB Extraction (ng)/m^2**
S. canadensis	4	10[a]	11.4	32,000	62	4,200,000
T. pratense	3	8000[b]	5.5	**96,000,000**	25	110,000,000
S. novae-angliae	2	144[c]	4.2	**1,432,000**	38	14,000,000
C. sanguinea	4	2.5[d]	13.1	12,000		
S. vulgaris	3	20[e]	4.3	54,000		
L. cardiaca	3	10[g]	5.1	11,000		
S. ptycanthum Dun	3	150[h]	1.5	**97,000**	22	2,500,000
V. thapsus	3	44[i]	6.0	**650,000**	35	990,000
C. pepo cv. Howden	3	1[j]	14.1	**57,000**		

[a]Zhang *et al*., 2009; [b]Black, 1960; [c]United States Department of Agriculture (USDA), 2003; [d]National Resources Conservation Service (NRCS), 2003; [e]Pawlowska *et al*., 2000; [f]Oakwoods Monarch Way station, 2008 ; [g]Petersen *et al*., 2005; [h]Trader, 2001; [i]Gucker, 2008; [j]OMAFRA, 2000.

Plant biomass is a key issue for phytoextraction as it directly affects total contaminant extraction. It has previously been shown that weed species can adapt to poor growing conditions (Cunningham and Ow, 1996; Ligenfelter and Hartwig,

2007), and should therefore have a negligible impact on plant growth. However, the time of harvest also has a great influence on plant growth, and maximum plant biomass is usually obtained when plants begin blooming. In this study, the plants were harvested in early summer; hence, maximum biomass was not achieved due to a short growing season.

Comparisons of DDT (present study) and PCB (Ficko *et al*., 2010) extractions between five native weed species common to both studies are also presented in (Table 3.1). In all cases, PCB shoot extractions were higher; however this is likely due to lower plant biomass in the present study. It was also determined that *T. pratense* and *V. thapsus* can take up similar amounts of DDT as PCBs. Clearly, native (or naturalized) weed species have the potential to phytoextract both PCBs and DDT.

3.5 Conclusion

S. novae-angliae (New England aster) and *V. thapsus* (mullein) achieved the highest root and shoot DDT extractions, respectively from the nine wild growing plant species sampled in Point Pelee National Park. *T. pratense* (red clover) had the maximum shoot DDT extraction capacity per square metre in contaminated soil. It is important to investigate these species in a more controlled environment to fully assess their true phytoextraction capability. As the plants were grown wild in a wide range of DDT-contaminated sites, DDT uptake and BAFs may vary due to: i) age of the plant, ii) soil properties (e.g., nutrient status, organic carbon content and contaminant concentration, soil pH), and iii) various environmental factors, (e.g., precipitation, temperature). In addition, microbial interactions in the rhizosphere and root exudates can affect phytoextraction.

This study has demonstrated that all of the plant species studied have the ability to phytoextract DDT, and hence may be used to assist with the remediation of Point Pelee National Park. However, as phytoextractors, these species will also serve to move DDT through the food chain and are hence not acceptable choices to plant without precaution in areas where sensitive organisms may be affected.

3.6 Acknowledgements

We thank the Point Pelee National Park authority, especially Park Ecologist Tammy Dobbie for providing access to the park, Brian Campbell for analytical and technical assistance, undergraduate research assistants for assisting with field work, staff of the Analytical Service Unit (ASU) of Queen's University for technical, and the Natural Sciences and Engineering Research Council of Canada for the Collaborative Research and Development Grant to Drs. Barbara Zeeb and Allison Rutter (NSERC CRDPJ 344456).

3.7 Supporting information

Wet weight, length, total DDT concentration and TLF of native and naturalized species have been included in the Table A1-A6, Appendix A.

Chapter 4

Phytoextraction of DDT-contaminated soil at Point Pelee National Park, Leamington, ON, using *Cucurbita pepo* cv. Howden and native grass species

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Abstract

A field investigation was conducted at three DDT-contaminated areas in Point Pelee National Park (PPNP), Leamington, Ontario. *Cucurbita pepo* cv. Howden and three native grass species, *Schizachyrium scoparium* (little bluestem), *Panicum virgatum* (switchgrass) and *Sporobolus cryptandrus* (sand dropseed) were grown at three different sites in the Park having low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) soil DDT contamination levels. A threshold soil DDT concentration was identified at ~5000 ng/g where the DDT uptake into *C. pepo* was maximized, resulting in plant shoot and root DDT concentrations of 16,600 ng/g and 45,000 ng/g, respectively. Two native grass species (*P. virgatum* and *S. scoparium*) were identified as potential phytoextractors with higher shoot extraction capabilities than that of the known phytoextractor *C. pepo* when optimal planting density was taken into account.

4.1 Introduction

Persistent organic pollutants (POPs) are organic compounds that are stable in the environment due to their resistance to photolytic, biological and chemical degradation. They include polychlorinated biphenyls (PCBs) and dioxins, as well as numerous pesticides. 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and its metabolites 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (DDE) and 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene (DDD), are POPs which have had major environmental impacts as a result of DDT's widespread use as an insecticide (Ritter *et al.,* 1995). The persistence of DDT is indicated by its half-life, which ranges from 2 to 35 years (ATSDR, 2002; Crowe and Smith, 2007; White *et al*., 2010). In addition, as DDT has a high hydrophobicity with an octanol-water partition coefficient (log K_{ow}) > 6, it binds strongly to organic matter in the soil (Alexander, 2000; White *et al*., 2005; Wang *et al*., 2004). These qualities can lead to progressive sequestration of DDT, with later bioaccumulation in lipid cells, and biomagnification through the food chain (CCME, 1999; Alexander, 2000). Numerous studies have now shown that DDT may lead to nervous system disorders, reproductive and developmental problems, immune response suppression, cancer, and endocrine disruption in humans (Wania and Makay, 1996; Kelly and Gobus, 2001; Buccini *et al*., 2004; Wang *et al*., 2004).

DDT was used on a large scale in agricultural areas of Point Pelee National Park (PPNP) for pest control between 1947 and 1948, in addition to its use for mosquito control on Park roadways, campgrounds, and picnic areas until 1967. Thirty years later, Russell and Haffner (1997) detected significant levels of DDT in the tissues of reptiles and amphibians from PPNP. In the same study, elevated concentrations of DDT (up to 15,573 ng/g) were found in the Park soil. In another study, DDT was detected in nestling tree swallows, as well as in their dietary constituents, including insects (Smits *et al.*, 2004). In 2007, Crowe and Smith conducted a comprehensive study of DDT in the soil and sediments of PPNP. A maximum soil DDT concentration of 316,000 ng/g was identified in an area referred to as 'Former Agricultural Land'. This level far exceeds the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health of 700 ng/g DDT in areas designated as residential or parkland (CCME, 1999).

Traditional remediation techniques for DDT, such as excavation and incineration, are costly and destroy the soil matrix as well as the surrounding ecology (US EPA, 2002; Arthur *et al*., 2005). Therefore, Parks Canada personnel are seeking more environmentally friendly and economically viable techniques for DDT remediation within the Park. Phytoextraction, whereby plants take up contaminants through their roots and transfer them to the harvestable portion of the plant (Porebska and Ostrowska, 1999; Hülster *et al*., 1994; Lunney *et al*., 2004;

Whitfield Åslund *et al*., 2007, 2008, 2010) has been identified as an ecologically-favourable and potentially cost-effective method for DDT remediation.

The viability of phytoextraction for a particular site depends on various parameters, including the contaminant type and concentration, the extraction capacities of the plant species, and various other physical parameters, such as climate and soil type (Ficko *et al*., 2010; Anderson *et al*., 1995; Porebska and Ostrowska, 1999). Using the basic principles proposed by Hülster *et al.* in 1994, researchers have compared the DDT and PCB phytoextraction capacity of different plant species (White, 2002; White *et al*., 2003; Zeeb *et al*., 2006; Whitfield Åslund *et al*., 2007, 2008 and Ficko *et al*., 2010). Under field conditions, three varieties of *Cucurbita pepo* ssp *pepo* (Goldrush, pumpkin) (White, 2002) and zucchini (Wang *et al*., 2004) have shown significant capacity to take up weathered soil *p,p'*-DDE.

Weed species are potential phytoextractors of organic compounds (Lunney *et al*., 2004; White *et al*., 2005; Ficko *et al*., 2010, 2011a, 2011b). Advantages of using weed over crop species include that they self-sustaining, easy to propagate, more cost effective, and often have less chance of being consumed by herbivores, thereby resulting in a lower possibility for contaminants to be transmitted through the food chain (Ficko *et al*., 2010). Recent research indicates perennial weed species have the potential to phytoextract quantities of PCBs greater than that of known the phytoextractor *C. pepo* (Ficko *et al*., 2010).

In a 2011-12 study of nine wild grown native and naturalized weed species at PPNP, four of nine species exceeded the shoot DDT extraction of the known POPs accumulator C. *pepo* (Paul *et al*., subm.). Based on that success, PPNP authorities are interested in pursuing the efficacy of using native grass species to assist with the remediation of contaminated sites within the Park. The present study reports on the phytoextraction of DDT by *C. pepo* cv. Howden and three native grass species, *S. scoparium* (little bluestem), *P.virgatum* (switchgrass) and *S. cryptandrus* (sand dropseed) grown in monoculture plots at three different field sites in PPNP with low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contamination. Each of theses is a common Ontario plant species found growing widely at PPNP.

4.2 Materials and Methods

4.2.1 Site description and selection

Point Pelee National Park (PPNP) located in Leamington, Ontario, Canada consists of a peninsula of land (16 km^2) made up of marsh and woodland habitats. PPNP is ecologically important due to its support of significant bird and butterfly migrations and breeding habitats (Crowe and Smith, 2007). For this study,

three sites were selected on the basis of their mean DDT soil concentration. ‘Sleepy Hollow’ (SH), ‘Anders Field’ (AF) and ‘Former Agricultural Land’ (FAL) are referred to as low (291 ± 69.9 ng/g (n = 10)), moderate (5083 ± 1635 ng/g (n = 10)), and high (10192 ± 4842 ng/g (n = 10)) DDT-contaminated sites, respectively based on their mean soil DDT concentrations. The soil concentration of each site represents the average values of 10 soil concentrations – 7 soils sample from the root system of the plants (4 pumpkin and 3 native species) and 3 soils randomly collected from the plot area. Soil DDT at these sites is a mixture of *o,p’*-DDT, *p,p’*-DDT, *o,p’*-DDE, *p,p’*-DDE, *o,p’*-DDD and *p,p’*–DDD. The soils are comprised predominantly of sand with a total organic carbon content of 1.8% at SH, 3.4% at AF and 4.2% at FAL.

4.2.2 Plant selection

C. pepo cv. Howden was selected for this comparative phytoextraction study with native plant species as it is a known successful phytoextractor of DDT (e.g., Lunney *et al*., 2004; White, 2001; White *et al*., 2003 A recently completed study (Paul *et al*., subm.) determined that several native and naturalized weed species showed potential as DDT phytoextractors. PPNP authorities were particularly interested in the DDT phytoextraction potential of native growing grass species in the Park as they are comparatively easier to propagate than other weed species. For this reason, *P. virgatum*, *S. scoparium* and *S. cryptandrus*, all of whom are found growing throughout the Park, were selected for monoculture field trials.

P. virgatum was chosen as it has high tolerance for a wide range of environmental conditions. Moreover, it grows at high density and produces a large amount of biomass. Studies have determined that *P. virgatum* efficiently enhanced biodegradation of the organic contaminants 1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine (atrazine) and polycyclic aromatic hydrocarbons (PAHs) (Murphy *et al*., 2011 and McCutcheon and Schnoor, 2003). *P. virgatum* can also be used as a bioenergy crop and hence may be useful for wide-scale or long-term phytoremediation.

S. scoparium containing a flat bluish basal shoot is adapted to living in well drained to medium, dry, and infertile soils where it effectively controls soil erosion (USDA, 2002). It is widely distributed in North America and has excellent drought and flood tolerance. Plant height can range from 1-3 feet and plants can produce 255,000 seeds per pound. Recently, *S. scoparium* was used for the assessment of phytotoxicity assays in petroleum-contaminated soils (Kirk *et al*., 2010).

S. cryptandrus, belonging to the Poacea family, is a warm season perennial bunchgrass distributed across North America. This species is well known as a prolific seed producer which allows it to pioneer new areas including sandy soils. It is known to be extremely drought tolerant and its fibrous root system stabilizes the soil in sand dunes or hilly areas. Plant height ranges from 11-40 inches, and the width and length of leaf blades vary from 0.08-0.25 inches and 3-10 inches, respectively (Tilly *et al*., 2009).

4.2.3 Field plot preparation and cultivation scheme

A 4 m^2 area *C. pepo* plot and a 2 m^2 native grass species plots were established adjacent to one another at each of the three DDT-contaminated sites in PPNP (Figure 4.1). *C.pepo* cv. Howden seeds were obtained from the Ontario Seed Company (OSC), Waterloo, ON and seeds of native species were collected from plants found in PPNP by Park staff and volunteers under the Parks Canada Research and Collection Permit # PP-2009-4232. Seeds were grown under contract by the St. Williams Ecology Group of St. Williams, Ontario. Three *C. pepo* seeds were planted in each of four mounds (with all but 1 seedling removed upon germination), such that each *C. pepo* was grown in a 1 m^2 area. Five seedlings of each of the three native weed species, *P. virgatum*, *S. scoparium*, and *S. cryptandrus,* were planted maintaining a distance of 15 cm between each plant and 50 cm between each species. *C. pepo* and native grass species were planted on 6 July, 2011 and harvested 83 days later on 28 September, 2011. Plants were monitored on a weekly basis and watered as required.

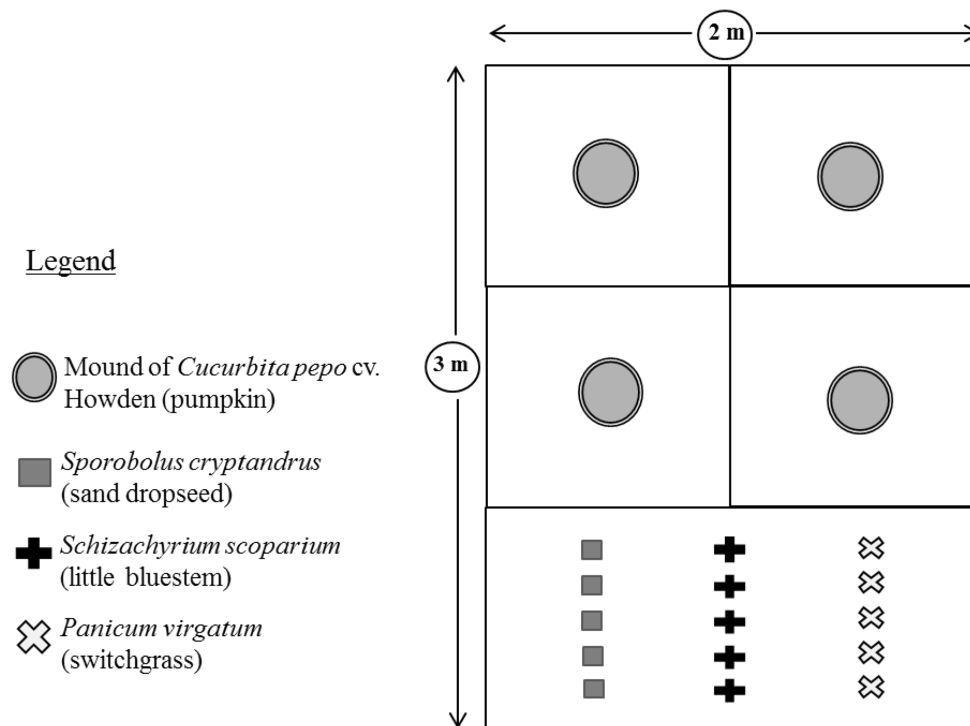

Figure 4.1: Schematic diagram of *C. pepo* and native grass species plots at each of the three DDT-contaminated sites in Point Pelee National Park.

4.2.4 Soil and plant sample collection

Soil collection: Thirty soil samples were collected from 0-10 cm depth from each experimental plot using a garden trowel. The trowel was cleaned between sampling. Soil samples from the root systems of *C. pepo* and native plants were collected at each site at the time of plant harvest. In addition, soil samples were collected randomly from the experimental plot area of each site. Samples were placed into labelled Whirlpak® bags and frozen at -20 ^{o}C until analysis.

Plant Harvesting: Prior to harvesting, a garden trowel was used to loosen the soil around the root, and extra soil was removed from the root by gently shaking the plant. The length of the plant's longest shoot was measured. The plants were separated into shoots and roots using scissors were rinsed with methanol or acetone between samples. Plant tissues were washed under clean running water, blotted dry with paper towel, and weighed. Finally, all plant tissues were placed individually into labelled Whirlpak® bags.

4.2.5 Selection of harvested plants for analysis

For analysis of *C. pepo* plants, 20 cm sections of shoots from the 'bottom', 'middle' and 'top' regions were collected. The subsampling procedure from Ficko *et al*. (2010) was used for native plants. Briefly, if the whole plant biomass was less than 50 g, the whole plant was homogenized with 10 to 15 g of the homogenate selected for analysis. When the whole plant biomass was greater than 50 g, a representative subsample of 10 to 15 g was selected for analysis and subsequently chopped and homogenized.

4.2.6 Analytical procedures for soil and plant samples

Plant samples were dried in an oven at 25-28 ^{o}C overnight prior to analysis and were then ground by mortar and pestle in preparation for extraction. The dry weights of soil and plant samples were recorded to determine their original moisture content. Twenty grams of each soil sample were air dried at room temperature for 24 h prior to analysis. Approximately 2 g of dried soil or plant sample were used for analysis. All pre-dried soil samples were extracted by Accelerating Solvent Extraction (ASE) using Hexane (H303-4; Fisher Scientific, USA) as a solvent. Plant samples were extracted using the Soxhlet method for at least 4 to 5 h (4-6 cycles per hour) using 250 ml dichloromethane (DCM) (D151-4, Fisher Scientific; USA) as a solvent. Samples were mixed with approximately 10 g of Ottawa sand and 10 g of sodium sulphate prior to extraction. The extracted sample was concentrated using a Buchi syncore to ~2 ml and solvent exchange was done by ~3 ml aliquots of hexane three times. The solution was then filtered using a florisil column and adjusted to 10 ml in a volumetric flask.

The extracted samples were analyzed by gas chromatography (Agilent 6890 Plus). The gas chromatograph was equipped with a ^{63}Ni electron capture detector GC/ECD), a SPB-1 methyl siloxane capillary column (30 m x 0.25 mm x 0.25 µm film thickness) with constant flow. The chromatograph was programmed to an initial temperature of 100 ^{o}C for 1 minute, ramped at 8 ^{o}C/min to 180 ^{o}C, followed by 3 ^{o}C/min to 220 ^{o}C, and held for 3 minutes at 220 ^{o}C. The chromatograph was finally ramped at 20 ^{o}C/min to 300 ^{o}C where the temperature was maintained at 300 ^{o}C for 5 minutes. The total run time was 36.33 minutes. Nitrogen was used as the makeup gas for ECD detector where temperature in ECD was maintained at 250 ^{o}C. Helium was used as a carrier gas.

4.2.7 Quality assurance and control

Nine samples were extracted at a time and subsequently processed with one analytical blank, one control sample and one analytical duplicate. The control consisted of Ottawa sand and sodium sulfate spiked with 100 µl of an organochlorine pesticide mixture at 2 ppm named Appendix IX, supelco. The surrogate standard was 1 ppm 100 µl decachlorobiphenyl (DCBP) (D442537; Sigma Aldrich, USA). Sample concentrations were corrected for surrogate recovery. All analytical blanks were less than 1.0 ng/g (below the detection limit) and the mean recovery of the internal surrogate standard was 97% for both soil and plant samples. The mean relative standard deviation between the samples and their analytical duplicates was 20% for plant samples and 10% for soil samples.

4.2.8 Statistical analysis

A one way Analysis of Variance (ANOVA) was employed to analyze the data following testing for normality (Kolmogorov-Smirnov test). The data was transformed using the natural logarithm ($\log_e$) to increase the normality of the data set. A one way ANOVA was used to compare shoot and root DDT concentrations as well as the shoot and root BAFs of *C. pepo* plants between sites. A one way ANOVA was also conducted to compare root and shoot DDT concentrations and root and shoot BAFs between the three native weed species at each site. All statistical analysis was carried out using TIBCO Spotfire S^+ software with a significance level of $p = 0.05$.

4.3 Result and Discussion

4.3.1 Plant growth and biomass of plant tissues

The three native grass species and *C. pepo* cv. Howden grew well at each of three DDT-contaminated sites in PPNP. *C. pepo* plants showed significantly higher shoot length and biomass at the high DDT-contaminated site in comparison to the low DDT-contaminated site (Table B 7, Appendix B), but no significant differences were found in shoot length or biomass between the moderate and high DDT-contaminated sites. Lower *C. pepo* biomass at the low DDT-contaminated site was likely due to very dry soil conditions. No significant differences were observed in shoot biomass and length for the native grass species at the different sites. In contrast to a study of plants grown in PCB-contaminated soil (Zeeb *et al.*, 2006), there was no indication that *C. pepo* or native grass species were stunted or otherwise stressed in the DDT-contaminated soil.

4.3.2 DDT concentration in *C. pepo* plant tissues.

Mean *C. pepo* root DDT concentrations of 14500 ng/g and 920 ng/g were found at the high and low DDT-contaminated sites, respectively whereas the highest mean root DDT concentration of 29600 ng/g was found at the moderate DDT-contaminated site (Figure 4.2). Mean shoot DDT concentrations were 8100 and 1350 ng/g at the high and the low DDT-contaminated sites, respectively. Again, the highest mean shoot DDT concentration of 12000 ng/g was found at the moderate DDT-contaminated site (Figure 4.2).

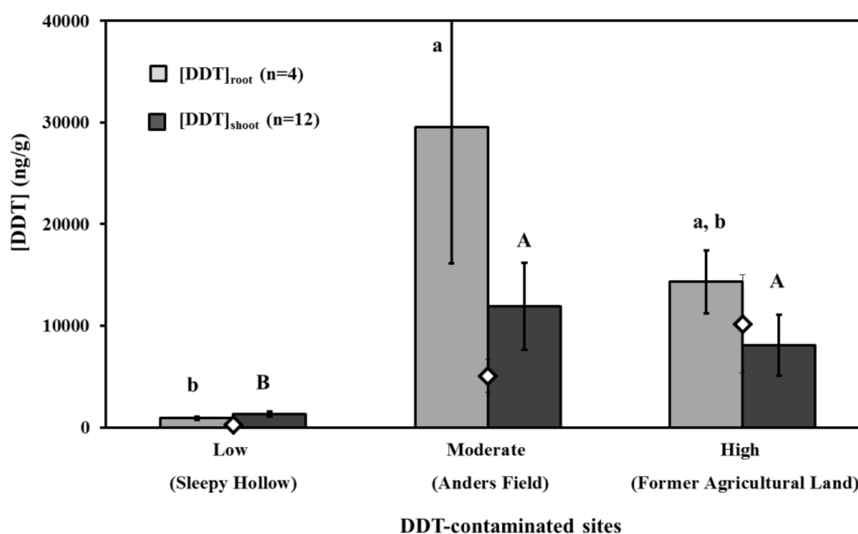

Figure 4.2: Mean [DDT] for *C. pepo* plants grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites in PPNP. The error bars represent one standard deviation. Lowercase and uppercase letters indicate root and shoot DDT concentrations, respectively which are significantly different between the sites ($p < 0.05$). ◊ indicates the mean soil DDT concentration at the site. Note that four plants were analyzed at each site. Three subsections of the shoots (n = 12) and root (one sample per plant) (n = 4).

To further investigate the effect of soil DDT concentration on plant DDT uptake, root and shoot DDT concentrations for all 12 plants were plotted with the soil DDT concentration of the corresponding plant. DDT uptake into plant tissues increased with increasing soil DDT concentration and reached peak levels of 45,000 ng/g and 16,600 ng/g in root and shoot tissues, respectively at a soil DDT

concentration of 4650 ng/g (Figure 4.3). Thereafter (at soil DDT concentrations >5000 ng/g), DDT uptake into *C. pepo* tissues declined sharply. Even at soil DDT concentrations of 20,000 ng/g (from the high DDT-contaminated site) both root and shoot uptake remained stable with tissue concentrations similar to those found in soil at 8000 ng/g DDT. This may indicate a threshold level for *C. pepo* uptake of DDT, or may be in response to the different soil types particularly the different soil organic contents (i.e. 1.8% at low, 3.4% at moderate and 4.2% at high) found at the three different contaminated sites.

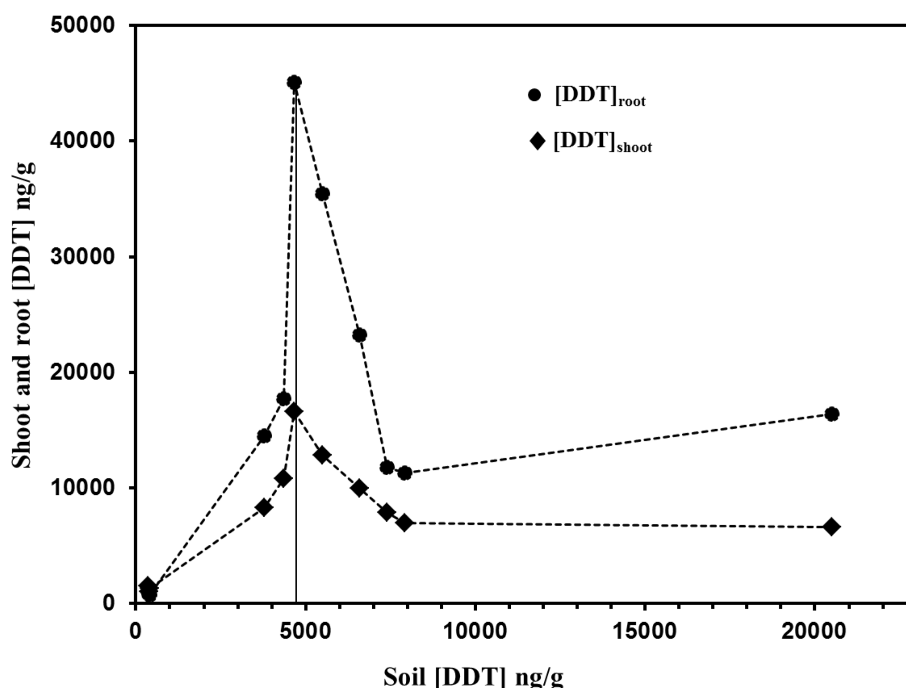

Figure 4.3: *C. pepo* shoot and root DDT concentrations in relation to DDT concentrations in soil. ♦ indicates the shoot DDT concentration and ● indicates the root DDT concentration. Solid line indicates threshold soil DDT concentration.

Previous investigators (Whitfield Åslund *et al*., 2007, 2008) have shown decreasing PCB concentrations with increasing distance from the root of *C. pepo.* Figure 4.4 shows shoot DDT concentrations along the stem of *C. pepo* using plant samples from each at the three DDT-contaminated areas in PPNP. DDT concentrations decrease with increasing distance from the root at the moderate and high DDT-contaminated sites, but showed a linear behavior at the low DDT-contaminated site. No significant differences were found between the shoot

subsections at the low DDT-contaminated site, but this is likely due to the short length of plant (mean shoot length 116 m) which is half that of the moderate and high DDT-contaminated sites. Similar observations were found for pumpkin plants grown in potting soil contaminated with PCBs (2700 ng/g) (Whitfield Åslund *et al*., 2008). The pattern of PCB uptake was explained by the presence of nodal adventious roots, however in the current DDT study there was no indication that adventious roots were present. At the moderate DDT-contaminated site, mean DDT concentrations of 29,250, 5530, 1010 ng/g were calculated for bottom, mid and top shoot sections, respectively for *C. pepo* (Figure 4.4). A one-way ANOVA confirmed that these DDT concentrations were statistically different ($p < 0.05$). At the high DDT-contaminated site, mean DDT concentrations of 13925, 7260, 3050 ng/g were measured for the bottom, mid and top shoot sections, respectively, where bottom and mid shoot DDT concentrations were significantly greater than the top shoot

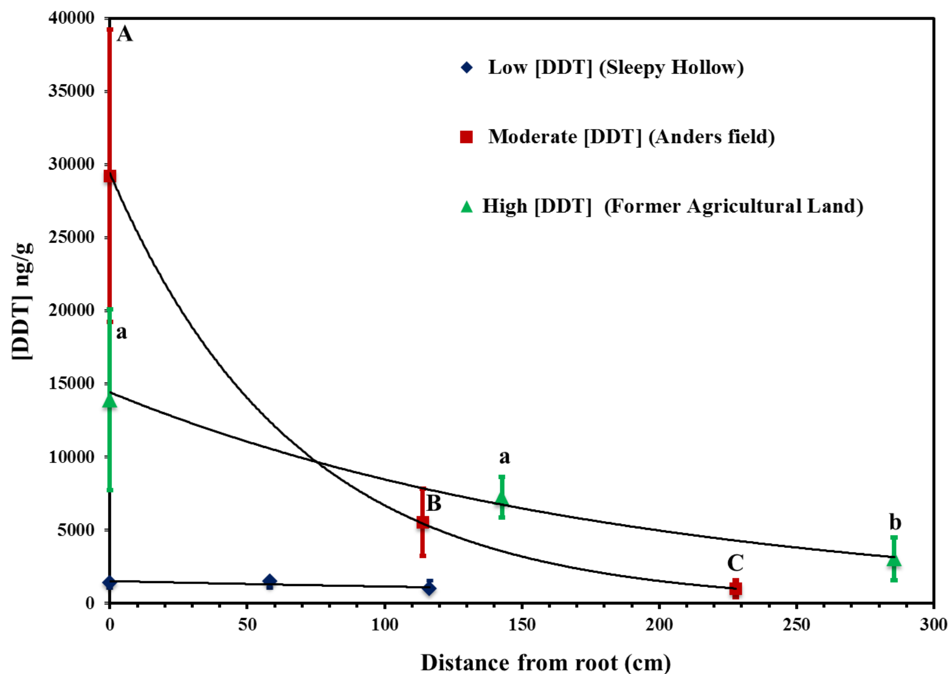

Figure 4.4: DDT concentrations in shoot segments of *C. pepo* at the low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. Uppercase and lowercase letters indicate DDT concentrations in shoot segments were significantly different at the moderate and high DDT-contaminated sites, respectively ($p < 0.05$).

4.3.3 Bioaccumulation Factors (BAFs) and Translocation Factors (TLFs) in *C. pepo*

The efficiency of phytoextraction may be calculated using bioaccumulation factors ($[\text{contaminant}]_{\text{plant tissue}}/[\text{contaminant}]_{\text{soil}}$) and translocation factors ($[\text{contaminants}]_{\text{shoot tissue}}/[\text{contaminants}]_{\text{root tissue}}$). A maximum mean root BAF of 5.9 was calculated in *C. pepo* grown at the moderate DDT-contaminated site, and was almost three times higher than at the high DDT-contaminated site. This root BAF is comparable with the field study conducted by White et al. (2003) where the mean root BAF of *C. pepo* was 7.2 for the weathered p,p'-DDE-contaminated soil. The root BAF of 2.7 in *C. pepo* at the low DDT-contaminated correlates well with Lunney *et al*. (2004) who found a root BAF of 2 in their low DDT-contaminated soil in a greenhouse study. In contrast, the highest mean shoot BAF of 3.8 was found in *C. pepo* grown at the low-DDT contaminated site, which was significantly greater than shoot BAF of 1.2 at the high DDT-contaminated site,

but not than the shoot BAF of 2.4 at the moderate DDT-contaminated site (Figure 4.5).

TLFs values were below one, except in the low DDT-contaminated soil (TLF 1.4), which was significantly greater than at the moderate and high DDT-contaminated sites (Figure 4.5). Here TLFs are similar to the TLFs of 1.2 recorded in low DDT (150 ng/g)-contaminated soil by Lunney *et al*. (2004). In contrast, lower TLFs were measured in *Cucumis sativus* (ranging from 0.02 to 0.05) in *p,p'*-DDE-contaminated soils (Wang *et al*., 2004).

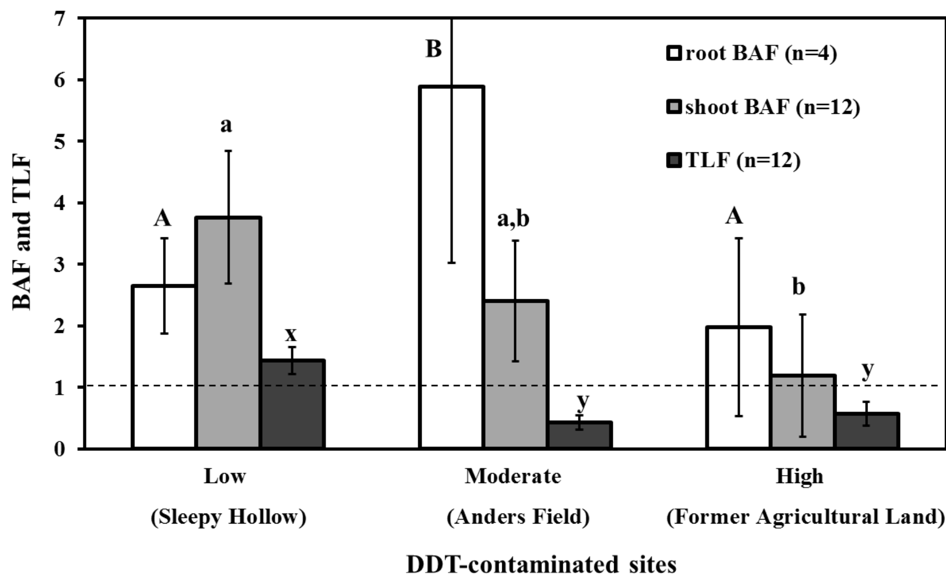

Figure 4.5: **Comparison of shoot and root BAFs and TLFs of *C. pepo* grown at the low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. The error bars represent standard deviation of the mean BAFs and TLFs. Uppercase letters (A, B) indicate root BAFs and lowercase letters (a, b) indicate shoot BAFs and lowercase letters (x, y) indicate TLFs which were significantly different at each of the DDT-contaminated sites (p < 0.05).**

4.3.4 DDT concentration in root and shoot tissues of native grass species

Root and shoot DDT concentrations in native grass species were below the detection limit at the low DDT-contaminated site. Root DDT concentration ranged

from 1100 ng/g (*P. virgatum*) to 5000 ng/g (*S. cryptandrus*) and shoot DDT concentration ranged from 370 ng/g (*S. cryptandrus*) to 770 ng/g (*S. scoparium*) at the moderate DDT-contaminated site (Figure 4.6a and Figure 4.6b). Root DDT concentration ranged from 1900 ng/g (*P. virgatum*) to 11,000 ng/g (*S. cryptandrus*) and shoot DDT concentration ranged from 1000 ng/g (*P. virgatum*) to 3600 ng/g (*S. scoparium*) at the high DDT-contaminated site (Figure 4.6a and Figure 4.6b).

The DDT concentrations for both root and shoot tissues of the native grass species increased in the high DDT-contaminated site in comparison to the moderate DDT-contaminated site, with shoot concentrations being significantly higher for all three species. This trend of increasing DDT accumulation with increasing DDT soil concentration does not follow the trend of DDT accumulation in *C. pepo*. Similarly Zeeb *et al*. (2006) observed that *Festuca arundinacea* (tall fescue), *Carex. normalis* (sedge) and *Glycine max* (soybean) grown in soil with 90,000 ng/g PCBs had lower PCB uptake in root and shoot tissues than that of the same plants grown in higher PCB-contaminated soil (4,150,000 ng/g). Likewise, Ficko *et al*. (2010) reported that weed species had greater PCB concentrations in root and shoot tissues when the plants were grown in 31000 ng/g PCB soil, than when grown in 4700 ng/g PCB.

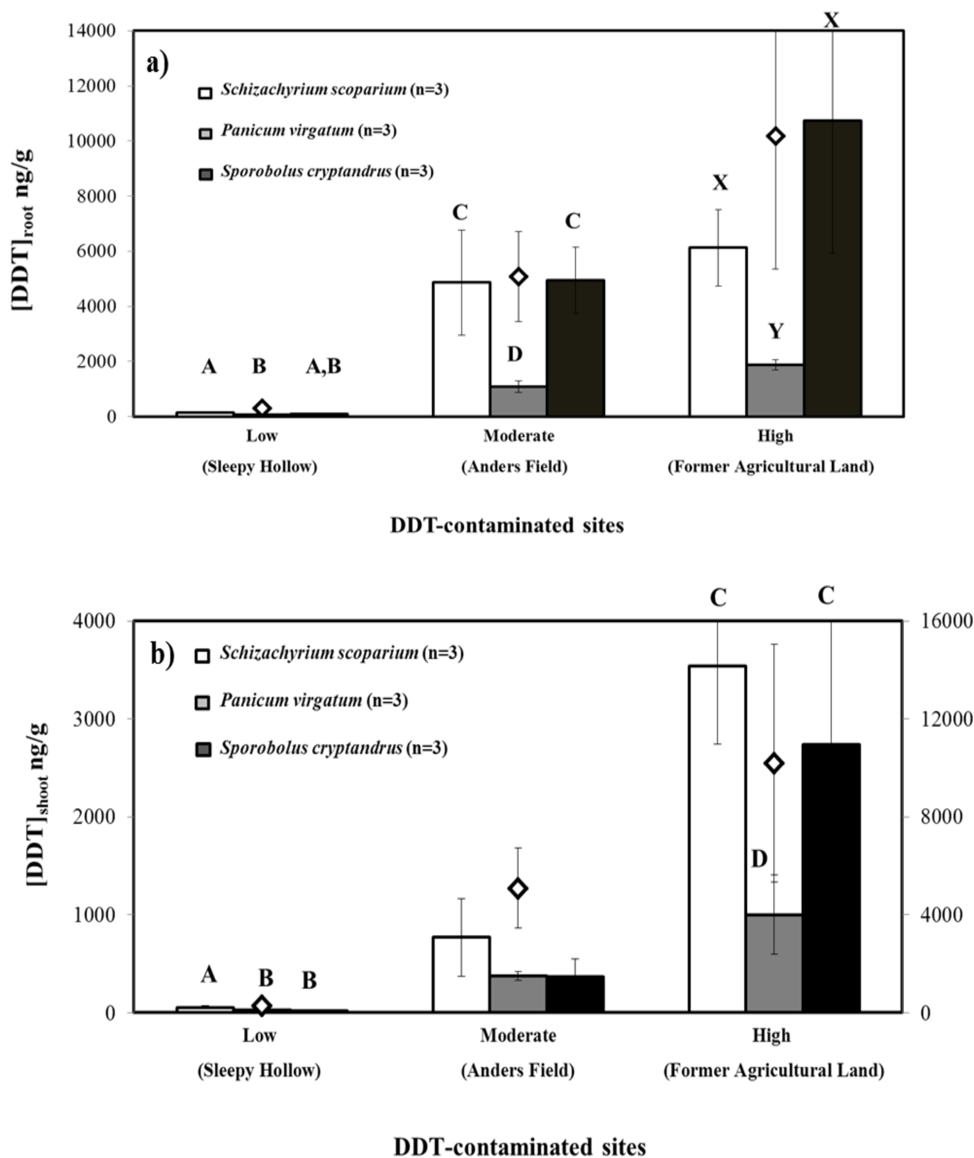

Figure 4.6: **Comparison of DDT concentrations between the native grass species at the low (291 ng/g), moderate (5083 ng/g) and high DDT (10192 ng/g)-contaminated sites in a) root and b) shoot tissues. Error bars indicate one standard deviation of the mean. Letters indicate that significant differences in root and shoot DDT concentrations were found between the species at each of DDT-contaminated site where ($p < 0.05$). ◊ indicates the soil DDT concentration at each site.**

4.3.5 BAFs in native grass species

In the current study, a maximum root BAF of 1.9 was found in *S. cryptandrus* at both the moderate and high DDT-contaminated sites. Root BAFs of 1.24 (*S. scoparium)* and 0.26 in (*P. virgatum*) were measured at the moderate and high DDT–contaminated sites, respectively (Figure 4.7a). Shoot BAFs of native grass species were slightly lower at the moderate DDT-contaminated site compared to the low DDT-contaminated site (Figure 4.7b). However, differences in shoot BAFs among the species were insignificant at the moderate and high DDT-contaminated sites.

Previous studies examining weed species for PCB phytoextraction have achieved shoot BAFs ≥1 (Whitfield Åslund *et al*., 2007; 2008; Zeeb *et al*., 2006; Ficko *et al*., 2010), indicating good performance for phytoextraction. However, no previous studies have found DDT shoot BAFs ≥1 in native grass species (Lunney *et al*., 2004; White *et al*., 2005). BAFs are primarily species and site dependent, with soil properties such as particle size and the concentration of organic compounds affecting them. For example, soils with smaller particle sizes (i.e. clay rich) may strongly adsorb organic contaminants reducing plant BAFs.

The native grass species exhibited TLFs values ranging from 0.23 (*S. cryptandrus*) to 0.42 (*S. scoparium*) and from 0.27 (*S. cryptandrus*) to 0.60 (*S. scoparium*) at the low and high DDT-contaminated sites, respectively (Table B 9, Appendix B). TLFs were higher in the high DDT-contaminated site in comparison to low and moderate DDT-contaminated sites. Only *S. scoparium* and *P. virgatum* had TLFs > 0.50 at the high DDT-contaminated site.

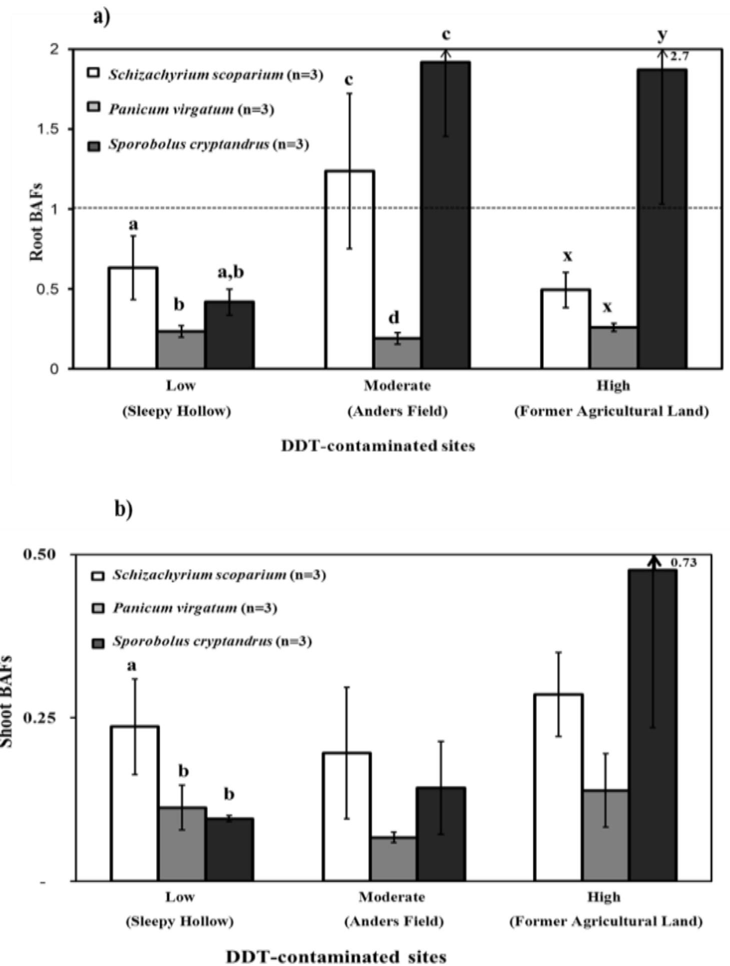

Figure 4.7: **Comparison of root a) and b) shoot BAFs between the native weed species at low (291 ng/g), moderate (5083 ng/g) and high DDT (10192 ng/g)-contaminated sites. Error bars indicate the standard deviation of the mean. The letters indicate that significant difference was found between the species at sites where (*p* <0.05).**

4.3.6 Phytoextraction potential based on optimal plant density

It is essential to compare total amounts of DDT (ng) extracted by different plant species for a given area or volume of soil to compare their actual phytoextraction ability. For example, *C. pepo* grows optimally at one plant per metre square (OMAFRA, 2000), while *S. scoparium* grows optimally at 50 plants

per metre square (Adler *et al*., 2004), and *P. virgatum* and *S. cryptandrus* at 170 (Gettle *et al.,* 1994) and 12 plants per metre square, respectively (Weaver *et al.,* 1954). Total shoot extraction per square metre of area at the high and moderate DDT-contaminated sites are presented in Table 4.1.

P. virgatum extracted more than double the amount of DDT (2,110,000 ng) per square metre compared to *C. pepo* (716,000 ng) at the high DDT-contaminated site (Table 4.1). *S. scoparium* also had a higher shoot DDT extraction (1,640,000 ng) than *C. pepo* but this difference was not statistically significant at the same site. *C. pepo* did however have the highest shoot extraction (1,380,000 ng) per square metre in at the moderate DDT-contaminated site.

Whitfield Åslund *et al*. (2007) observed that sedge can take up higher PCB concentrations (4,800,000 ng) per square metre compared to other species, such as *C. pepo* and tall fescue. Similarly, Ficko *et al.* (2010) that 18 of 27 native weed species took up similar or greater quantities of PCBs than *C. pepo* cv. Howden on a per square metre basis. Therefore, native weed species appear to be useful for both the phytoextraction of PCBs and DDT.

Table 4.1: Comparison of shoot DDT extraction per square metre for plants at the moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites in Point Pelee National Park.

DDT-contaminated site	Plant species	Mean shoot dry wt. of plant (g)	Mean shoot [DDT] (ng/g)	Plant density/m^2	Total mean shoot DDT extraction/m^2 (ng)
High (Former Agricultural Land)	*C.pepo* cv. Howden (pumpkin)	88.4	8100	1[a]	**716,000**A
	S. scoparium (little bluestem)	9.10	3600	50[b]	**1,640,000**A,B
	P. virgatum (switchgrass)	12.4	1000	170[c]	**2,110,000**B
	S. cryptandrus (Sand dropseed)	6.60	2700	12 [d]	214,000C
Moderate (Anders Field)	*C.pepo* cv. Howden (pumpkin)	115	12000	1[a]	1,380,000A
	S. scoparium (little bluestem)	8.60	770	50[b]	331,000A,B
	P. virgatum (switchgrass)	13.3	380	170[c]	860,000A
	S. cryptandrus (Sand dropseed)	16.4	370	12 [d]	73000B

[a]OMAFRA, 2000; [b]Adler *et al*., 2004; [c]Gettle *et al.,* 1994; [d]Weaver *et al.,* 1954

4.4 Conclusions

DDT uptake by native grass species increased with increasing soil DDT concentration. In contrast, DDT uptake by *C. pepo* increased from the low to medium soil DDT concentration, but did not increase further in the high soil DDT concentration. Further investigation is required to determine whether the threshold $[DDT]_{soil}$ of ca. 5000 ng/g is related to total organic content in the soil and/or other factors. Two native grass species, *P. virgatum* (switchgrass) and *S. scoparium* (little bluestem), have higher shoot DDT extraction potential than *C. pepo* on a per square metre basis at the high DDT-contaminated site. A follow up investigation is required to determine whether the optimal planting density for each of the species can be achieved in PPNP. These data indicate that DDT phytoextraction using native resident grass species may be a viable remediation strategy in Point Pelee National Park.

4.5 Acknowledgements

We thank the Point Pelee National Park authority, especially Park Ecologist Tammy Dobbie for providing access to the park, Brian Campbell for analytical and technical assistance, undergraduate research assistants for assistance in field work, staff members of the Analytical Service Unit (ASU) of Queen's University for technical help in experiment, Alexis Pietak for proof read of the manuscript and the Natural Sciences and Engineering Research Council of Canada for the Collaborative Research and Development Grant to Drs. Barbara Zeeb and Allison Rutter.

4.6 Supporting information

Wet weight, length, total DDT concentration, total mean shoot extraction and TLF of *C. pepo* and native weed species at low, moderate and high DDT-contaminated site have been included in the Table B1-B9, Appendix B.

Chapter 5

Phytoextraction of weathered DDT by perennial Native and Naturalized weed species under greenhouse conditions

Abstract

DDT-uptake and shoot translocation capacities of three native weed species (*Solidago Canadensis* (Canada goldenrod), *Symphyotrichum novae-angliae* (New England aster), *Chrysanthemum leucanthemum* (ox- eye daisy) and one naturalized species, *Trifolium pratense* (red clover) of Point Pelee National Park were investigated in a greenhouse study. Of the four species, *C. leucanthemum* showed the highest root and shoot DDT uptake (132,000 and 5000 ng/g, respectively). In contrast, when extraction per square metre was calculated based on optimal plant density, *T. pratense* attained the highest shoot DDT extraction efficiency and was 1- 2 orders of magnitude higher than that of the known DDT phytoextractor *Cucurbita pepo* ssp. *pepo* cv. Howden (pumpkin). The uptake patterns of DDT metabolites of the four species were also investigated. The root and shoot DDT metabolite uptake patterns were similar to those in the soil. Overall, it was observed that 4,4' metabolites have the highest uptake rate (from soil to root and shoot tissues), whereas 2,4' metabolites uptake was negligible. Mechanisms of uptake need to be further investigated to understand these patterns and determine how they can be used to enhance DDT uptake into plants.

5.1 Introduction

Point Pelee National Park (PPNP), situated in Leamington, ON, is an ecologically sensitive area that supports significant bird and butterfly migrations and bird breeding habitats. Until the mid-1970s, DDT was extensively used for mosquito control in Park campsites and picnic areas, and was used to control pests in an agricultural area of the Park. Elevated DDT levels far exceeding the level of 700 ng/g of soil recommended by CCME guidelines (CCME, 1999) are still found in the soils of PPNP today. A maximum DDT level of 316,000 ng/g in soil was documented at a Former Agricultural Land (FAL) site in 2007 (Crowe and Smith, 2007). In 2011, the phytoremediation research group at the Royal Military College (RMC) measured up to 108,000 ng/g of DDT at this site. Furthermore a significant level of DDT have accumulated in the tissues of PPNP amphibians, reptiles and birds (Russell and Haffner, 1997; Smits *et al*., 2004). Hence, the existing levels of soil DDT still pose a threat to the ecology and environment of PPNP. Conventional remediation techniques involving excavation and treatment of the soil which will disturb the ecological integrity of the Park are not in line with the Parks Canada mandate.

One potentially viable technique for DDT remediation is phytoextraction, which involves root uptake of the soil contaminant followed by its translocation to the harvestable portion of the plant. The success of phytoextraction depends on the efficiency of the phytoextracting plant, which is influenced by various parameters including in (i) the soil DDT concentration, (ii) the cultivation environment, (iii) the achievable plant biomass, (iv) the supported planting density of the phytoextracting plant, and (v) the inherent extraction and shoot translocation capacities of the plant.

DDT and its metabolites (e.g. *p,p'*-DDE) have been phytoextracted using a number of crop species including *Cucurbita pepo* ssp. *pepo* (White, 2002; White *et al.,* 2003; Whitfield Åslund *et al*., 2010; Lunney *et al*., 2004, 2010) and *Cucumis sativus* (Gent *et al*., 2006). However, weed species, or native colonizing species, may offer advantages over the use of crop species, especially in a location such as PPNP. Many weed species are native to the park, and they are easily propagated, self-sustaining, and can tolerate unfavorable growing conditions. In addition, native weed species do not pose any human health risk as they do not produce edible crops for human consumption. Weed species have been effectively used for inorganic contaminant remediation, and are widely accepted by remediation specialists (Aboulroos *et al*., 2006; McGrath and Zhao, 2003). To date, weed species have been found capable of successful remediation of PCBs from contaminated soil, under both field and greenhouse conditions (Ficko *et al*., 2010, 2011a, 2011b), but very few studies have investigated the use of weed species for DDT remediation (Lunney *et al*., 2004; White *et al*., 2005).

When using weed species, the extraction efficiency may be underestimated based on the significant biomass differences between these species and crop species, such as *C. pepo* (Ficko *et al*., 2010). Hence, plants must be compared based on their extraction efficiency unit area (Whitfield Åslund*et al*., 2007, Ficko *et al*., 2010 and Ficko *et al*., 2011b). A comprehensive study including 27 wild-grown weed species for PCB remediation demonstrated that 18 species (i.e. 67%) had similar or higher PCB shoot extraction capabilities than the known phytoextractor *C. pepo* ssp. *pepo* cv. Howden (pumpkin). Another study demonstrated that three perennial weed species (*Chrysanthemum leucanthemum* (ox-eye daisy), *Rumex crispus* (curly dock) and *Solidago canadensis* (Canada goldenrod)) extracted higher quantities of PCBs than *C. pepo* even at sub-optimal planting densities (Ficko *et al*., 2011b).

DDT consists of six isomers, and the isomeric composition of soil depends on the age of the DDT and the degradation environment (*i.e.* aerobic or anaerobic). However, the accumulation and translocation of these isomers from the soil varies from plant species to species. Structural variation in DDT isomers can be influential for plant uptake. For example, it was observed that 2,4'-DDD was preferentially translocated to *Medicago sativa* (alfalfa) shoot tissue, but not to *C. pepo* ssp. *pepo* cv. Howden (pumpkin) and *C. pepo* L. cv. Senator hybrid (zucchini) stem tissue (Lunney *et al*., 2004). To date, no studies have investigated the mechanism of DDT uptake by weed species.

This greenhouse study reports on the DDT uptake and extraction capability of four perennial plant species native and naturalized to PPNP (*S. canadensis, S. novae-angliae, C. leucanthemum and T. pratense*) at low (2300 ng/g) and high (17,500 ng/g) soil DDT levels. Growing plants in the controlled environment of a greenhouse minimizes the variation of environmental factors and allows for the use of controlled soil concentrations to be used. A comparison between the phytoextraction capability per square metre of i) greenhouse grown weed species, ii) wild grown weed species, and iii) field cultivated *C. pepo* ssp. *pepo* cv. Howden (pumpkin) grown in historically DDT-contaminated soil from PPNP is presented. In addition, DDT isomer uptake pattern in the four perennial species are investigated.

5.2 Methodologies

5.2.1 Soil selection and processing

DDT contaminated soil was collected from Point Pelee National Park, Leamington, Ontario in 2011. Additional soil was collected from the same contaminated site in 2012. As the DDT concentration of PPNP soils was found to be heterogeneous, the collected soils were thoroughly homogenized using the

process described in Low *et al*. (2010) and Ficko *et al*. (2011a). Briefly, soil collected in 2011 was sieved through a 1 cm^2 sieve and consolidated in one pile (~100 L). The original pile of soil was quartered on the table by random scooping using a flat-bottom scoop. Each of the four piles was manually mixed and reconstructed into a central pile by scooping from the four piles. The procedure was repeated 30 times for complete homogenization. The soil collected in 2012 (~120 L) was homogenized using the same method. Homogenized soil DDT concentrations of 2011 and 2012 were ~17500 ng/g and ~2300 ng/g, respectively, and are referred to as the 'high' (17500 ng/g) and 'low' (2300 ng/g) DDT-contaminated soil groups with TOCs of 7.4% and 2.7%, respectively. Both soils are predominantly composed of sand and silt.

5.2.2 Species selection, seed collection, and germination

Four plant species were selected based on their previous performance as PCB phytoextractors (Ficko *et al*., 2010; 2011b), as well as their performance observed in initial field work in PPNP (Chapter 3). *S.canadensis* (Canada goldenrod)*, S. novae-angliae* (New England aster)*, C. leucanthemum* (ox-eye daisy) were chosen from the Asteraceae family, and *T. pratense* (red clover) was chosen from the Fabaceae family. *S. canadensis* and *C. leucanthemum* were chosen based on their high biomass and previously demonstrated shoot extraction capabilities for both for PCBs (Ficko *et al*., 2010) and DDT. *S. novae-angliae* showed excellent capability to accumulate DDT in root tissue and can grow at high density. *T. pratense* was similarly selected as it grows at high density (Black, 1960), and has demonstrated capability to extract both DDT (chapter 3) and PCBs (Ficko *et al*., 2010). Seeds of *S. canadensis, S. novae-angliae* and *T. pratense* species were purchased from the Ontario Seed Company (OSC) Ltd. in Waterloo, ON and *C. leucanthemum* seeds were purchased from Richter Herbs, Goodwood, ON. Approximately 600 seeds per species were sown in clean moistened potting soil in eight germination trays (50.8 cm x 25.4 cm x 6 cm). Each tray's surface was wrapped with clear plastic and placed in the greenhouse at 25 ºC. *T. pratense* seeds germinated after seven days, *C. leucanthemum* and *S. novae-angliae* germinated in eight to nine days, and *S. canadensis* germinated in six days. Approximately 45 day old seedlings of *S. canadensis* and 60 to 65 day old seedlings of the other three species were transplanted into the low and high DDT-contaminated homogenized soils.

5.2.3 Experimental design

Homogenized DDT-contaminated soil was distributed amongst 16 containers (8 low and 8 high) (Kainos Hybrid Plastic) measuring 30 cm x 15 cm x 15 cm, each containing 3 litres of soil. Each of the four species was planted in both low and high DDT-contaminated soil. Four replicates (n = 4) of each species in

each container were transplanted into the low and high DDT-contaminated soil (n = 8) containers (Figure 5.1). Each species had duplicate containers of each of the two soil concentrations. Before transplantation, the soil of each container was moistened with water.

All of the containers were placed into an enclosure in the greenhouse with 12 hours of light and a room temperature of 26 ºC. Containers were watered on a regular basis as required, usually at least three days per week. Any non-specific weeds found growing in the containers were removed. One month after transplantation, plant containers were removed from the enclosure and placed into the greenhouse to access natural sunlight. Plant growth, stem and leaf appearance were monitored on a weekly basis.

Low DDT-contaminated soil (2300 ng/g)

SN1 SN2 SN3 SN4	SN5 SN6 SN7 SN8	TP1 TP2 TP3 TP4	TP5 TP6 TP7 TP8	CL1 CL2 CL3 CL4	CL5 CL6 CL7 CL8	SC1 SC2 SC3 SC4	SC5 SC6 SC7 SC8

High DDT-contaminated soil (17500 ng/g)

SN1 SN2 SN3 SN4	SN5 SN6 SN7 SN8	TP1 TP2 TP3 TP4	TP5 TP2 TP3 TP4	CL1 CL2 CL3 CL4	CL5 CL6 CL7 CL8	SC1 SC2 SC3 SC4	SC5 SC6 SC7 SC8

SN- *Symphyotrichum novae-angliae* TP- *Trifolum pratense* CL- *Chrysanthemum leucanthemum* SC- *Solidago canadensis*

Figure 5.1: Schematic representation of the placement of weed seedlings (n = 4) in containers of low and high DDT-contaminated soil, respectively. Each of the species was grown in duplicate containers for each contamination group.

5.2.4 Harvesting

Plant species were harvested at the end of their representative growth cycles. *T. pratense* was harvested in the flowering stage after 80 days, *S. canadensis* was harvested after 116 to 140 days, *S. novae-angliae* was harvested in the flowering stage after 137 days, and *C. leucanthemum* was harvested after 133 days. Four to five randomly selected plants per species were harvested from each of the low and high DDT-contaminated soils. The harvesting procedure is described in detail in Ficko *et al*. (2010). Briefly, soil was loosened around the root and the extra soil was removed. Plants were separated into root and shoot tissues,

the length of the root tissues were measured (cm), they were cleaned under running water to remove all particles, blotted dry with paper towel and then the tissues were weighed to a hundredth of a gram. Samples were placed into labelled Whirl-Pak® bags and kept at -15 ºC until analysis.

5.2.5 Analytical procedure

Soil Sample Extraction by Soxhlet Extraction

Soil samples were extracted using the soxhlet extractor which was described in the earlier chapter. Briefly, 20 g of soil was air dried at room temperature for twenty-four hours and 2 g of soil was used for analysis. Approximately, 250 ml of dichloromethane (DCM), 10 g of Ottawa sand, and 10 g of sodium sulphate were mixed with the sample for an extraction period of at least 4 to 5 hours (4 to 6 cycles per hour). The extraction procedure progressed through several condensation and solvent vaporization cycles. The extracted volume was concentrated in a (Büchi Rotavapor, R-114, 500 psi: DCM, 320 psi: Hexane, with temperature set 37-40 ºC) to approximately 2 ml. At least ~3 ml aliquots of hexane were added to solvent exchange the concentrated extraction. This was completed a total of 3 times to ensure the solvent was completely hexane. Finally, the concentrated extract was filtered through a Florisil column and diluted to 10 ml by adding hexane in a volumetric flask.

Plant Tissue Extraction by Milestone Microwave Assited Solvent Extraction (MAE)

Approximately 10 g of representative plant sample was finely chopped with scissors. Scissors were wiped down with methanol or acetone before chopping. The sample was then placed into a paper bag and kept in a vented oven at 25 ºC overnight with the oven door slightly ajar. Both the wet and dry weights of the plant tissue were taken to determine the wet to dry weight ratio which was also used to calculate the total dry weight of plant tissue. Approximately 1 g of sample was then ground with a mortar and pestle and was placed into a pre-cleaned microwave thimble. A 1:1 hexane to acetone mixture (30 ml) was added to each thimble. Lids were firmly connected to thimbles and they were transferred to the Automatic Temperature Control (ATC) vessel to protect the temperature probe of the microwave. The thermowell was rinsed three times with hexane before insertion through the thimble lid. The corresponding lid of each ATC vessel was carefully added. All of the vessels were placed in the MAE apparatus and extracted for 55 minutes. After cooling, the extract was transferred to syncore tube through glass funnels lined with 15 cm diameter Fisherbrand® filter paperwith. approximately 5 g of sodium sulphate in the filter paper.The thimbles were rinsed with hexane at least four times. Approximately 90 ml of hexane was used to rinse

the thimbles and funnels. The filter paper and sodium sulphate was rinsed 3 times with approximately 10 ml total of hexane.

The extract was concentrated to approximately 2 ml in the Büchi syncore (with temperature set up to 60 ºC, 550-200 psi, 250 RPM). Finally, the concentrated extract was filtered through a Florisil column and diluted to 10 ml by adding hexane in a volumetric flask. The extracted concentration was analyzed by gas chromatography (Aglient 6890 Plus) with a ^{63}Ni electron capture detector (GC/ECD) where operation method of the gas chromatograph was described in the previous chapter. Each run consisted a vial of clean hexane, a 4,4' DDT 100 ppb (to test for degradation) and Pesticide 6 standards in concentrations of 2 ppb, 20 ppb and 200 ppb, three separate DCBP standards followed by the samples including the duplicate, blank and QC sample.

5.2.6 Quality assurance and control

QA/QC was retained by using one control sample (spiked with organochlorine), one analytical duplicate, and one blank for every nine-sample run. The control was spiked with100 µl of an organochlorine pesticide mixture at 2 ppm described in Appendix IX, and demonstrated a mean recovery of 98%. The 100 µl at 1 ppm surrogate standard was used with a mean recovery of 92%. The analytical blank was below the detection limit of 1.0 ng/g. The mean relative standard deviation between the analytical duplicate and samples was 23%.

5.2.7 Statistical analysis

All of the plant and soil DDT concentrations are reported based on the dry weight of the original samples with the standard deviation of the mean. The plant data set was tested for normality using the Kolmogrov-Smirnov test. A one-way analysis of variance (ANOVA) was used to compare the DDT concentration, DDT extraction and BAFs of the four native species in each group (i.e. low and high DDT-contaminated soil groups). A level of $p < 0.05$ was considered statistically significant. Statistical analysis was conducted using TIBCO Spotfire S^+ software.

5.3 Results and Discussion

5.3.1 Plant growth characteristics

All of the plants survived well both in low and high DDT-contaminated soil. Mean biomass of the wet shoots ranged from 12 to 19 g for all species except *C. leucanthemum* which had a mean biomass of 47 g when grown in high DDT-contaminated soil (Table C 7, Appendix C). Notably, all of the species obtained slightly higher mean biomasses when grown in the high DDT soil compared to the

low DDT soil, however, the differences were not statistically significant, and mean shoot length of each species was comparable between high and low DDT-contaminated soils.

The maximum height of *S.canadensis* (93 cm) observed in this study is comparable with that of *S. canadensis* (102 cm) grown at a PCB-contaminated field site (Ficko *et al*., 2011b). Likewise, Ficko *et al*. (2011b) observed *S. canadensis* to have progressive growth over the growing season with the development of a fibrous and adventitious root system. Plants of *C. leucanthemum* were composed of fibrous and shallow root systems with a number of basal leaves originating from the root system. To date, few studies have documented growth patterns of weed species used as phytoextractors of persistent organic pollutants and hence further comparisons are not available.

5.3.2 DDT concentrations in root and shoot tissues

Plants of *C. leucanthemum* exhibited the highest mean $[DDT]_{root}$ of all of the weed species both in both the low and high DDT-contaminated soils, respectively (Figure 5.2a). The DDT root concentration of 132,000 ng/g was approximately three times higher than that of the root grown in the low DDT-contaminated soil. A one way ANOVA analysis indicated that $[DDT]_{root}$ of *C. leucanthemum* was significantly higher than in the other three species (*S. canadensis*, *T. pratense,* and *S. novae-angliae*) for both low and high DDT-contaminated soils.

C. leucanthemum had a maximum mean $[DDT]_{shoot}$ of 5000 ng/g in the high DDT-contaminated soil, which was ~1.2 orders of magnitude higher than that in the low DDT-contaminated soil (Figure 5.2b). *S. canadensis* obtained the highest $[DDT]_{shoot}$ of 1150 ng/g in the low DDT-contaminated soil (Figure 5.2b). The $[DDT]_{shoot}$ of *S. canadensis* was significantly higher than in the other three species (*T. pratense, C. leucanthemum* and *S. novae-angliae*) in the low DDT soil (p = 0.001). In contrast, the $[DDT]_{shoot,}$ of *C. leucanthemum* was significantly higher than *S. novae-angliae* (p = 0.03) in the high DDT-contaminated soil.

Generally, DDT uptake in plant tissues is dependent on soil DDT concentration. As expected, higher DDT uptake was observed in root and shoot tissues for plants grown in high DDT-contaminated soils. Ficko *et al*. (2010) also documented higher PCB uptake in higher soil PCB concentrations for wild-grown weed species. Higher root than shoot DDT uptake is a common phenomenon observed in phytoextraction of organic contaminants (White, 2001; Zeeb *et al*., 2006 and Ficko *et al*., 2010).

Notably, all of the weed species grown in high DDT-contaminated soil (17500 ng/g) achieved $[DDT]_{root}$ 2.2- 9.1 times higher than that achieved by the

known phytoextractor *C. pepo* ssp. *pepo* cv. Howden grown in the Former Agricultural Land (FAL) in similar soil (10192 ng/g) (Chapter 4). Similarly, *C. leucanthemum* and *S. novae-angliae* grown in the low DDT-contaminated soil (2300 ng/g) in this study also exceeded root DDT uptake of *C. pepo* grown at the FAL site. However, no species exceeded the shoot DDT uptake of *C. pepo* in either high or low DDT soil.

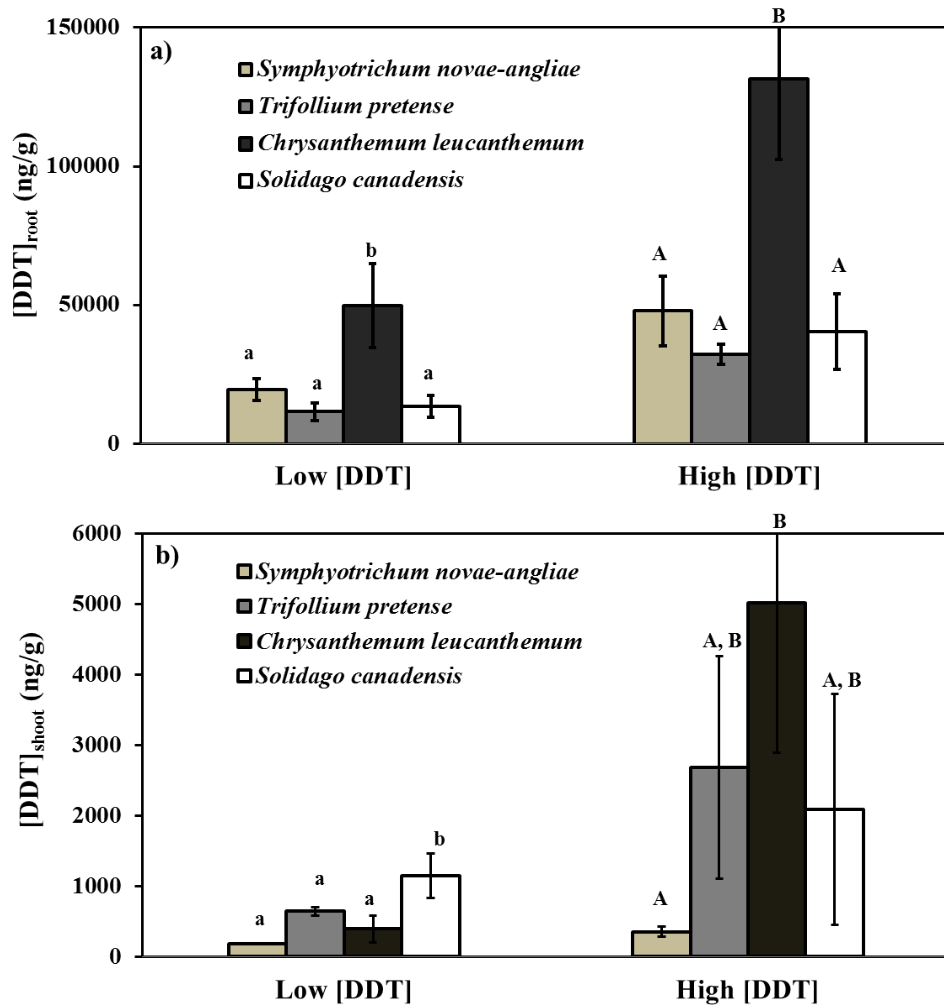

Figure 5.2: Mean DDT concentrations in a) root tissue and b) shoot tissue of four plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soils. Error bars represent one standard deviation of the mean (n = 3 for each plant species). Lower case and upper case letters indicate significant differences between the species in low and high soil DDT contamination, respectively.

5.3.3 Bioaccumulation factors (BAFs)

Root BAFs of the four species ranged from 5 (*T. pratense*) to 22 (C. *leucanthemum*) in the low DDT-contaminated soil, and from 1.8 (*T. pratense*) to 7.5 (C. *leucanthemum*) in the high DDT-contaminated soil (Figure 5.3a). Plants of *C. leucanthemum* achieved a statistically higher root BAF than the other plant species at the low DDT-contaminated soil (p = 0.001), and at the high DDT-contaminated soil (p = 0.0003), respectively. Root BAFs for all species were significantly greater in the low DDT-contaminated soil than the high DDT contaminated soil.

Root BAFs of 5 and 1.8 in *T. pratense* in the low and high DDT-contaminated soil, respectively (present study) were significantly higher than that of *T. pratense* (0.62) under field conditions (chapter 3). Likewise*, S. canadensis* had significantly higher root BAFs (6 and 2.3) in the present study than the root BAF of 0.58 in same species grown in the field (chapter 3). In the present study, the root BAF of 5 in *T. pratense* grown in low DDT soil (2300 ng/g) was similar to the root BAF of 6 observed previously in *Trifollium incarnatum* in *p,p*-DDE-contaminated soil (610 ng/g) under field conditions (White *et al*., 2005). In the current study, higher root BAFs were found for both low and high DDT soil contamination levels in comparison to those observed in PCB-contaminated soil (4700 to 31000 ng/g) for similar species (Ficko *et al*., 2010). The exception is the root BAF of *S. canadensis*, which was similar for both DDT and PCB contaminated soils. Additionally, the maximum root BAF of 22 reported in *C. leucanthemum* was more than three times higher than the root BAF of 5.9 in *in situ C. pepo* (pumpkin) grown in soil from the same DDT-contaminated site (chapter 4).

Shoot BAFs ranged from 0.07 (S. *novae-angliae)* to 0.50 (S. *canadensis*) in low DDT-contaminated soil, and from 0.02 (*S. novae-angliae*) *to* 0.3(C. *leucanthemum*) in the high DDT soil group (Figure 5.3b). Shoot BAFs followed a similar trend to those of root BAFs, with higher shoot BAFs found for plants grown in the low DDT-contaminated soil. The highest shoot BAF was documented in *S. canadensis* which was significantly higher than the remaining three species for the low DDT-contaminated soil (BAF_{shoot} ,p = 0.001). In the high DDT soil, the shoot BAF of *C. leucanthemum* was significantly higher than that of *S. novae-angliae* ($BAF_{shoot,}$ p = 0.03).

Ideally, shoot BAFs should be greater than one, however to date, no studies using native weed species as DDT phytoextractors have achieved such high shoot BAFs (Lunney *et al*., 2004; White *et al*., 2005). For PCBs, some studies have reported higher shoot BAFs than one under greenhouse condition (Ficko *et al*., 2011a). There were no significant differences in shoot BAFs of *T. pratense* and *S.*

novae-angliae in both low and high DDT-contaminated soil (present study) and field grown plants in DDT-contaminated soil (chapter 3). *S. canadensis* showed shoot BAFs of 0.50 and 0.12 in the low and high DDT-contaminated soil, respectively which were significantly greater than the shoot BAF (0.01) in the same species under field condition (chapter 3).

In the current study, shoot BAFs of *T. pratense* (0.28) and *S. novae-angliae* (0.08) in the low DDT soil levels were comparable with the PCB shoot BAFs of 0.12 and 0.08, respectively in the same species (Ficko *et al*., 2010). *S. canadensis* plants had a similar DDT shoot BAF (0.50) and PCB shoot BAF (0.62) (Ficko *et al*. 2011b). In contrast, *C. leucanthemum* had a higher PCB shoot BAF (1.43) (Ficko *et al*., 2011b) than the DDT shoot BAF (0.3) observed in the present study.

Translocation factors (TLFs = $[DDT]_{shoot}/[DDT]_{root}$) are used to determine how efficiently DDT can be transferred from root to shoot tissue. TLFs in this study were comparatively lower than those observed in recent PCB studies using weed species (Ficko *et al*., 2010; 2011b). The exception is the TLF of *S. canadensis,* which was similar to the TLF observed for PCB phytoextraction (Ficko *et al*., 2010). TLF values are presented in (Table C 9, Appendix C).

In summary, the four species accumulated high DDT levels in the root tissue, but were not able to translocate high levels of DDT to the shoot tissue. While this study was conducted under greenhouse conditions which controlled environmental factor such as light, precipitation, and soil heterogeneity of DDT, other factors, such as small container sizes, dense cultivation, inhibition of root penetration depth, shoot diameter, and leaf size may have affected DDT translocation (Ficko *et al*., 2010).

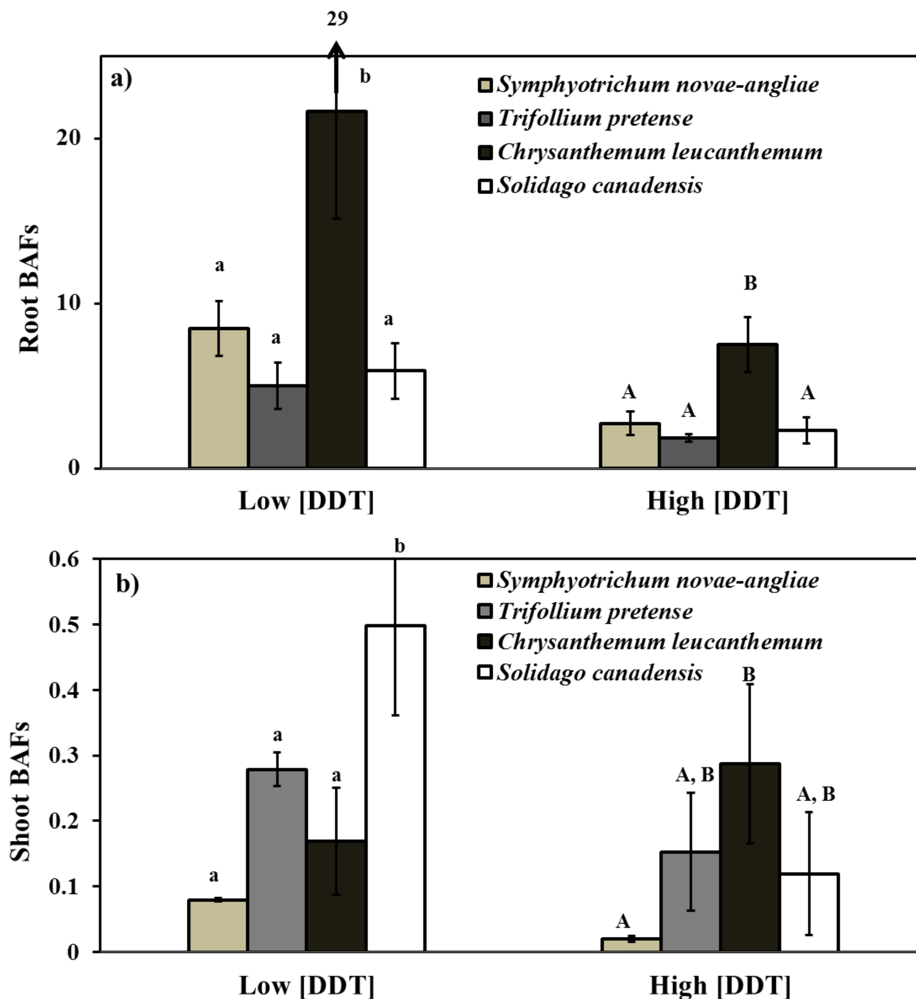

Figure 5.3: Comparison of BAFs in a) roots and b) shoots of four plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soils. Error bars represent one standard deviation of the mean (n = 3 for each plant species). Lower case and upper case letters indicate significant differences between the species in low and high soil DDT contamination, respectively.

5.3.4 Root and shoot DDT extraction

All of the species had higher root extractions in the high versus the low DDT-contaminated soil. A significant difference in root extractions was observed

amongst the species in the low DDT soil where the highest root extraction was 30000 ng measured in *S. novae angalie* (p = 0.003) (Figure 5.4a). The highest root extraction of 87000 ng was found in *S. canadensis* in the high DDT soil, but there was no significant difference observed between the species (p = 0.13) (Figure 5.4a). Although root DDT concentration of *S. canadensis* was significantly lower than *C. leucanthemum* in the high DDT-contaminated soil, *S. canadensis* had higher root extraction than *C. leucanthemum* because of its higher root biomass.

Although lower amounts of DDT were observed in the shoot tissues, the shoots still extracted DDT efficiently due to their high biomass. Higher shoot extraction was documented in high DDT-contaminated soil than the low DDT-contaminated soil. The highest shoot DDT extraction of 34000 ng was determined in *C. leucanthemum* in the high DDT soil (Figure 5.4b). In contrast, *S. novae-angliae* had a significantly lower shoot extraction than the other species in the high DDT-contaminated soil because of its lower shoot biomass. The shoot DDT extraction in the low DDT-contaminated soil ranged from 500 ng (*C.leucanthemum*) to 2100 ng (*T. pratense*) (Figure 5.4b). The highest shoot biomass of *C. leucanthemum* corresponds to 87% and 92% of total wet biomass of the plant in the high and low DDT-contaminated soil, respectively (Table C 7, Appendix C). A significantly higher shoot extraction than root extraction was found in *C. leucanthemum* as it had a much higher shoot biomass (89% of total plant biomass) (Ficko *et al*., 2010). Other studies have also documented higher shoot extraction due to greater shoot biomass despite lower shoot contaminant concentrations (White, 2001; Zeeb *et al*., 2006; Ficko *et al*., 2010).

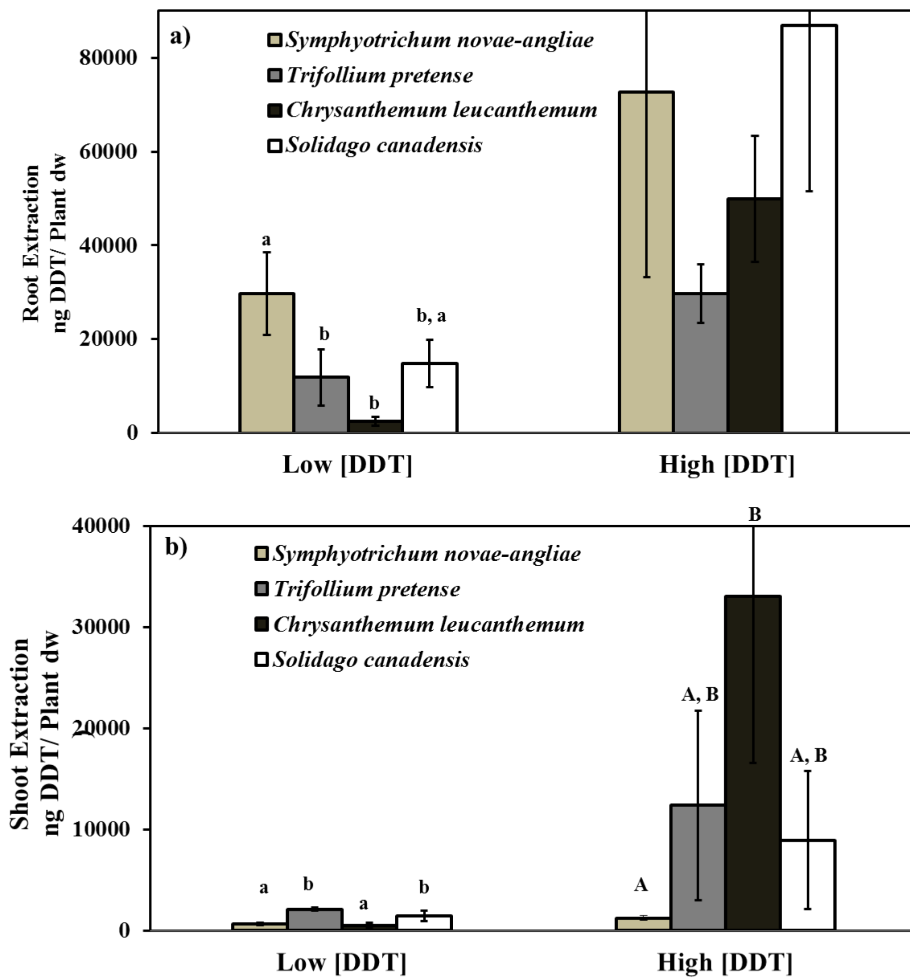

Figure 5.4: Total mean extractions in a) roots and b) shoots for each plant species grown under greenhouse conditions in low (2300 ng/g) and high (17500 ng/g) DDT-contaminated soil. Error bars represent one standard deviation of the mean (n = 3 for each plant species). Lower case and upper case letters indicate significant differences between the species in low and high soil DDT contamination, respectively.

5.3.5 Phytoextraction efficiency

The phytoextraction efficiency of species can be compared using the shoot extraction values calculated per unit area of soil. To compare the capabilities, a theoretical density value (obtained from the literature) was applied. For example, the known phytoextractor *C. pepo* cv. Howden (pumpkin) is optimally grown at one plant per square metre (OMFERA, 2000; Whitfield Åslund *et al*., 2007), whereas weed species are typically grown at much higher densities (Canola Council of Canada, 2013). A phytoextraction comparison (per square metre) between greenhouse grown species, wild grown species, and field cultivated *C. pepo* ssp. *pepo* (Howden) is presented in (Table 5.1). *T. pratense* grown under greenhouse conditions in high DDT-contaminated soil (17500 ng/g) and under field conditions at PPNP (21000 ng/g) showed the highest DDT extraction ability. In contrast, *S. novae-angliae* grown under field conditions extracted a significantly greater quantity of DDT per square metre than plants grown under greenhouse conditions in the high DDT-contaminated soil. Greenhouse grown *S. canadensis* in the high DDT-contaminated soil level extracted twice the amount of DDT on a per square metre basis than *S. canadensis* grown under field conditions. *C. pepo* grown under field conditions in PPNP (10192 ng/g) extracted 716,000 ng DDT per square metre (Chapter 4). In the same study, the two native grass species, *S. scoparium* (little bluestem) and *P. virgatum* (switchgrass), extracted two and three times the amount of DDT than *C. pepo* per square metre. Likewise, in this greenhouse study *T. pratense* grown in the low or high DDT-contaminated soils extracted 1 to 2 orders of magnitude more DDT than *C. pepo* when theoretical optimal density was taken into account.

Table 5.1: A comparison of mean shoot [DDT], and shoot DDT extraction between greenhouse and field studies using weed species and *C. pepo* shoot extractions those exceeded *C. pepo* were shown in bold.

	Soil conc. (ng/g)	Species	Mean shoot DDT ng/g	Mean shoot dry wt (g)	Theoretical planting density /m²	Shoot extraction ng/m²
Green house study (2012)	High [DDT] (17500)	*S. novae-angliae*	350	3.52	144[a]	178,100
		T. pratense	2700	4.29	8000[b]	**91,807,000**
		C. leucanthemum	5020	6.72	20[c]	675,000
		S. canadensis	2100	4.31	10[d]	90000
	Low [DDT] (2300)	*S. novae-angliae*	180	3.51	144[a]	92500
		T. pratense	650	3.25	8000[b]	**16,678,000**
		C. leucanthemum	400	1.36	20[c]	10600
		S.canadensis	1200	1.24	10[d]	14800
Survey study (2011 & 2012)	Field [DDT] (21000)	*S. novae-angliae*	2400	4.20	144[a]	**1,460,000**
		T. pratense	2200	5.45	8000[b]	**96,000,000**
		S. canadensis	290	11.38	10[d]	32000
Field study (2011)	Field [DDT] (10200)	*C. pepo* ssp. *pepo* cv. Howden (pumpkin)	8100	88.4	1[e]	**716,000**

[a]United States Department of Agriculture (USDA); [b] Black, 1960; [c]Pill *et al*., 1994; [d] Zhang *et al*.,2009; [e]OMAFRA, 2000.

5.3.6 DDT metabolite accumulation and translocation pattern in root and shoot tissues

In the present study, it was determined that the initial commercial formulation of DDT that was applied to areas of PPNP has largely transformed to DDE resulting in an soil composition of 34.6% 4,4'-DDT, 5.4% 2,4'-DDT, 56.1% 4,4'-DDE, 0.4% 2,4'-DDE, 2.7 % 4,4'-DDD and 0.7 % 2,4'-DDD in the low DDT-contaminated soil collected from the FAL site in PPNP (Figure 5.5a). The high DDT-contaminated soil group contained of 45.6% 4,4'-DDT, 5.6% 2,4'-DDT, 45.4% 4,4'-DDE, 0.2% 2,4'-DDE, 2.4% 4,4'-DDD and 0.8% 2,4'-DDD (Figure 5.5a). Hence, the soil composition with respect to ratio of DDT metabolites was very similar between the low and high contaminated soils, and these in turn were consistent with Crowe and Smith (2007) as the soil was collected from the same site in PPNP. The uptake patterns of DDT metabolites for the four plant species are presented in (Figure 5.5b-c). The root uptake of DDT metabolites appeared in similar proportions to those found in the soil. It was similar for all four species, with the percentage of metabolites ranging from 56 to 60% of 4,4'-DDE and 31 to 38% of 4,4'-DDT whereas 4,4'-DDD was found in comparatively low percentages.

DDT metabolite uptake into shoot tissue was in turn the same as for the root and soil. The overall uptake of 4,4' isomers were higher and a lower uptake of 2,4' isomers was observed in shoot tissues. In this study, the uptake of 4,4'-DDE ranged from 40% (*S. canadensis*) to 54% (*C. leucanthemum*) in the shoot. Approximately 54% 4,4'-DDE accumulation might be explained by the fact that 4,4'-DDE is comparatively more water soluble and has a lower K_{ow} value than 4,4'-DDT metabolites (ASTDR, 2002; Howard and Meylan 1997). Whitfield Åslund *et al*. (2010) found that 57- 63% of DDT metabolites consisted of 4,4'-DDT in the shoot tissue of pumpkin, despite the fact that 4,4'-DDT is less soluble in water than other metabolites.

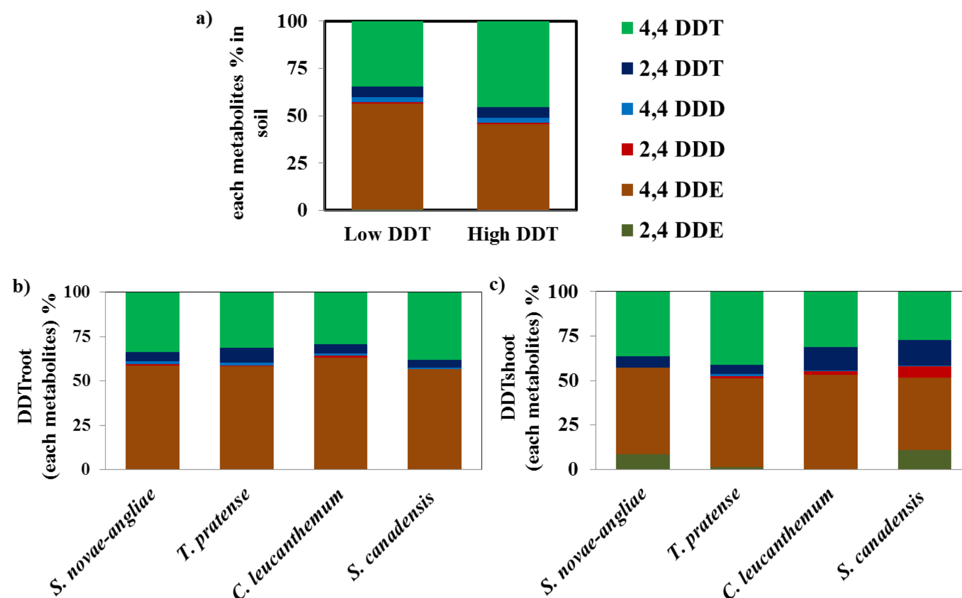

Figure 5.5: a) Percentages of DDT metabolites in low and high DDT-contaminated soil. Percentages of DDT metabolites in b) root and c) shoot tissue in high DDT-contaminated soil.

5.4 Conclusions

The DDT phytoextracton capabilities of four weed species in Point Pelee National Park soil were investigated in a greenhouse study. It was determined that *C. leucanthemum* and *S. canadensis* have the highest shoot and root DDT extractions, respectively. However, a maximum shoot DDT extraction per square metre was measured in *T. pratense* grown in both low and high DDT-contaminated soil, and these extractions exceeded the shoot DDT extraction of *C. pepo* ssp. *pepo* (Howden). The shoot BAFs of two species (*S. novae-angliae* and *T. pratense*) were not significantly different under greenhouse and field conditions, however, *S. canadensis* showed significantly higher root and shoot BAFs under greenhouse conditions. In general, the DDT isomer uptake in root and shoot tissues appeared similar to that of the soil profile with an abundance of 4,4’-metabolites found in the root and shoot tissue. In contrast, negligible amount of 2,4’-metabolites were found in root and shoot tissues of the plant. These findings will assist in determining the probable DDT metabolite uptake patterns of other species. In addition, these findings might help shed light on the phytoextraction mechanism taking place in native (or naturalized) plants. Further research is required to determine how the mobility of highly chlorinated or less water soluble DDT

isomers can be enhanced within plant shoots in order to contribute to phytoextraction capability.

Chapter 6

Final Discussion and Summary

In the emerging field of phytoextraction research, plants of *Cucurbita pepo* ssp *pepo* (pumpkin and zucchini) have demonstrated success in the phytoextraction of DDT and its metabolites (Lunney *et al*., 2004, 2010; Whitfield Åslund*et al*., 2010; White, 2002, White *et al*., 2003). Nonetheless, as a phytoextractor *C. pepo* has some disadvantages; the plant is a human food crop, it may be consumed by herbivores and as an annual species, it needs considerable agronomic care to grow. For these reasons, research has been carried out to identify native (or naturalized) plant species (weeds) capable of phytoextracting organic contaminants. These species have a number of requirements including: (i) they must grow with minimum maintenance and cost, (ii) they should be easily propagated, (iii) they should tolerate a wide range of environmental conditions, (iv) they should not appeal to herbivores, and (v) they should be non-crop plants. Many native (or naturalized) weed species fulfill these requirements. This thesis establishes the DDT phytoextraction capability of several native and naturalized weed species at Point Pelee National Park (PPNP). The motivation for this work stemmed from recent success in remediation of PCBs with weed species (Ficko *et al*., 2010, 2011a, 2011b).

In Chapter 3, knowledge of DDT phytoextrction by weed species was expanded based on a screening study of phytoextraction among nine wild/naturally grown native and naturalized weed species including *S. canadensis* (Canada goldenrod), *S. ptycanthum Dun* (eastern black nightshade), *S. vulgaris* (bladder campion), *A*. syriaca (milkweed), *L. cardiac* (motherwort), *S. novae-angliae* (new England aster) (native) and *T. pratense* (red clover) *V. thapsus* (mullein), and *C. sanguinea* (dogwood) (naturalized) at PPNP. The root and shoot tissues of these species were analyzed to determine their phytoextraction capabilities and it was found that all the species were able to accumulate DDT into their root tissues and translocate it into the harvestable portion of the plants. Interestingly, four species out of nine (i.e. 44%) showed higher DDT extractions per square metre than the known phytoextractor *C. pepo*. Moreover, two species common to the present study and a PCB phytoextraction study by Ficko *et al*. (2010) were found to have similar capabilities for DDT extraction.

In chapter 4, a comparative *in situ* phytoextraction study was undertaken between *C. pepo* (pumpkin) and three native grass species (*S. scoparium* - little

bluestem, *P. virgatum* - switchgrass and *S. cryptandrus* - sand dropseed). These grass species were chosen as they are native, abandant and colonize everywhere without any human intervention. The plants were grown at three DDT-contaminated sites within PPNP where DDT contaminations in soil was labeled as 'low' (291 ng/g), 'moderate' (5083 ng/g) and 'high' (10192 ng/g). Two of the three native species (*P. virgatum* and *S. scoparium*) extracted higher DDT amounts (ng) per square metre than the proven phytoextractor, *C. pepo* at the high DDT-contaminated site. However, at the moderate DDT-contaminated site, *C. pepo* had higher extractions per square metre than any of the native grass species. It was also noted that the DDT uptake into *C. pepo* was the highest at the moderate DDT-contaminated site. A threshold soil DDT concentration of ~5000 ng/g was observed where the root and shoot DDT uptake were at a maximum. This is the first study which introduces the concept of soil contaminant concentration as a limiting factor of phytoextraction and identifies a threshold soil DDT concentration for *C. pepo*. Further research is required to establish the soil DDT concentration effect by using one soil type with multiple soil DDT concentrations under controlled conditions. In contrast, no threshold soil DDT concentration was found for the three native grass species as root and shoot DDT concentrations of these species increased from low to high DDT-contaminated sites. Follow-up studies for the three native grass species in the same field condition where a theoretical/optimal density factor can be applied in practice is recommended.

Phytoextraction can be affected by soil properties (soil heterogeneity, organic carbon content and pH) and environmental factors (precipitation, temperature). Therefore, in chapter 5, the phytoextraction capability of three native and one naturalized weed species was further studied in a more controlled greenhouse study. Perennial native weed species selected for this study were *S. canadensis* (Canada goldenrod), *S. novae-angliae* (New England aster), *C.leucanthemum* (ox-eye daisy), and *T. pratense* (red clover). Each of these species were grown in two levels of DDT-contaminated soil collected from PPNP, termed 'high' (17500 ng/g) and 'low' (2300 ng/g). *T. pratense* had the maximum shoot DDT extraction per square metre in both the the low and high DDT-contaminated soil and exceeded DDT extraction of *C. pepo*. *C. leucanthemum* had the highest root BAF of all species in the low DDT-contaminated soil, and it also exceeded the maximum root BAF of *C. pepo*. To compare the extraction achieved between field and green house studies, the shoot BAFs of two species (*T. pratense* and *S. novae-angliae*) obtained in the field (chapter 3) and in the greenhouse (chapter 5) were compared and found to be not statistically different. The uptake pattern of DDT metabolites in the species was also investigated. A similar pattern of DDT metabolites were observed in soils, roots and shoots of all four species with an abundance of 4,4' metabolites.

This study is the first comprehensive investigation of DDT phytoextraction by native and naturalized weed species in naturally-grown, field-grown and greenhouse-grown conditions. Hence, it indicates that native weed species are potential phytoextractors for DDT. However, planting density of weed species and soil DDT concentration are key factors to determining the efficiency of this technology. For example, *P. virgatum* with a maximum planting density 170 plants/m^2 at a soil DDT concentration 10192 ng/g is a more efficient phytoextractor than *C. pepo*. In contrast, *C. pepo* has a better shoot DDT extraction per square metre than any native species at the moderate DDT-contamination (~5000 ng/g) level. As PPNP soil is heterogeneously contaminated with DDT, the use of multiple species (*C. pepo* at moderate DDT contamination levels and native weed species at high DDT-contamination levels – i.e. > 5000 ng/g) may reduce the number of required seasons to reach acceptable DDT concentration levels (i.e. < 700 ng/g). Although, the mechanism of phytoextraction is still largely unknown, the knowledge gained in this thesis furthers our understanding of DDT uptake within native plant species. Further research is required to fully understand the behavior of DDT metabolites within plants and to develop a method to enhance the transportation or mobilization of these compounds in shoot tissues. Ultimately, this thesis has identified a number of native and naturalized weed species that are efficient phytoextractors of DDT. The findings will assist in developing a phytoextraction database that can be valuable to contaminant site owners and site investigators.

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8. Appendices

Contents:

Appendix A
Additional information for chapter 3: An Evaluation of Wild-Grown Native (or Naturalized) weed species for the Phytoextraction of DDT at Point Pelee National Park, Leamington, ON.

Appendix B
Additional information for chapter 4: Phytoextraction of DDT-contaminated soil at Point Pelee National Park, Leamington, ON, using *Cucurbita pepo* cv. Howden and native grass species.

Appendix C
Additional information for chapter 5: Phytoextraction of weathered DDT-contaminated soil by perennial native (or naturalized) weed species under greenhouse conditions.

Appendix A

Additional information for chapter 3: An Evaluation of Wild-Grown Native (or Naturalized) weeds species for the Phytoextraction of DDT at Point Pelee National Park, Leamington, ON

Table A 1: Wet and dry weights, and total DDT concentrations (ng/g) in root, shoot tissues of native and naturalized weed species and soil samples (arranged alphabetically by Latin species name) at Point Pelee National Park (PPNP). 93

Table A 2: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples in chapter 3. 96

Table A 3: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of soil samples reported in chapter 3. 98

Table A 4: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 3. 99

Table A 5: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of soil samples reported in chapter 3. 101

Table A 6: TLFs of the native weed species for DDT (current study) and PCB (Ficko *et al*., 2010). 103

Table A 1: Wet and dry weights, and total DDT concentrations (ng/g) in root, shoot tissues of native and naturalized weed species and soil samples (arranged alphabetically by Latin species name) at Point Pelee National Park (PPNP).

Sample Number	Latine Name	Common Name	Tissue	Total ww (g)	Total dw (g)	wet/dry ratio	[DDT] (ng/g)	Run	Corresponding Soil Samples no	[DDT] ng/g)
062a	*Asclepias syriaca*	Milkweed	shoot	20.7	3.9	5.3	1140	120425	29245	30800
062b			root	2.8	0.8	3.6	1290	120515		
065a	*Asclepias syriaca*	Milkweed	shoot	7.3	1.4	5.2	1340	120425	29248	64800
065b			root	2.4	0.7	3.2	1720	120515		
112A	*Asclepias syriaca*	Milkweed	shoot	13.4	3.0	4.5	1640	120425	29257	108000
112B			root	1.7	0.6	2.7	1180	120515		
80	*Cornus sanguinea*	Dogwood	shoot	24.5	15.6	1.6	124	120502	29854	7360
79			root	7.7	4.4	1.8	7380	120515		
060a	*Cornus sanguinea*	Dogwood	shoot	18.3	8.2	2.2	115	110817	29252	4330
060b			root	3.8	2.0	1.9	1743	110812		
32	*Cornus sanguinea*	Dogwood	shoot	27.1	17.4	1.6	79.9	110817	29952	15200
31			root	9.6	5.5	1.7	2020	110817		
069a	*Cornus sanguinea*	Dogwood	shoot	21.9	11.3	1.9	468	110817	29953	27300
069b			root	42.8	28.7	1.5	8400	110812		
092 A	*Leonurus cardiaca*	Motherwort	shoot	19.9	7.3	2.7	311	120425	29240	19600
092B			root	5.5	1.4	4.0	5250	120515		
091A	*Leonurus cardiaca*	Motherwort	shoot	11.4	6.0	1.9	124	120425	29240	19600
091B			root	2.6	0.8	3.4	1340	120515		
12	*Leonurus cardiaca*	Motherwort	shoot	17.7	2.1	8.2	66.6	130228	29290	8300
11			root	6.4	1.2	5.3	6190	130228		
099a	*Silene vulgaris*	Bladder Campion	shoot	6.5	6.8	0.9	1280	110812	29955	22400
099b			root	1.8	6.1	0.3	5240	110817		

Sample Number	Latine Name	Common Name	Tissue	Total ww (g)	Total dw (g)	wet/dry ratio	[DDT] (ng/g)	Run	Corresponding Soil Samples no	[DDT] ng/g)
14	*Silene vulgaris*	Bladder Campion	shoot	15.9	2.1	7.7	139	130228	29291	4300
13			root	5.7	0.4	14.7	1080	130228		
29	*Silene vulgaris*	Bladder Campion	shoot	20.2	9.4	2.1	48.2	110817	29983	2020
28			root	8.8	1.6	5.4	2140	110812		
066a	*Solanum ptycanthum Dun*	Eastern black nightshade	shoot	5.8	0.9	6.3	1600	120502	29249	6230
066b			root	1.0	0.1	7.1	4000	120515		
098a	*Solanum ptycanthum Dun*	Eastern black nightshade	shoot	9.0	1.8	5.1	462	120502	29250	2390
098b			root	1.5	0.4	3.3	4750	120515		
34	*Solanum ptycanthum Dun*	Eastern black nightshade	Shoot	14.2	7.9	1.8	280	110812	29982	14000
33			root	2.9	6.4	0.5	4950	110812		
063a	*Solidago canadensis*	Goldenrod	shoot	38.8	14.6	2.7	633	120502	29246	27300
063b			root	17.6	5.5	3.2	7790	120515		
064a	*Solidago canadensis*	Goldenrod	shoot	32.1	11.5	2.8	166	120502	29247	12500
064b			root	8.4	2.8	3.0	11900	120515		
76	*Solidago canadensis*	Goldenrod	shoot	22.3	9.0	2.5	112	110812	29850	7200
75			root	5.5	2.2	2.5	6580	110817		
37	*Solidago canadensis*	Goldenrod	shoot	28.4	10.5	2.7	213	110817	29984	53000
36			root	6.5	2.0	3.2	5570	130228		
310A	*Symphyotrichum novae-angliae*	New England aster	shoot	15.9	2.6	6.0	451	130228	29242	6320
310B			root	4.7	2.5	1.9	17200	130228		
311A	*Symphyotrichum novae-angliae*	New England aster	shoot	6.7	5.6	1.2	504	130228	311	3040
311B			root	17.0	7.1	2.4	8940	130228		
405 A	*Trifollium pratense*	Red clover	shoot	11.1	5.1	2.2	36.9	130228	29260	602
405B			root	4.6	1.6	2.9	344	130228		
406 A	*Trifollium pratense*	Red clover	shoot	6.3	2.4	2.7	127	130228	29260	602

Sample Number	Latine Name	Common Name	Tissue	Total ww (g)	Total dw (g)	wet/dry ratio	[DDT] (ng/g)	Run	Corresponding Soil Samples no	[DDT] ng/g)
406B	*Trifollium pratense*		root	1.9	0.6	3.1	334	130228		
407 A		Red clover	shoot	16.0	8.8	1.8	28.6	130228	29260	602
407B			root	6.9	2.0	3.4	439	130228		
113A	*Verbascum thapsus*	Mullein	shoot	31.3	5.4	5.8	43.1	120502	29270	227
113B			root	1.5	0.4	3.6	98.0	120515		
401A	*Verbascum thapsus*	Mullein	shoot	60.1	11.6	5.2	700	130228	29485	5070
401B			root	6.3	1.9	3.3	1470	130228		
87	*Verbascum thapsus*	Mullein	shoot	9.8	1.2	8.5	170	120502	29811	1200
86			root	0.7	0.1	6.9	525	120515		

Table A 2: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples in chapter 3.

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120622
Control	<1.0	17	<1.0	21	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		105		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120524
Control	<1.0	19	<1.0	20	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		100		100	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130225
Control	<1.0	17	<1.0	18	<1.0	17	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		85	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120926
Control	<1.0	18	<1.0	22	<1.0	15	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		110		75	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	110622
Control	<1.0	17	<1.0	16	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		80		95	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	110614
Control	<1.0	17.5	<1.0	17.1	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		85		95	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	110614

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Control	<1.0	17.7	<1.0	17.9	<1.0	18.1	110614
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		90		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120502
Control	<1.0	17	<1.0	18	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		95	
Blank	<1.0	<0.5	<1.0	<1.0	<1.0	<1.0	120425
Control	<1.0	18	<1.0	18	15	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		90		75	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120515
Control	<1.0	20	<1.0	24	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		120		100	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120515
Control	<1.0	19	<1.0	28	<1.0	11	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		140		55	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130228
Control	<1.0	17	<1.0	18	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130301
Control	<1.0	17	<1.0	18	<1.0	17	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		85	

Table A 3**: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of soil samples reported in chapter 3.**

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120502
Control	<1.0	17	<1.0	18	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		95	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120425
Control	<1.0	18	<1.0	18	15	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		90		75	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120515
Control	<1.0	20	<1.0	24	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		120		100	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	120515
Control	<1.0	19	<1.0	28	<1.0	11	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		140		55	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130228
Control	<1.0	17	<1.0	18	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130301
Control	<1.0	17	<1.0	18	<1.0	17	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		85	

Table A 4: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 3.

Sample	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)	Mean RSD %	Total [DDT] (ng/g)
407A	<1.0	21	<1.0	<1.0	1.1	2.30		27.4
407A DUP	<1.0	26.2	<1.0	<1.0	1.1	5.40		35.7
Mean	<1.0	23.6	<1.0	<1.0	1.1	3.90		
STD	n/a	3.70	n/a	n/a	n/a	2.20		
RSD (%)	n/a	15.6	n/a	n/a	n/a	56.9	12.1	
311B	27.5	7700	232	92.7	226	890		9170
311B duplicate	26.1	7170	348	83.6	219	867		8710
Mean	26.8	7440	290	880	223	879		
STD	1.0	375	82.0	6.40	4.90	16.3		
RSD (%)	3.7	5.0	28.3	7.30	2.20	1.90	8.1	
SG Sh D3 site 6/7	10.6	158	17.4	17.4	7.00	23.7		234
Dup SG Sh D3 site 6/7	0.40	127	17.3	15.2	5.70	19.2		185
Mean	5.50	143	17.4	16.3	6.30	21.4		
STD	7.20	21.4	0.10	1.60	0.90	3.20		
RSD (%)	131	15.0	0.40	9.70	15.0	14.9	30.9	
Rt Gr 063 B	18.4	4950	27.2	102	317	1640		7060
Dup Rt Gr 063B	16.8	6540	27.1	99.6	255	1580		8510
Mean	17.6	5740	27.1	101	286	1610		
STD	1.10	1120	0.10	1.50	43.4	44.5		
RSD (%)	6.40	19.5	0.30	1.50	15.2	2.80	7.6	

Sample	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)	Mean RSD %	Total [DDT] (ng/g)
GR-063A	7.10	286	130	53.0	18.3	140		634
Dup GR-063A	1.00	166	250	242	10.0	142		812
Mean	4.10	226	190	148	14.1	141		
STD	4.30	84.8	85.0	134	5.80	2.00		
RSD (%)	107	37.6	44.7	90.7	41.4	1.40	53.7	
Motherwort-091A	<1.0	78.9	<1.0	0.50	5.10	26.7		112
Motherwort-091A dup	0.20	95.5	1.10	0.90	5.80	31.5		135
Mean	0.10	87.2	1.00	0.70	5.40	29.1		
STD	0.10	11.7	0.10	0.30	0.50	3.40		
RSD (%)	141	13.4	6.10	42.8	9.20	11.6	37.4	
Rt Gr 063 B	18.4	6722	27.2	102	317	2160		9350
Dup Rt Gr 063B	16.8	7814	27.1	99.6	255	2640		10900
Mean	17.6	7268	27.1	101	286	2400		
STD	1.10	772	0.10	1.50	43.4	340		
RSD (%)	6.40	10.6	0.30	1.50	15.2	14.2	8.0	

Table A 5: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of soil samples reported in chapter 3.

Sample	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 DD (ng/g)	Mean RSD %	Total [DDT] (ng/g)
29804	1.30	306	1.60	4.5	11.2	92.4		417
Dup29804	1.80	318	1.90	5.3	10.8	103		441
Mean	1.60	312	1.80	4.9	11.0	97.7		
STD	0.40	8.50	0.20	0.6	0.30	7.50		
RSD (%)	22.8	2.70	12.1	11.5	2.60	7.70	9.9	
29811	4.40	753	5.80	22.5	47.2	434		1200
DUP29811	3.80	668	5.40	22.5	43.8	395		1140
Mean	4.10	710	5.60	22.5	45.5	415		
STD	0.40	60.0	0.30	0.0	2.40	27.9		
RSD (%)	9.10	8.50	4.60	0.2	5.30	6.70	5.7	
29290	30.6	5670	24.6	73.9	297	18800		7980
29290 dup	33.2	6360	32.2	72.8	296	1930		8720
Mean	31.9	6020	28.4	73.3	297	1910		
STD	1.80	488	5.40	0.8	0.70	35.4		
RSD (%)	5.70	08.1	19.0	1.0	0.20	1.90	6.0	
2011-PP-29970	2.40	613	4.70	18.3	25.6	255		919
2011-PP-29970 Duplicate	2.50	655	3.80	16.6	26.7	274		978
Mean	2.40	634	4.20	17.4	26.1	265		
STD	0.10	29.5	0.70	1.2	0.70	13.3		
RSD (%)	3.30	4.70	15.8	7.0	2.80	5.00	6.4	
300110	27.2	3770	113	142	220	982		5250
dup 300110	27.3	3620	39.0	151	216	902		4950
Mean	27.2	3690	76.0	146	218	942		

Sample	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 DD (ng/g)	Mean RSD %	Total [DDT] (ng/g)
STD	0.10	107	52.4	6.30	2.8	56.8		
RSD (%)	0.20	2.90	69.0	4.30	1.3	6.00	14.0	
29950	26.7	5500	37.0	91.3	397	2910		8960
Dup29950	37.3	6770	48.0	113	555	3710		11230
Mean	32.0	6130	42.5	102	476	3310		
STD	7.50	898	7.80	15.0	111	566		
RSD (%)	23.5	14.6	18.3	14.7	23.4	17.1	18.6	
29257	216	29660	752	4550	8340	77680		121200
Dup 29257	157	22470	587	3490	6710	61580		94990
Mean	187	26060	670	4020	7520	69630		
STD	42	5080	117	755	1150	11390		
RSD (%)	22.6	19.5	17.4	18.8	15.3	16.4	17.5	

Table A 6: TLFs of the native weed species for DDT (current study) and PCB (Ficko *et al*., 2010)

Species	DDT Mean TLFs	PCB (Ficko *et al*., 2010)
Solidago canadensis	0.04 (0.01-0.81)	0.1 (0.06-0.19)
Cornus sanguinea	0.04 (0.01-0.06)	
Silene vulgaris	0.13 (0.2-0.24	
Asclepias syriaca	0.10 (0.07-0.13)	
Leonurus cardiaca	0.05 (0.01-0.09)	
Solanum ptycanthum Dun	0.16 (0.05-0.32)	0.08 (0.04-0.12)
Verbascum thapsus	0.29 (0.11-0.51)	0.16 (0.11-0.21)
Trifolium pratense	0.18 (0.06-0.38)	0.09 (0.04-0.18)
Symphyotrichum novae-angliae	0.04 (0.02-0.05)	0.11 (0.02-0.25)

Appendix B

Additional information for chapter 4: Phytoextraction of DDT-contaminated soil at Point Pelee National Park, Leamington, ON, using *Cucurbita pepo* cv. Howden and native weed species

Table B 1: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of *C. pepo* were grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated site in PPNP. 105

Table B 2: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of three native grass species grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated site in PPNP 110

Table B 3: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples reported in chapter 4. 115

Table B 4: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of soil samples 117

Table B 5: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 4. 118

Table B 6: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of soil samples reported in chapter 4. 120

Table B 7: Wet weight and length of *C .pepo* and native weed species at low, moderate and high DDT-contaminated site. 121

Table B 8: Total mean shoot extraction (ng) per plant species at low (291.3 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. 122

Table B 9: Translocation factors (TLFs) of plant species at low (291.3 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites. 123

Table B 1: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of *C. pepo* were grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated site in PPNP.

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Length of tissues	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
Sl_Pum_A_bsh_313a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Bottom shoot	20	14.5	2.7	1210	120116	397
Sl_Pum_B_bsh_314a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Bottom shoot	20	17.9	3.2	1140	120116	392
Sl_Pum_C_bsh_315a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Bottom shoot	20	12.3	2.0	1520	120116	293
*SL-P-D-bsh-316a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Bottom shoot	20	24.4	2.3	1820	120312	348
Sl-P-A-M sh-313a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Mid shoot	20	5.6	1.2	1370	120123	397
Sl-P-B-M sh-314a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Mid shoot	20	4.6	0.7	1170	120123	392
Sl-P-C- M sh-315a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Mid shoot	20	7.9	1.4	1750	120123	293
SL-P-D-msh-316a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Mid shoot	20	9.3	0.8	1710	120312	348
Sl-P-A-Tsh-313a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Top shoot	20	7.5	1.1	1280	120203	397
Sl-P-B-Tsh-314a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Top shoot	20	3.9	0.4	795	120203	392

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Length of tissues	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
Sl-P-C-Tsh-315a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Top shoot	20	7.3	1.0	939	120203	293
*SL-P-D-bsh-316a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Top shoot	20	10.3	0.9	1820	120312	348
Sl-P-A-Rt_-313b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Root	43.5	4.7	0.8	758	120207	397
Sl-P-B-Rt_-314b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Root	78.5	7.5	1.5	818	120207	392
Sl-P-C-Rt_-315b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Root	45	8.5	1.4	1030	120207	293
SL-PunD-Root-316B	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Sleepy Hollow	Low DDT-contaminated soil	Root	78	14.6	1.8	1080	120329	348
*An_Pum_A_bsh_350a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Bottom shoot	20	26.9	4.7	41180	120116	4653
An_Pum_B_bsh_351a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Bottom shoot	20	20.7	4.2	31900	120116	5485
An_Pum_C_bsh_352a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Bottom shoot	20	14.8	3.1	26600	120116	6583
An_P_D_bSh_353a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Bottom shoot	20	17.5	2.7	17300	120312	3775
*An_Pum_A_M sh_350a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Mid shoot	20	10.1	2.1	5200	120123	4653

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Length of tissues	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
An_Pum_B_M sh_351a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Mid shoot	20	9.1	2.2	5130	120123	5485
An_Pum_C_M sh_352a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Mid shoot	20	7.5	1.7	2650	120123	6583
An_P_D_mSh_353a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Mid shoot	20	10.2	1.2	6050	120312	3775
An_Pum_A_TSh_350a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Top shoot	20	5.1	0.8	379	120203	4653
*An_Pum_A_tsh_351a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Top shoot	20	9.7	1.7	1350	120203	5485
An_Pum_C_Tsh_352a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Top shoot	20	10.5	1.4	659	120203	6583
An_P_D_tSh_353a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Top shoot	20	7.9	0.7	1650	120312	3775
An_Pum_A_Rt_350b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Root	60.5	9.9	1.8	45100	120207	4653
*An_Pum_B_Rt__351b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Root	84.5	15.9	3.0	35500	120207	5485
An_Pum_C_Rt__352b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Root	133	11.7	2.2	23.3	120207	6583
An-PunD-Root-353B	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Anders Field	Moderate DDT-contaminated soil	Root	182	13.0	2.1	14500	120329	3775

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Length of tissues	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
Ag_Pum_B_bSh_344a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Bottom shoot	20	10.8	1.1	10200	120122	7406
Ag_Pum_C_bsh_345a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Bottom shoot	20	13.5	1.7	22900	120122	20507
Ag_Pum_A_bsh_343a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Bottom shoot	20	24.2	1.9	13100	120122	4343
Ag_P_D_bsh_346a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Bottom shoot	20	16.9	1.3	9470	120312	7922
Ag_P_A_M sh_343a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Mid shoot	20	8.5	1.3	6670	120123	7406
Ag_P_B_ M sh_344a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Mid shoot	20	8.8	1.2	5590	120123	20507
Ag_P_C_M sh_345a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Mid shoot	20	16.5	1.8	8580	120123	4343
Ag_P_D_msh_346a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Mid shoot	20	17.6	1.1	8180	120312	7922
Ag_P_A_Tsh_343a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Top shoot	20	5.6	0.5	3890	120203	7406
Ag_P_B_Tsh_344a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Top shoot	20	9.8	1.2	4130	120203	20507
Ag_P_C_Tsh_345a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Top shoot	20	11.8	0.9	964	120203	4343

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Length of tissues	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
Ag_P_D_tsh_346a	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Top shoot	20	14.1	0.9	3210	120312	7922
Ag_P_A_Rt__343b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Root	56	3.9	0.7	11800	120207	7406
Ag_P_B_Rt__344b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Root	69	7.0	1.1	16500	120207	20507
Ag_P_C_Rt__345b	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Root	88	6.5	1.2	17800	120207	4343
Ag-PunD-Root-346B	*Cucucbita pepo* ssp. *pepo* Howden	Pumpkin	Former Agricultural Land	High DDT-contaminated soil	Root	85	12.6	2.0	11300	120329	7922

Table B 2: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of three native grass species grown at low (291 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated site in PPNP

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run	Soil [DDT] ng/g
SL_Switch grass_317A-Sh	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Shoot	28.0	6.1	25.0	120309	248
SL_SG-Root-317B	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Root	12.5	4.8	63.0	120515	
SL_Switch grass_318A-Sh	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Shoot	22.2	4.5	32.0	120309	
SL_SG-Root-318B	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Root	6.7	2.3	46.0	120515	
SL_Switch grass_319A-Sh	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Shoot	10.0	2.4	17.0	120309	
SL_SG-Root-319B	*Panicum virgatum*	Switchgrass	Sleepy Hollow	Low DDT-contaminated soil	Root	9.1	3.7	48.0	120515	
An_Switch grass_304A-Sh	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated soil	Shoot	35.5	14.0	391	120212	5660
An_Switch grass_304B-Rt	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated soil	Root	20.0	8.8	848	120216	
An_Switch grass_305A-Sh	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated soil	Shoot	40.0	13.9	328	120212	
An_Switch grass_305B-Rt	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated	Root	18.7	5.6	1230	120216	

soil

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
An_Switch grass_306A-Sh	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated soil	Shoot	40.0	12.1	415	120212
An_Switch grass_306B-Rt	*Panicum virgatum*	Switchgrass	Anders Field	Moderate DDT-contaminated soil	Root	11.9	4.1	1170	120216
Ag-Switchgrass-340A-Sh	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Shoot	49.5	15.0	1470	120207
Ag-Switchgrass-340B-Rt	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Root	16.0	3.6	1680	120315
Ag-Switchgrass-341A-Sh	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Shoot	46.6	12.8	788	120207
Ag-Switchgrass-341B-Rt	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Root	8.9	3.2	1890	120315
Ag-Switchgrass-342A-Sh	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Shoot	35.0	9.5	744	120207
Ag-Switchgrass-342B-Rt	*Panicum virgatum*	Switchgrass	Former Agricultural Land	High DDT-contaminated soil	Root	9.5	2.5	2040	120315
SL-Little blueStem_320A-sh	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Shoot	17.3	5.1	64.0	120309
SL-Little blueStem _320B-Rt	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Root	5.7	2.3	144	120515
SL-Little blueStem_321A-sh	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Shoot	25.2	7.5	34.0	120309

7210

221

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
SL-Little blueStem_321B-Rt	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Root	3.5	1.6	182	120515
SL-Little blueStem_322A-sh	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Shoot	37.6	16.1	59.0	120309
SL-Little blueStem _322B-Rt	*schizachyrium scoparium*	Little bluestem	Sleepy Hollow	Low DDT-contaminated soil	Root	4.6	2.2	94.0	120515
An_Little blueStem_300A_Sh	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Shoot	11.3	3.4	1130	120216
An_Little blueStem-300B_Rt	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Root	12.8	2.9	6520	120329
An_Little blueStem_301A_Sh	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Shoot	44.5	11.8	837	120216
An_Little blueStem-301B_Rt	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Root	7.8	1.8	2784	120329
An_Little blueStem_302A_Sh	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Shoot	41.0	10.8	345	120216
An_Little blueStem_302B_Rt	*schizachyrium scoparium*	Little bluestem	Anders Field	Moderate DDT-contaminated soil	Root	10.2	2.3	5450	120329
Ag-Little blueStem-337A-Sh	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Shoot	67.3	16.0	2770	120123
Ag-Little blueStem-337B-Rt	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Root	4.3	1.0	4820	120315

3920

1250

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
Ag-Little blueStem-338A-Sh	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Shoot	27.3	6.1	4350	120123
Ag-Little blueStem-338B-Rt	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Root	1.9	0.5	5970	120315
Ag-Little blueStem-339A-Sh	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Shoot	21.2	5.0	3490	120123
Ag-Little blueStem-339B-Rt	*schizachyrium scoparium*	Little bluestem	Former Agricultural Land	High DDT-contaminated soil	Root	2.3	0.5	7570	120315
SL_Sanddropseed_326A-sh	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Shoot	18.9	5.2	21.0	120309
SL_Sanddropseed_326B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Root	1.8	0.5	74.0	120425
SL_Sanddropseed_327A-sh	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Root	41.6	14.8	23.0	120309
SL_Sanddropseed_327B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Shoot	2.4	0.6	109	120425
SL_Sanddropseed_328A-sh	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Shoot	22.4	11.8	16.0	120309
SL_Sanddropseed_328B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Sleepy Hollow	Low DDT-contaminated soil	Root	3.3	1.1	102	120425
An_Sanddropseed_307A-sh	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Shoot	54.0	23.0	160	120216

227

2580

Sample Number	Latin Name	Common Name	Site	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
An_Sanddropseed_307B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Root	3.4	0.9	3800	120515
An_Sanddropseed_308A-sh	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Shoot	35.6	13.8	415	120216
An_Sanddropseed_308B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Root	3.0	1.7	5080	120515
An_Sanddropseed_309A-sh	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Shoot	31.5	12.3	451	120216
An_Sanddropseed_309B_Rt	*Sporobolus cryptandrus*	Sand dropseed	Anders Field	Moderate DDT-contaminated soil	Root	3.1	1.4	6070	120515
Ag_Sand dropseed_331A-Sh	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Shoot	24.8	7.9	1460	120207
Ag_Sand dropseed_331B-Rt	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Root	1.5	0.3	5780	120315
Ag_Sand dropseed_332A-Sh	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Root	2.1	0.6	4210	120207
Ag_Sand dropseed_332B-Rt	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Shoot	23.8	7.2	11050	120315
Ag_Sand dropseed_333A-Sh	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Shoot	13.7	4.6	2540	120207
Ag_Sand dropseed_333B-Rt	*Sporobolus cryptandrus*	Sand dropseed	Former Agricultural Land	High DDT-contaminated soil	Root	2.5	0.6	15400	120315

5750

Table B 3: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples reported in chapter 4.

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	19	<1.0	19	<1.0	16	120122
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		95		80	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	18	<1.0	21	<1.0	12	120123
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		105		60	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	17	<1.0	19	<1.0	12	
Control Target	<1.0	20	<1.0	20	<1.0	20	120201
Recovery %		85		95		60	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	16	<1.0	18	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	120214
Recovery %		80		90		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	19	<1.0	14	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	120309
Recovery %		95		70		95	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	17	<1.0	16	<1.0	16	
Control Target	<1.0	20	<1.0	20	<1.0	20	120216
Recovery %		85		80		80	
Blank	<1.0	<0.5	<1.0	<0.5	<1.0	<0.5	
Control	<1.0	18	<1.0	17	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	120312
Recovery %		90		85		95	
Blank	<1.0	<1.0	<0.5	<1.0	<0.5	<0.5	
Control	<1.0	17	<1.0	15	<1.0	17	
Control Target	<1.0	20	<1.0	20	<1.0	20	120315
Recovery %		85		75		85	

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	17	<1.0	16	<1.0	15	
Control Target	<1.0	20	<1.0	20	<1.0	20	120329
Recovery %		85		80		75	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	22	<1.0	20	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	120425
Recovery %		110		100		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	18	<1.0	18	<1.0	15	
Control Target	<1.0	20	<1.0	20	<1.0	20	120515
Recovery %		90		90		75	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	19	<1.0	18	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		90		100	

Table B 4: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of soil samples

reported in chapter 4.

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	17	<1.0	18	<1.0	17	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		85	111129
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	18	<1.0	17	<1.0	15	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		90		85		75	111214
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	17	<1.0	18	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		85		90		95	111214
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
Control	<1.0	18	<1.0	18	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	120210

Table B 5: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 4.

Sample	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)		Total [DDT] (ng/g)
LBS-Root-302B	10.2	4.27	92.6	25.6	97.7	597		5090
LBS-Root-302B Dup	10.9	4541	60.5	26.3	119	691		5450
Mean	10.5	4403	76.5	25.9	108	644		
STD Dev	0.50	195	22.7	0.50	14.9	66.4		
RSD (%)	4.80	4.40	29.7	2.00	13.8	10.3	10.8	
An_Pum_B_M sh_351a	91.4	3410	105	99.5	741	680		5120
DupAn_Pum_B_M sh_351a	89.9	3460	145	96.0	761	725		5280
Mean	90.6	3430	125	97.8	751	703		
STD Dev	1.10	35.4	28.3	2.50	14.2	31.9		
RSD (%)	1.20	1.00	22.6	2.60	1.90	4.50	5.6	
SL-P-D-bsh-316a	3.20	1270	82.0	57.3	43.5	28.4		1470
Dup SL-P-D-bsh-316a	5.30	2060	24.7	0.80	54.3	29.3		2170
Mean	4.30	1657	53.3	29.1	48.9	28.8		
STD Dev	1.50	566	40.5	39.9	7.7	0.70		
RSD (%)	34.5	34.2	75.9	137	15.7	2.40	50.0	
An_Pum_A_bsh_350a	471	30750	1760	269	4170	2710		40130
Dup An_Pum_A_bsh_350a	528	32680	1000	534	4420	3060		42220
Mean	500	31710	1380	402	4290	2880		
STD Dev	39.9	1370	537	187	177	248		
RSD (%)	8.00	4.30	38.9	46.6	4.10	8.60	18.4	
SL_Sanddropseed_328A-sh	0.20	14.0	0.50	0.40	0.10	0.30		15.6
Dup SL_Sanddropseed_328A-sh	0.10	15.5	0.70	0.40	0.10	0.30		17.2
Mean	0.20	14.7	0.60	0.40	0.10	0.30		
STD Dev	0.10	1.00	0.10	0.0	0.0	0.0		
RSD (%)	34.4	7.00	22.5	6.4	13.8	0.30	14.1	
Ag_SS_331A-Sh	8.00	1090	23.7	98.6	77.6	360		1660
Dup Ag_SS_331A-Sh	4.90	827	23.7	83.6	54.5	263		1260
Mean	6.50	958	23.7	91.1	66.1	312		
STD Dev	2.20	185	0.0	10.6	16.4	70.6		
RSD (%)	34.0	19.3	0.0	11.7	24.8	22.6	18.7	

Sample	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)		Total [DDT] (ng/g)
An_Sanddropseed_308A-sh	2.00	363	6.30	37.1	2.60	3.50		415
Dup An_Sanddropseed_308A-sh	5.70	414	7.60	39.7	6.60	13.0		487
Mean	3.90	389	7.00	38.4	4.60	8.30		
STD Dev	2.60	36.1	0.90	1.90	2.80	6.70		
RSD (%)	68.1	9.30	12.6	4.80	60.2	81.1	39.3	
Ag-SG-340B-Rt	4.00	787	7.60	5.50	55.9	317		1180
DupAg-SG-340B-Rt	5.40	1624	16.9	9.20	79.9	451		2190
Mean	4.70	1206	12.3	7.30	67.9	384		
STD Dev	0.90	592	6.60	2.60	17.0	95.1		
RSD (%)	19.8	49.1	53.6	35.8	25.1	24.8	34.7	
SDS-Root-307B	9.70	3040	111	10.6	57.8	247		3470
SDS-Root-307 B Dup	9.70	3450	68.4	13.1	69.2	298		3910
Mean	9.70	3240	89.8	11.8	63.5	273		
STD Dev	0.00	291	30.2	1.70	8.10	35.9		
RSD (%)	0.30	9.00	33.7	14.6	12.7	13.2	13.9	
An_Pum_B_Tsh_351a	28.4	546	40.0	21.4	329	369		1340
DupAn_Pum_B_Tsh_351a	30.0	561	37.0	20.8	337	383		1370
Mean	29.2	554	38.5	21.1	333	376		
STD Dev	1.10	10.5	2.10	0.50	5.40	9.60		
RSD (%)	3.80	1.90	5.40	2.20	1.60	2.60	2.90	
An_Pum_B_Rt__351b	371	26500	305	61.3	3200	2100		32540
DupAn_Pum_B_Rt__351b	437	31500	352	37.1	3700	2400		38430
Mean	404	29000	329	49.2	3450	2250		
STD Dev	46.2	3540	33.2	17.1	354	212		
RSD (%)	11.4	12.2	10.1	34.8	10.2	9.40	14.7	

Table B 6: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of soil samples reported in chapter 4.

Sample	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)	Mean RSD (%)	Total [DDT] (ng/g)
29270	0.80	165	2.00	4.50	10.3	40.9		223
DUP29270	0.90	169	2.30	3.80	10.7	42.8		229
Mean	0.80	167	2.20	4.10	10.5	41.8		
STD Dev	0.10	2.90	0.20	0.50	0.30	1.40		
RSD (%)	6.80	1.70	7.90	12.6	2.90	3.20	5.9	
29274	0.80	193	3.00	3.00	7.70	31.0		238
DUP29274	0.80	208	6.90	3.40	8.00	32.8		259
Mean	0.80	200	5.00	3.20	7.80	31.9		
STD Dev	0.0	10.6	2.70	0.30	0.20	1.20		
RSD (%)	2.50	5.30	54.4	8.50	2.90	3.80	12.9	
29271	23.3	2240	81.6	49.4	112	546		3060
DUP29271	19.8	2660	85.9	44.3	122	604		3530
Mean	21.6	2450	83.7	46.9	117	575		
STD Dev	2.50	291	3.10	3.60	7.60	40.8		
RSD (%)	11.8	11.8	3.70	7.70	6.50	7.10	8.1	
300108	0.30	95.8	3.40	1.10	4.40	16.6		122
DUP300108	0.40	111	1.30	1.20	4.80	15.3		135
Mean	0.40	104	2.40	1.20	4.60	15.9		
STD Dev	0.10	11.1	1.50	0.10	0.30	0.90		
RSD (%)	25.1	10.7	62.0	7.00	6.10	5.50	19.4	
29269	37.8	4010	112	123	463	2890		7180
DUP29269	36.7	3560	118	119	413	2530		6430
Mean	37.3	3790	115	121	438	2710		
STD Dev	0.70	322	4.5	2.7	35.4	250		
RSD (%)	2.00	8.50	3.90	2.20	8.10	9.20	5.7	
29263	32.9	3810	111	154	519	2970		7600
DUP29263	24.7	3620	115	132	404	2920		7220
Mean	28.8	3715	113	143	462	2950		
STD Dev	5.7	133	3.1	15.5	81.5	33.9		

Table B 7: Wet weight and length of *C .pepo* and native weed species at low, moderate and high DDT-contaminated site.

DDT-contaminated sites	Name of the species	# of plant s	Wet weight of root (g)	Length of root (cm)	Wet weight of shoot (g)	Length of shoot (cm)
Low (Sleepy Hollow)	*S. scoparium* (little bluestem)	3	4.6	30.8	26.7	29.7
	P. virgatum (switchgrass)	3	9.4	30.3	20.1	54.3
	S. cryptandrus (sand dropseed)	3	2.5	20.3	27.6	67.2
	Cucurbita pepo cv. Howden (pumpkin)	4	13.0	61.3	391	116.5
Moderate (Anders field)	*S. scoparium* (little bluestem)	3	10.3	25.0	32.3	72.0
	P. virgatum (switchgrass)	3	16.9	35.7	38.5	75.8
	S. cryptandrus (sand dropseed)	3	3.2	22.3	40.4	80.0
	Cucurbita pepo cv. Howden (pumpkin)	4	15.0	115.0	624	228
High (Former Agricultural Land)	*S. scoparium* (little bluestem)	3	2.8	25.0	38.6	87.3
	P. virgatum (switchgrass)	3	11.5	35.7	43.7	88.2
	S. Cryptandrus (sand dropseed)	3	2.0	22.3	20.8	58.2
	Cucurbita pepo cv. Howden (pumpkin)	4	12.3	74.5	951	286

Table B 8: Total mean shoot extraction (ng) per plant species at low (291.3 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites.

DDT-contaminated sites	Plant species	# of plants	Mean Dry weight of root (g)	Mean Dry weight of shoot (g)	Mean shoot DDT extraction (ng)	Mean root DDT extraction (ng)
Low (Sleepy Hollow)	*S. scoparium* (little bluestem)	3	2.1	9.6	502	290
	P. virgatum (switchgrass)	3	3.6	4.3	110	208
	S. cryptandrus (sand dropseed)	3	0.7	10.6	230	68
	Cucurbita pepo cv. Howden (pumpkin)	4	1.4	53.2	70000	1300
Moderate (Anders field)	*S. scoparium* (little bluestem)	3	2.3	8.6	6700	11000
	P. virgatum (switchgrass)	3	6.2	13.3	5000	6700
	S. cryptandrus (sand dropseed)	3	1.3	16.4	6100	6600
	Cucurbita pepo cv. Howden (pumpkin)	4	2.3	114.8	1,380,000	68000
High (Former Agricultural Land)	*S. scoparium* (little bluestem)	3	0.7	9.1	32000	4000
	P. virgatum (switchgrass)	3	3.1	12.4	13000	5800
	S. Cryptandrus (sand dropseed)	3	0.5	6.6	18000	5700
	Cucurbita pepo cv. Howden (pumpkin)	4	1.3	88.4	716,000	18000

Table B 9: Translocation factors (TLFs) of plant species at low (291.3 ng/g), moderate (5083 ng/g) and high (10192 ng/g) DDT-contaminated sites.

TLFs	Low [DDT] (Sleepy Hollow)	Moderate [DDT] (Anders Field)	High [DDT] (Former Agricultural Land)
Schizachyrium scoparium (n=3)	0.42 (±0.22)	0.18 (±0.12)	0.59 (±0.13)
Panicum virgatum (n=3)	0.42 (±0.08)	0.36 (±0.10)	0.55 (±0.28)
Sporobolus cryptandrus (n=3)	0.23 (±0.04)	0.07 (±0.02)	0.27 (±0.11)

Appendix C

Additional information for chapter 5: Phytoextraction of weathered DDT by perennial native weed species under greenhouse conditions

Table C 1: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at low (2300 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5. 125
Table C 2: DDT metabolite concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at the low (2300 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5. 127
Table C 3: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at high (17500 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5. 129
Table C 4: DDT metabolite concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at the high (17500 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5. 131
Table C 5: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples.. 133
Table C 6: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 5 134
Table C 7: Root and shoot wet and dry weight percentages of each compartment to total biomass both for High DDT-contaminated soil and Low DDT-contaminated soil. 136
Table C 8: Shoot length of the native species 137
Table C 9: Translocation factors (TLFs) of the species at low and high DDT soil 137

Table C 1: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at low (2300 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5.

Sample Number	Latin Name	Common Name	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
O.E. Daisy 76A	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	shoot	17.0	1.2	1500	130228
O.E. Daisy 76B	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	Root	1.3	0.0	67000	133005
O.E. Daisy 78A	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	shoot	18.8	1.5	909	130228
O.E. Daisy 78B	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	Root	1.4	0.1	40100	133005
O.E. Daisy 80A	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	shoot	18.0	1.4	1020	130228
O.E. Daisy 80B	*chrysanthemum leucanthemum*	Ox-eye daisy	Low DDT-contaminated soil	Root	1.5	0.0	42200	133005
NEA 54A	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	shoot	9.4	2.5	186	130227
NEA 54B	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	Root	6.9	1.2	16000	133001
NEA 55A	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	shoot	11.8	3.7	176	130227
NEA 55B	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	Root	9.3	1.5	23600	133001
NEA 56A	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	shoot	15.5	4.3	188	130227
*NEA 56B	*Symphyotrichum novae-angliae*	New England aster	Low DDT-contaminated soil	Root	9.2	1.7	18870	133001
CGR 58A	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	shoot	9.9	1.3	534	130228
CGR 58B	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	Root	4.8	1.1	10500	133005
CGR 59A	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	shoot	9.1	1.1	178	130228

Sample Number	Latin Name	Common Name	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
CGR 59B	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	Root	5.0	1.0	12180	133005
*CGR62A	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	shoot	15.4	1.3	451	130228
*CGR 62B	*Solidago canadensis*	Canada goldenrod	Low DDT-contaminated soil	Root	4.4	1.1	18000	133005
RC 41A	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	shoot	13.4	2.8	698	130227
RC 41B	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	Root	4.1	0.9	10600	133001
RC 42A	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	shoot	16.8	3.5	579	130227
RC 42B	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	Root	6.7	1.2	15100	133001
*RC 46A	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	shoot	15.4	3.5	648	130227
RC 46B	*Trifollium pratense*	Red clover	Low DDT-contaminated soil	Root	4.4	0.8	8850	133001

Table C 2: DDT metabolite concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at the low (2300 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5.

Sample Number	Latine Name	2,4 DDE	4,4 DDE	2,4 DDD	4,4 DDD	2,4 DDT	4,4 DDT
GR58A	*Solidago canadensis*	58.2	306	<1.0	<1.0	<1.0	170
GR 58B	*Solidago canadensis*	30.2	7290	67.1	115	502	2530
GR 59A	*Solidago canadensis*	<1.0	108	<1.0	<1.0	<1.0	70.6
GR 59B	*Solidago canadensis*	<1.0	8490	<1.0	130	430	3120
GR62A	*Solidago canadensis*	<1.0	284	<1.0	<1.0	<1.0	170
GR 62A Dup	*Solidago canadensis*	<1.0	260	<1.0	<1.0	<1.0	188
GR 62B	*Solidago canadensis*	<1.0	15600	<1.0	70.0	454	3670
GR 62B Dup	*Solidago canadensis*	<1.0	11800	96.5	<1.0	412	3920
NEA 54A	*Symphyotrichum novae-angliae*	36.1	85.7	<1.0	7.00	10.8	46.3
NEA 54B	*Symphyotrichum novae-angliae*	316	1200	161	261	760	2457
NEA 55A	*Symphyotrichum novae-angliae*	70.1	60.8	<1.0	5.10	7.10	32.4
NEA 55B	*Symphyotrichum novae-angliae*	230	16600	380	1060	751	4590
NEA 56A	*Symphyotrichum novae-angliae*	58.7	66.3	<1.0	4.70	13.8	44.6
NEA 56B	*Symphyotrichum novae-angliae*	144	8350	190	535	382	3770
OED 76A	*chrysanthemum leucanthemum*	<1.0	1160	<1.0	<1.0	49.2	297

Sample Number	Latine Name	2,4 DDE	4,4 DDE	2,4 DDD	4,4 DDD	2,4 DDT	4,4 DDT
OED 76B	*chrysanthemum leucanthemum*	205	45500	580	1200	2600	16960
OED 78A	*chrysanthemum leucanthemum*	<1.0	694	<1.0	<1.0	34.2	181
OED 78B	*chrysanthemum leucanthemum*	63.4	26360	364	855	1430	10990
OED 80A	*chrysanthemum leucanthemum*	<1.0	648	221	<1.0	<1.0	158
OED 80B	*chrysanthemum leucanthemum*	188	22770	884	2890	1860	13580
RC 41A	*Trifollium pratense*	42.7	444	<1.0	10.9	29.3	171
RC 41B	*Trifollium pratense*	21.6	7480	46.3	108	375	2630
RC42A	*Trifollium pratense*	49.8	357	<1.0	3.00	26.4	142
RC 42B	*Trifollium pratense*	33.1	10450	79.6	287	530	3710
RC 46A	*Trifollium pratense*	73.1	373	<1.0	11.5	28.7	160
RC 46B	*Trifollium pratense*	19.0	6310	38.9	117	290	2080

Table C 3: Wet and dry weights, total DDT concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at high (17500 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5.

Sample Number	Latin Name	Common Name	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
CGR 48A	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Shoot	17.8	4.2	3980	130205
*CGR 48B	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Root	11.3	1.6	54000	130211
*CGR 63A	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Shoot	20.4	5.1	1200	130205
CGR 63B	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Root	15.9	3.0	40810	130211
CGR 64A	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Shoot	14.6	3.6	1080	130205
CGR 64B	*Solidago canadensis*	Canada goldenrod	High DDT-contaminated soil	Root	9.3	2.0	26500	130211
NEA 50A	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Shoot	14.6	3.6	279	130204
NEA 50B	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Root	8.5	1.2	44100	130208
NEA 51A	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Shoot	10.4	3.4	345	130204
NEA 51B	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Root	5.1	1.3	37700	130208
NEA 52A	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Shoot	11.3	3.5	430	130204
NEA 52B	*Symphyotrichum novae-angliae*	New England aster	High DDT-contaminated soil	Root	11.0	1.9	61900	130208
O.E. Daisy 68A	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Shoot	42.5	4.7	5280	130205
O.E. Daisy 68B	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Root	6.4	0.3	140000	130211
O.E. Daisy 71A	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Shoot	52.1	8.0	2770	130205

Sample Number	Latin Name	Common Name	Treatment	Section	Total ww (g)	Total dw (g)	[DDT] (ng/g)	Run
O.E. Daisy 71B	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Root	7.4	0.3	156000	130211
O.E. Daisy72A	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Shoot	46.6	7.4	7000	130205
O.E. Daisy 72B	*chrysanthemum leucanthemum*	Ox-eye daisy	High DDT-contaminated soil	Root	7.6	0.7	99300	130211
RC 31A	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Shoot	20.6	4.6	2090	130204
RC 31B	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Root	7.6	0.8	29600	130208
*RC 36A	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Shoot	23.0	5.1	4500	130204
*RC 36B	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Root	6.4	1.0	36400	130208
RC 38A	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Shoot	13.5	3.1	1470	130204
RC 38B	*Trifollium pratense*	Red clover	High DDT-contaminated soil	Root	5.8	0.9	30500	130208

Table C 4: DDT metabolite concentrations (ng/g) in shoot and root tissues of four native and naturalized weeds grown at the high (17500 ng/g) DDT-contaminated soil under greenhouse condition reported in chapter 5.

Sample Number	Latine Name	2,4 DDE	4,4 DDE	2,4 DDD	4,4 DDD	2,4 DDT	4,4 DDT
GR 48A	*Solidago canadensis*	196	470	265.0	<1.0	280	330
GR 48B	*Solidago canadensis*	<1.0	31400	<1.0	<1.0	2170	18400
GR 48B Dup	*Solidago canadensis*	<1.0	33900	<1.0	<1.0	2290	19700
GR 63A	*Solidago canadensis*	45.3	741	<1.0	5.60	72.1	430
GR 63A Dup	*Solidago canadensis*	227.3	448	<1.0	<1.0	102	328
GR 63B	*Solidago canadensis*	<1.0	23200	<1.0	240	1930	15500
GR 64A	*Solidago canadensis*	78.8	383	65.4	<1.0	264	291
GR 64B	*Solidago canadensis*	<1.0	14200	<1.0	<1.0	1420	10900
NEA 50A	*Symphyotrichum novae-angliae*	33.1	139	<1.0	<1.0	13.1	94.5
NEA 50B	*Symphyotrichum novae-angliae*	132	26000	420	613	2410	14500
NEA 51A	*Symphyotrichum novae-angliae*	7.80	178	<1.0	<1.0	23.1	136
NEA 51B	*Symphyotrichum novae-angliae*	118	20050	300	431	1880	14900
NEA 52A	*Symphyotrichum novae-angliae*	49.5	198	<1.0	<1.0	28.7	153
NEA 52B	*Symphyotrichum novae-angliae*	233	37970	651	1140	3190	18700
OE Daisy 68A	*chrysanthemum leucanthemum*	<1.0	3360	<1.0	46.1	253	1630
OE Daisy 68B	*chrysanthemum leucanthemum*	317	78700	1210	1294	6480	51600

Sample Number	Latine Name	2,4 DDE	4,4 DDE	2,4 DDD	4,4 DDD	2,4 DDT	4,4 DDT
OE Daisy 71A	*chrysanthemum leucanthemum*	<1.0	1170	170	7.20	850	575
OE Daisy 71B	*chrysanthemum leucanthemum*	386	85500	1660	2650	77900	57600
OE Daisy 72B	*chrysanthemum leucanthemum*	151	54700	999	2347	4210	36900
RC 31A	*Trifollium pratense*	83.5	1010	21.5	24.5	106	847
RC 31B	*Trifollium pratense*	54.7	15700	177	487	1280	11900
RC 36A	*Trifollium pratense*	30.4	2460	42.4	64.4	238	1950
RC 36A Dup	*Trifollium pratense*	36.5	1980	49.4	63.9	223	1790
RC 36B	*Trifollium pratense*	90.5	21700	221	509	1790	11800
RC 38A	*Trifollium pratense*	20.1	777	19.2	12.8	73.3	572
RC 38B	*Trifollium pratense*	71.2	17300	219	412	1730	10800
RC 36B Dup	*Trifollium pratense*	81.8	18400	300	849	1760	15300

Table C 5: Total DDT concentrations in (ng/g) in spiked control samples and analytical blanks for DDT metabolites analysis of plant samples reported in chapter 5.

Sample	2,4-DDE	4,4-DDE	2,4-DDD	4,4-DDD	2,4-DDT	4,4-DDT	Run
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130204
Control	<1.0	20	<1.0	19	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		95		100	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130205
Control	<1.0	22	<1.0	22	<1.0	23	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		110		110		115	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130208
Control	<1.0	24	<1.0	22	<1.0	22	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		120		110		110	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130211
Control	<1.0	21	<1.0	20	<1.0	20	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		105		100		100	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130227
Control	<1.0	20	<1.0	19	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		95		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130228
Control	<1.0	20	<1.0	19	<1.0	19	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		95		95	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130301
Control	<1.0	19	<1.0	18	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		95		90		90	
Blank	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	130305
Control	<1.0	20	<1.0	19	<1.0	18	
Control Target	<1.0	20	<1.0	20	<1.0	20	
Recovery %		100		95		90	

Table C 6: Total DDT concentrations (ng/g) and relative standard deviations (RSD) (%) of analytical duplicates for total DDT metabolites analysis of plant samples reported in chapter 5

Plant	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 DD (ng/g)	Mean RSD (%)	Total [DDT] ng/g
Goldenrod 63A	45.3	741	<1.0	5.6	72.1	430		1290
Goldenrod 63A Duplicate	227	448	<1.0	0.0	102	328		1100
Mean	136	594	<1.0	2.8	87.2	380		
STD	129	207	<1.0	4.0	21.3	72.1		
RSD (%)	94.4	34.9	<1.0	141	24.5	19.0	52.4	
Red Clover 36B	90.5	2200	221	509	1790	11800		36100
Red Clover 36B Duplicate	81.8	18400	300	849	1760	15300		36700
Mean	86.1	20100	260	679	1770	13600		
STD	6.10	2320	55.8	241	20.3	2470		
RSD (%)	7.10	11.5	21.4	35.4	1.1	18.2	15.8	
GoldenRod 48B	<1.0	31400	<1.0	<1.0	2170	18300		51900
GRod 48B Dup	<1.0	33900	<1.0	<1.0	2280	19700		55900
Mean	<1.0	32600	<1.0	<1.0	2220	19000		
STD	<1.0	1740	<1.0	<1.0	81.8	970		
RSD (%)	<1.0	5.30	<1.0	<1.0	3.70	5.10	2.30	
Red Clover 46A	73.1	373	<01.0	11.5	28.7	160		650
Red Clover 46A Dup	65.9	362	11.5	14.1	28.6	168		650
Mean	69.5	367	5.80	12.8	28.6	164		
STD	5.10	8.1	8.20	1.80	0.1	5.90		
RSD (%)	7.30	2.2	141	14.4	0.2	3.60	28.2	
Goldenrod 62A	<1.0	284	<1.0	<1.0		169		454
Goldenrod 62A Duplicate	<1.0	259	<1.0	<1.0		188		448
Mean	<1.0	272	<1.0	<1.0		179		
STD	<1.0	17.4	<1.0	<1.0		13.3		

Plant	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 DD (ng/g)	Mean RSD (%)	Total [DDT] ng/g
RSD (%)	<1.0	6.40	<1.0	<1.0	<1.0	7.40	2.30	
NEA 56B	144	13200	260	605	608	3350		
NEA 56B Duplicate	172	13700	241	566	695	4190		
Mean	158	13400	251	585	651	3770		
STD	19.8	292	13.4	27.6	60.9	592		
RSD (%)	12.5	2.2	5.30	4.7	9.4	15.7	8.30	
GR 62B	<1.0	15600	<1.0	68.8	454	3660		18200
GR 62B Dup	<1.0	11800	96.5	<1.0	411	3920		19500
Mean	<1.0	13700	48.3	34.4	433	3790		
STD	<1.0	2660	68.3	48.7	29.8	182		
RSD (%)	<1.0	19.5	141.4	141	6.9	4.80	52.3	

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Table C 7: Root and shoot wet and dry weight percentages of each compartment to total biomass both for High DDT-contaminated soil and Low DDT-contaminated soil.

[DDT]	Species	Mean root wet weight	mean root dry weight	% Root	Mean shoot wet weight	Mean shoot dry weight	% Shoot
High [DDT]	*Symphyotrichum novae-angliae*	8.2	1.5	40.4	12.1	3.5	59.6
	Trifollium pratense	6.6	1.0	25.7	19.0	4.3	74.3
	Chrysanthemum leucanthemum	7.1	0.4	13.1	47.1	6.7	86.9
	Solidago canadensis	12.2	2.2	40.9	17.6	4.3	59.1
Low [DDT]	*Symphyotrichum novae-angliae*	8.4	1.5	40.9	12.2	3.5	59.1
	Trifollium pratense	5.0	1.0	24.8	15.2	3.3	75.2
	Chrysanthemum leucanthemum	1.4	0.1	7.2	17.9	1.4	92.8
	Solidago canadensis	4.7	1.1	29.1	11.5	1.2	70.9

Table C 8: Shoot length of the native species

[DDT]	Species	Length of shoot (cm)
High [DDT]	*Symphyotrichum novae-angliae*	59
	Trifollium pretense	51
	Chrysanthemum leucanthemum	23
	Solidago canadensis	82
Low [DDT]	*Symphyotrichum novae-angliae*	70
	Trifollium pretense	33
	Chrysanthemum leucanthemum	22
	Solidago canadensis	56

Table C 9: Translocation factors (TLFs) of the species at low and high DDT soil

Species	TLFs (Low DDT soil) 2300 ng/g	TLFs (High DDT soil) 17500 ng/g
Symphyotrichum novae-angliae	0.01(±0.002)	0.007(±0.001)
Trifollium pratense	0.06(±0.018)	0.08 (±0.038)
Chrysanthemum leucanthemum	0.007(±0.003)	0.04 (±0.026)
Solidago Canadensis	0.10(±0.045)	0.05 (±0.023)