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DDT in aged, contaminated soils from Swedish forest nurseries - treatment with white-rot fungi

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Cover: Watercolour compilation of various white-rot fungi studied within this thesis work. Artist: Daisy Charlsworth, 2022.

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Abstract

Dichlorodiphenyltrichloroethane (DDT) was used across the world for over 50 years but was banned due to its ecotoxic effects. It is classed as a persistent organic pollutant (POP) because its highly hydrophobic nature allows it to bioaccumulate and biomagnify up the food chain. The transformation products of DDT that appear due to natural processes are also harmful environmental pollutants. Many strategies have been used to manage DDT contaminated soils, sediments, and waters, but no *in-situ* environmentally safe remediation strategy exists.

This thesis tests the capacity of multiple white-rot fungi to degrade DDT and its transformation products (DDX) in aged, contaminated soils from former forest nursery sites in Sweden. White-rot fungi can degrade a variety of POPs by producing high redox potential oxidative extracellular enzymes, such as peroxidases and laccases. Their bioremediation capacity was tested on soils of varying DDX concentrations, across various scales, using fungus-inoculated grain, straw, and spent mushroom substrate. Surfactant was applied due to the limited bioavailability of hydrophobic compounds that partition to organic matter. To improve risk assessment and comparison of soil treatment alternatives, partitioning coefficients for DDX were determined for a passive sampling material (polyoxymethlylene; POM). The method was applied and validated for measurements of freely dissolved DDX concentrations in pore water of contaminated soil.

Overall, this thesis demonstrates the effectiveness of white-rot fungi in degrading DDT and its transformation products. It also explores limitations of upscaling, and how surfactants can mitigate the effects of soil hydrophobic pollutant aging, namely partitioning into organic matter.

Keywords: White-rot fungi; bioremediation; contaminated soil; dichlorodiphenyltrichloroethane; DDT; polyoxymethylene; POM; passive sampling; partitioning coefficients; pilot reactor; spent mushroom substrate

DDT i historiskt förorenad jord från svenska skogsplantskolor - behandling med vitrötesvampar

Sammanfattning

Diklordifenyltrikloretan (DDT) användes över hela världen i över 50 år men förbjöds på grund av sina ekotoxikologiska effekter. DDT klassificeras som en persistent organisk förorening (POP) eftersom dess starkt hydrofoba natur gör att det kan bioackumuleras och biomagnifieras uppåt i näringskedjan. DDT:s omvandlingsprodukter, som bildas genom naturliga processer, är också skadliga miljöföroreningar. Många strategier har använts för att hantera DDT-förorenade jordar, sediment och vatten, men någon miljövänlig saneringsstrategi har ännu inte etablerats.

Denna avhandling testar utvalda vitrötesvampars förmåga att bryta ner DDT och dess omvandlingsprodukter (DDX) i historiskt förorenad jord från tidigare skogsplantskolor i Sverige. Vitrötesvampar kan bryta ner en mängd olika POP:ar genom att producera oxidativa extracellulära enzymer med hög redoxpotential, såsom peroxidaser och lackaser. Deras kapacitet för bioremediering testades på jordar med varierande DDX-koncentrationer och i olika skalor, med användning av svampinokulum, halm och förbrukat svampsubstrat. Ett ytaktivt ämne tillsattes på grund av den begränsade biotillgängligheten av hydrofoba föreningar som binder starkt till organiskt material. För att förbättra riskbedömning och möjliggöra jämförelser mellan olika saneringsalternativ bestämdes fördelningskoefficienter för DDX för ett passivt provtagningsmaterial (polyoximetylen; POM). Metoden tillämpades och validerades för mätning av fritt lösta DDX-koncentrationer i porvatten i förorenad jord.

Sammantaget visar denna avhandling effektiviteten hos vitrötesvampar vid nedbrytning av DDT och dess omvandlingsprodukter. Den belyser också begränsningar med uppskalning samt hur tensider kan minska effekterna av att hydrofoba föroreningar binder starkt till organiskt material.

Nyckelord: Vitrötesvampar; bioremediering; förorenad jord; diklordifenyltrikloretan; DDT; polyoximetylen; POM; passiv provtagning; fördelningskoefficienter; bioreaktor; förbrukat svampsubstrat

Dedication

To Rachel Carson, and all the environmental whistleblowers who changed the world.

"We are stuck with technology when what we really want is just stuff that works."
— Douglas Adams, The Salmon of Doubt: Hitchhiking the Galaxy One Last Time

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Enell A.*, **Casey S.***, Au Musse A., Josefsson S., Kikuchi-McIntosh J., Nilén G., Wiberg K, Dahlberg A.-K., Larsson M. (2025), Determination of polyoxymethylene (POM) water partition coefficients for DDT and its degradation products, with inter-laboratory comparison of the passive sampling methodology and bioaccumulation in earthworm (*Eisenia fetida*). *Environmental Chemistry* 22, https://doi.org/10.1071/EN25011 *shared 1st authorship

II. **Casey, S.,** Wiberg, K., Lindahl, B. D., Dahlberg, A. K., & Hultberg, M. (2026). Enhanced DDT degradation in aged soils via fungal treatment and surfactant application. *Chemical Engineering Journal: Green and Sustainable* 2, https://doi.org/10.1016/j.cejgas.2026.100051

III. **Casey S.**, Wiberg K., Dahlberg A.-K., Hultberg M., Volchko Y. Evaluation of two applied approaches for treatment of aged DDT-contaminated soil with white-rot fungi. *Submitted to:* PLOSOne

IV. **Casey S.**, Hultberg M., Wiberg K., Sarra M. A pilot study on use of *Gandoderma lucidum* in bioreactor for treatment of aged soil contaminated with DDT. *Submitted to:* Applied Microbiology

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The contribution of Stephanie Casey to the papers included in this thesis was as follows:

I. Planned and executed field work to collect soils from nine forest nursery sites across Sweden, laboratory work, data extraction and analysis, writing, editing, revision of the manuscript

II. Planning the study together with supervisors, literature search, design and execution of the study, data extraction and analysis, writing, editing, revision, submission of manuscript.

III. Planning and advising on study design, laboratory work, extraction and analysis of data, writing, editing and revision and submission of manuscript.

IV. Design of study together with researchers, assembly and running of studies, data analysis, writing, editing, revision and submission of manuscript.

Abbreviations

DBP	bis(4-chlorophenyl)methanone
DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
DDE	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
DDM	1-chloro-4-[(4-chlorophenyl)methyl]benzene
DDMU	1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene
DDT	1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene
DDX	DDT and its transformation products
GC-MS/MS	gas chromatography coupled to tandem mass spectrometry
K_{OW}	partitioning coefficient for octanol-water
K_{POM}	partitioning coefficient for POM-water
LiP	lignin peroxidase
MnP	manganese peroxidase
POM	polyoxymethylene
SMS	spent mushroom substrate
WRF	white-rot fungi

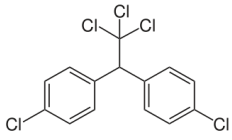
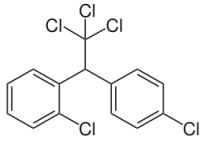
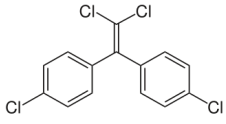
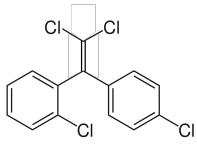
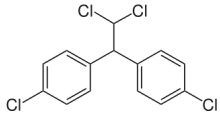
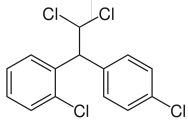
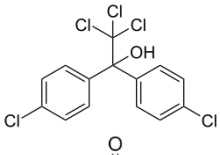
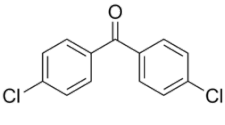
1. Background

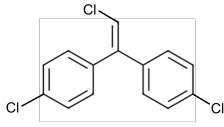
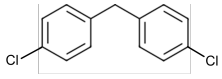
1.1 DDT - Uses, transformation products, and harms

DDT was first synthesized in 1874, but only after 65 years was it discovered to be a highly effective insecticide. It was then implemented as a disease vector control, substantially altering the management of malaria and typhus (Mansouri et al., 2017). The World Health Organisation (WHO) relied heavily on its use, and by the 1950s it was a household item across the world. DDT has transformed both the agricultural industry and disease vector management globally, but concerns for its environmental safety have always existed. In 1962, one of the first major environmental whistle-blowing events took place in the form of the book "Silent Spring" by marine biologist and conservationist Rachael Carlson (Carson, 1962). Focusing on the United States, Carson demonstrated the catastrophic effects that DDT was having on aquatic creatures and birds, raised concerns regarding its potential carcinogenicity, and argued the irresponsibility of releasing untested chemicals into the environment for both human and non-human exposure. This sent ripples across the world and Sweden banned DDT for agricultural use in 1969, preceding the US by two years (Hayes, 1969). A 30-year campaign culminated in the eventual global ban of DDT in agriculture, as formalised by the Stockholm convention of persistent organic pollutants (POPs) in 2001, with the exemption of use in disease vector management within WHO guidelines (Stockholm Convention, 2001).

DDT is a trichlorinated aromatic hydrocarbon with five chlorines and two aromatic rings, joined with an ethane backbone, capable of forming either the *p,p′*- or *o,p′*-isomers (Table 1). The desired compound is *p,p′*-DDT, and *o,p′*-DDT is the isomeric impurity. It is highly hydrophobic with an octanol-water partitioning coefficient (K_{OW}) of log K_{OW} 5.92 (ARCHem SPARC 2010). Its intended mode of action is opening Na^+ channels in insect neurons, leading to seizure and death (Dong, 2007). Unfortunately, DDT does not only act on the intended pests but many insects that serve critical ecosystem functions such as pollination.

Table 1: Molecular structures, molecular weight (MW) and logarithm of octanol-water partitioning coefficient (K_{OW}) (ARCHem SPARC 2010), of the target DDX in their *p,p′*- and *o,p′*-isomers. Adapted from Paper I, Table S1.

Chemical structure	Name	ID	MW	log K_{OW}
	1,1,1-trichloro-bis-2,2(4-chlorophenyl)ethane	*p,p*'-DDT	354.49	6.84
	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene	*o,p*'-DDT	354.49	6.68
	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene	*p,p*'-DDE	318.03	6.89
	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene	*o,p*'-DDE	318.03	6.82
	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene	*p,p*'-DDD	320.04	6.11
	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene	*o,p*'-DDD	320.04	5.98
	2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol	dicofol	370.49	5.83
	4,4'-dichlorobenzophenone	*p,p*'-DBP	251.11	4.34

	1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene	*p,p*'-DDMU	283.58	6.16
	1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene	*p,p*'-DDM	237.12	5.34

Alongside this, its major transformation products, dichloro-diphenyldichloroethylene (DDE) and dichlorodiphenyl-dichloroethane (DDD), which have log K_{OW} values between 6.0 and 6.9 respectively (ARCHem SPARC 2010), are also classed as persistent organic pollutants (POPs) and have shown toxicity to rats, birds, fish, and as probable human carcinogens (Mansouri et al., 2017). For example, DDE acts as an endocrine disrupter (anti-androgenic), that causes eggshell thinning in fish and birds, leading to their parents crushing them instead of incubating them. Examples of species which have been threatened by this are the peregrine falcon, bald eagle, and many songbirds and waterfowl (ATSDR, 2022). DDD is in turn classed as a probable human carcinogen, linked to lung, liver and thyroid tumours in various animals (IARC, 2020). In the transformation pathway of DDT, many of the intermediates produced are also more mobile, which can increase risk of leaching into groundwater or surface water. The main toxic compounds, DDT, DDE and DDD, all have high hydrophobicity; therefore, once in the food chain they can bioaccumulate in lipid tissues and biomagnify, causing catastrophic damage at many trophic levels (European Food Safety Authority (EFSA), 2006; Hellou et al., 2013).

1.2 The Swedish situation

In Sweden, the use of DDT was highly effective in combating the risk posed by the pine weevil (*Hylobius abietis L.*) in monoculture spruce and pine plantations by the forestry industry (Lalík et al., 2021; EFSA, 2006). Despite well documented risks to both workers' health and the surrounding ecosystem, DDT was used throughout the 50s and 60s to dip saplings before planting, effectively protecting them. Due to its tenacity in soils and recalcitrant structure, over 750 forest nursery sites where DDT was used remain contaminated to this day (Swedish Geotechnical Institute (SGI), 2017). Within the soil structure, DDT can occupy both the pore space in the water fraction, or sorb tightly to the organic matter in the soil and particulate matter in pore water, where it is less bioavailable and can evade degradation for decades (Guo et al., 2014; Fought et al., 2001; Mansouri et al., 2017).

Across Sweden, there are many sites still exceeding the Swedish Environmental Protection Agency's guideline of 1 mg kg^{-1} dry weight (d.w.), measured as a weighted sum of *p,p′*- and *o,p′*-DDT, DDD and DDE, and requiring remediation (Naturvårdsverket, 2016). The sites are primarily managed by Swedish state-owned company Sveaskog, and the Geological Survey of Sweden (SGU), who are now making efforts to remediate the contaminated land. A map of the sites managed by these actors are shown in Figure 1. The contamination of the soils measured in our study ranges from 3 to 130 mg kg^{-1} d.w., far exceeding the safety guidelines limit. This far, remediation has often entailed the dig-and-dump method of excavation and transport to landfill sites. Unfortunately, this damages the excavated sites, with many decades needed to replenish the organic layer, soil structure, and soil community, and also leaves the DDT in the environment. From landfill sites, the DDT may still leach into ground water, becoming a risk for aquatic beings, or spread to the terrestrial environment and food chain (Mansouri et al., 2017).

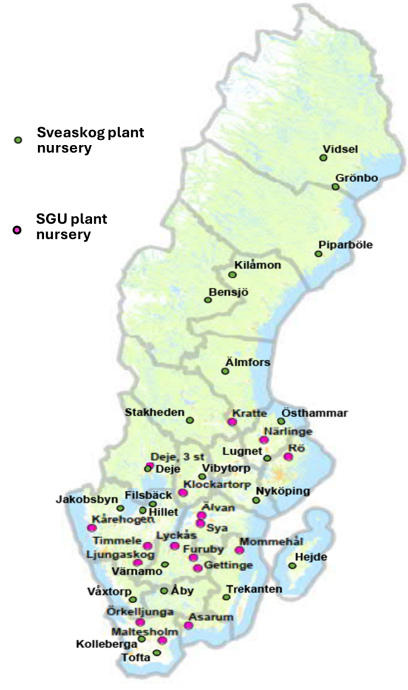


Figure 1: Former forest plant nursery sites managed by Sveaskog (green) and SGU (pink) that have been assessed for DDT contamination and found to have areas exceeding 5 mg kg^{-1} d.w. Source: Hanna Wåhlen, SGU.

Biochar appears to be a promising remediation strategy to immobilise the DDT, as it sorbs tightly to the biochar and thereby becomes much less bioavailable (Drenning et al., 2024; Harwood et al., 2022). Uptake into plant and invertebrate life may be reduced below a safe threshold after treatment. However, this does not remove the DDT from the environment, nor degrade it, thus leaving potential for desorption and assimilation into the food chain. While this strategy may work for immobilisation on large areas of land, small scale high contamination sites are still excavated. Consequently, sustainable remediation strategies that can work on large scale are still urgently necessary to develop. This thesis takes part in this development, exploring the various contexts and conditions under which bioremediation using white-rot fungi (WRF) can degrade DDT and its transformation products, hereafter collectively referred to as DDX.

Additionally, risk assessment for many contaminated sites in Sweden remains incomplete, even though ecotoxicological assessments exist for a many plant species, soil invertebrates, mammals, and avian fauna (U.S. EPA, 2007). Although regulation thresholds for DDT concentrations in soil are available, the environmental behaviour and fate of DDX are complex and variable (Agency for Toxic Substances and Disease Registry, 2022). The mobile and bioavailable fractions of DDX cannot be reliably inferred just from total soil concentrations, as factors such as soil organic matter content strongly influence bioavailability, adding uncertainty to risk assessment techniques (van Hall et al., 2023). Furthermore, while the toxicity of DDX-contaminated soils has been tested for soil fauna, a knowledge gap remains as to whether observed biological effects correlate better with the bioavailable concentration of DDX rather than with total soil concentrations. Passive sampling can be used for site-specific risk assessment of bioavailable concentrations in soil pore water, as it measures freely dissolved DDX, which corresponds to the bioavailable concentration (Hawthorne et al., 2011; Wang et al., 2018).

Remediation approaches that involve the need to mobilise hydrophobic contaminants, such as DDT, often use surfactants, which may unintentionally mobilise contaminants into groundwater or enable uptake by soil organisms (Gonzales et al., 2019; Sudharshan et al., 2012). Consequently, there is a need for accurate methods capable of detecting and quantifying DDX compounds in the different soil compartments and tracking changes in contaminant mobility and bioavailability during and after treatment, even at low levels. Some DDX are also taken up by a variety of crops and tree species (Lunney et al., 2004; Mermer et al., 2020), so assessing whether land is safe for human or livestock exposure requires reliable measurements of the bioavailable fraction. Therefore, one component of this thesis investigated the use of passive samplers (polyoxymethylene (POM) strips) to estimate the freely dissolved, bioavailable pore-water concentrations of DDX in contaminated soils.

1.3 White-rot fungi

1.3.1 Lifestyle and degradation mechanisms

White-rot fungi (WFR) are saprotrophs within the mushroom-forming class Agaricomycetes (Floudas et al., 2012) that inhabit woody tissues of plants and have capacity to degrade all the macromolecules of the plant cell wall, including lignin, to access energy from cellulose, and scavenge their required nitrogen from nutrient-poor environments (Keiluweit et al., 2015). Lignin is a complex, non-uniformly linked macromolecule composed of three main types of phenolic aromatic monomers: coniferyl, sinapyl and paracoumaryl alcohol. To decay lignin, the fungi rely on extracellular oxidative processes involving enzymes such as class II peroxidases, laccases, and dye-decolorizing peroxidases (Lundell et al., 2014).

Class II peroxidases such as manganese dependent peroxidases, lignin peroxidases, and versatile peroxidases act through long-range attacks on lignin using high redox potential, low molecular weight intermediates (Lundell et al., 2014). These can be veratryl alcohol, oxalic acid, or other cofactors, and require H_2O_2 produced by the fungus to remain active. Furthermore, manganese dependent and versatile peroxidases oxidise Mn^{2+} to Mn^{3+} which diffuse through wood as low molecular weight redox agents, oxidising bonds in phenolic structures. They are consequently reduced to Mn^{2+}, which can then be reused.

Laccase has lower redox potential than peroxidases but oxidises lignin aromatic rings by reducing oxygen to create OH radicals, which then introduce OH groups onto the ring. This can then leave targeted compounds vulnerable to bond cleavage between aromatic carbons (Hildén et al., 2013). Within the CAZy database (Drula et al., 2022), laccases and laccase-like multicopper oxidases fall within the auxiliary activity (AA) family AA1, while class II peroxidases are within AA2. In general, a higher number of AA1 and AA2 genes implies higher capacity for ligninolytic enzyme activity by the fungus, in a wider range of conditions. Importantly, actual expressed enzyme activity is strongly influenced by environmental and substrate-specific cues, so expression can manifest very differently from what may be inferred from genomes. Environmental triggers, such as the presence of

lignin, veratryl alcohol, manganese, or an absence of available nitrogen, or amendment with woody biomass can enhance the expression and activity of ligninolytic enzymes (Buswell et al., 1995; Heinzkill et al., 1998; Ikehata et al., 2004; Janusz et al., 2013).

Brown rot fungi are also capable of degrading lignin to a lesser extent, using Fenton chemistry (Purnomo et al., 2008; Purnomo et al., 2010b). This uses Fe^{2+} to crease hydroxyl radicals from H_2O_2, fulfilling a similar purpose to class II peroxidases. These enzymatic and regulatory characteristics provide a mechanistic basis for selecting fungal species capable of remediating soil under environmentally relevant conditions.

The production of ligninolytic enzymes may vary throughout the fungal lifecycle. Spent mushroom substrate (SMS), or spent mushroom waste, is a by-product of mushroom cultivation when the fungus is post-fruiting and largely senescent. Although there is a risk that production of ligninolytic enzymes has declined in the exploited substrate (Bellettini et al., 2019), it may still exhibit high ligninolytic capacity depending on the species (Ghose, 2022; Grimm & Wösten, 2018), and mycelium can potentially reactivate if supplied with fresh substrate, aeration, and favourable temperature and moisture.

1.3.2 White-rot fungi in DDT remediation

Remediation using WRF has been applied to many environmental pollutants, from heavy metals, to pharmaceuticals, xenobiotics and pesticides. Various mechanisms have been proposed and there is general agreement that the detoxification of the environment relies on extracellular high oxidative capacity enzymes producing radicals. In the microbial transformation pathway of DDT, a sequence of reactions occurs that yields several persistent transformation products, as depicted in Figure 2 (Aislabie & Richards, 1997).

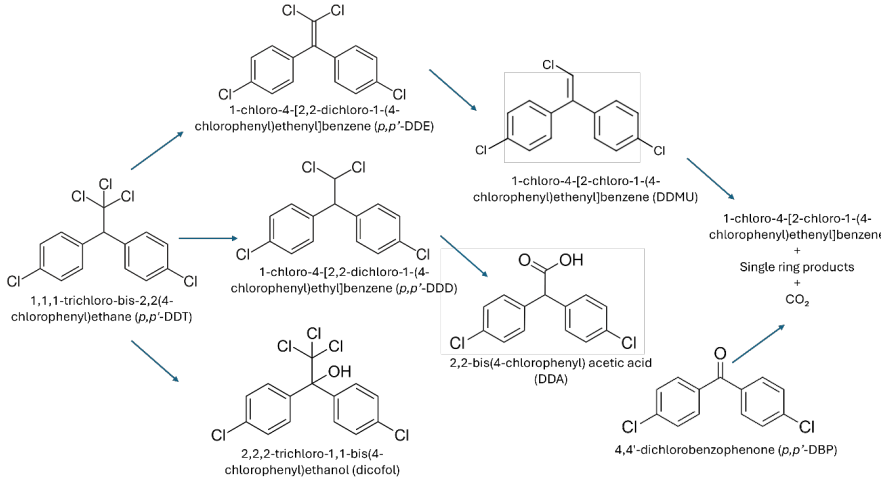


Figure 2: Proposed pathway of DDT transformation by white-rot fungi (adapted from Mohapatra et al., 2018). Credit: R. Biella, 2026

The initial transformation of DDT in soils is reductive dechlorination, where one of the aliphatic chlorines is removed to form DDE, or replaced with a hydrogen atom to form DDD, depending on whether the conditions are anaerobic or aerobic (Aislabie et al., 1997; EFSA, 2006). Further oxidation of DDT, replacing the other two aliphatic chlorines with a carboxyl group in its place forms bis(*p*-chlorophenyl)acetic acid (DDA). The carboxyl group increases polarity and solubility and is therefore a key step in enhancing bioavailability and enabling further degradation by soil microorganisms, including both bacteria and fungi (Mansouri et al., 2017; Sudharshan et al., 2012).

WRF have demonstrated the capacity to transform DDT and its metabolites in laboratory systems, through extracellular oxidative mechanisms associated with ligninolytic enzyme systems (Barr et al., 1994; Bumpus et al., 1987; Zhao et al., 2010). The reactive radicals that are generated by laccase and class II peroxidases to attack lignin molecules are used to oxidise transformation products and hydroxylate and partially cleave aromatic rings, resulting in smaller chlorinated products, such as dichlorobenzophenone (DBP) and dicofol (Mohapatra et al., 2018; Xiao et al., 2010). Complete mineralisation of DDT to carbon dioxide has been demonstrated in

controlled laboratory experiments, most notably using *Phanerochaete chrysosporium* in liquid cultures (Bumpus et al., 1987).

Some research suggests that the cytochrome P450 detoxification system (CytP450) is also involved in DDT degradation (Purnomo et al., 2011; Xiao et al, 2010). Fungal CytP450s are NADPH-dependent monooxygenases that act as intracellular redox sinks, mitigating oxidative pressure (Chen et al., 2014; Shin et al., 2018; Lah et al., 2011; Subramanian & Yadav, 2009). The CytP450 system contributes to lignin degradation (Črešnar & Petrič, 2011; Tome et al., 2024), and their involvement in pollutant degradation has been demonstrated in *P. chrysosporium* (Subramanian & Yadav, 2009; Coelho-Moreira et al., 2013), and *Ganoderma lucidum* (Hu et al., 2020; Tayar et al., 2025).

Many studies have had varying levels of success in degrading DDT with WRF, but this far, often under highly optimised conditions and at small scale. For example, previous studies have relied on liquid cultures (Ortíz et al., 2013; Purnomo et al., 2010a; Fan et al., 2013) or used artificially spiked soil as opposed to aged, contaminated soil. A summary of key relevant studies and their contexts is shown in Table 2. While WRF in soil remediation certainly show promise, there is yet to be developed an up-scalable treatment for aged, contaminated soils.

Table 2: Selected studies applying white-rot fungi to DDT remediation

Fungal species	Medium	Scale	Reported outcome	Major transformation products	Reference
Phanerochaete chrysosporium	Liquid culture	250 mL, 60 days	DDT degradation (99%); mineralisation of DDT to CO_2 (13%)	CO_2	Bumpus et al., 1987
Pleurotus ostreatus (SMS)	Spiked soil	2 g, 28 days	48% (80%) degradation of DDT	DDE, DDD, CO_2	Purnomo et al., 2010(a)
Flammulina velutipes	Spiked soil	30 days, 15 g	53% DDT degradation	DDMS, DDOH	Fan et al., 2013
Ganoderma lingzhi	Liquid culture, spiked	7 days, 10 mL	82% DDT degradation	DDMU, pyrene, DDD	Boelan & Purnomo, 2018;
Ganoderma lingzhi	Liquid culture, spiked	7 days, 10 mL	53% DDT degradation	DDMU, pyrene, DDE, DDD	Boelan et al., 2024
Phelbia lindtneri	Liquid cultures, spiked	21 days, 10 mL	70% DDT degradation	DDD, DDA, DBP, DBH	Xiao et al. (2011)
Trametes versicolor	Spiked soil	30 days, 30g	54% DDT degradation	DBP, DDE, DDD	Sari, 2013

SMS: spent mushroom substrate

There is some evidence that native soil microorganisms can contribute to DDT degradation when stimulated by nutrient or organic matter amendment (Purnomo et al., 2011). For example, Ortiz et al. (2013) reported up to 67% mineralisation of DDT in liquid cultures containing indigenous microbial communities, while Betancur-Corredor et al. (2015) observed up to 94% degradation following phosphate and urea amendment. However, these studies were conducted using relatively small quantities of aged, contaminated soil (5 g and 2 kg, respectively) under optimal conditions so their applicability to field remediation remains unknown.

1.4 Surfactants in remediation

In soils, the high hydrophobicity of DDT results in strong sorption to organic matter, which limits its bioavailability and consequently constrains microbial degradation. Surfactants are used to mobilise hydrophobic contaminants. Guo et al. (2014) tested multiple surfactants for their ability to desorb DDT from soil particles and found Tween 80 to be not only the most effective but also the least ecotoxic. In that study, Tween 80 was applied at concentrations of up to 1 g L^{-1}, which is approximately 60 times higher than its critical micelle concentration (CMC; 0.012 mM or 0.016 g L^{-1}) and resulted in highly effective DDT mobilisation. Similarly, Ríos et al. (2013) demonstrated that surfactants could be used to effectively wash DDT from soils at field-relevant contamination levels of 25 mg kg^{-1} d.w., although this was carried out in only 10 g aliquots of soil. Biosurfactants produced by bacteria have also been explored for co-culturing with WRF and have increased DDT degradation (Purnomo et al., 2017; Rizqi et al., 2023).

Tween 80 is biodegradable, and low toxicity to fungi has been reported (Lee et al., 2023; Wei et al., 2016). Conversely, there is evidence that some fungi can utilise the fatty acid tails of Tween 80 as a carbon source (Lee et al., 2013; Wei et al., 2016). Its addition may also stimulate ligninolytic enzyme production, proposed mechanisms for which include enhancing fungal growth or the release of phenolic compounds that induce expression (Usha et al., 2014). For example, MnP activity in *P. chrysosporium* increased in the presence of Tween 80 (Ürek & Pazarlioğlu, 2005), while *Pleurotus sajor-caju* produced higher laccase activity. Increased biomass production has also been observed in *Pleurotus eryngii* in the presence of Tween 80, which may be conducive to successful bioremediation through sustained enzyme production and colonisation (Teodoro et al., 2018; Wu et al., 2016).

Overall, Tween 80 is a promising amendment for the remediation of DDT-contaminated soils, combining effective mobilisation with low fungal toxicity and potential stimulation of fungal growth and ligninolytic activity. However, most applications to date have been conducted at small scales under controlled conditions. In this thesis, Tween 80 was evaluated both in flask-scale trials and in an up-scalable bioreactor, assessing its compatibility with WRF degradation strategies under increasingly realistic conditions.

2. Objectives and Research Questions

The objective of this research was to evaluate and optimise fungal-based approaches for the remediation of soils contaminated with DDT and its transformation products (collectively referred to as DDX). To achieve this, the project investigates both the behaviour of DDX in soil compartments through pore water measurement and when washed with surfactants, and the potential for white-rot fungi to remediate contaminated systems. The specific research questions driving this work are outlined below.

1. **Quantification of contamination:**
   How can the truly dissolved (bioavailable) pore-water concentration of DDX in contaminated soil be measured?

2. **DDX degradation potential of white-rot fungi:**
   Which white-rot fungal species can degrade DDT in aged, contaminated soils? Which demonstrate the highest degradation efficiency?

3. **Optimisation and scalability:**
   Can the degradation process be optimised and successfully upscaled to support practical, field-relevant bioremediation strategies?

4. **Use of spent mushroom substrate (SMS):**
   Can spent mushroom substrate − a by-product of commercial mushroom production − serve as a cost-effective alternative fungal material for DDT degradation?

5. **Integration with soil washing:**
   Can soil washing be used to remove DDX from aged, contaminated soils and concentrate it into a contained liquid phase, and if so, can fungi subsequently degrade DDX within this closed aqueous system?

3. Methodology

3.1 Overall methodological framework

The work presented in this thesis progressed from controlled laboratory tests to increasingly complex and environmentally realistic applications. Fungal growth, ligninolytic activity, and DDX degradation were evaluated, as were the limitations of fungal-based remediation strategies across multiple experimental scales.

Initial laboratory experiments and pre-studies were carried out to establish fungal cultivation methods, substrate composition, and inoculation strategies. Ligninolytic enzyme activity and respiration were also monitored under different growth conditions and for a variety of species. These experiments informed subsequent choices, such as substrate type, and experiment duration. Soil microcosm experiments were then created to test fungal transformation of DDX in aged, contaminated soil.

Mesocosm experiments were conducted to assess fungal establishment in soils by assessing the potential of spent mushroom substrate for DDX degradation over a longer time period and at higher soil volume. These systems were designed to introduce greater spatial heterogeneity and biological complexity, particularly soil depth, while conditions were sufficiently controlled to allow comparison between treatments. Field trials were then conducted to evaluate fungal DDX degradation under realistic conditions, including natural climate variability.

In addition, DDX in the pore water fraction of the soil was quantified for nine sites across Sweden, and the method validated in an inter-lab comparison study. This enabled characterisation of contaminant fate and bioavailability, and informed selection of the field site and test soils.

Limitations observed in soil-based systems, in particular, contaminant sorption, and fungal growth heterogeneity, led to experimentation with surfactants. A novel pilot-scale bioreactor system was developed, combining

soil washing using pre-tested surfactant with WRF degradation. This separated contaminant mobilisation and biological transformation.

The methodological framework of the thesis is reflected in the four papers. Paper I focuses on site characterisation and analytical methods for DDX concentration, including determination of the partitioning of DDX between passive sampling material and water. Paper II investigates fungal-mediated transformation in microcosms under controlled laboratory conditions. Paper III evaluates mesocosm- and field-scale applications, and Paper IV explores upscaling using soil washing through a prototype pilot bioreactor system.

3.2 Forest plant nursery soil sampling

Soils were collected from nine former forest plant nursery sites across Sweden in May 2022: Klockatorp, Ljungaskog, Deje Nord, Deje Syd, Kolleberga, Åby, Stackheden, Sya, and Jakobsbyn. Site coordinates and background information are provided in Paper I. At each site, a 50 × 50 m sampling grid was established in the area of highest reported contamination. A soil pit was dug at the centre of each grid to document soil profiles, and composite soil samples were collected by combining multiple soil cores from each grid point taken to a depth of approximately 20 cm (Figure 3).

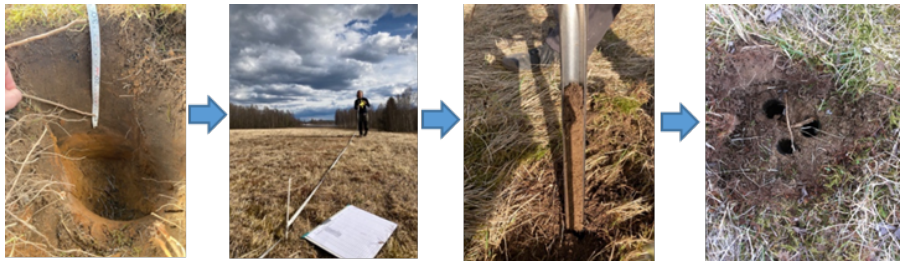


Figure 3: Soil profiling examination and sampling procedure. Photo credit: S. Casey

Collected soils were homogenised on site and subsampled for chemical analysis in triplicate. Concentrations of *p,p′*- and *o,p′*-DDT and transformation products *p,p′*- and *o,p′*-DDD and -DDE, dicofol, *p,p′*-DBP, *p,p′*-DDMU, and *p,p′*-DDM were determined by Soxhlet extraction followed by gas chromatography-tandem mass spectrometry (GC–MS/MS) analysis,

as described in Paper I. The sites represented a range of land-use histories, including agricultural soils, managed forest plantations, clear-cut areas, and sites undergoing rewilding. Details of land usage at each site can be found in Paper I, Table S7. Soils from all sites were characterised for total DDX concentrations, freely dissolved DDX in pore water, and toxicity to earthworms.

Soil from the Kolleberga site was selected for use in future laboratory, mesocosm, and field experiments (Papers II–IV). This site was chosen based on moderate contamination levels, high organic matter content, and practical considerations including site accessibility and access to a field station and watering systems.

3.3 Selection of fungal species

Species selection for this thesis was guided by three conceptual limitations: (i) ligninolytic potential sufficient to transform DDT and its metabolites, (ii) the ability to establish and remain active in non-sterile soils, and (iii) practical suitability for upscaling beyond laboratory systems. All species chosen for this thesis belong to Agaricomycetes (Polyporales or Agaricales) orders which include many WRF fungi capable of oxidative degradation of persistent organic pollutants. Remediation at environmentally relevant scales will involve soils that already host indigenous microbial communities. Consequently, fungal species adapted to growth in the competitive environment of non-sterile soils are more likely to establish successfully (Tornberg et al., 2003).

Pleurotus ostreatus and *Trametes versicolor* are well established model organisms in bioremediation. They both have well documented high expression of ligninolytic enzymes capable of degrading DDX (Mohapatra et al., 2018; Purnomo et al., 2011; Sadiq et al., 2015; Sari et al., 2013). *P. ostreatus* is fast growing, produced commercially for oyster mushroom harvesting and has low substrate specificity and high versatility (El-Ramady et al., 2022). *T. versicolor* is a wood decaying polypore which produces high levels of class II peroxidases and laccase (Drula et al., 2022).

G. lucidum is another white-rot polypore which grows as brackets on hardwood trees, and has a well profiled, strong ligninolytic system (CAZy database, Drula et al., 2022; D'Souza et al., 1999). Given that its natural habitat is not soil, *G. lucidum* was chosen for a liquid degradation study. Previous studies have reported *G. lucidum* cultures degrading lindane, which was attributed to peroxidases (Kaur et al., 2016), and isolated laccases from *G. lucidum* degrading chlorophenols (Deng et al. 2022). While not tested on DDT, the closely related species *Ganoderma lingzhi* showed success in degrading DDT to DDE and DDD (Boelan et al., 2024; Boelan & Purnomo, 2018).

While ligninolytic activity is primarily expressed by wood-dwelling fungi, the DDT contamination exists in soil. Therefore, successful remediation requires fungus able to survive in, colonise, and produce wood-degrading enzymes in soil. Isolated enzymes have a short half-life in soil; 1-4 days for laccase and Mn-peroxidase (Margot et al., 2013; Wu et al., 2014). Therefore, the live fungus should be present and continuously produce new enzymes (Mohapatra et al., 2018).

Hypholoma fasciculare was selected based on its lifestyle as a wood and litter decomposer that can grow into soils on partially degraded woody substrates at the base of trees while still producing ligninolytic enzymes (Voříšková et al., 2011). It had already shown the ability to degrade various herbicides with chlorinated phenolic groups (Bending et al., 2002). *Pholiota adiposa* was recommended by mushroom growers (Ecofungi, Malmö, Sweden), for its white-rot lifestyle, fast growth rate, and variable habitat. Though untested for remediation, *Ph. adiposa* has been found to secrete laccase, manganese peroxidase, and lignin peroxidase (Liu et al., 2009).

Stropharia coronilla was originally selected as a white-rot litter decomposer that grows in grasslands – as are many of the DDT contaminated forest nursery sites. However, after pre-trials it was substituted with *Stropharia rugosoannulata* - a closely related species that showed more aggressive soil colonisation. This species was then used in subsequent field trials.

Similarly, *Agaricus bisporus* is also a soil inhabiting species, preferentially growing on partially decomposed leaf litter, and producing ligninolytic enzymes on lignocellulosic substrates (Kerrigan et al. 2013; Drula et al., 2022; Hilden et al., 2013). It is a commercially available species with a history of use in remediation (Gracia-Delgado et al., 2015; Hildago et al., 2023).

Spent mushroom substrate (SMS) from *Pleurotus* sp. and from *Agaricus* sp. has shown promise in degrading persistent organic pollutants. In laboratory, pilot-scale and field scale experiments, soils amended with SMS resulted in significant reductions in endosulfans, PCBs, petroleum hydrocarbons and PAHs (Sadiq et al., 2019; Siracusa et al., 2017; Di Gregorio et al., 2016; Gasecka et al., 2015; Mayans et al., 2024). The only reported successful treatment of DDT, used *P. ostreatus* SMS in a very small amount (2 g) of aged, DDT contaminated soil (Purnomo et al., 2010(a)).

3.4 Fungal cultivation

3.4.1 Media and culturing

Mycelial cultures were obtained from established culture collections and collaborators. Species obtained from Mycelia BVBA (Belgium) included *P. ostreatus* M2191, *H. fasciculare* MUCL 047611, *A. bisporus* MUCL 031615, *T. versicolor* M9911, *S. rugosoannulata* MUCL 28162, and *S. coronilla* MUCL 044835. *Ph. adiposa* M4200 was obtained from Ecofungi (Sweden) in the form of grain spawn, and *G. lucidum* FP-58537-Sp was obtained from Dr. C.A. Reddy (Michigan State University, USA).

Cultures were maintained by regular subculturing and stored at 25 °C. Most species were grown on modified Melin–Norkrans (MMN) agar medium (Islam & Ohga, 2013), while *G. lucidum* was maintained on malt extract agar. Full media compositions and culturing procedures are described in Papers II and IV.

3.4.2 Spawn production

Grain spawn was produced by propagating fungal cultures on sterile rye grain amended with calcium carbonate and incubated at 25 °C until full colonisation. This grain spawn was used to inoculate secondary substrates, including birch woodchip and sterilised straw pellets. For experiments involving *G. lucidum*, woodchip substrates were prepared using a mycelial suspension method (Baccar et al., 2011; Beltrán-Flores et al., 2021) Further details of substrate preparation, inoculation ratios, and incubation times is provided in Papers II and IV.

3.5 Analytical methods

3.5.1 Enzyme assay

Ligninolytic enzyme activity was assessed using a colorimetric assay based on the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-dimethylaminobenzoic acid (DMAB), originally developed by Daniel et al. (1994) and applied as described by Kyaschenko et al. (2017). The assay was used to estimate oxidative enzyme activity of manganese peroxidase (MnP) and laccase in liquid samples and in soil sample extracts. Full assay protocols are provided in Papers II and III.

3.5.2 Measurement of DDX concentrations

The concentrations of DDT and its transformation products (collectively referred to as DDX) were determined in solid and liquid samples, fruit bodies and in passive sampling material (POM-strips for pore water concentrations) using established extraction and analytical methods. Full protocols for extraction, clean-up, and instrumental analysis are described in detail in Paper I (pore water and soil samples), Papers II and IV (liquid samples in Tween 80 experiments) and Paper III (fruit bodies) and are summarised here.

3.5.3 Extraction and sample preparation of solids

Solid samples were freeze-dried prior to extraction, and DDX was extracted using Soxhlet extraction with dichloromethane (DCM) for 24 hours. Internal standards (7 isotope-(^{13}C)-labelled internal standards) were added prior to extraction to enable quantification and quality control. Extracts were subjected to clean-up using copper to remove sulphur, and a column of aluminium silica to remove co-extracted compounds. Solvent was exchanged to isooctane and was then spiked with recovery standard (^{13}C-labelled PCBs). An aliquot was transferred to a GC vial for instrumental analysis. Details of internal and recovery standards are provided in Papers I and II (Table S2).

3.5.4 Extraction and sample preparation of liquid samples in Tween 80 experiments

For the apparently dissolved DDX concentrations in the water suspension in the Tween 80 experiments, liquid-liquid extraction with DCM was used. The sample was pre-filtered, separation funnels were used to shake the liquid sample with DCM and ^{13}C-labelled internal standards were added. Two solvent layers solvent layers were formed (DCM:water), and the lower one (DCM) was drained into a conical flask. This extraction procedure was repeated once, and the solvent layers combined for dehydrating and filtering. Solvent was changed to isooctane, recovery standard was added, and an aliquot was transferred to a GC vial for instrumental analysis. For full details, see section 2.4.2 in Paper II.

3.5.5 Measurement of pore water DDX concentrations

Passive sampling using polyoxymethylene (POM) strips was used to measure freely dissolved concentrations of DDX in soil pore water from contaminated forest nursery soils, with strips equilibrated with soil and water prior to extraction. This approach was included in the thesis to complement bulk concentration measurements and to provide an estimate of the bioavailable concentrations of DDX, which is not captured by total solid-phase concentrations alone. The experiment was conducted in co-ordination with the Dept. of Science and Technology at Örebro University, which

analysed total soil concentration and pore water content of the same samples for comparison and validation of results.

Deriving K_{POM}: Partition coefficients were determined as part of Paper I, and validated in the interlaboratory comparison with soil from nine DDX contaminated sites across Sweden, as presented in Paper I. In brief, $K_{polyoxymethylene/water}$ (K_{POM}) values were derived from equilibrium experiments according to Equation 1 in Paper I:

$$C_{w,free} = \frac{C_{POM}}{K_{POM}}$$

Where C_{POM} is the concentration measured in the polymer phase.

Sampling: POM strips (76-µm, 4 x 5 cm) were cleaned with acetone and hexane (1:1 v/v) and weighed before usage. A soil-water suspension was created in amber glass bottles, composing 25 g (calculated d.w.) of homogenised soil from each site, and 93 mL of water containing $CaCl_2$ and NaN_3. Each suspension was created in triplicate per site, and POM strips were placed in each. These were then tumbled end-over-end for 28 days to equilibrate before POM removal, rinsing (ultra-pure water), weighing, and freezing until extraction.

Extraction: Following equilibration, POM strips were extracted twice for 24 h with 40 mL acetone:*n*-hexane (1:1, v/v), with 7 ^{13}C-labelled internal standards. Combined extracts were cleaned using scaled down clean-up columns, and solvent exchanged to isooctane before recovery standards were added. An aliquot was decanted into a GC vial and analysed for DDX using the same GC–MS/MS methods applied to other sample types. Full experimental procedures are described in detail in Paper I.

Calculating freely dissolved concentrations ($C_{W,free}$): These were calculated based on the concentration of DDX measured in the POM and experimentally derived compound-specific POM-water partition coefficients (K_{POM}; L_w kg_{POM}^{-1}), using Eqn1 in Paper I.

Inter-lab comparison: As part of Paper I, a second research laboratory (Örebro University) also analysed both the bulk soil and pore water concentrations of soils from the nine sites, using the same experimentally derived K_{POM} values. The POM strips from two sites in both labs (6 per lab) were cut in half after the equilibration step (the 28-day shaking), and half were exchanged between the laboratories, and variation in results assessed. The study aimed to capture differences in results caused be differences in each laboratory's extraction procedures or analytical steps. This way potential effects of differences in conducting the shaking experiment or heterogenous contaminant dispersal in the soils could be excluded. The extraction and analysis of half of each strip for two sites therefore took place at the opposite lab, and the results compared.

3.5.6 GC-MS/MS analysis

Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was used to analyse all extracts, following procedure described by Enell et al. (2025) (Lab B), and in papers II, III and IV. The same instruments and instrumental conditions (injector, oven program, column, and MS/MS settings) were used to those described (Enell et al., 2025). Quantification was performed using an 11-point calibration curve from 0.5 to 2000 ng mL^{-1}, and the isotope dilution method, and concentrations were reported for *p,p′*- and *o,p′*- isomers of DDT, DDE and DDD, along with dicofol, *p,p′*-DBP, *p,p′*-DDMU and *p,p′*-DDM.

3.5.7 Quality assurance and quality control

Internal standards were added to all samples prior to the extraction and recovery standards were added to sample extracts prior to GC-MS/MS analysis to be able to correct analytes losses using the isotope dilution method. Recovery limits were set to >50% and <200%. Any individual data with recovery falling outside this range were excluded.

Further quality control used throughout the thesis experiments are outlined fully in Paper III. In summary, from Paper II onwards, a *p,p′*-DDT quality control solution was run separately to monitor degradation of DDT to DDD in the GC-MS injector, with a limit of 10% degradation deemed acceptable

to report DDT and DDD separately (Budde et al., 1995; EPA, 1996). According to Foreman et al. (1997), best practise would have been to add multiple mass-labelled internal standards in the samples to check for degradation of DDT in each individual sample. However, the method of degradation before and after each run was deemed sufficient.

Throughout the thesis, the limit of detection (LOD) was set to a S/N ratio of ≥3. The limit of quantification (LOQ) was defined as the lowest calibration solution that could be reliably quantified by the instrument and fulfilling a S/N criterion of ≥10. Solvent blanks were also included to an average of one per 6 samples across soil and liquid extraction, to control for contamination. If contamination was present in the blanks, the LOQ was defined as follows:

LOQ = average procedural blanks + (10 x stdev procedural blanks).

Maximum retention time (min) deviation in relation to peaks in standard run for each compound never exceeded 0.1 minutes, with an average of 0.023 across all studies, for any data point not subject to previous exclusion criteria. This threshold is deemed acceptable for DDT within the studies by Frenich et al (2003) and Frias et al (2003).

3.5.8 Data handling

All analyses were performed using R (version 1.1-37), and both R and Microsoft excel (version 2606) for figure creation in Papers II, III and IV. In Paper I, JMP (ver. 17, JUMP Statistical Discovery LLC, NC) was used.

In Paper I, analyses were mainly ANOVA followed by Tukey's HSD test, with Pearson correlations employed to define the relationships between C_{POM}, C_{SOIL}, and between the various sites. For Paper II, linear mixed-effects modelling was employed for organic matter and DDX changes over time and between species, with Tukey HSD post-hoc testing, alongside PERMANOVA to assess overall compound proportions. To assess differences in respiration levels, AUC analysis was performed. Similarly, in Paper III, linear mixed-effects modelling was used with Tukey HSD post host testing, and PERMANOVA for overall compound profiles. In Paper IV, no statistical analysis was used to interpret the pilot bioreactor results due to

lack of replication, and data are presented descriptively. The pre-experiment used linear mixed-effects modelling and Tukey. Details on all statistical modelling are outlined fully in each paper. R packages: ggplot2, tidyverse, dplyr, corrplot, lmr, lme4 and emmeans for analysis and visualisation.

Replacement values were used for statistical purposes only in Paper I and Paper III when S/N<3 (LOD) or S/N<10 (LOQ). Replacement values were calculated as (mean(blank) + 3·SD(blank))/2 for LOD, and (mean(blank) + 10·SD(blank))/2 for LOQ.

3.6 Pre-studies: Woodchip, straw, and soil colonisation

Pre-studies were conducted to inform substrate selection and experimental design for fungal application in soil systems. The suitability of straw and woodchip as substrates for fungal inoculation into soil, and the spatial heterogeneity of fungal growth and enzyme activity in soil were assessed.

Woodchip provides a more persistent lignocellulosic substrate, whereas straw contains lower lignin content and more readily available nutrients that may support faster fungal establishment in already colonised soils. Fungal cultures were propagated on millet grain spawn and used to inoculate straw and woodchip substrates, which were subsequently incubated until colonised and mixed with soil at 20% w/w ratio. Ligninolytic activity was measured in colonised substrates and in substrate–soil mixtures (Figure 4).

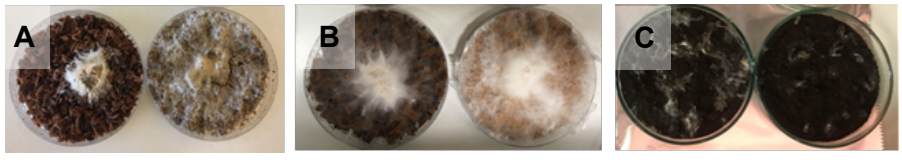


Figure 4: Panel A: *A. bisporus* growing on woodchip (left) and straw (right) at 14 days. Panel B: *P. ostreatus* growing on woodchip (left) and straw (right) at 14 days. Panel C: *P. ostreatus* colonised straw (left) and woodchip (right) mixed with soil at 20%. Photo credit: S. Casey

Substantial spatially heterogeneous of growth and enzyme activity was observed, particularly on woodchip, and some species visibly had better

success on straw (Figure 4; Panel A). The ligninolytic activity at 14 days was also higher in *P. ostreatus, H. fasciculare* and *A. bisporus* on straw than on woodchip. When secondary spawn was applied to soil, straw was more fibrous and therefore spread easier, allowing for better colonisation.

The outcomes of these pre-studies guided the design of later microcosms, mesocosms, and field experiments, including decisions regarding substrate type, amendment rates, and sampling strategies.

3.7 Experimental systems

3.7.1 Microcosms

The initial experiments consisted of petri-dish soil microcosms combining contaminated soil (60%), hydrated straw pellets (20%), and fungal grain spawn (20%). These microcosms were designed to test fungal establishment and DDX transformation under controlled conditions and to allow direct comparison between fungal species. Treatments were *P. ostreatus, H. fasciculare, A. bisporus, Ph. adiposa,* and *T. versicolor*, along with an autoclaved *P. ostreatus* control.

Microcosms of 50 g (30 g soil d.w., 10 g fungal spawn w.w., 10 g straw w.w.) were incubated at 20 °C controlled temperature conditions for 28 days. Subsamples were collected for analysis of DDX concentrations and basic soil parameters, including pH, organic matter content, moisture content, and inorganic nitrogen. Full details of microcosm preparation, incubation, and sampling procedures are provided in Paper II.

3.7.2 Surfactant amended liquid culture flasks

To investigate the influence of contaminant mobilisation on fungal degradation, liquid microcosms were created using liquid straw extract combined with contaminated soil, surfactant and enzyme suspension in conical flasks. Tween 80 was used as a non-ionic surfactant to release organic matter bound DDX into the liquid phase, where it could be degraded

by fungal enzymes. The fungal enzyme suspension was pre-made using *P. ostreatus* grain spawn (40 g L^{-1}) grown in sterilised straw solution (10 g L^{-1}).

The liquid microcosms were created in triplicate, and all contained 50 mL of liquid with 5 g of soil. Experimental treatments included controls without surfactant or fungal material, systems amended with Tween 80 (1 g L^{-1}) only, fungal enzyme suspensions only, and combined Tween 80 and fungal enzyme suspension. At the end of the 8-day incubation period, liquid fractions were collected for DDX analysis. The full experimental design and treatment composition are described in Paper II.

3.7.3 Respiration pots

In parallel with the microcosm tests, a respiration pot experiment was conducted to visually assess colonisation and observe respiration levels over time of the entire soil system, including both indigenous microbes and the introduced fungi. Pots containing Kolleberga soil were amended with grain spawn of *P. ostreatus, T. versicolor, H. fasciculare,* or *S. rugosoannulata* in triplicate. Soil amended with autoclaved fungal material and unamended soil were used as controls.

Carbon dioxide production was monitored continuously using CO_2 loggers positioned above gas-exchange filters on the pot lids, allowing respiration rates of the combined soil–fungus systems to be tracked hourly for 14 days. Colonisation of the soil matrix was also observed visually, and representative differences can be seen in Figure 5. These experiments provided complementary information on fungal activity and are described in detail in Paper II.

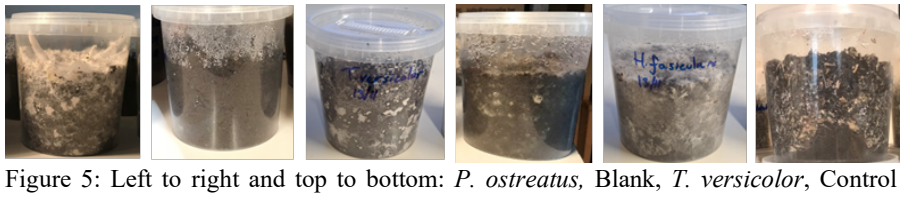


Figure 5: Left to right and top to bottom: *P. ostreatus,* Blank, *T. versicolor*, Control (autoclaved *P. ostreatus*), *H. fasciculare, S. rugosoannulata.* Photo credit: S. Casey

3.7.4 Spent mushroom substrate buckets

Pot-scale experiments were designed to evaluate the potential of spent mushroom substrate (SMS) from commercial mushroom production to enhance DDT degradation when mixed with contaminated soil. Fresh SMS from *P. ostreatus* production was obtained from the mushroom producer Fungigården AB (Harplinge, Sweden).

Contaminated soil from the Kolleberga site was mixed with SMS and incubated in buckets under controlled indoor conditions for four months. Experimental treatments included soil (4.5 kg) amended with fresh SMS (900 g), soil amended with fresh SMS and additional straw substrate (450 g), soil amended with autoclaved SMS (900 g) as a control for organic matter addition, and unamended soil as an experimental blank. Moisture content was maintained at ~60% throughout the incubation, with a fabric layer for insulation. The mixed and unmixed pots can be seen in Figure 6.

Soil–substrate mixtures were sampled before and after incubation to determine DDX concentrations, organic matter content, water content, and mass loss. Composite samples (100 g) were collected using soil cores and homogenised prior to analysis. Full details of experimental setup, sampling, and analytical procedures are provided in Paper III.

Figure 6: Spent mushroom substrate buckets A) pre-mixing; B) post-mixing; C) incubating. Photo credit: Y. Volchko

3.7.5 Field trial

A field experiment was conducted at the Kolleberga forest plant nursery in Skåne during autumn 2022 to evaluate fungal treatment under realistic environmental conditions. The site was previously characterised in Paper I.

Six experimental plots (2 × 2 m) were excavated to a depth of approximately 40 cm and refilled with homogenised soil (~1600 L per plot). Plots were lined with permeable barrier fabric before refilling. Colonised straw substrate of either *P. ostreatus* or *S. rugosoannulata* was tilled into the soil in triplicate plots (20 L per plot) to a depth of approximately 15–20 cm. Substrate preparation and inoculation procedures are described in detail in Paper III. The desired proportion of substrate to soil was approximately 20 L substrate to 800 L soil (2.5% (v/v)). Photos of the field site are shown in Figure 7.

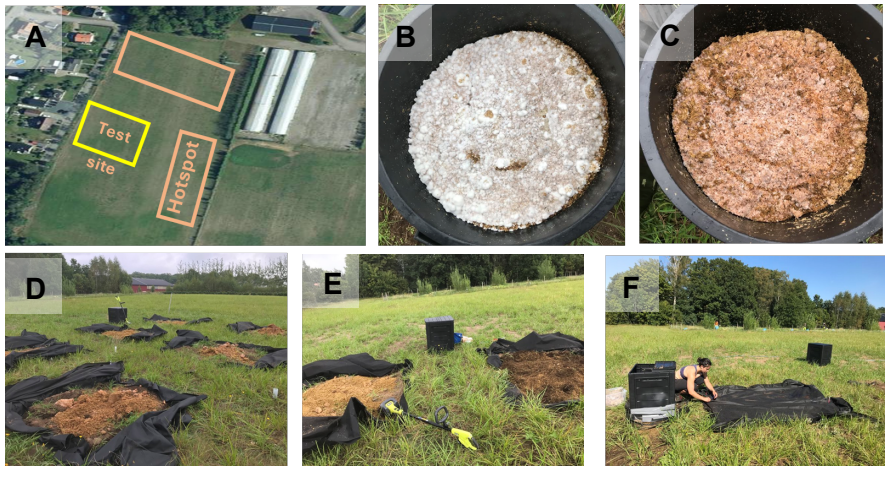


Figure 7: A) Kolleberga forest plant nursery site. Hotspot was excavated and soil homogenised before used to fill plot in the mycoremediation test site. B) *P. ostreatus* straw substrate after one week colonisation time at the field site. C) *S. rugosoannulata* straw substrate. D) Six of the plots pre-mixing E) unmixed (left) and mixed (right) soil and substrate with hand tiller. F) covered field plot. Photo credit: S. Casey

Soil samples were collected immediately after substrate incorporation and after six weeks of incubation. Composite samples were obtained from 12 soil cores per plot, sieved, and homogenised prior to chemical analysis.

Fruiting of *P. ostreatus* was observed in treated plots during the experimental period (Figure 8), indicating successful fungal establishment, and these fruit bodies were also collected for DDX analysis. Further details of site preparation, experimental design, and sampling are provided in Paper III.

Figure 8: Fruit bodies of *P. ostreatus* emerged after five weeks in all *P. ostreatus* treated plots at Kolleberga forest plant nursery. Photo credit: S. Casey

3.7.6 Pilot bioreactor

Limitations observed in soil-based fungal treatments, particularly related to contaminant sorption and fungal establishment, motivated the development of a pilot-scale bioreactor system combining soil washing with fungal degradation. This system was developed in collaboration with BioRem, UAB (Autonomous University of Barcelona, Spain), and represents an exploratory step towards upscaling remediation.

Two bench-scale bioreactors were constructed, comprising a soil chamber and a downstream fungal degradation chamber. Contaminated soil was placed in the upper chamber, allowing a washing solution to percolate through the soil before passing into a secondary chamber containing *G. lucidum* colonised woodchip. The solution flowed through this chamber, into a glass collection bottle, and then pumped back to the top to re-circulate. A photo and schematic are shown in Figure 9 and presented in Paper IV. The circulating washing solution containing Tween 80 mobilised sorbed DDX from the soil and transported it through the fungal chamber for degradation. One bioreactor served as a treatment system, and the other as a control, with autoclaved woodchip. The bioreactor enabled separation of contaminant mobilisation and biological transformation processes and allowed evaluation of fungal degradation of DDX in liquid phase.

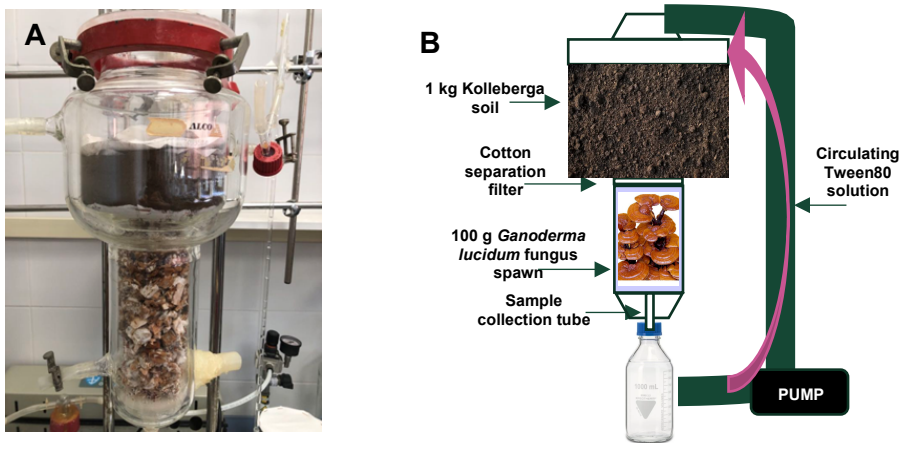


Figure 9: Photograph (A) and schematic (B) of the bioreactor set-up with soil chamber, fungus inoculated woodchip chamber, and circulating Tween 80. Photo and illustration credit: S. Casey

4. Results and Discussion

4.1 Characterization of forest nursery soils – ∑DDX and pore water concentrations

Soil from nine of the forest nursery sites were characterized with regard to DDX content by both laboratories involved in the research project presented in Paper I. The mean ratio between the concentrations found by our laboratory (Lab B) and Lab A was 1.2 ± 0.5, if considering the sum of DDT and DDD (and not individual DDD values). This provided confidence that the analytical workflow was robust and that the site-to-site differences observed reflect real variation in contamination rather than variation in laboratory measurements.

A limitation of this study was related to the analytical separation of DDT and DDD. In Paper I, no degradation standard was included to quantify thermal degradation of DDT to DDD in the GC- injector. As a result, DDT and DDD were combined in reporting to avoid overestimation of DDD and underestimation of DDT.

In high contamination sites, only small soil masses could be extracted, and multiple dilution steps were required to reach the analytical range of the GC-MS/MS calibration curve. For fungal treatment, a soil with lower concentrations of DDX was therefore preferable, allowing larger extraction masses and reducing analytical error introduced through dilution.

Kolleberga soil was selected for future experiments due to its moderate ∑DDX concentrations, and relatively high organic matter content. This was important given the need for treatment that could overcome partitioning of DDX to the organic matter.

4.2 Passive sampling and estimation of freely dissolved concentrations of DDX in pore water

In parallel with bulk soil analysis, passive sampling using polyoxymethylene (POM) was applied to estimate freely dissolved pore water concentrations of DDX. POM–water partition coefficients (K_{POM}) were experimentally derived in the first part of Paper I and used to calculate the freely dissolved concentrations of DDX in pore water in the second part. This part of the work directly addresses Research Question 1 by providing a method to quantify the apparently bioavailable concentration of DDX in pore water of soils.

The measured concentration in the POM phase (C_{POM}) for the combined ∑DDT/DDD signal is presented with both isomers of DDE, DBP and dicofol in Paper I.

Agreement between laboratories was generally lower for C_{POM} values than for bulk soil values, with our laboratory (Lab B) consistently reporting lower concentrations. However, mean RSD for Lab A and Lab B were 5.1 and 11% respectively, and several compounds (e.g., *o,p'*-DDT and *p,p'*-DDE) matched closely enough between laboratories to be statistically indistinguishable. Mean ratios between laboratories for both isomers of DDT/DDD and DDE fell between 1.3 and 2.4.

4.3 Interlaboratory validation and relevance for remediation

A key validation step in Paper I was the interlaboratory comparison based on split POM strips, and results are presented in Figure 10. For two sites (Klockatorp and Ljungaskog), POM strips were cut in half after equilibration, and halves were exchanged between laboratories for extraction and analysis. The exceptionally close agreement between labs for C_{POM} in observation of both isomers of ΣDDT/D and DDE provides strong support for the passive sampling method and suggests that variability introduced by extraction and instrumental analysis was low relative to the underlying differences between soils. Mean relative standard deviation for our

laboratory was 11% for all interlaboratory validation data. For lab A the RSD was 4.9%. C_{POM} values for dicofol and *p,p'*-DBP were significantly different for every strip both sites, and Lab A found consistently higher values than our laboratory.

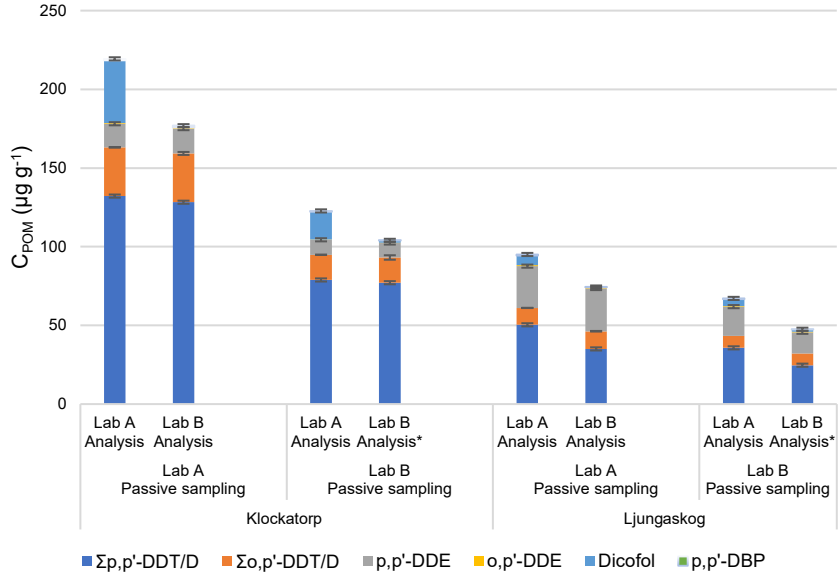


Figure 10: Interlaboratory comparison of C_{POM} (µg g^{-1}) measured by analysis of the same strips by two laboratories for sites Klockatorp and Ljungaskog

Whatever remediation approach is ultimately used for these soils, it would be preferable to find not only reductions in total concentrations but also for changes in contaminant bioavailability and mobility. Many treatment strategies (including surfactant washing) can increase the freely dissolved fraction of hydrophobic contaminants, potentially increasing short-term risk even if total concentrations decrease. Passive sampling therefore provides an important complementary tool for risk assessment, allowing sensitive detection of changes in the mobile DDX pool across treatments and experimental scales.

4.4 Growth and respiration test in Kolleberga soil

Soil from the Kolleberga site was selected for the experimental work due to its moderate bulk ∑DDX concentration (~10 mg kg^{-1}) and relatively high organic matter content (2.7%). Preliminary tests were performed to evaluate whether the selected fungal species could colonise the soil under realistic conditions, i.e., in unsterilised soil with competition from native microbial communities, and where DDX contamination is aged. The respiration study (Section 3.7.3) was therefore conducted as a screening step. Results are presented in Figure 11, where panels are shown in pairs to reflect experimental treatments that were run in parallel rather than sequentially.

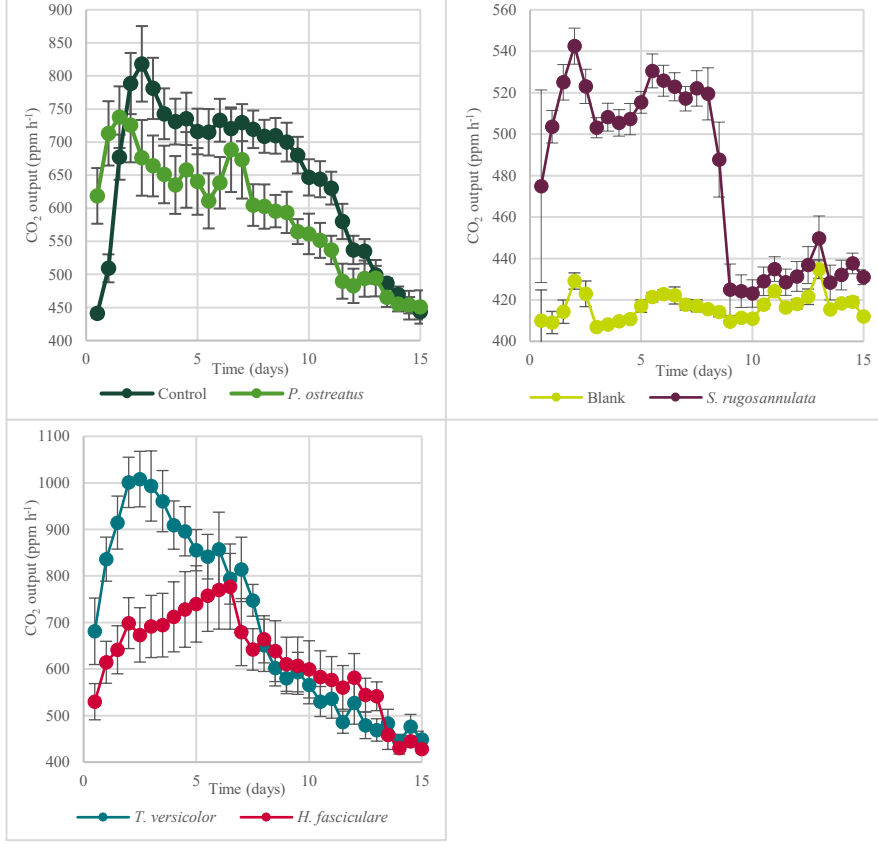


Figure 11: Respiration over time in untreated soil (blank), soil amended with autoclaved spawn (control) and soil amended with *P. ostreatus*, *T. versicolor, H. fasciculare* and *S. rugosoannulata*. Mean ± std, *n*=3.

All the tested species could colonize the soil to a depth of 15 cm, based on visual assessment (Figure 5). Visual colonisation by *T. versicolor* (a strict wood-decay species) was the lowest, yet respiration still increased comparably to other fungi.

Respiration for all treatments including the control increased after inoculation but returned to the baseline rate of the blank soil within ~15 days, with the peak occurring around days 2 and 3, and the decline beginning by day 7. Presumably the decline in activity represents then point where easily available nutrients were depleted. Interestingly, the addition of autoclaved spawn (i.e., a high organic matter input without live fungus) resulted in an increase in respiration comparable to that of live *P. ostreatus* substrate. Using AUC analysis these were not found to be significantly different to each other. This implies that the native soil microbial community is actively exploiting available nutrients, and that an unknown proportion of the respiration signal in other treatments may also be attributable to native soil microbes. The competition posed by native soil microbes for nutrients is likely to influence fungal establishment, affecting DDX degradation outcomes. The differences in magnitude of each pair of treatments can be partially attributed to differences in abiotic conditions such as temperature during the incubation, as only each of the pairs in the panels of Figure 11 were run in parallel. *S. rugosoannulata* was ultimately replaced by *A. bisporus* in the degradation experiments due to production rates of secondary spawn.

Overall, this screening experiment showed that all species were able to establish at a field-relevant depth under controlled conditions, in contaminated field soils, but that sustained activity did not exceed 20 days. This informed the design of the subsequent degradation experiments, which were set to a duration of 30 days to capture both the initial colonisation phase and the later decline in activity. For further upscaling, the implication is that additional carbon input may be necessary for sustained growth.

As described in Table 2, most previous studies on fungal degradation of DDX have been performed in liquid culture. A few studies have focused on soil and then mostly artificially contaminated soils. In these studies, the fungal growth was rarely quantified, except in Bumpus et al. (1987) where glucose utilisation was followed and was fully depleted within 30 days.

4.5 Microbial degradation of DDX in soils

The capacity of fungal treatment to degrade DDX directly in soil were evaluated on different levels including laboratory scale experiments in petri dishes, intermediate size experiments with 4.5 kg of soil up to a field trial. In the intermediate experiment, spent mushroom substrate (SMS) was evaluated as fungal inoculum, while grain spawn or straw inoculated with grain spawn were used in the other experiments.

4.5.1 Microcosms – Petri-dish scale

The microcosm experiments form the main foundational backbone of this thesis, demonstrating that multiple white-rot fungal species can achieve substantial degradation of DDX in aged, contaminated soil. Full experimental details are provided in Paper II. In summary, over 30-days, *Pleurotus ostreatus* (69% ± 3), *Trametes versicolor* (72% ± 8) and *Pholiota adiposa* (80% ± 3) achieved the highest relative reductions in ∑DDX (Figure 12).

The strong performance of *P. adiposa* is consistent with its ecological strategy, as it colonizes woody substrates at the base of trees overlapping with the soil environment (Holec & Jan 1998). The effectiveness of *T. versicolor* was more surprising, given its relatively low visual colonization in soil, but can plausibly be attributed to its high ligninolytic enzyme capacity. *P. ostreatus* also performed very well and showed extensive visible colonisation of the soil. In contrast, *A. bisporus* and *H. fasciculare* performed less effectively, which is consistent with their comparatively lower ligninolytic profiles seen in pre-trials, and slower observed secondary substrate colonization.

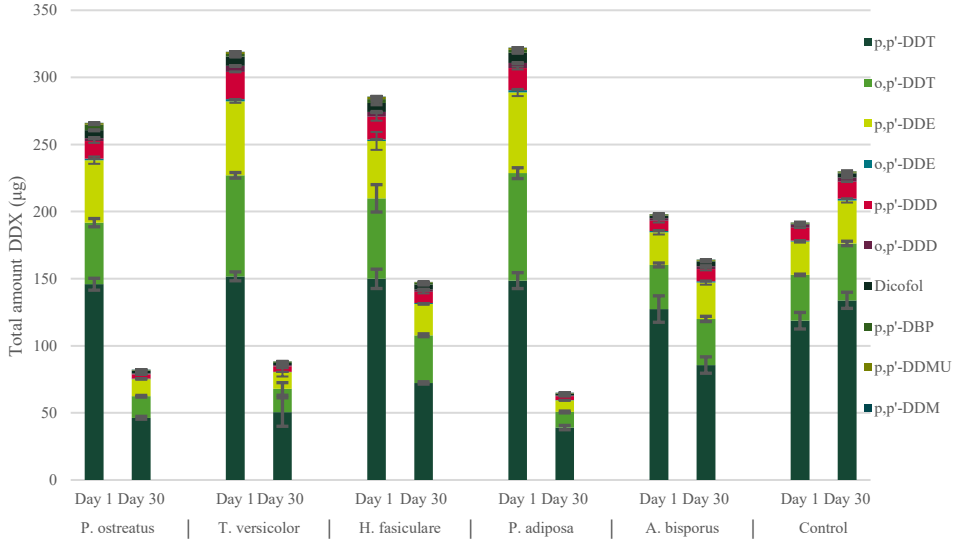


Figure 12: Total amount (µg) of DDX pre- (day 1) and post treatment (day 30) in the petri dish experiment. Data shown are mean ± std (*n*=4). Reproduced from Paper II.

In addition to ∑DDX concentrations, the microcosm systems were characterised by measurements of organic matter content and ligninolytic capacity (Table 3).

Ligninolytic enzyme activity measurements were not used as a main explanatory variable in this study. This was because it represents a snapshot of enzymatic activity at a single time point from a subsample of the system (unhomogenised at day-1 to avoid mycelial destruction) and therefore do not capture special or temporal variation in ligninolytic capacity.

Changes in organic matter (OM) content between the beginning and end of incubation provided a more informative indicator of system-wide decomposition. OM reduction was proportionally highest in the treatments that also achieved the highest ∑DDX degradation, supporting the hypothesis that decomposition processes are linked to DDX transformation (Table 3). Still, it should be pointed out that there are additional enzyme systems besides the ligninolytic enzymes, such as cellulases, which would also have contributed to OM degradation.

Table 3: Microcosm material parameters and characteristics before and after treatment. Subsamples were taken on day 1 (T0) and day 30 (T1). Data is given as mean ± std (*n*=4). Adapted from Paper II.

Treatment	**OM** (%)	**Relative reduction ΣDDX** (%)	**Ligninolytic capacity (U g^{-1} d.w.)**
Control (day 1)	13 ± 5		4.1 ± 3.1
Control (day 30)	10 ± 0.5	-20 ± 8	6.4 ± 6.6
P. ostreatus (day 1)	29 ± 6		27 ± 12
P. ostreatus (day 30)	16 ± 1*	69 ± 3*	120 ± 30
T. versicolor (day 1)	20 ± 0.4		1.8 ± 0.5
T. versicolor (day 30)	9 ± 1*	72 ± 8*	37 ± 7.3
H. fasciculare (day 1)	22 ± 0.4		6. 3 ± 2.1
H. fasciculare (day 30)	10 ± 0.8*	45 ±15*	300 ± 55
Ph. adiposa (day 1)	22 ± 8.0		16 ± 6.3
Ph. adiposa (day 30)	9 ± 0.5*	80 ± 3*	22 ± 4.0
A. bisporus (day 1)	16 ± 1		0.3 ± 14
A. bisporus (day 30)	10 ± 1*	16 ± 16	34 ± 11

OM: fraction of organic matter in the soil (%); *indicates significant reduction compared to T0 in OM and relative reduction of ΣDDX

Taken together, the microcosm results provided proof-of-concept that WRF can substantially transform aged DDX in real contaminated soil. This provided the foundation for moving to mesocosm and field experiments, with the additional challenges of microbial competition, substrate limitation, and environmental variability.

4.5.2 Mesocosms - SMS experiment

Spent mushroom substrate (SMS) from commercial *P. ostreatus* production was tested as an amendment for remediation of aged DDX-contaminated soil, on bucket scale. SMS was mixed into soil in bucket mesocosms and incubated for four months. In contrast to the strong degradation observed in the microcosm experiments, the SMS treatment did not result in a statistically significant reduction in ∑DDX, nor was significant ligninolytic activity observed (Figure 13).

Previous studies have reported promising results using *P. ostreatus* SMS for degradation of persistent organic pollutants, including DDT and related compounds, but either at very low weights (1 g) and with spiked soils (Purnomo et al., 2010) or with less hydrophobic compounds (Mayas et al., 2024; Siracusa et al., 2017; Di Gregorio et al., 2016). This literature provided a strong rationale for testing whether SMS could act as a low-cost and readily available amendment for bioremediation under controlled mesocosm conditions. The mesocosm experiment was designed as an intermediate step between laboratory microcosms and field application, with sampling performed at the start of the experiment (T0) and after four months of incubation (T1).

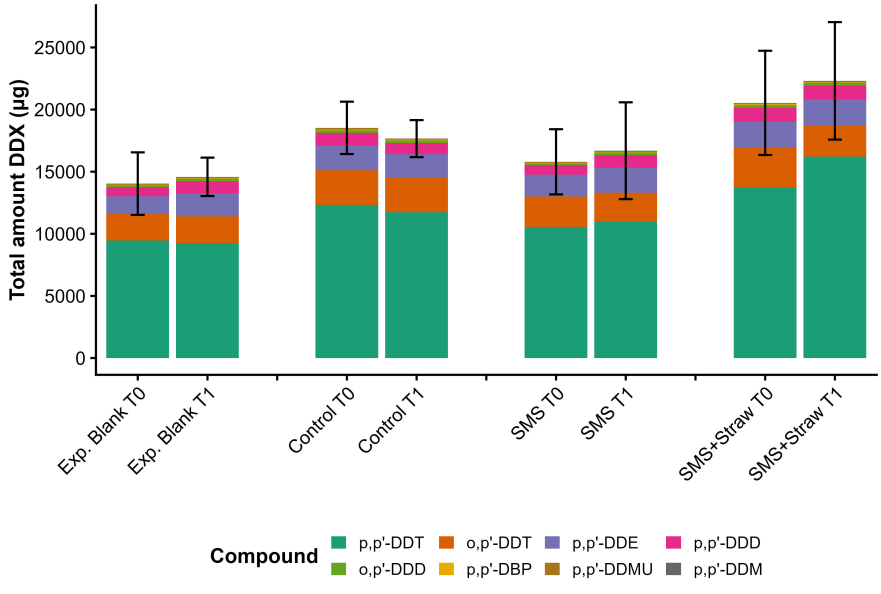


Figure 13: Total amount of DDX (µg) in soil only (Exp. Blank), in soil with added autoclaved SMS (Control), SMS treatment, and treatment with SMS and an additional carbon source (SMS+Straw) in the mesocosms at the beginning of the experiment (T0) and after four months of incubation (T1). Values are means with error bars showing standard deviation. *n*=3 except for *p,p'*-DDM in Blank T0, Control T1 and SMS T0 where *n*=2.

The characteristic fluffy white growth associated with active *P. ostreatus* was not present, suggesting that the fungal biomass may have been inactive. A pretreatment step to reactivate the SMS (e.g., aeration and mixing with fresh organic substrate) may therefore have improved performance.

Potentially, if this had been done before mixing with the unsterile soil, native microbes would have had less chance to colonise the SMS before *P. ostreatus* could re-establish and begin to colonise the soil. No details are provided in Purnomo et al. (2010) regarding pretreatment of the SMS used in their experiments. This is particularly relevant because ligninolytic enzymes generally have short half-lives in soil (Margot et al., 2013; Wu et al., 2014), meaning that DDX degradation is unlikely unless a metabolically active fungus is present and producing enzymes continuously. In this sense, the SMS treatment may have failed before it even had a chance to become a fungal treatment.

4.5.3 Field scale trial

The *in-situ* field trial, where inoculated mushroom substrate was tilled into the upper layer of DDX-contaminated soil, did not result in a reduction in soil DDX concentrations after six weeks. Neither *P. ostreatus* nor *S. rugosoannulata* produced measurable treatment effects under field conditions, despite the degradation observed in simplified microcosm systems by *P. ostreatus* (Figure 14).

The lack of treatment effect is likely explained by a combination of ecological constraints on fungal establishment and persistent physicochemical limitations related to the aged, strongly sorbed nature of DDX in agricultural soil. Observations from soil cores suggest one limitation was restricted colonisation. In *P. ostreatus* plots, visible mycelial growth was largely confined to the top 2–3 cm, whereas *S. rugosoannulata* showed deeper vertical colonisation, reaching up to ~7 cm after six weeks. However, in both treatments, colonisation remained patchy and concentrated immediately around amended substrate, with minimal spread into surrounding soil. This implies that fungal activity was too spatially limited to influence the DDX pool.

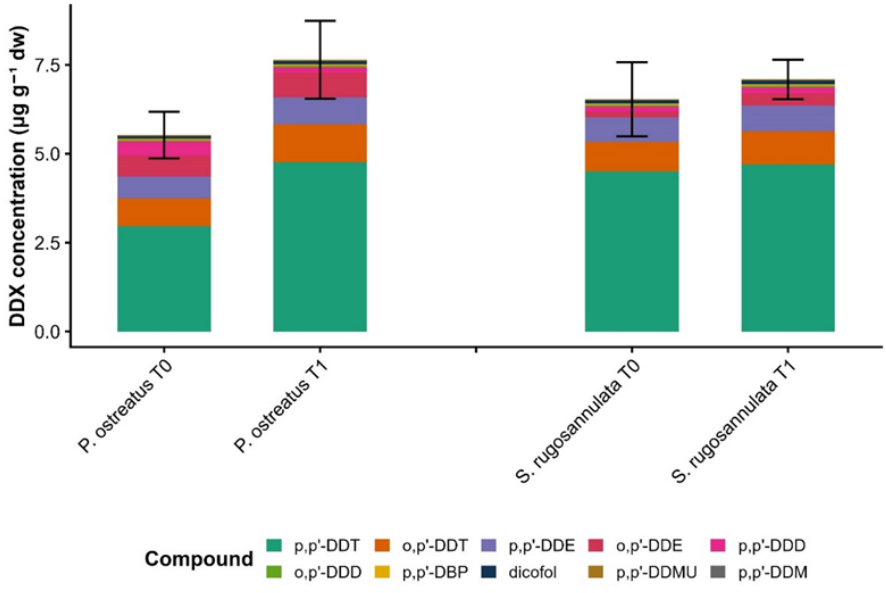


Figure 14: Concentration (ng g^{-1} d.w.) of DDX in soils of the field plots at the beginning of the experiment (T0) and after 6 weeks (T1). *n*=3 except for *S. rugosoannulata*: *p,p'*-DDE at T0 and T1 where *n*=1 and *n*=2, respectively. *p,p'*-DDM was excluded from *S. rugosoannulata* as levels were <LOQ. Reproduced from Paper III.

Interestingly, *P. ostreatus* started fruiting at week 4, and following a period of heavy rainfall and cooler temperatures, all three *P. ostreatus* treated plots produced fruit bodies by week 5. This confirms that the inoculated substrate remained biologically active. However, fruiting also suggests that the fungus may have allocated resources towards reproduction rather than exploration and nutrient scavenging in an unfavourable habitat. Although DDX accumulation was detected in fruit bodies, the total mass removed via harvesting was negligible relative to the soil contaminant burden and cannot be considered a meaningful remediation pathway.

4.5.4 Considerations and limitations – scaling up treatment, spawn quality and habitat

The SMS trial and the field trial illuminated many of the same issues that arise when upscaling mycoremediation from microcosms towards more realistic systems.

A first shared limitation is that both experiments created a deeper and less aerated soil-substrate system than the successful petri-dish microcosms. This could create oxygen limitation which is harmful for WRF which normally colonises wood above the soil level. A shallow tray-based mesocosm may have aligned better with fungal ecology, for example, Betancur-Corredor et al. (2015) reported promising results in a shallow system with only surfactant addition and nutrient stimulation of the native microbial community.

Second, both systems used aged, contaminated soil. In contrast to many successful studies using freshly spiked soils (Table 2), DDX in this system is likely strongly sorbed to soil organic carbon, reducing bioavailability. Fungal remediation for aged DDX-contaminated soils may therefore require strategies that promote mobilisation. However, in an open system careful risk assessment would be required regarding the risk for DDX leaching in groundwater.

Third, the amount of spawn and substrate applied may have been insufficient for *in-situ* conditions, even though inoculation ratios were based on comparable *ex-situ* biopiling systems (Di Gregorio et al., 2016). In the mesocosms, the fungus faced immediate competition from native soil microbes. In the field, the fungus additionally faced variable rainfall, temperature shifts, soil fauna activity, and vegetation dynamics. Under these conditions, a higher inoculum proportion may have been needed to create sustained colonisation of sufficient magnitude.

These two experiments also highlight a fundamental challenge of applying WRF in soil; soils are heterogeneous, and fungal growth is inherently uneven. Unlike aqueous systems, where treatment conditions can be homogenised, soil contains microhabitats that differ in oxygen, moisture, nutrient availability, and microbial competition. These constraints differ substantially from the controlled conditions of the earlier microcosm experiments.

Importantly, these results illustrate a key scaling challenge in fungal remediation; a treatment that performs well in microcosms can fail at mesocosm and field scale if fungal activity is not maintained and contaminant accessibility remains low. In this thesis, the SMS and field

experiments therefore serve as a critical transition point, showing that the main barrier is not only whether fungi can degrade DDX, but whether fungal activity can be established and sustained in soil long enough for transformation to occur. Additionally, even if fungi establish, the strongly sorbed hydrophobic fraction of DDX may remain largely unavailable, which may be why such high success was seen in the spiked soils experiments of previous studies (Table 2). As a result, successful field-scale remediation may require combined strategies that improve bioavailability. Further discussion and details on this matter can be found in Paper III.

These outcomes directly motivate the next set of experiments, where surfactant-assisted mobilisation, using Tween 80, was tested as a strategy to increase accessibility of the aged DDX pool.

4.6 Microbial degradation of DDX - Soil washing experiments

Two soil washing experiments using Tween 80 were conducted in this thesis. The first was an experiment at flask scale, while the second was an upscaled pilot bioreactor combining soil washing with a two-phase treatment system. Together, these experiments address the key issue observed in the mesocosm and field trials; that aged DDX is strongly sorbed and poorly accessible, even if fungal activity can be established.

4.6.1 Flask-scale experiment - mobilisation and enzyme-assisted degradation

The first experiment is outlined fully in Paper II. DDX-contaminated Kolleberga soil was treated with Tween 80 and/or enzyme extract from *P. ostreatus* in flasks. The purpose was twofold: (i) to test whether Tween 80 alone could mobilise DDX from the solid phase into the liquid phase, and (ii) to test whether a fungal enzyme suspension could degrade mobilised DDX, both with and without Tween 80 present. The results indicated that Tween 80 did indeed mobilise DDX into the liquid phase, and that this was

subsequently subject to degradation by the enzyme suspension of *P. ostreatus* (Figure 15).

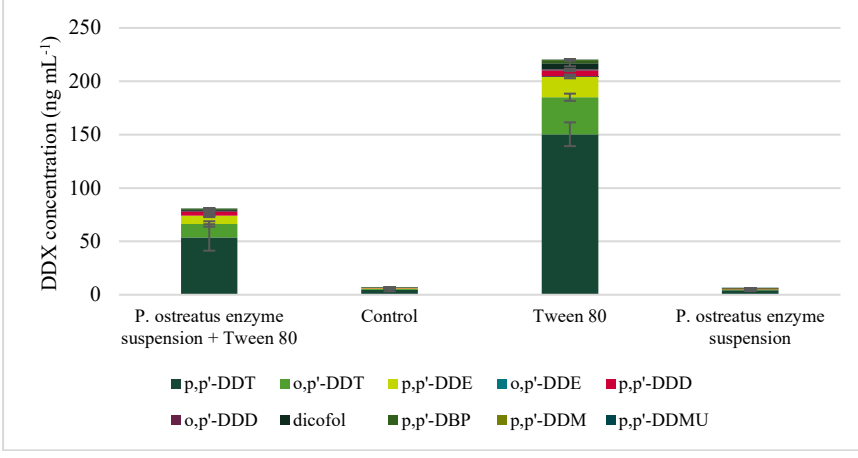


Figure 15: DDX concentrations in the liquid phase (ng mL^{-1}) in Tween 80 mobilisation / degradation experiments in flasks. Reproduced from Paper II.

Additionally, the ligninolytic enzyme activity was measured daily for the duration of the 8-day experiment, showing a stimulatory effect of Tween 80 on ligninolytic enzyme activity. This has been attributed in literature mostly to indirect mechanisms where Tween 80 mobilizing soil compounds such as phenolics that stimulate ligninolytic activity (Kotterman et al., 1998; Zhang et al., 2011). The activity decreased from day 1, reaching baseline levels by day 4 for all treatments.

Overall, this experiment supported the hypothesis that surfactant addition can increase DDX mobility and bioavailability and simultaneously influence fungal enzymatic capacity.

4.6.2 Liquid culture pre-study - DDX degradation by *Ganoderma lucidum*

A pre-study was conducted to test the degradation of DDX in mycelial suspension of *Ganoderma lucidum* (Paper IV). Over the course of 10 days, *G. lucidum* treatment decreased ∑DDX amounts, corresponding to 73 ± 6% reduction. This pre-study provided a practical basis for selecting *G. lucidum* for the pilot bioreactor work.

4.6.3 Pilot bioreactor - Two-phase soil washing and fungal treatment

The pilot bioreactor experiment (Paper IV) was designed to test an upscaled approach combining surfactant-assisted mobilisation of DDX with fungal treatment. The system consisted of soil, a liquid phase containing Tween 80, and woodchips inoculated with *G. lucidum*. This design allowed mobilisation of DDX from soil into solution while simultaneously exposing the mobilised fraction to fungal degradation. The total DDX amount in the starting soil added to both the control and treatment reactors is shown as "Starting Soil". Final DDX amounts in treatment and control soil, and woodchips were calculated after the 28-day treatment period (Figure 16).

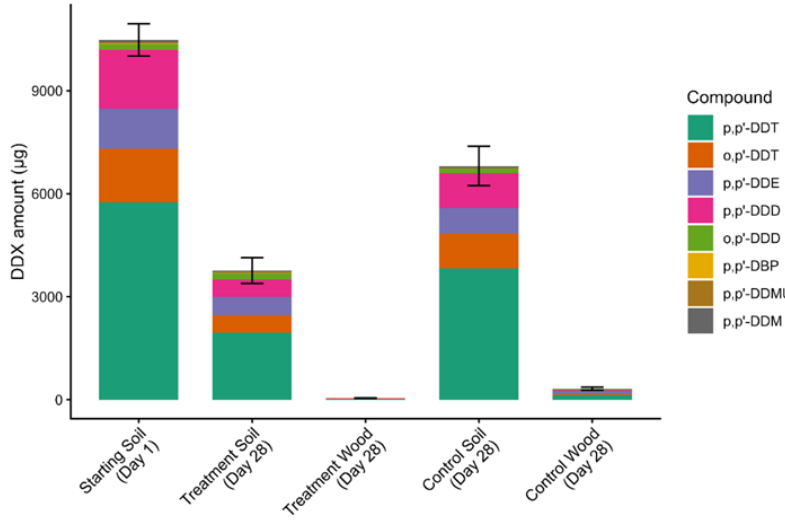


Figure 16: Amounts of DDX (µg) present in the solid phases of the bioreactor at day 1 and day 28 of treatment.

The amount of DDX in the soil decreased in both the control and treatment systems, though by substantially more in the treatment. The starting soil contained 10 500 ± 990 µg total DDX. After 28 days, this reduced to 7 600 ± 920 µg DDX in the control, and 4 300 ± 610 µg DDX in the treatment, indicating *G. ludicum* degradation. Notably, in the treatment, the degradation was higher for more abundant and recalcitrant parent compounds (DDT, DDE, DDE) but was more even in the control (Paper IV, Table 1).

One observed difference between the treatment and control was the amount of DDX released into the liquid phase over time. Despite identical Tween 80 circulation, the control system released slightly less DDX. One possible explanation is that ligninolytic enzymes produced by *G. lucidum* acted on soil organic matter, mobilising sorbed DDX. In addition, some WRF such as *T. versicolor* are known to produce biosurfactants, which could also enhance mobilisation (Lourenço et al., 2017). Although *G. lucidum* has not been tested for this capability, it remains a possible contributing mechanism. Finally, Tween 80 is known to stimulate ligninolytic enzyme production in WRF (Ürek & Pazarlioğlu, 2005; Teodoro et al., 2018; Wu et al., 2016), which may create a combined effect where mobilisation and degradation are linked through increased decomposition of organic matter.

At the end of the experiment, DDX partitioning among soil, woodchip and liquid phases was 93%, 1.3% and 5.5%, respectively, in the treatment, and 90%, 4.9% and 4.5%, respectively, in the control. Notably, a higher proportion of DDX was sorbed to the woodchips in the control, presumably due to the absence of fungal degradation in this phase. Absolute amounts at the final day of sampling (Solution: day 24; Soil and Woodchips: day 28) are shown in Table 4.

Table 4: Amounts of ∑DDX (µg) in each phase of the bioreactor (Experiment 1) at final day of sampling.

∑DDX	Control (µg)	Treatment (µg)
Total	8 300	4 600
Soil	7 600	4 300
Woodchips	410	62
Solution	370	250

4.6.4 Soil washing in bioreactor – biodegradation, sorption, and scaling up

In the system described above, the mobilised DDX partitioned between the liquid, soil and woodchip phases, with losses attributable to fungal degradation, native microbial activity, and sorption to reactor surfaces.

The reduction in DDX in the control soil may partly be ascribed to the activity of the native microbial community, which has been reported to possess limited capacity to transform certain DDX compounds (Ortiz et al., 2013; Purnomo et al., 2010). After the initial increase, a decrease in DDX concentration in the liquid phase was observed between days 4 and 6 in both the control and treatment. A likely explanation is that once Tween 80 mobilised DDX into solution, the compounds also partially adsorbed onto reactor surfaces, including plastic tubing and fittings (Champion & Olsen, 1971; Zhang et al., 2021). After this initial loss, increases in DDX concentration in the liquid phase of both systems were observed, potentially reflecting saturation of materials sorption and re-equilibration. Rapid degradation by enzymes present in the inoculated woodchip matrix may also have contributed to early decreases in the system.

Similar systems (e.g., trickle-bed reactors, fluidised reactors, and packed-bed channel systems) have been applied for removal of hydrophobic pollutants by WRF, with considerable success (Beltran-Flores et al., 2023; Hu et al., 2022). For example, Tayar et al. (2025) applied a trickle-bed reactor for organophosphate flame-retardant degradation by *G. lucidum*. Their system, originally developed for wastewater treatment, was well suited for treating the contaminated soil effluent, and fungal establishment lasted long enough to achieve 85% removal. This supports the idea that technologies developed for wastewater treatment may be promising for scaling combined soil washing and fungal treatment for DDX-contaminated soils.

In the context of this thesis, the Tween 80 and bioreactor experiments shift the direction of remediation potential from direct soil remediation in the field, overcoming competition, establishment and spatial challenges, to a soil cleaning system where the DDT can then be degraded by a water treatment step. The pilot reactor results suggest that coupling mobilisation with a separate fungal degradation phase may be a more realistic pathway to remediation than direct *in-situ* fungal application. While replication was limited at pilot scale, the observed reductions support further development of two-phase systems where accessibility and treatment can be optimised independently.

5. Conclusions and Future Perspectives

Across the experiments in this thesis, a consistent pattern emerged: WRF can achieve substantial transformation of DDX in aged, contaminated soil, but this is difficult to translate to larger, more heterogeneous systems. The results therefore shift the focus from whether fungi can degrade DDX at all, to what conditions are required for degradation to occur reliably in aged soils, and how these conditions might be engineered at relevant scales.

The microcosm experiments provided proof-of-concept for soil with aged DDX contamination. Multiple species, *P. ostreatus*, *T. versicolor* and *Ph. adiposa*, achieved large reductions in ∑DDX over 30 days in unsterilised, soil directly from a contaminated field site. These results demonstrate that fungal degradation of DDX is not limited to freshly spiked laboratory systems, and that aged contaminated soil can be remediated when fungal activity is sustained. The relationship observed between DDX reduction and organic matter loss supports the idea that transformation occurs as part of broader decomposition processes by ligninolytic enzyme activity.

However, when the experimental scale increased to pots and field trials, treatment was no longer successful. Both trials failed to produce measurable DDX reduction. Importantly, these outcomes should not only be viewed as negative results, but as evidence of the limitations to remediation in realistic soil environments. The SMS experiment highlighted that fungal remediation requires good inoculum quality, potentially requiring a reactivation step, to withstand competition from native microbiota and survive in soil. The field trial further showed that even when inoculated substrate remains biologically active (as indicated by fruiting in *P. ostreatus* plots), colonisation in soil remains patchy and largely confined to amended material, limiting enzyme exposure to the soil contaminant pool.

Together, these results indicate that in aged DDX-contaminated soils, contaminant accessibility is as much of a challenge as fungal survival. The sorption to organic matter limits microbial uptake and transformation and this is compounded by the spatial heterogeneity of fungal growth, and the difficulty of maintaining ligninolytic activity.

The Tween 80 experiments targeted this problem by mobilising DDX from the solid phase and increasing exposure to ligninolytic enzymes. At flask scale, Tween 80 increased mobilisation of DDX into solution and was associated with increased ligninolytic enzyme activity and DDX degradation. The novel pilot bioreactor extended this concept by combining soil washing with Tween 80, followed by a separate fungal treatment of the effluent using *G. lucidum*. While there was no replication at pilot scale, the treatment system showed substantially greater reductions in soil DDX compared with the control. The bioreactor results are particularly important because they represent a shift in remediation strategy. A two-phase approach separates the mobilisation and degradation of sorbed contaminants It moves the exposure of the mobilised DDX to a context where there can be sustained fungal growth and better dispersal of ligninolytic enzymes.

Overall, this thesis demonstrates that fungal remediation of DDX is feasible but depends on sustaining fungal activity and increasing accessibility of the aged contaminant pool. The failure of the mesocosm and field treatments highlights why direct *in-situ* approaches are unlikely to be reliable. In contrast, the Tween 80 and pilot bioreactor experiments suggest that soil washing followed by fungal water treatment may provide a more realistic pathway for remediation of historically contaminated soils. Five different fungal species showed success in degrading DDX across the course of this thesis, each with different ecological traits and habitat preferences. This provides many potential avenues for future research, as the versatility and variety of WRF could make many remediation strategies possible.

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Popular science summary

DDT was a commonly used and highly effective insecticide but was eventually banned due to its harmful effects on the environment. It is highly hydrophobic and therefore accumulates in fatty tissues and magnifies up the food chain. After major environmental activism in the 1960s revealed that DDT was harming birds, fish, and other wildlife, the pesticide was progressively banned across the global north. However, there are still many polluted areas across Sweden that require treatment.

White-rot fungi grow on wood and can break down lignin, a plant molecule which is very complex and hard to degrade, by producing extracellular oxidative enzymes that work over long distances. These enzymes are very unspecific which means they can also act on DDT and its transformation products (collectively referred to as DDX) and other environmental pollutants. For this reason, there has been extensive research into using white-rot fungi to remediate polluted soils, water, and sediments. Although much previous research demonstrates their success in degrading a wide variety of pollutants, their use for treatment of aged DDT contaminated soils has only been explored in a few, and often small-scale, studies. This thesis therefore sets out to answer the following research questions:

- Can we accurately quantify how much DDT there is in soils, and how much is present in the soil pore-water (the bioavailable concentration)?
- Can white-rot fungi degrade DDT in aged, contaminated soils?
- Which fungi are the most effective, and why?
- Can the degradation process be optimised and upscaled?
- Can addition of a waste product from the mushroom production industry serve as a cost-effective treatment technique?
- Can soil washing transfer DDT from soil into a closed liquid system where the contaminants can then be degraded by fungi?

To answer the first question, we collected soils from nine contaminated sites (old forest nurseries) across Sweden and analysed them for both total and pore-water DDX concentrations, the latter determined using a polymeric sampling material polyoxymethylene (POM). To be able to do so, we had prior to the measurements determined the partitioning coefficients between the pore-water and the sampling material for the DDX compounds, helping establish this passive sampling technique for future risk assessments.

To address the second and third questions, we selected five white-rot fungi and mixed them with soils from one of the sampled sites. We then measured DDX concentrations before and after 28 days of incubation to quantify degradation. Most of the fungi showed promising degradation potential. We also investigated the use of the biodegradable surfactant Tween 80 to increase efficiency, which could support future upscaling.

The optimisation and upscaling were attempted through both a pot-scale and a field-scale trial but were ultimately unsuccessful. The field trial used oyster mushroom (wood-growing) and wine-cap mushroom (wood- and soil-growing), were applied onsite at a contaminated Swedish forest nursery. DDX concentrations in the soil were measured before treatment and after six weeks. The pot-scale trial used spent mushroom substrate (waste from industrial oyster mushroom production) mixed with contaminated soil, and DDX content of the soil was assessed before and after 4 months. In both these experiments, no effect on DDX concentration in the soil of the fungal treatment was observed. This highlights the challenges that realistic treatment conditions provide, namely that the hydrophobic pollutants are strongly sorbed to the soil and not accessible to the fungus, and the difficulty of maintaining ideal fungal growth conditions.

Finally, to address these two challenges, the use of surfactant was explored. We developed a combined soil-washing and fungal treatment process where the DDX was washed from the soil by the surfactant Tween 80, into a liquid phase, where it was more bioavailable for degradation by fungi. This was first tested in small scale flasks, and then to a larger, bench-scale pilot treatment. We created a novel bioreactor system that circulated Tween 80 solution through contaminated soil, then through a cylinder containing

fungus grown on woodchip for degradation. In contrast to the experiment when the fungus was grown directly in the field or in buckets, the result from the soil washing technique was very promising. Further research is needed to scale this up but the technique of soil washing followed by remediation of the effluent was demonstrated to be effective, and developing this would be the natural progress of this thesis work.

Populärvetenskaplig sammanfattning

DDT var ett vanligt använt och effektivt insektsmedel som användes globalt i stor skala. Det är mycket hydrofobt och ansamlas därför i fettvävnader och transporteras upp i näringskedjan. Under 1960-talet blev det tydligt att DDT skadade fåglar, fiskar och annat vilt, och bekämpningsmedlet förbjöds efterhand. Det finns dock fortfarande många förorenade områden i Sverige som kräver sanering.

Vitrötesvampar växer på trä och kan bryta ned lignin, en växtmolekyl som är mycket svårnedbrytbar. Svamparna bryter ner ligninet genom att producera nedbrytande enzymer som skickas ut ur cellen och som kan verka över långa avstånd. Dessa enzymer är mycket ospecifika, vilket innebär att de också kan påverka DDT och dess nedbrytningprodukter (benämnda som DDX) samt andra miljöföroreningar. Av denna anledning har det bedrivits mycket forskning kring hur vitrötesvampar kan användas för att sanera förorenade jordar, vatten och sediment. Mycket av den tidigare forskningen visar att vitrötesvampar framgångsrikt kan användas för att bryta ned en mängd olika föroreningar. När det gäller svampbehandling av historiskt DDT-förorenade jordar har bara ett fåtal, och ofta småskaliga, studier genomförts. Denna avhandling syftar därför till att besvara följande forskningsfrågor:

• Kan vi noggrant kvantifiera hur mycket DDT som finns i jordar och hur mycket som finns biotillgängligt i porvattnet?

• Kan vitrötesvampar bryta ned DDT i historiskt, förorenade jordar?

• Vilka svamparter är mest effektiva, och varför?

• Kan behandlingsprocessen optimeras och skalas upp?

• Kan tillsats av en restprodukt från svampodlingsindustrin fungera som en kostnadseffektiv behandling?

• Kan jord tvättas för att överföra DDT från jord till ett slutet vätskesystem där föroreningarna sedan kan brytas ned av svampar?

För att besvara den första frågan samlade vi in jord från nio förorenade platser (tidigare skogsplantskolor) runt om i Sverige och analyserade dem både för totalhalter av DDX i jorden och för koncentrationer av DDX i porvattnet. Den senare bestämdes med hjälp av ett polymert provtagningsmaterial (polyoxymetylen; POM).

För att kunna analysera porvattnet hade vi först bestämt hur DDX-ämnena fördelar sig mellan porvattnet och provtagningsmaterialet, så kallade fördelningskoefficienter. Detta gör det möjligt att använda den passiva provtagningsmetoden för DDX och därmed förbättra framtida riskbedömningar.

För att besvara den andra och tredje frågan valde vi ut fem vitrötesvampar och använde dem för att behandla förorenad jord. DDX-koncentrationer före och efter 28 dagars analyserades för att kvantifiera nedbrytningen. De flesta av svamparna visade lovande nedbrytningspotential. Vi undersökte även användningen av den biologiskt nedbrytbara tensiden Tween 80 för att öka effektiviteten.

Optimering och uppskalning undersöktes genom både ett krukförsök och ett fältförsök, men dessa blev i slutändan inte framgångsrika. I fältförsöket användes ostronskivling (vedväxande) och jättekragskivling (som växer på både ved och jord). De tillsattes direkt i jorden på en förorenad svensk skogplantskola. DDX-koncentrationer i jorden mättes före behandlingen och efter sex veckor. I krukförsöket användes en restprodukt från kommersiell odling av ostronskivling som blandades med förorenad jord, och jordens DDX-innehåll analyserades vid start och efter fyra månaders behandling. I inget av experiment sågs någon effekt av svampbehandlingen på DDX-koncentrationen i jorden. Detta belyser två stora utmaningar vid behandling under verkliga förhållande: dels att hydrofoba föroreningar, som DDT, är starkt bundna till jordpartiklar och därför inte är tillgängliga för svampen, dels att det är svårt att skapa goda tillväxtförhållanden för svamparna.

Slutligen undersöktes en möjlighet att hantera dessa två utmaningar i en kombinerad process med både jordtvättning och svampbehandling. DDX tvättades ut från jorden till en vätskefas med hjälp av tensiden Tween 80, där

föroreningarna sedan bröts ner av vitrötesvamp. Detta testades först i enkla flaskförsök och därefter i en större behandling i bioreaktor. Bioreaktorsystemet baserades på en Tween 80-lösning som cirkulerades genom förorenad jord och sedan genom en cylinder som innehöll svamp odlad på träflis. Till skillnad från experimenten där svampen odlades direkt i jorden var resultaten från den kombinerade processen mycket lovande. Ytterligare forskning krävs för att skala upp metoden, men tekniken med jordtvättning följt av svampbehandling av den förorenade vätskan är ett lovande system, och att utveckla detta skulle vara den naturliga fortsättningen på detta avhandlingsarbete.

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“A learning experience is one of those things that says, 'You know that thing you just did? Don't do that.”
― Douglas Adams, The Salmon of Doubt

I

RESEARCH PAPER
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Environmental Chemistry

Determination of polyoxymethylene (POM) water partition coefficients for DDT and its degradation products, with inter-laboratory comparison of the passive sampling methodology and bioaccumulation in earthworm (*Eisenia fetida*)

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Environmental context. The widespread use of the insecticide DDT has left a legacy of pollution that still threatens ecosystems today. This study presents a method to accurately measure the bioavailability of DDT and its breakdown products in contaminated soils. This will improve risk assessments and guide sustainable land management practices, helping to protect both the environment and human health.

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ABSTRACT

Rationale. The insecticide dichlorodiphenyltrichloroethane (DDT) and its degradation products (collectively DDX) are persistent organic pollutants that pose significant environmental risks due to their persistence and bioaccumulation in ecosystems. Accurate quantification of DDX bioavailability in soil systems is crucial for effective land management and risk assessment. **Methodology.** This study utilised equilibrium passive sampling with polyoxymethylene (POM) to determine the bioavailability of DDX in soil. The sorption dynamics of 10 DDX compounds were investigated (*p,p′*-DDT, *o,p′*-DDT, *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD), *o,p′*-DDD, *p,p′*-dichlorodiphenyldichloroethene (*p,p′*-DDE), *o,p′*-DDE, *p,p′*-dichlorodiphenylmethane (*p,p′*-DDM), *p,p′*-dichlorobenzophenone (*p,p′*-DBP), 1-chloro-2,2-bis(4-chlorophenyl)ethylene (*p,p′*-DDMU) and dicofol) and their POM–water partition coefficients (K_{POM}) were determined. The study involved interlaboratory comparisons, using soils from nine historically contaminated sites and ecotoxicology assessments (mortality, reproduction and bioaccumulation in earthworms, *Eisenia fetida*) to validate the POM method. **Results.** K_{POM} values for 9 of the 10 DDX compounds were successfully determined, allowing for accurate quantification of freely dissolved pore water concentrations of DDX in historically contaminated soils. The interlaboratory study highlighted important considerations in extraction and gas chromatography–mass spectrometry analysis, and the ecotoxicology study demonstrated the potential of POM passive sampling as a reliable tool for assessing DDX bioavailability (bioaccumulation in *Eisenia fetida*). **Discussion.** The POM method proved to be a robust and reliable approach for quantifying freely dissolved DDX, with implications for improving the accuracy of risk assessments and guiding sustainable land management practices. The study also highlighted the need for careful consideration of analytical challenges, such as the potential degradation of DDX compounds during gas chromatography analysis, to ensure accurate quantification.

Keywords: aged soil contamination, bioavailability, earthworm toxicity and uptake, equilibrium passive sampling, persistent organic pollutants, POPs, pore water concentration, risk assessment.

Introduction

From the 1940s to the early 1970s, the insecticide dichlorodiphenyltrichloroethane (DDT) was widely used to control the spread of vector-borne diseases, particularly mosquito-borne malaria, and lice, and to prevent pests in forestry and agriculture (Mansouri *et al.* 2017). Technical DDT products contain primarily *p,p′*-DDT (65–80%) and *o,p′*-DDT (15–21%) and

small amounts of dichlorodiphenyldichloroethene (DDE) and dichlorodiphenyldichloroethane (DDD) (European Food Safety Authority 2006). These compounds and their degradation products, e.g. 1-chloro-2,2-bis-(4-chlorophenyl)ethylene (*p,p′*-DDMU), *p,p′*-dichlorodiphenylmethane (*p,p′*-DDM), dicofol and *p,p′*-dichlorobenzophenone (*p,p′*-DBP), hereafter collectively referred to as DDX, are persistent organic pollutants that can still be found at elevated levels in the environment, e.g. at previously treated agricultural and forest soils (Drenning *et al.* 2024). In addition, dicofol is not only a transformation product of DDT, but also a commercial acaricide, which has been widely used in controlling mites after DDT was banned in 1983 in China (Huang *et al.* 2018).

Being hydrophobic, DDX tend to sorb to natural organic matter (NOM) in the organic-rich top layers of soil, where soil-dwelling organisms are exposed, which may cause secondary poisoning of higher trophic levels of the ecosystem (European Food Safety Authority 2006; Chattopadhyay and Chattopadhyay 2015). Toxic effects of DDTs in secondary consumers include eggshell thinning in birds of prey and endocrine disruption in fish (Tubbs 2016), whereas long-term (chronical) effects for humans include endocrine disruption, cancer and immunodeficiency (Mansouri *et al.* 2017).

In Sweden, technical DDT has been extensively used in forestry to control pine weevils (*Hylobius abietis*), for example, treating plants in forest nurseries. Over time, as the insects developed resistance to DDT, gradually higher doses were required and applied until DDT was eventually fully banned in 1975, which has resulted in more than 750 old forest nurseries being classified as polluted (Ekelund and Hamilton 2001). Many of these sites are, besides the contamination, arable land of high quality, consisting of mainly sandy soils. Conventional remediation, i.e. excavation and landfilling, is not an environmentally sustainable option, considering the large volumes of contaminated soil and the need for extensive masses of uncontaminated soil for restoration. Sustainable remediation alternatives and accurate risk assessments are thus highly needed. Generic risk assessments, where soil contaminant concentrations are compared with national soil quality standards (also known as soil guideline values, soil screening values or soil target levels) (Swartjes 2011) may, however, result in overestimation of risks and unnecessary soil excavations. By considering the bioavailability of the contaminants, more accurate assessments can be made (Alexander 2000; Volchko *et al.* 2020). Several tools for assessing the bioavailability and mobility of hydrophobic organic compounds (HOC) have been developed, and their use in site-specific risk assessment projects is evolving. Equilibrium passive sampling allows for measurement of the freely dissolved concentration in pore water ($C_{W,free}$), which is considered to reflect the bioavailable concentration (Hawthorne *et al.* 2011; Gomez-Eyles *et al.* 2012; Mayer *et al.* 2014), in contrast to the total porewater concentration ($C_{W,total}$), which also includes compounds sorbed to dissolved organic matter (DOM) and particulate and colloidal matter (Enell *et al.* 2016).

By the use of pre-determined compound-specific equilibrium polymer–water partition coefficients, $K_{polymer/water}$ (L kg^{-1}), and the quantification of the concentration of HOC in the polymer ($C_{polymer}$) the water concentration ($C_{W,free}$) can be calculated using Eqn 1:

$$C_{W,free} = C_{polymer} \div K_{polymer/water} \quad (1)$$

In order to turn equilibrium passive sampling into a standardised and routine analysis of $C_{W,free}$, it is crucial to have well-calibrated $K_{polymer/water}$ values and to use a polymer that is commercially available, inexpensive and robust during handling, e.g. does not tear, is easy to clean and is chemically stable in both aqueous media and organic solvents. Polyoxymethylene (POM) meets these criteria (Jonker and Koelmans 2001; Hawthorne *et al.* 2009) and is already used in commercial laboratories in Sweden for equilibrium passive sampling of polyaromatic hydrocarbons (PAHs), using 76-µm POM strips and the method described in Arp *et al.* (2014). POM–water partition coefficients (K_{POM}) have been determined for 76-µm strips for several HOC, e.g. polychlorinated biphenyls (PCBs) (Hawthorne *et al.* 2009; Perron *et al.* 2013*a*), polycyclic aromatic compounds (PACs) (Hawthorne *et al.* 2011; Kupryianchyk *et al.* 2011; Perron *et al.* 2013*a*; Josefsson *et al.* 2015) and polybrominated diphenyl ethers (PBDEs) (Perron *et al.* 2013*b*), but also for more polar substances, e.g. organophosphate esters (OPEs) (Qin *et al.* 2023). However, for DDX, K_{POM} values have, to our knowledge, only been reported for *p,p′*-DDE and *p,p′*-DDT (Endo *et al.* 2011). To meet the need for tools that can be used to improve risk assessments of DDX contaminated sites, we therefore opted to use POM in this study and to investigate this polymer's applicability as a prediction tool for bioavailability for 10 environmentally relevant DDT related compounds: *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD, *o,p′*-DDD, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDM, *p,p′*-DBP, *p,p′*-DDMU and dicofol. For IUPAC names see Supplementary Table S1.

The objectives of the study were to (i) derive K_{POM} values for the selected DDX (using 76-µm POM strips) to enable determination of $C_{W,free}$, (ii) perform an interlaboratory comparison study for determining $C_{W,free}$ in historically contaminated soils using the derived K_{POM} values, and (iii) compare the results with bioaccumulation and ecotoxicological studies (bioaccumulation of DDX, mortality, growth and reproduction) of the earthworm *Eisenia fetida* to verify POM as a biomimetic method. The results are discussed in terms of practical strategies for improving the risk assessment of historically DDT-contaminated soils by considering bioavailability.

Materials and methods

Part 1 of this study involved determining the K_{POM} values in Lab A. In Part 2, the reproducibility and applicability of the POM method were tested in an interlaboratory study

involving two labs (Lab A and Lab B) analysing DDX in contaminated soils. Part 3 assessed the POM method as a biomimetic method by comparing it to the bioaccumulation of DDX by *Eisenia fetida* (Lab A). Detailed descriptions for the three parts of the study are given in the 'Materials and Methods' in the Supplementary material.

Part 1 – determination of K_{POM} (Lab A)

Chemicals and standards

K_{POM} was determined for 10 DDX: *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *p,p′*-DDMU, *p,p′*-DDM, *p,p′*-DBP and dicofol (Supplementary Table S1). Fortification standards of native DDX compounds were prepared in methanol to obtain four mixtures (C_{f1}–C_{f4}; Supplementary Table S2). The aqueous solutions for the POM–water experiments were prepared using Milli-Q Ultrapure water, NaN_3 and salts of NaH_2PO_4 and Na_2HPO_4. Two internal standard (IS) toluene solutions were prepared: (i) ^{13}C-labelled *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE and *p,p′*-DDE and (ii) deuterium-labelled dicofol (Supplementary Table S3). ^{13}C-labelled PCB101 was used as a recovery standard (RS) (Supplementary Table S3).

Partitioning experiments

The influence of analyte concentrations on K_{POM} was tested using 28-day end-over-end tumbling (Gerhardt Laboshake, Germany) with four different DDX concentration levels, chosen to cover the expected range of concentrations to be found in soils at historical contaminated sites but not exceeding aqueous solubilities: C1, C2, C3 and C4 in methanol (Table 1). The time required for the analytes to reach equilibrium between the aqueous phase and the POM (at pH 7.0), and the potential effect of pH (at 28 days tumbling), were investigated using the C2-level concentration, at tumbling times of 3, 7, 14, 28 and 56 days, and in buffer solutions titrated to pH 4.1 and pH 8.4 with 3 mol L^{-1} HCl and 1 mol L^{-1} NaOH (Table 1). All tests were performed in triplicate.

In brief, 76-µm-thick POM (CS Hyde, Lake Villa, IL, USA) was cut into 4- × 5-cm strips, weighed and placed in a 100-mL bottle with 100 g of 1 g L^{-1} NaN_3 and 10 mmol L^{-1} PO_4-buffer solution. For tests using concentration levels C1 and C2, 50 µL of the DDX fortification mixture (C_{f1}–C_{f2}; Supplementary Table S2) was added. For concentration levels C3 and C4, a staggered fortification approach was used to avoid exceedance of water solubilities. Once fortified, bottles were tumbled end-over-end. At the end of each test, POM strips were removed, wiped dry and placed in glass amber vials. POM strips and sample solutions were stored at +4°C until analysis.

Extraction of water and POM strips

Water samples were extracted twice with *n*-hexane using liquid–liquid extraction, with IS added prior to extraction. Extracts were combined, evaporated to 2 mL, treated with anhydrous Na_2SO_4 and concentrated to 100 µL. RS solution (Supplementary Table S3) was added, the solvent exchanged to toluene, and the sample stored in gas chromatography (GC) vials at −20°C for gas chromatography–mass spectrometry (GC-MS) analysis. POM strips were extracted twice with 40 mL of acetone–*n*-hexane (1:1, v/v) using ultra-sonication, with IS added beforehand. Combined extracts were evaporated to ~0.5 mL and extracts of low concentrations (C1, C2) were cleaned up with deactivated silica. All extracts were concentrated to 100 µL, solvent was exchanged to toluene, RS was added, and the sample stored at −20°C for GC-MS analysis. Further details are in 'POM Concentrations, C_{POM} (Part 1)' in the Supplementary material.

Table 1. Testing scheme to determine K_{POM}; tumbling times (days), and pH and spiking concentrations (µg L^{-1}) at the start of the test.

Parameters	Concentration levels			
	C1	C2	C3	C4
Concentration of DDX (µg L^{-1})				
p,p′-DBP	0.003	0.9	10	100
p,p′-DDM	0.03	1.6	9	90
Dicofol	0.01	0.4	9	90
o,p′-DDD	0.2	0.5	9	90
p,p′-DDD	0.2	0.5	9	90
p,p′-DDMU	0.04	3	9	90
o,p′-DDT	0.3	0.6	9	90
o,p′-DDE	0.2	0.5	9	90
p,p′-DDT	0.3	0.6	9	90
p,p′-DDE	0.2	0.4	9	90
Testing scheme				
Tumbling time (days) at pH = 7	28	3, 7, 14, 28 and 56	28	28
pH at 28 days of tumbling	7.0	4.1, 7.0 and 8.4	7.0	7.0

GC-MS analysis

All measurements were performed in the selected ion monitoring mode. Identification and quantification of the target compounds were done using quantification standard solutions that included all compounds, in addition to IS and RS. More details are given in 'POM Concentrations, C_{POM} (Part 1)' in the Supplementary material.

Quality assurance and quality control (QA+QC)

Target compounds were quantified by use of a 10-point calibration curve. If a calibration point was below 80% or above 120% of expected linearity, the point was excluded to a minimum of eight calibration points. Quantification standards were analysed after every tenth sample to monitor instrumental performance and ensure calibration validity,

with solvent blanks run at the start and end of each sequence to check for carry-over effects. Liners were replaced after 50 injections or upon sign of decomposition. Concentrations were calculated using isotope dilution. Owing to lack of an IS for *p,p′*-DDMU, *p,p′*-DDM and *p,p′*-DBP, calculations were based on 13C-*o,p′*-DDE peak areas (Supplementary Table S3). The limit of detection (LOD) was defined as the mean blank concentration plus 3 × standard deviation (s.d.) and, in the absence of blank contamination, the LOD was set to the lowest concentration of the calibration curve. Samples with concentrations exceeding the range of the calibration curve were diluted, re-spiked and reanalysed. Sample blanks (n = 3) and process blanks (aqueous and POM) were processed in parallel to the experiments.

No DDX compounds were detected in the blanks, except for *p,p′*-DDMU (found in both sample blanks and process blanks), traces of *p,p′*-DDM (in one sample blank) and *p,p′*-DBP (in one process blank) (Supplementary Table S4). If the amount in the blank was >10% of the amount in the corresponding sample, the data for this sample was excluded from further evaluation (Supplementary Table S5).

Since the concentrations of DDX in both the POM and the water phase were measured, K_{POM} can be obtained even if there was a mass loss of the analyte during the experiment (e.g. volatilisation, transformation, glassware sorption). Nevertheless, to ensure good data quality, mass balance calculations were performed (amount in water + amount in POM) ÷ added amount), and if the recovery was <50 or >130%, the data were excluded (Supplementary Table S6). To check for potential degradation of DDT (to DDD) and dicofol (to DBP), which can occur in the GC injector (Foreman and Gates 1997; Fujii *et al.* 2011), mass balance calculations (recovery, i.e. added *v*. measured amounts) were compared between *p,p′*-DDT and Σ*p,p′*-DDT/D, *o,p′*-DDT and Σ*o,p′*-DDT/D, and dicofol and Σ*p,p′*-dicofol + DBP. These calculations indicated very low, or acceptably low, degradation in all experiments, except for dicofol at pH 8.4 as expected, due to the instability of this substance under alkaline conditions (Yin *et al.* 2017). The recovery was generally higher for ΣDDT/D than DDT, but the difference between DDT and ΣDDT/D recoveries were on average only 7% (s.d. ± 3) and 10% (s.d. ± 3) for *p,p′*- and o,*p′*-DDT/D respectively, which is well below the 20% threshold set by the United States Environmental Protection Agency (1995) guidelines for methods for the determination of organic compounds in drinking water. The difference between the recovery of dicofol and Σ*p,p′*-dicofol + DBP were on average 16% (s.d. ± 16), indicating a higher degradation of dicofol to DBP than DDT to DDD.

Statistical evaluations

Statistical comparisons between treatments were performed in *JMP* (ver. 17, JMP Statistical Discovery LLC, NC), using ANOVA followed by Tukey's HSD test at α = 0.05. Values given in the text are arithmetic means ± s.d.

Part 2 – interlaboratory comparison of the POM method (Lab A and B)

In this exercise, we opted to use in-house methods without harmonisation and with special attention to potential degradation of DDX (in the injector) and large differences in concentration levels between different DDX compounds. The two laboratories performed parallel passive sampling with POM to determine $C_{W,free}$ (in triplicate) in soil samples from nine DDX-contaminated forest plant nurseries in Sweden (Supplementary Table S7), together with analyses of C_{soil}.

Soil sampling and characterisation of soils

Soil samples were collected at a depth of 0–20 cm. The samples were sieved (<2 mm) and homogenised in the field before dividing into two subsamples sent to Lab A and B. After arrival at the lab, the soil sample was further homogenised and divided into subsamples for POM tests and analyses for soil characterisation. Details on the sampling, sample preparation and determination of soil properties (total organic carbon, TOC, content; pH; water holding capacity, WHC; and grain size distribution) and levels of potential soil contaminants other than DDX (pesticides and metals) are described in section 'S1.3.1 Soil sampling and analyses of soils' in the Supplementary material. Soil properties are listed in Table 2. None of the soils contained elevated levels of inorganic contaminants, but low to medium levels of pesticides other than DDT were found at some sites (Supplementary Table S8).

Extraction and clean-up of soils

Lab A. In brief, 1 g of wet soil was mortared with anhydrous Na_2SO_4, IS was added and the soil extracted using accelerated solvent extraction (ASE) with in-cell clean up based on the method in Kim *et al.* (2019). A procedure blank containing anhydrous Na_2SO_4 and active silica was included in every batch of samples. The sample extract was evaporated to near dryness using nitrogen and solvent exchanged to toluene (final volume 0.4 mL). RS (Supplementary Table S3) was added to GC vials after transferring the extracts. More details are given in section 'S1.3.1 Soil sampling and analyses of soils' in the Supplementary material.

Lab B. Freeze-dried soil (0.5–1 g) was Soxhlet extracted with dichloromethane (DCM) for 24 h, similar to Huang *et al.* (2018). Prior to extraction, IS was added to the sample (Supplementary Table S3) and a procedural solvent blank was included in every batch of samples. One of the soil samples (Deje Nord) was analysed in triplicate. After Soxhlet extraction, the extract was evaporated to near dryness using nitrogen and the solvent changed to *n*-hexane (1 mL). Activated granular copper was added to remove sulfur. The sample was then further cleaned up on an alumina–silica column and analytes were eluted with DCM–*n*-hexane similar to Huang *et al.*

Table 2. Properties of soil samples from the nine forest plant nurseries.

Site	Deje Syd	Jakobsbyn	Ljungaskog	Kolleberga	Deje Nord	Sya	Stakheden	Klockatorp	Åby
Sample depth (cm)	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20
Sand 2–0.063 mm (%)	29	88	84	91	20	89	62	81	85
Silt 63–2 µm (%)	67	7.0	12	6.2	75	7.7	34	16	12
Clay <2 µm (%)	4.7	4.7	3.7	3.2	4.9	3.7	3.8	2.7	2.9
pH (H_2O)	5.98	5.99	5.76	6.32	5.61	5.45	5.25	4.73	5.63
Total-N (%)	0.12	0.11	0.12	0.081	0.14	0.074	0.092	0.12	0.088
Total-C (%)	1.64	2.04	1.97	1.92	1.94	1.07	1.73	2.66	1.58
TOC (%)	1.63	2.04	1.97	1.91	1.93	1.07	1.73	2.65	1.57
WHC (%)	73.1	51.0	53.3	40.8	53.4	37.4	78.9	59.7	48.3

TOC, total organic carbon; WHC, water holding capacity.

(2018). The sample was evaporated to near dryness using nitrogen, and the solvent changed to isooctane (final volume 2 mL). RS was added (Supplementary Table S3), and the sample was vortexed before an aliquot was transferred to a GC vial for analysis. More details are given in section 'S1.3.1 Soil sampling and analyses of soils' in the Supplementary material.

Determination of $C_{W,free}$ using the POM method

The tests were performed the same way in Lab A and B. In brief, 76-µm POM strips (4 × 5 cm) were placed in amber glass bottles together with 25 g of homogenised soil (calculated as dry weight, DW) and then mixed with 93 mL of water containing $CaCl_2$ and NaN_3. Vials were tumbled end-over-end for 28 days in the dark. Thereafter, the POM strips were removed, rinsed with ultrapure water, and wiped dry before being placed in clean scintillation vials and frozen (−20°C) until extraction.

To study variance caused by differences in the extraction and analysis steps of the two labs and thereby exclude any other variance (e.g. caused by heterogeneous contaminant dispersion in the soil samples and differences in conducting the shaking experiment), six of the POM strips at each lab were cut into halves ('I' and 'II') after the shake-test (in total 24 halves). The 'I' halves ($n = 12$) were then extracted and analysed by Lab B whereas Lab A extracted and analysed the 'II' halves ($n = 12$) (Supplementary Table S9).

The $C_{W,free}$ (ng L^{-1}) of the DDX were calculated using the derived K_{POM} (L kg^{-1} POM) (part 1 of this study) and Eqn 2:

$$C_{W,free} = C_{POM} \div K_{POM} \quad (2)$$

where C_{POM} is the concentration of DDX in the POM strip (ng kg^{-1}), calculated from the amount of DDX in the strip divided by the weight of the POM strip (Supplementary Table S9).

To avoid underestimation of $C_{W,free}$ during equilibrium passive sampling, a criterion of <5% depletion of the HOC from the soil or sediment has been recommended (Mayer *et al.* 2014). With the exception of dicofol, the highest depletion (defined in the Supplementary material, see 'S1.3.3 Control of depletion of DDX from the soil during the POM-test') reported for Lab A and B was 31 and 36%, both found for *o,p′*-DDE, for which the C_{soil} was close to the limit of quantification (LOQ). Soils with TOC content >2% came close to meeting the 5% criterion for all DDX (Supplementary Table S10), except for dicofol analysed by Lab A, for which the depletion was 35%. Lab A also obtained very high depletion of dicofol (85 to >100%) for four forest plant nursery soils, whereas Lab B received ≤17% for all soils. Dicofol in these four samples was thus excluded from further evaluation in both Parts 2 and 3.

Extraction of POM strips

POM strips were extracted as described above (Lab A) with minor adjustments for Lab B; using 24 h of shaking at low speed instead of sonication, and isooctane instead of toluene as final solvent. Lab A performed clean up of all POM extracts using deactivated silica. More details are given in 'S1.3 Interlaboratory comparison of the POM-method (Part 2)' in the Supplementary material.

Instrumental analysis

Lab A analysed soils and POM extracts using GC-MS as described in Part 1. Lab B analysed soil and POM extracts using GC-MS/MS and multiple reaction monitoring (MRM) mode. Identification and quantification were performed using authentic reference standards (Supplementary Table S3). For data evaluation, the software Agilent MassHunter Quantitative Analysis (for QQQ) was used.

Quality assurance and quality control

In Lab A, the QA + QC of the GC-MS analysis is described in Part 1. POM sample blanks (i.e. POM in aqueous solution, with no soil added; $n = 3$) and a process blank per nine POM samples were included in the sample preparation stage and run together with the POM samples. Three process blanks were included in the preparation and analysis of the soil samples.

The LOQ was defined as the mean blank concentration plus 10× s.d. and, in the absence of blank contamination, the LOQ was set to a signal-to-noise (S/N) ratio of 10. The average + s.d. of the recoveries for IS added to the POM samples was 98 ± 24% (n = 252). For IS added to soil samples, the average + s.d. of the recovery was 103 ± 18% (n = 63).

In Lab B, POM sample blanks (i.e. POM in aqueous solution, with no soil added; n = 3) and a process blank per six POM samples were included in the sample preparation stage and run together with the POM samples. Target compounds were quantified using an 11-point calibration curve. If a calibration point was below 80% or above 120% of expected linearity, the point was excluded to a minimum of seven calibration points. Concentrations of target compounds were calculated as described for Lab A. If the amount in the blank was 10% of the amount in the corresponding sample, the sample was excluded from further evaluation. Recoveries for IS added to the POM samples were occasionally found to be high, with six exceptional values. Excluding these six, the average ± s.d. was 106 ± 55 (n = 246). The average ± s.d. of the recovery for the soil samples was 104 ± 58 (n = 98). When samples were below the LOQ, replacement values were used for statistical analysis. The LOQ replacement values were half of the lowest point used on the calibration curve. If the compound was below the limit of detection (S/N ratio <3), the replacement value was three times the value reported, divided by the S/N ratio.

The statistical evaluation was performed using *Excel* (ver. 2407 Build 16.0.17830.20210, Microsoft) and the *Excel* add-in statistical program *Data Analysis Toolpack*.

Part 3 – verification of POM as a biomimetic method (Lab A)

Ecotoxicity tests with *Eisenia fetida* as model organism following the protocol of ISO 11268-1:2012 (mortality after 28 days of exposure) and ISO 11268-2:2023 (reproduction after 56 days of exposure) were performed at room temperature (20 ± 2°C) on sub-samples for the nine soils from the field sites and one ISO control soil (in single samples), and the earth worm bioaccumulation of DDX was analysed. The surviving worms from the mortality test had their gut content purged, and their bodies analysed for lipid content and bioaccumulation of DDX. GC-MS analysis was carried out as described in Part 1. Details are given in 'S1.4 Verification of POM as a biomimetic method (Part 3)' in the Supplementary material.

Results and discussion

Part 1 – determination of K_{POM}

Analysed DDX concentrations in water (C_W), in POM (C_{POM}) and calculated K_{POM} values for the 10 studied DDTs are given in Supplementary Tables S11, S12 and S13. At the end of the experiment, the water concentrations were always <0.3% of the saturated water solubilities (Supplementary Table S1), even in high level treatments.

Potential effect of pH levels

No correlation between pH and log K_{POM} was observed (Supplementary Fig. S1). There was an increase in mean K_{POM} between pH 7.0 and 8.4 for *p,p′*-DDM and *p,p′*-DDT, but the reverse was found for *p,p′*-DDD, and no other significant differences were observed at $P < 0.05$ (Supplementary Table S14). The pH effect was not evaluated for *p,p′*-DDMU and dicofol due to lack of data (blank/sample ratios >10% and C_W < LOD respectively). Given the small sample size (n = 3) and varying results, no trend was deemed strong enough to separate the data set. Thus, data from the tests at the three pH levels (pH 4.1, 7.0 and 8.4) were combined into one data set for the evaluation of equilibrium time and the potential effect of analyte concentrations on K_{POM}.

Time to reach equilibration

At 28 days, after shaking POM with DDX-spiked water (using the C2 concentration), a steady state indicating chemical equilibrium (i.e. no apparent change in K_{POM} values over time) was achieved for all the studied compounds (Fig. 1). Previous kinetic studies on POM of 76-µm thickness have shown that HOC with log K_{OW} < 4.5 can reach equilibrium with POM after 14 days of shaking, whereas compounds with higher hydrophobicity (log K_{OW} between 4.5 and 6.8) may need approximately double the time (Hawthorne *et al.* 2011; Josefsson *et al.* 2015). The same trend was observed in our experiment, where compounds with high K_{OW} values seemed to need longer to reach equilibrium, although most of the compounds appeared to have reached equilibrium already at 14 days. For example, *p,p′*-DDM, with the second lowest K_{OW} value of the studied DDX (log K_{OW} 5.3), may have reached equilibrium already after 7 days, whereas *p,p′*-DDE, which has the highest K_{OW} value (log K_{OW} 6.9) needed at least 28 days (statistics shown in Supplementary Table S15). There was also no difference between mean log K_{POM} values for *p,p′*-DDD, and *o,p′*- and *p,p′*-DDT derived at 14 and 28 days, indicating that 14 days of shaking will be enough for these compounds too. Dicofol (log K_{OW} 5.8) and DDMU (log K_{OW} 6.2) could not be statistically evaluated due to lack of data at several or all time points (Supplementary Fig. S2), but as the hydrophobicity of these compounds are lower than many of the other DDX compounds studied, they are assumed to reach equilibrium within the same time frame (i.e. within 28 days). Consequently, it was concluded that an exposure time of 28 days should be sufficient to reach equilibrium for all the investigated DDX. For evaluation of the potential effect of analyte concentrations on K_{POM}, the replicates with equilibration times of 28 and 56 days were used.

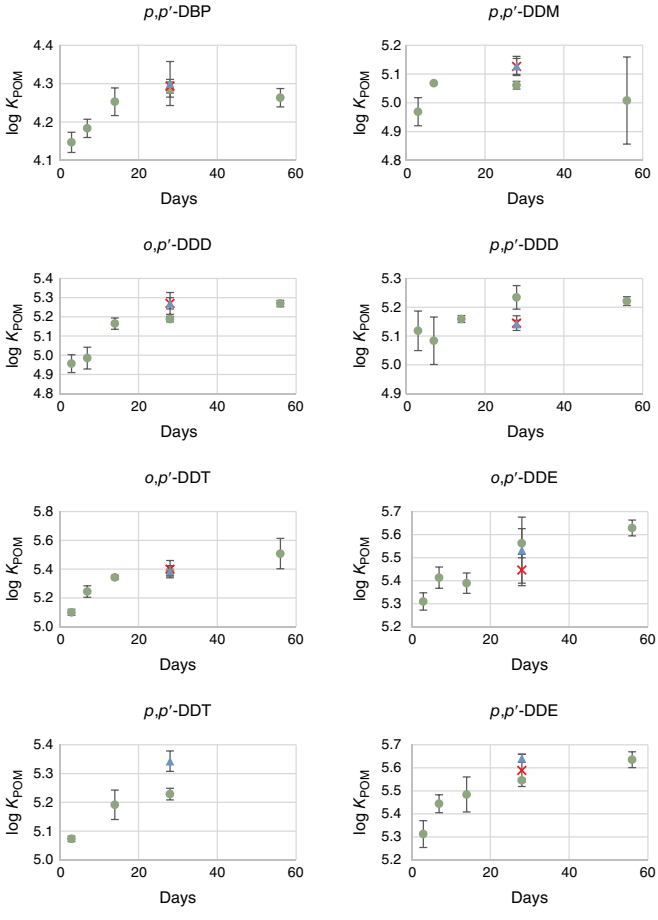

Fig. 1. Mean log K_{POM} for the DDX as a function of tumbling time (days) derived for the C2 concentration level. Error bars represent the standard deviation (n = 3). Green circles, blue triangles, and red crosses indicate the pH of the test; 7.0, 8.4 and 4.1 respectively. Dicofol was excluded due to lack of data. DDMU is shown in Supplementary material; Fig. S2.

Effect of analyte concentrations on K_{POM}

There were no consistent trends in K_{POM} values for three of the four tested concentrations (using the 28- and 56-day replicates; Supplementary Fig. S3 and Supplementary Table S16). The lowest concentration level (C1) gave, however, a noticeably lower mean log K_{POM} for the DDDs and DDEs but not for the DDTs (the other DDX could not be evaluated at this level due to data not meeting quality requirements or having C_W < LOD). These lower K_{POM} values at the C1 level for some compounds were likely due to overestimation of areas for peaks with low signal-to-noise ratios. The DDTs had 50% higher starting concentrations than DDDs and DDEs in C1 (Table 1) and should theoretically reach a higher C_W at equilibrium than the compounds with higher hydrophobicity (i.e. *o,p′*- and *p,p′*-DDE). Since the opposite was observed ($C_{Wo,p'\text{-DDE}} > C_{Wo,p'\text{-DDT}}$ and $C_{Wp,p'\text{-DDE}} \approx C_{Wp,p'\text{-DDT}}$ (Supplementary Table S11), this indicates overestimation of peak area, leading to overestimation of C_W at this very low concentration level. Therefore, it was decided to exclude C1 data for DDE and DDD in the final determination of log K_{POM}.

The mean K_{POM} values obtained at C3 did not differ significantly from those obtained at C4 for all compounds that could be evaluated at these two levels (i.e. dicofol, *p,p′*-DDM, and *p,p′*- and *o,p′*-DDE). Between C2 and C3, *o,p′*- and *p,p′*-DDE had an increased log K_{POM} by 0.38 and 0.39 log units respectively. Between C2 and C4, there was a slightly lower mean log K_{POM} for *p,p′*-DDM (−0.24 log unit), but a slightly higher value for *p,p′*-DDE (+0.25 log unit). Regressions of log C_{POM} *v.* log C_W for the compounds with valid data (i.e. data that met our stated quality criteria) from three C levels (Supplementary Fig. S4) gave linear isotherms,

with r^2 values of 0.99, 0.97 and 0.96, for *p,p′*-DDM, *p,p′*-DDE and *o,p′*-DDE respectively. Hence, we concluded that K_{POM} is not dependant on the analyte concentrations within our investigated interval. Since no general trend with concentration levels was observed, data from all concentration levels were used to calculate the final K_{POM} values.

Final K_{POM} values

The final log K_{POM} values for the 10 DDX compounds studied are listed in Table 3. Our measurements of log K_{POM} were highly repeatable (relative standard deviation, r.s.d., was 0.9–3.1%) except for *p,p′*-DDMU (r.s.d. = 16%), for which the final mean log K_{POM} was based on only three valid measurements. The final K_{POM} values for *p,p′*-DDT and *p,p′*-DDE are in the same range as values previously reported by Endo *et al.* (2011), with a deviation of −0.3 and +0.3 log units (i.e. a factor 0.5 and 2) respectively, but with better or equal r.s.d. (Table 3).

The relationship between the final log K_{POM} and the log K_{OW} of the DDX (DDMU excluded) conformed to a linear regression (log K_{POM} = 0.44 × log K_{OW} + 2.6) with an explanatory power of r^2 = 0.81 (Supplementary Fig. S5). This regression has a lower slope (and different intercept) than the single-parameter linear free energy relationship (SP-LFER) model derived by Endo *et al.* (2011), which was based on log K_{POM} values for 43 compounds (not including any organochlorine pesticides (OCPs). Endo *et al.* also derived K_{POM} for 13 OCPs, including *p,p′*-DDT and *p,p′*-DDE, and noted that these deviated from the SP-LFER model; their experimentally determined K_{POM} values for *p,p′*-DDT and *p,p′*-DDE were lower (Table 3). However, their determined K_{POM} values matched well with our final values, and our regression between log K_{POM} and log K_{OW} displayed a good linearity. The K_{OW} values used here are from the SPARC model (ARCHem, see http://www.archemcalc.com/HTML/index.html). Few reliable experimental log K_{OW} data exist for DDX (Pontolillo and Eganhouse 2001). The choice of using SPARC is based on the results in Eganhouse *et al.* (2018), who evaluated five of the most widely used and currently available computational methods and concluded that SPARC provided results that best matched the few available controlled experimental DDX data. However, we note that values derived from SPARC in 2024 gave higher values for *o,p′*-DDT (+0.8 log unit) and *p,p*-DDT (+0.9 log unit) compared to those reported by Eganhouse *et al.* (2018).

As experimental data are generally considered more reliable than predicted data, we recommend using the experimental final log K_{POM} values rather than the ones derived from log K_{POM} − log K_{OW} regressions. The K_{POM} for DDMU should, though, be used with caution as the value is based on only n = 3 and the r.s.d. is high.

Other equilibrium passive sampler materials have been used for the determination of $C_{W,free}$ of DDX, such as low-density polyethylene (PE) (Hale *et al.* 2009; Borrelli *et al.* 2018) and poly(dimethylsiloxane) (PDMS) (Maruya *et al.* 2009; Xing *et al.* 2009; Eganhouse 2016). The precision we obtained (s.d. < 0.18 and s.e. < 0.05 log units, excluding DDMU), was of equal quality to data compiled in the study by Eganhouse (2016), where s.d. was generally <0.2 for log $K_{PDMS/water}$ derived from PDMS between 7 and 100 µm thick. It also compared well to data from Hale *et al.* (2009) where s.e. <0.16 for log $K_{PE/water}$, using 51-µm-thick PE. The

Table 3. Comparison of log K_{POM} determined within this study with those derived by Endo *et al.* (2011).

	Average log K_{POM} this study ± s.d.	Average log K_{POM} reported by Endo *et al.* (2011)	log K_{POM} derived using the linear regression derived by Endo *et al.* (2011); log K_{POM} = 1.01 × log K_{OW} − 0.60 (±difference to our study)
p,p′-DBP	4.30 ± 0.038 (*n* = 13; r.s.d. = 0.88%)		3.78 (+0.51)
p,p′-DDM	5.04 ± 0.11 (*n* = 16; r.s.d. = 2.1%)		4.80 (+0.24)
Dicofol	5.47 ± 0.061 (*n* = 5; r.s.d. = 1.1%)		5.29 (+0.19)
o,p′-DDD	5.26 ± 0.048 (*n* = 13; r.s.d. = 0.92%)		5.44 (−0.18)
p,p′-DDD	5.18 ± 0.050 (*n* = 11; r.s.d. = 1.0%)		5.57 (−0.39)
p,p′-DDMU	4.43 ± 0.71 (*n* = 3; r.s.d. = 16%)		5.63 (-1.2)
o,p′-DDT	5.40 ± 0.065 (*n* = 14; r.s.d. = 1.2%)		6.15 (−0.75)
o,p′-DDE	5.63 ± 0.17 (*n* = 16; r.s.d. = 3.1%)		6.29 (−0.66)
p,p′-DDT	5.32 ± 0.078 (*n* = 8; r.s.d. = 1.5%)	5.66 ± 0.24 (*n* = 6; r.s.d. = 4.2%)	6.31 (−0.99)
p,p′-DDE	5.70 ± 0.18 (*n* = 16; r.s.d. = 3.1%)	5.44 ± 0.17 (*n* = 6; r.s.d. = 3.1%)	6.36 (−0.65)

For the average log K_{POM} reported by Endo *et al.* (2011), the sorption isotherms for the average log K_{POM} used a batch method where POM (76 µm thick) was mixed with water fortified with organochlorine pesticides (end-over-end at 10 rpm) for 37 days at a concentration range covering three orders of magnitude. Data derived for log K_{POM} derived using the linear regression derived by Endo *et al.* (2011) used the single-parameter linear free energy relationship (SP-LFER) model, i.e. log K_{POM} = 1.01 × log K_{OW} - 0.60, and K_{OW} values obtained with the SPARC model (Supplementary Table S1).

higher s.d. of our DDMU data, along with the much lower K_{POM} value than $K_{polymer/water}$ values reported by both Eganhouse (2016) and Hale *et al.* (2009) for this compound, indicates a potential underestimation. A comparison of our log K_{POM} data against log K_{PDMS} and log K_{PE} data from these studies is shown in Supplementary Fig. S6. In addition, all three materials are also regarded equivalent in terms of the affinity for hydrophobic non-polar chemicals (Endo *et al.* 2011). However, POM offers benefits when aiming to extract polar compounds from environmental phases since POM has H-bond acceptor sites in its molecular structure in contrast to PDMS and PE, and thus strong H-bond donor compounds generally have higher $K_{POM/water}$ than $K_{PDMS/water}$ and $K_{PE/water}$ (Endo *et al.* 2011). Hence, POM is expected to be a more sensitive sorbent than PDMS and PE for, e.g. dicofol, which possesses an OH group, and thus has H-bond donor properties.

Part 2 – application of the POM method on soil samples from DDX-contaminated sites – interlaboratory comparison

As no monitoring of potential degradation of DDT to DDD in the GC injector was done in this part of the study, and as comparative Lab A and Lab B data suggested that degradation occurred (Supplementary Tables S20 and S22), sum levels of DDT and DDD (ΣDDT/D) were used for both labs. This approach is also motivated by findings in Foreman and Gates (1997), which showed that even with monitoring through a Performance Evaluation Standard (PES) containing DDT but not DDD or DDE (US EPA methods, see references 14–17 in Foreman and Gates 1997), the degree of degradation and formation is uncertain and particularly DDT and DDD cannot be quantified individually, i.e. only as a sum, as has been applied in e.g. Wiberg *et al.* (2002). In the case of dicofol and DBP, comparative Lab A and Lab B data did not show consistent trends and the compounds were therefore not summed.

DDX concentrations in soil samples (C_{soil})

Soil concentrations of Σ*p,p′*-DDT/D, Σ*o,p′*-DDT/D, *p,p′*-DDE, *o,p′*-DDE, dicofol and *p,p′*-DBP are shown in Fig. 2 (C_{soil} for all individual compounds can be found in Supplementary Tables S17–S18). The C_{soil} values of *p,p′*-DDMU and *p,p′*-DDM were below the LOQ for all soils in Lab B's analyses, and just above the LOQ in Lab A's analyses. These two compounds were thus excluded from further evaluation. The mean C_{soil} (μg g^{-1} DW) of ΣDDX of Lab A and B ranged from ~5 to ~30 μg g^{-1} DW for eight of the nine studied sites, whereas one site (Åby) showed significantly higher concentrations (110 μg g^{-1} DW). The most dominant DDX in all soil samples was Σ*p,p′*-DDT/D, which accounted for 60–76% of the total concentration, followed by Σ*o,p′*-DDT/D (10–23%), *p,p′*-DDE (2.1–18%), dicofol (0.77–8.0%), *p,p′*-DBP (0.30–3.7%) and *o,p′*-DDE (0.0006–1.9%) (Supplementary Table S19).

Both labs quantified similar C_{soil} for Σ*p,p′*-DDT/D, Σ*o,p′*-DDT/D, *p,p′*-DDE and *p,p′*-DBP, with concentration ratios of the labs ($C_{soil,LabA}/C_{soil,LabB}$) between 0.5 and 2 (Supplementary Table S20). Higher ratios, up to 4.8, were found for dicofol, but with a mean of 2.3. For this compound, Lab A found mostly higher levels (9 out of 10 sites), whereas for *p,p′*-DBP, Lab B found higher levels in 4 of 10 sites. This implies that a higher level of degradation in the

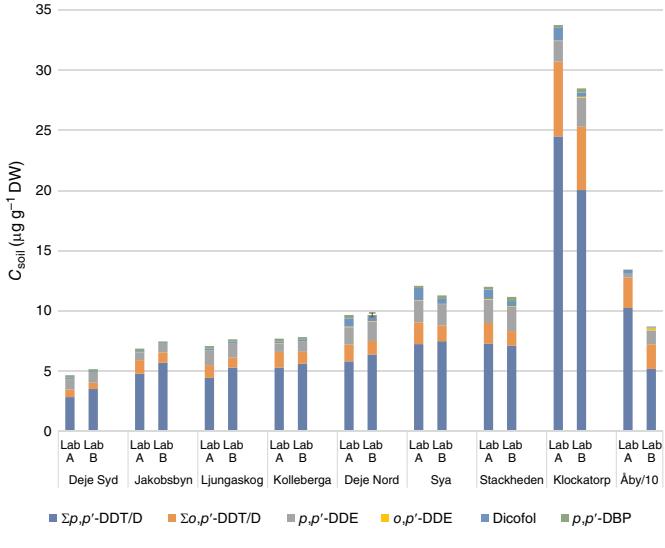

Fig. 2. Concentrations (μg g^{-1} DW) of DDX in soil samples from nine forest plant nurseries analysed by Lab A and Lab B. Åby concentrations as shown are divided by 10. Deje Nord in Lab B was analysed in triplicate (s.d. = 0.34 μg g^{-1} DW).

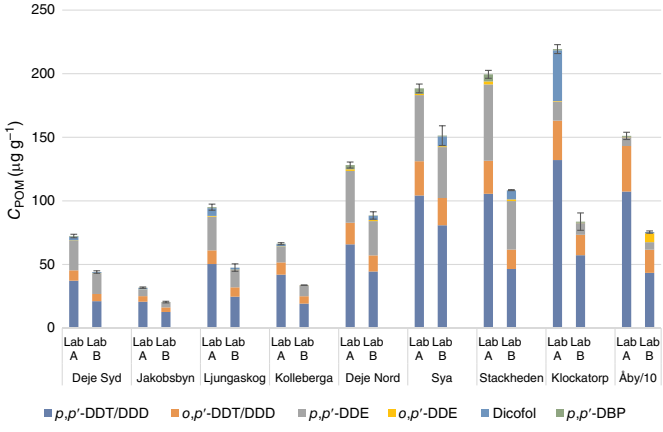

Fig. 3. C_{POM} values and s.d. (n = 3 except for Ljungaskog Lab B, n = 2) for nine different soils analysed by Lab A and Lab B. Åby values as shown are divided by 10 for easier visualisation.

GC injector of dicofol to *p,p′*-DBP occurred in Lab B. However, Lab B seemed to have had less conversion of DDT into DDD compared to Lab A. Differences in extraction procedure, clean up method, reference compound solutions and instrumental analysis can also be reasons for the different C_{soil}, which are expanded upon in Materials and methods, Part 2. For *o,p′*-DDE, one outlier was identified (negligible levels detected at Lab B) and excluded from the comparison. Overall, results showed relatively good agreement with mean ratios (Supplementary Table S20) ranging from 0.8 to 2.3, and the precision of the analysis (Lab B triplicate) was high, with r.s.d. ranging from 2.2 to 11%, with a mean of 6.4%.

DDX concentrations in POM (C_{POM})

The levels of DDX in the POM strips (C_{POM}) are shown in Fig. 3, all C_{POM} data, means and standard deviations are in Supplementary Table S21, and ratios between C_{POM} analysed by Lab A and Lab B are in Supplementary Table S22. Lab A consistently quantified equal or higher C_{POM} than Lab B for all compounds. For the POM analyses, both labs experienced major challenges with very high MS signals. This required dilution of the extracts, further addition of IS and reanalysis, often more than once. This procedure is not optimal for good accuracy and is likely to have caused some of the observed differences between the labs. The C_{POM} mean ratios between labs were below 2, except for dicofol and *p,p′*-DBP with C_{POM} mean ratios of 35 and 10 respectively. Although the degradation of dicofol to DBP in the injector was deemed to be at an acceptably low level for soil samples, the ratios for POM suggest that this could have been an issue in these analyses. This highlights the importance of monitoring the degradation or adjusting the injection method to avoid such degradation, e.g. as suggested by Yin *et al.* (2017). Another potential explanation for the differences could be that ^{13}C-labelled internal standards were not available for these two compounds; a deuterated internal standard was used for dicofol, and DBP was quantified using ^{13}C-*o,p′*-DDE. In spite of analytical challenges, the precision of the analyses was good for both labs, with a mean r.s.d. of 5.1 and 11% respectively for Lab A and B (Supplementary Table S21).

Focused interlab comparison study of C_{POM}

The strips shaken in soil from Klockatorp and Ljungaskog (by both labs) were cut in half and sent to the other lab for DDX analysis (n = 3 for each soil except Klockatorp shaken by Lab B, n = 2). This way, there were no differences in homogenisation and subsampling of the soil, and the comparison was focused on the analytical procedure from POM extraction onwards. Concentrations, standard deviations and lab data ratios are shown in Fig. 4 and Supplementary Table S22. The results showed that analysing the same POM strip resulted in similar precision, with a mean r.s.d. of 4.9 and 11% for Lab A and B respectively (Supplementary Table S23) (5.1 and 11% in the full C_{POM} comparison). Lab ratios improved, however, some high ratios were still found. The C_{POM} results for both isomers of ΣDDT/D and DDE were not significantly different between labs (*t*-test: $P > 0.05$), with all ratios between labs ranging from 0.8 to 1.4 (as compared with 1.2–2.6). C_{POM} values for dicofol and *p,p′*-DBP, however, were significantly different for every strip for nearly every site, with Lab A consistently quantifying higher values than Lab B, i.e. the same trend as for C_{soil}. This could be attributed to the analytical procedure from POM extraction onwards. As the agreement between labs was good for most substances (ratios close to one), and only unacceptably high for the low level compounds, dicofol and DPB, this points to the instrumental analysis as the major cause for the differences, e.g. the need for several dilution

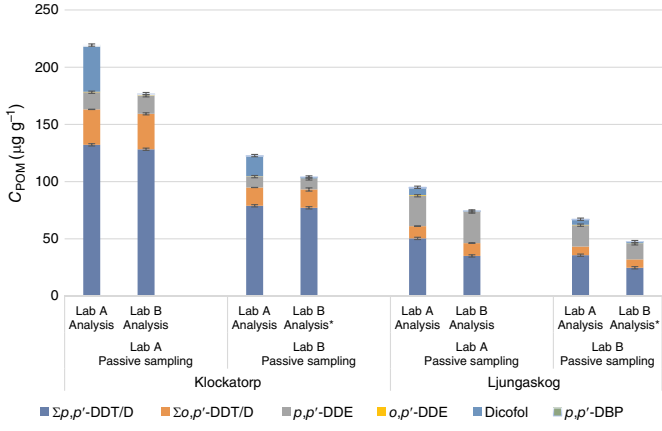

Fig. 4. Mean and standard deviation of DDX concentrations in the POM (C_{POM}) in the focused interlaboratory comparison study. Samples were analysed in triplicate, except where indicated with an asterisk (*), where n = 2.

steps and reanalysis, lack of some ^{13}C-labelled IS, and the potential degradation of dicofol to DPB in the GC injector. The somewhat higher precision of Lab A might partly be attributed to well established homogenisation and subsampling procedures.

Freely dissolved pore water concentrations ($C_{W,free}$)

Based on the concentrations in the POM strips (C_{POM}), the $C_{W,free}$ values for the nine field sites were determined (Supplementary Table S24 and Fig. 5) using K_{POM} established in Part 1 of this study (Table 3). The compound pairs *p,p′*-DDT/*p,p′*-DDD and *o,p′*-DDT/*o,p′*-DDD have different K_{POM} values, with DDTs having higher values. In our conversions of C_{POM} into $C_{W,free}$, we opted for using individual levels of DDT and DDD and their respective K_{POM}. Consequently, the following $C_{W,free}$ results for DDT and DDD are approximations, with potential underestimation of DDT and overestimation of DDD. As we cannot be sure of the exact deviation from a true ΣDDT/D, the quantification results are to be seen as relatively valid with respect to each other, but not accurate. For every compound, Åby showed, as expected, significantly higher $C_{W,free}$ than the other soils, due to the much higher C_{soil}, which reflects the land use history of the sampling area as a DDT dipping station for saplings. This, however, also resulted in higher variation in the analysis due to multiple dilution of the sample being necessary for the value to be within the range of the calibration curve. Åby also generally showed bad lab ratios for C_{soil} and C_{POM}. Although there was some variation in reported concentrations, the $C_{W,free}$ data were significantly correlated between labs ($P < 0.05$, $r^2 > 0.96$ for log–log relationships) (Fig. 5), excluding *p,p′*-DBP (r^2 = 0.93) and dicofol (r^2 = 0.43) (Supplementary Fig. S5). Overall, the r.s.d. remained low for both labs (mean r.s.d. Lab A = 3.9%; mean r.s.d. Lab B = 10%).

Part 3 – verification of POM as a biomimetic method (Lab A)

POM as a prediction tool for bioaccumulation of DDX in earthworms

Normalising worm DDX concentrations (C_{worm}) to the individual fat content of the worms resulted in lipid concentrations (C_{worm_lipid}) ranging for ΣDDX-10 from 558 to 3840 µmol kg^{-1} lipid with Jakobsbyn at the low end and Sya at the top (Table 4). As mortality was 100% in the soil with the highest C_{soil} (Åby), no worms could be analysed for this soil. All C_{worm} and C_{worm_lipid} data are presented in Supplementary Tables S25 and S26.

log–log correlations between C_{worm_lipid} *v.* C_{POM}, C_{soil} and C_{TOC} (i.e. the soil concentration normalised to the fraction of TOC content; C_{soil}/f_{TOC}), were conducted for the ΣDDX-10 (Fig. 6), the individual DDX, Σ*p,p′*-DDT/D and Σ*o,p′*-DDT/D (Supplementary Table S27). The worst log–log correlations were observed for C_{worm_lipid} *v.* C_{soil} (r^2 of 0.60–0.93), and the best for C_{worm_lipid} *v.* C_{POM} (r^2 of 0.82–0.97) and C_{worm_lipid} *v.* C_{TOC} (r^2 from 0.89 to 0.97), excluding results for *p,p′*-DBP, the compound with the lowest K_{OW} value, which had r^2 of 0.84, 0.24 and 0.00012 for C_{POM}, C_{TOC} and C_{soil} respectively.

In addition to the strong linear log–log correlations between C_{worm_lipid} and C_{POM}, the slopes of these relationships were also close to one (between 0.80 and 1.05) for all DDX, except for dicofol and *o,p′*-DDD, which had slightly lower slopes of 0.66 and 0.77 respectively (Fig. 6 and Supplementary Table S27). The fact that the linear regression slopes were near one and exhibited better r^2 values than log–log correlations of C_{worm_lipid} *v.* C_{soil} demonstrates that the POM method has the capacity to accurately predict the bioavailability of DDX in soil and is superior for predicting bioaccumulation compared to analysing total DDX content in the soil.

The strong correlations and feasibility of using POM as a prediction tool for bioaccumulation align with other studies

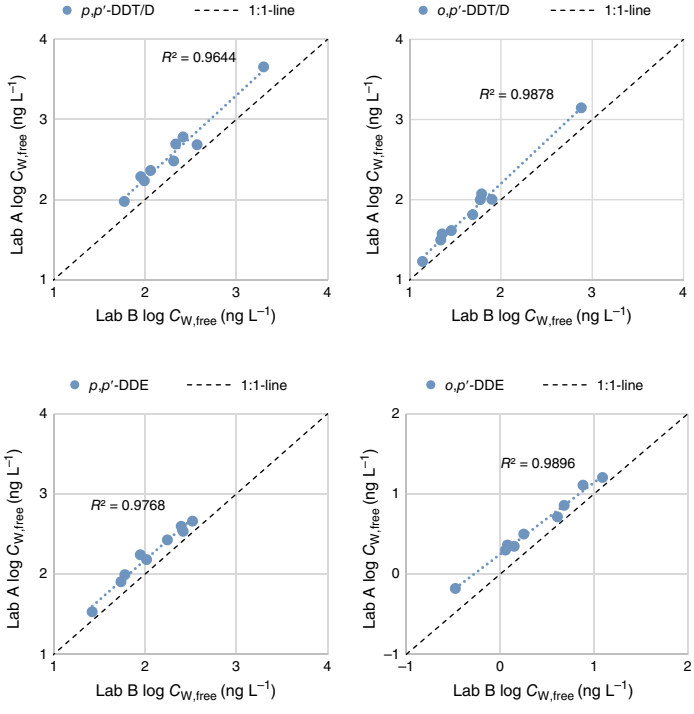

Fig. 5. Comparison of freely dissolved pore water concentrations (log values) of DDX compounds from nine sampling sites analysed by two labs.

Table 4. Ecotoxicity results; mortality, growth (adult biomass) and reproduction, together with Σ10-DDX C_{soil} and C_{worm_lipid}.

Σ10-DDX	C_{soil} (mg kg^{-1} soil DW)	C_{worm_lipid} (µmol kg^{-1} lipid)	Mortality after 28 days (%)	Adult biomass after 28 days (%)	Reproduction 56 days Number of juveniles hatched
ISO control	0	0	0	112	81
Deje Syd	4.7	760	0	130	146
Jakobsbyn	6.9	560	0	133	49
Ljungaskog	7.1	1 030	0	118	93
Kolleberga	7.7	79	0	123	32
Deje Nord	9.7	907	75	118	15
Sya	12	3 800	33	118	47
Stakheden	12	2 200	0	135	83
Klockatorp	34	2 900	0	120	86
Åby	130	NA	100	NA	3
Pearsons correlation for C_{soil} *v.* C_{worm_lipid} & ecotoxicity results		0.60	0.72	-0.28	-0.48
Pearsons correlation for C_{worm_lipid} *v.* ecotoxicity results			0.09	0.08	-0.03

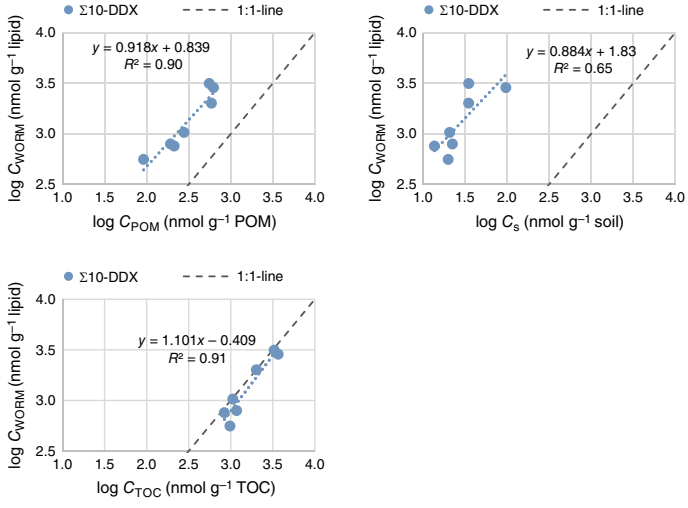

Fig. 6. Correlations of log C_{worm_lipid} with various chemical measurements, including log C_{POM} (top left), log C_{soil} (top right) and log C_{TOC} (bottom left).

on POM and HOC bioaccumulation in earthworms. Arp *et al.* (2014) reported a log–log correlation (r^2 = 0.94) for PAH bioaccumulation by *Enchytraeus crypticus* using 76 µm POM membranes, as in this study. Similarly, Wang *et al.* (2018) found strong correlations for DDX bioaccumulation by *Eisenia fetida* using PE (r^2 = 0.93) and solid-phase microextraction (SPME) (r^2 = 0.91). By contrast, poorer r^2 values were observed for bioaccessibility methods such as Tenax (r^2 = 0.79), isotope dilution (r^2 = 0.77) and supercritical fluid extraction (r^2 = 0.47). The only study, to our knowledge, that has investigated the correlation between POM and earthworms is the study by Denyes *et al.* (2016), who also used 76 µm POM to predict *E. fetida* bioaccumulation of *p,p′*-DDT and *p,p′*-DDE. They did not report any r^2 values but concluded that their mean worm bioaccumulation factors (BAFs) were within 50% of the POM-derived BAFs.

It can be argued, based on the good correlation between C_{worm_lipid} and C_{TOC} (Fig. 6), that this analysis can be used instead of POM analyses. One possibility, however, is that the good correlation can be attributed to the fact that all soils included in the study originated from plant nurseries situated in rural–agrarian regions with comparable NOM and similar contaminant age. Inclusion of soils from more urban environments would likely have resulted in greater variation and a poorer correlation, as anthropogenic carbon, such as soot, has been demonstrated to have a significantly greater sorption capacity for HOC than NOM (Cornelissen *et al.* 2005). The presence of types of black carbon, such as soot and charcoal, may therefore reduce the predictive power of C_{TOC} and lead to overestimation of bioavailability. It is therefore recommended that POM analysis and the here derived C_{worm_lipid} *v.* C_{POM} correlations are used in preference to C_{soil} and TOC determinations to predict the bioaccumulation of DDX in worms.

POM as a prediction tool for ecotoxicity

As illustrated in Fig. 6, C_{POM} can serve as a prediction tool for C_{worm_lipid}. Consequently, passive sampling with POM may also be employed to predict ecotoxicity, provided that a correlation exists between the organism's bioaccumulation of DDX and the toxic response. However, the endpoints investigated here (growth, reproduction and mortality) were not sufficiently sensitive for the concentration range investigated. No significant correlation was found for mortality or reproduction against C_{worm_lipid} or C_{POM}. It should be noted, though, that the sample with the highest expected C_{worm_lipid} (Åby; with C_{soil} of 134 mg DDX kg^{-1} DW), was not analysed, due to 100% mortality of worms, and that the C_{soil} of Σ*p,p′*-DDX showed a moderate and significant (P < 0.05) linear correlation with mortality percentage at 28 days (Pearsons correlation: 0.72, r^2 = 0.52).

DDT is an insecticide, and low levels of DDT in soil are expected to have toxic effects on terrestrial organisms, for example, an acute 50% lethal concentration (LC_{50}) for the cricket *Gryllus pennsylvanicus* has been reported to be 10 mg DDT kg^{-1} DW in soil with 10% NOM. There are, however, few data for terrestrial organisms available in ecological databases. Available data for *Eisenia fetida* in the scientific literature suggest that this species can tolerate high DDX concentrations, which is in line with our results (Table 4); the half maximal effective concentration (EC_{50}) for reproduction has been reported to be 588 mg DDT kg^{-1} DW, in a sandy soil with 1% TOC (Hund-Rinke and Simon 2005) and Shi *et al.* (2016) reported an acute LC_{50} of 274 mg DDT kg^{-1} DW (14 days exposure) and a chronic LC_{50} of 146 mg kg^{-1} for historical DDT-contaminated soils. In addition, Shi *et al.* (2016) showed that DDT in concentrations lower than 100 mg kg^{-1}, in both historical contaminated soils and

DDT-spiked natural soils, had minimal effects on the mortality of mature earthworms (*Eisenia fetida*), whereas few survivors were found in spiked artificial soils at DDT soil concentrations >200 mg kg^{-1}. Further investigations with more sensitive species or endpoints are thus required to determine whether C_{POM} can be used to predict toxicity.

POM as a tool in risk assessment and feasibility studies of remediation

POM can also be a tool to investigate the feasibility of remediation options and to check the achievement of objectives after remedial actions have been taken, especially in the case of stabilisation measures, which involve the sorption of contaminants onto a sorbent, e.g. biochar (Denyes *et al.* 2016). As the amount of contaminant is not removed from the soil, but stabilised, it is not possible to measure the effectiveness of remediation by C_{soil} analysis, and methods are needed to demonstrate how well the sorbent is working and whether the solubility, bioavailability and dispersion of the contaminants have been reduced to acceptable levels (Wang *et al.* 2018). Complementary measurements of $C_{W,free}$ and TOC enable calculations of soil–porewater distribution coefficients (K_d) and TOC–normalised soil–water distribution coefficients (K_{TOC}), see Supplementary Eqn S3, which are useful for this purpose, i.e. to assess how the sorption of DDX can improve after stabilisation measures, but also for risk assessment purposes, e.g. in modelling transport between different environmental compartments.

The K_{TOC} values of this study can be found in Supplementary Table S28, and ratios of log K_{TOC} derived by Lab A and Lab B are presented in Supplementary Table S28. The ratios were close to one in experiments where depletion was <10%, with the exception of the thermolabile compounds (mainly dicofol and its degradation product *p,p′*-DBP). The thermoinstability and potential matrix effect (Foreman and Gates 1997) may result in overestimations and underestimations of log K_{TOC}, which is reflected in the absence of good linear correlations between log K_{TOC} and log K_{OW} in our study (examples given in Supplementary Fig. S7).

In conclusion, this study shows that POM is a reliable tool for measuring $C_{W,free}$, and can be used for further calculations of bioaccumulation, using the here derived correlations for earthworms. The importance of controlling for degradation during GC analysis to accurately determine individual DDX analytes is highlighted, which is important when deriving site and compound-specific distribution coefficients needed for dispersion modelling.

Conclusions

In this study, K_{POM} values were determined for 10 environmentally relevant DDX compounds. The novel K_{POM} data presented here will allow for assessing freely dissolved pore water concentrations in soils and sediments, as well as other environmental media using POM as a passive sampler, which will facilitate investigations and risk assessments on the bioavailability and ecotoxicity of DDX. As the POM method is similar for other organic contaminants (e.g. PAH), the same POM sampling of contaminated soil or sediment can be used for measuring a multitude of compounds simultaneously.

Our experiments show that POM, as a quantitative method for freely dissolved DDX, seems to be a robust and reliable method with generally high precision (low r.s.d.) and linear isotherms. By contrast, the interlaboratory study showed that the analysis of DDX in the POM strip can be challenging, as several of the DDX compounds are thermolabile and can be converted or degraded during GC analysis, which can lead to both under- and overestimations of the levels. We also found that another major challenge is when DDX compounds occur at high or highly different levels, which may require pre-screening and adjustment of the analytical method so as to avoid dilution and reanalysis of sample extracts.

Soil-to-POM ratios of 100–200 have previously been proven to be enough for meeting the suggested criterion of <5% depletion when analysing HOC in historical urban contaminated soils and sediments. In this study we used a soil-to-POM ratio of 110:1 (on DW soil basis), which led to ≤36% depletion (excluding dicofol). To meet the <5% criterion in future tests of agricultural soils, where the sorbent domain consists of NOM and little or no anthropogenic carbon is present, we recommend that the soil-to-POM ratio is increased to 800:1. Owing to the strong sorption of POM for polar compounds, even higher ratios may be needed to bring down the depletion of dicofol to <5%.

Supplementary material

Supplementary material is available online.

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Data availability. The data used to generate the results in this paper are available in the Supplementary material and at the Swedish National Data Service's (SND) research data catalogue (Researchdata.se; doi:10.5878/efnj-xv18).

Conflicts of interest. The authors declare that they have no conflicts of interest.

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Environmental Chemistry

Supplementary Material

Determination of polyoxymethylene (POM) water partition coefficients for DDT and its degradation products, with inter- laboratory comparison of the passive sampling methodology and bioaccumulation in earthworm (*Eisenia fetida*)

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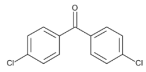
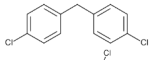
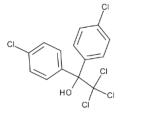
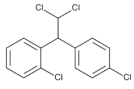
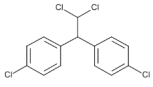
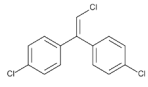
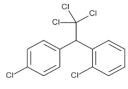
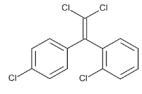
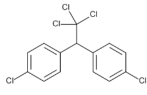
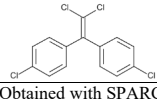
S1 Materials and Methods

S1.1 Molecular properties of target compounds

Table S1 Molecular structure, CAS-number, molecular weight (MW), logarithm of octanol-water partitioning coefficient (K_{OW}) and water solubility of the target DDX compounds of the study.

	Name (IUPAC)	ID	CAS	MW	log K_{OW}	Water solubility (mg/L at 25°C)
	bis(4-chlorophenyl)methanone	*p,p'*-DBP	90-98-2	251.11	4.34[A]	3.8 [B]
	1-chloro-4-[(4-chlorophenyl)methyl]benzene	*p,p'*-DDM	101-76-8	237.12	5.34[A]	
	2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol	dicofol	115-32-2	370.49	5.83[A]	
	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene	*o,p'*-DDD	53-19-0	320.04	5.98[A]	0.1 [C]
	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene	*p,p'*-DDD	72-54-8	320.04	6.11[A]	0.05 [C]
	1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene	*p,p'*-DDMU	1022-22-6	283.58	6.16[A]	
	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene	*o,p'*-DDT	789-02-6	354.49	6.68[A]	0.085 [C]
	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene	*o,p'*-DDE	3424-82-6	318.03	6.82[A]	0.14 [C]
	1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene	*p,p'*-DDT	50-29-3	354.49	6.84[A]	0.025 [C]
	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene	*p,p'*-DDE	72-55-9	318.03	6.89[A]	0.12 [C]

[A]Obtained with SPARC 2024-02-28 log K_{OW} (25°C, ionic strength 0), (ARCHem SPARC 2010).
[B]Estimated by EPIWEB 4.1; reported in (Huang et al. 2018)
[C]From the report "Toxicological Profile for DDT, DDE, DDD" by Agency for Toxic Substances and Disease Registry (2022)

S1.2 Determination of K_{POM} (Part 1)

S1.2.1 Chemicals and standards

Lab A.

Fortification standards (400 mg/L in methanol) of native DDX compounds were prepared from powders (Dr Ehrenstrofer from LGC Labor GmbH, Augsburg, Germany), then mixed and serially diluted with methanol to obtain a set of four mixtures (C_{f1}-C_{f4}) with different strengths (Table S2).

Table S2. Compound concentrations in each of the four fortification mixtures.

Compound	C_{f1}	C_{f2}	C_{f3}	C_{f4}
	(mg/L in methanol)			
o,p′-DDT	0.50	1.1	1.8	18.0
p,p′-DDT	0.60	1.2	1.8	18.0
o,p′-DDD	0.30	1.0	1.8	18.0
p,p′-DDD	0.30	1.0	1.8	18.0
o,p′-DDE	0.30	1.0	1.8	17.9
p,p′-DDE	0.30	0.90	1.8	18.0
p,p′-DDMU	0.090	5.2	1.8	18.0
p,p′–DDM	0.050	3.2	1.8	18.1
p,p′-DBP	0.0060	1.8	2.0	20.0
Dicofol	0.030	0.90	1.7	18.0

Anhydrous sodium sulfate (Na_2SO_4, 99% purity), *n*-hexane (≥98%), and toluene (≥ 99.7%) were purchased from VWR (Stockholm, Sweden). Silica gel 60 high-purity grade, and dichloromethane (DCM, 99.8%) were purchased from Sigma–Aldrich (Stockholm, Sweden) and acetone (≥99.8%) was purchased from Fisher Scientific (Gothenburg, Sweden). Ethanol (96%) was purchased from Solveco (Rosersberg, Sweden). Suppliers and purity of the native, internal and recovery standards are provided in Table S3.

The aqueous solutions were prepared from Milli-Q Ultrapure water (Merck Millipore IQ 7000), sodium azide (NaN_3, AnalR NORMAPUR) and salts of sodium dihydrogen phosphate (NaH_2PO_4, Merck EMSURE) and disodium hydrogen phosphate (Na_2HPO_4, Merck EMSURE) to yield 10 mmol/L of PO_4-buffer containing 0.1% NaN_3. HCl and NaOH were from Merck (Titrisol). All glassware and laboratory tools were pre-cleaned and heated (450°C, 3 h) or cleaned with ethanol, *n*-hexane and DCM prior to use.

Silica gel was cleaned and activated for 3 h at 450°C and then stored at 105°C prior to deactivation. The silica gel was cooled to room temperature in a desiccator and then deactivated by addition of 10% milli-Q water (w/w) for ~2–3 h using end-over-end shaking. The prepared deactivated silica gel was stored in a desiccator and considered valid for 1 week, if properly sealed.

Table S3 Target compounds, internal standards (IS), and recovery standard (RS) used in part 1 (Lab A) and part 2 (Lab A and B) of this study along with vendors, purities and MS ions.

Compounds	**Vendor**	**Chemical purity (%)**	**Quantifier ion (Lab A)/ Precursor ion (Lab B)**	**Qualifier ion (Lab A)/ Product ion (Lab B)**	**Internal standard (IS)**
Target compounds					
o,p′-DDT	Dr Ehrenstrofer	97.6	235	165	^{13}C-*o,p′*-DDT
p,p′-DDT	from LGC Labor	99.8	235	165	^{13}C-*p,p′*-DDT
o,p′-DDD	GmbH, Augsburg,	99.13	235	165	^{13}C-*o,p′*-DDD
p,p′-DDD	Germany	98.9	235	165	^{13}C-*p,p′*-DDD
o,p′-DDE		99.3	246	248 (176)	^{13}C-*o,p′*-DDE
p,p′-DDE		99.7	246	248 (176)	^{13}C-*p,p′*-DDE
p,p′-DDMU	(Sigma–Aldrich)	99.0	212	247	^{13}C-*o,p′*-DDE
p,p′-DDM		99.7	201	165	^{13}C-*o,p′*-DDE
p,p′-DBP	(Sigma–Aldrich)	98.2	139	111	^{13}C-*o,p′*-DDE
Dicofol		99.48	251	139	D_8-dicofol (^{13}C-dicofol)
Internal standards (IS)					
DDX solution					
^{13}C-*o,p′*-DDT	Cambridge Isotope Laboratories, Inc., Massachusetts, USA.	99.5	247	(177)	
^{13}C-*p,p′*-DDT		>98	249 (247)	177	
^{13}C-*o,p′*-DDD		96.2	247	(177)	
^{13}C-*p,p′*-DDD		98.2	247	177	

Compounds	Vendor	Chemical purity (%)	Quantifier ion (Lab A)/ Precursor ion (Lab B)	Qualifier ion (Lab A)/ Product ion (Lab B)	Internal standard (IS)
^{13}C-*o,p'*-DDE		99.6	258	188	
^{13}C-*p,p'*-DDE		>98	258	(188)	
D_8-dicofol (^{13}C-dicofol)	Dr Ehrenstrofer from LGC Labor GmbH, Augsburg, Germany	99.5	259 (263)	(145)	
Recovery standard (RS)					
^{13}C-PCB 81, (97), 101, (188)	Cambridge Isotope Laboratories, Inc., Guelph, ON, Canada.	NA	304, (340), 338, (406)	(268), (336)	

Vendors in parentheses indicate alternate vendor used by Lab B. Ion in parentheses indicates different ion used by Lab B, and IS and RS in parentheses indicate use at Lab B. NA, not applicable.

S1.2.2 POM-water partitioning experiments

The POM-water partitioning tests were performed in pre-baked, pre-rinsed (3 × ethanol, 3 × *n*-hexane and 3 × DCM) 100-mL amber glass bottles with PTFE-lined screw caps (Identipack, Someren, Netherlands). The POM strips were cleaned two times with acetone:hexane (1:1, v/v, 24 + 24h) and two times with methanol (24 + 24 h) and finally with Milli-Q water (24 h) before adding to the test.

For each test and replicate, a cleaned POM-strip (76 µm POM; CS Hyde, Lake Villa, IL, US) was weighed and placed in a 100-mL bottle and 100 g of 1 g/L NaN_3 10 mmol/L of PO_4-buffer solution (density assumed to be 1 g cm^{-3}) was added. The procedure follows the method in ISO-EN ISO 21268 but with PO_4-buffer solution instead of $CaCl_2$solution to enable test of solutions with different pH.

For tests using concentration level C1 and C2 (28-day-concentration dependent tests, time-dependent tests and pH-tests), 50 µL of the DDX fortification mixture was added to each 100-mL bottle. For tests with higher concentration levels (C3 and C4), a staggered fortification approach was used to avoid exceeding water solubilities. At the beginning of each week for a total period of 4 weeks, 125 µL of fortification mixture was added to the bottles, yielding a total fortification mixture addition of 500 µL. The start of the fifth week, from the initial DDX addition, was considered the start of the 28-day testing period for these two concentration levels. To ensure similar conditions between the different concentration levels with regards to solvent addition (methanol), 450 µL of methanol was added right after C1 and C2 addition, resulting in a total methanol addition of 500 µL, which was the same as for the higher concentration level additions. In relative terms, the fortification with DDX-compounds yielded an organic solvent content of 0.5% in the systems, which should not significantly influence compound solubility. Aimed concentrations in the aqueous solutions at the start of the test (before any sorption of DDX to POM) for the different testing schemes are shown in Table 1.

Extraction of water.

Water samples (100 mL) were extracted by liquid-liquid extraction using 30 mL of *n*-hexane (30 min and 2 h). IS were added (Table S3), flasks were covered with aluminium foil and shaken on an end-over-end tumbler. The extraction was repeated, and the extracts were then combined and evaporated to 2 mL by use of a rotary evaporator. Anhydrous Na_2SO_4 was added to each extract to remove any remaining water. Extracts were then filtered through glass wool in glass pipettes into 8 mL amber glass vials and concentrated to approximately 0.1 mL under a gentle stream of nitrogen. RS solution (Table S3) was added, and extracts were solvent exchanged into 100 µL in toluene by addition of 200 µL of toluene and further evaporation to 100 µL. Extracts were transferred to GC-vials with a 100-µL insert and then stored at -20°C until GC-MS analysis.

Extraction of POM strips.

POM strips were extracted in the 40-mL amber glass vials by 2 × 40 mL of acetone:*n*-hexane (1:1, v/v). IS solutions (Table S3) were added to the vials before the first extraction. The vials were placed in an ultra-sonication bath for 3 h, after which the first extract was collected and the second aliquot of solvent added, followed by another 3-h extraction. The two extracts were combined in glass flasks with glass stoppers and evaporated to ~0.5 mL by use of a rotary evaporator and transferred to 8-mL amber vials.

To minimise matrix effects from the POM strips in the GC-MS analysis of extracts with lower test concentrations, that is extracts of the two lowest test concentrations (C1 and C2) were cleaned-up using 10% deactivated mini-silica

columns. Small glass Pasteur pipettes were plugged with glass wool and packed with 4 cm of 10% deactivated silica and 1 cm of anhydrous Na_2SO_4 at the top. The columns were prewashed with 4 mL of *n*-hexane, before the extracts were transferred to the columns with glass Pasteur pipettes. The vials were rinsed three times with *n*-hexane:DCM (3:1, v/v), which were also transferred to the columns. Analytes were eluted with 4 mL of *n*-hexane:DCM (3:1, v/v) (included rinse volumes), followed by 4 mL of DCM into 8-mL amber glass vials. All extracts (C1 to C4) were then concentrated to ~100 µL under a gentle stream of nitrogen and 200 µL of toluene was added to each vial, followed by RS solution. The extracts were further evaporated to 100 µL of toluene and transferred to GC-vials with a 100-µL insert. The vials were stored at –20°C prior to GC-MS analysis.

GC-MS analysis.

Extracts were analysed by use of an Agilent 7890A gas chromatograph coupled to a 5975C low-resolution mass spectrometer (GC-LRMS). The injector temperature was maintained at 250°C and extract aliquots of 1–2 µL were injected in splitless mode (2 min). A splitless, single taper, deactivated liner (Agilent Technologies) was used. Separation of target compounds was achieved on a capillary column (30 m × 0.25 mm, 0.15-µm film thickness) (Select PAH; Agilent Technologies). Initial oven temperature was 100°C (1 min), 15°C/min to 250°C (2 min), 10°C/min to 280°C where it was held for 12 min. All measurements were performed in the selected ion monitoring mode. Identification and quantification of the target compounds were done by use of quantification standard solutions including all compounds in addition to IS and RS.

S1.2.3 Quality assurance/quality control (QA/QC)

Table S4 Amount DDX (pg) in blanks.

Amount (pg)	*p,p'*-DDM	*p,p'*-DBP	*p,p'*-DDMU	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT	Dicofol
PBA (Water)	ND	86	13	ND	ND	ND	ND	ND	ND	ND
PBB (Water)	ND	ND	1498	ND	ND	ND	ND	ND	ND	ND
PB (POM)	ND	21	40	ND	ND	ND	ND	ND	ND	ND
SB3d (Water)	ND	ND	702	ND	ND	ND	ND	ND	ND	ND
SB7d (Water)	ND	ND	371	ND	ND	ND	ND	ND	ND	ND
SB14d (Water)	60	ND	86	ND	ND	ND	ND	ND	ND	ND
SB3d (POM)	ND	ND	418	ND	ND	ND	ND	ND	ND	ND
SB7d (POM)	ND	ND	0	ND	ND	ND	ND	ND	ND	ND
SB14d (POM)	ND	ND	1423	ND	ND	ND	ND	ND	ND	ND

PB, process blanks; SB, sample blanks (3, 7 or 14 days of tumbling). ND, not detected

Table S5 Amount DDX in blank divided with amount found in the corresponding sample; ratios shown in percentage (%).

(%)	*p,p'*-DDM $m_{W\ blank}/m_{W\ sample} \times 100$; $m_{POM\ blank}/m_{POM\ sample} \times 100$	*p,p'*-DDMU
C1(28d;pH7)A	20; 0	179; 14
C1(28d;pH7)B	20; 0	16; 12
C1(28d;pH7)C	23; 0	298; 12
C2(3d;pH7)A	0; 0	132; 0
C2(3d;pH7)B	0; 0	10; 0
C2(3d;pH7)C	0; 0	26; 0
C2(7d;pH7)A	0; 0	5; 0
C2(7d;pH7)B	0; 0	28; 0
C2(7d;pH7)C	0; 0	12; 0
C2(14d;pH7)A	14; 0	29; 0.62
C2(14d;pH7)B	16; 0	13; 0.72
C2(14d;pH7)C	14; 0	9.8; 0.67
C2(28d;pH7)A	4.2; 0	103; 0
C2(28d;pH7)B	4.1; 0	133; 0
C2(28d;pH7)C	4.0; 0	17; 0
C2(56d;pH7)A	2.8; 0	11; 0
C2(56d;pH7)B	NA; 0	NA; 0
C2(56d;pH7)C	4.6; 0	183; 0
C2(28d;pH4.1)A	0.39; 0	19; 0
C2(28d;pH4.1)B	0.39; 0	19; 0
C2(28d;pH4.1)C	0.40; 0	18; 0
C2(28d;pH8.4)A	0.42; 0	2.6; 0
C2(28d;pH8.4)B	0.40; 0	18; 0
C2(28d;pH8.4)C	0.40; 0	0.15; 0
C3(28d;pH7)A	0.50; 0	7,5; 0
C3(28d;pH7)B	0.54; 0	28; 0
C3(28d;pH7)C	0.71; 0	41; 0
C4(28d;pH7)A	0; 0	2; 0
C4(28d;pH7)B	0; 0	6; 0
C4(28d;pH7)C	0; 0	7; 0

First position is the ratio for water samples; Second position is the ratio for POM-samples. For treatments conducted with tumbling times >14 days an average of amounts found in blanks at 3, 7 and 14 days was used for the ratio calculation. Results leading to ratios >10% (highlighted in red) were excluded from further evaluations. NA, not available due to values below limit of detection.

Table S6 Recovery of the different treatments, i.e. amount recovered in water and POM ÷ amount added × 100.

(%)	*p,p'*-DDM	*p,p'*-DBP	*p,p'*-DDMU	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT	Dicofol
					$(m_W + m_{POM}) \div m_{TOT\_added} \times 100$					
C1(28d;pH7)A	88%	449%	105%	93%	101%	99%	70%	110%	97%	91%
C1(28d;pH7)B	122%	808%	169%	97%	112%	105%	77%	107%	97%	52%
C1(28d;pH7)C	112%	583%	116%	96%	110%	107%	78%	110%	97%	95%
C2(3d;pH7)A	75%	109%	86%	90%	98%	83%	60%	87%	60%	104%
C2(3d;pH7)B	96%	118%	87%	86%	91%	72%	58%	72%	60%	89%
C2(3d;pH7)C	90%	134%	100%	94%	119%	84%	61%	83%	63%	106%
C2(7d;pH7)A	81%	100%	85%	91%	106%	66%	55%	66%	45%	99%
C2(7d;pH7)B	80%	88%	74%	82%	100%	75%	52%	69%	41%	99%
C2(7d;pH7)C	80%	101%	82%	86%	101%	66%	50%	76%	42%	103%
C2(14d;pH7)A	79%	104%	89%	90%	98%	84%	52%	72%	48%	99%
C2(14d;pH7)B	77%	93%	77%	83%	95%	82%	54%	76%	58%	85%
C2(14d;pH7)C	82%	100%	83%	85%	96%	83%	52%	75%	51%	79%
C2(28d;pH7)A	78%	111%	87%	88%	96%	74%	54%	68%	48%	71%
C2(28d;pH7)B	82%	111%	87%	90%	103%	77%	56%	70%	54%	90%
C2(28d;pH7)C	79%	108%	79%	85%	102%	75%	55%	70%	52%	84%
C2(56d;pH7)A	76%	110%	86%	94%	101%	71%	56%	66%	41%	NA
C2(56d;pH7)B	80%	107%	89%	93%	104%	75%	56%	62%	41%	86%
C2(56d;pH7)C	82%	105%	83%	85%	100%	72%	50%	69%	32%	85%
C2(28d;pH4.1)A	91%	123%	92%	86%	104%	89%	54%	54%	45%	63%
C2(28d;pH4.1)B	101%	121%	104%	101%	104%	83%	59%	60%	46%	64%
C2(28d;pH4.1)C	99%	120%	88%	100%	113%	92%	58%	57%	44%	68%
C2(28d;pH8.4)A	99%	137%	105%	108%	122%	80%	61%	57%	51%	29%
C2(28d;pH8.4)B	88%	114%	88%	97%	102%	73%	56%	57%	48%	32%
C2(28d;pH8.4)C	91%	130%	104%	108%	107%	82%	62%	61%	52%	6%
C3(28d;pH7)A	88%	106%	81%	78%	71%	31%	24%	30%	28%	59%
C3(28d;pH7)B	91%	96%	75%	77%	73%	35%	27%	29%	31%	54%
C3(28d;pH7)C	84%	98%	75%	83%	71%	30%	23%	29%	29%	53%
C4(28d;pH7)A	65%	47%	49%	77%	78%	70%	46%	51%	34%	62%
C4(28d;pH7)B	57%	47%	44%	65%	69%	68%	43%	47%	28%	59%
C4(28d;pH7)C	75%	46%	49%	68%	77%	64%	41%	48%	28%	66%

If the recovery was <50% or >130% (red values), the data were not used for the evaluation of K_{POM}.

S1.3 Interlaboratory comparison of the POM-method (Part 2)

S1.3.1 Soil sampling and analyses of soils

The soil samples were collected from nine Swedish sites, all previously used, or still in use, as plant nurseries (Table S7). The soil sampling was conducted in 50- × 50-m plots, with soil cores taken at each point in a 2- × 2-m grid system. Soil cores were taken to 20-cm depth and sieved at 2 mm, before homogenisation by mixing in a bucket. Subsamples were then taken randomly from the mix, then immediately stored in amber glass jars with PTFE lids, and stored at –20°C by end of the day.

The grain size distribution analysis was carried out by Lab B's Soil Physics Laboratory, using their particle size distribution method of dry sieving, wet sieving, and integral suspection pressure (Messing et al. 2024). Tot-C and OC was determined according to ISO 20236:2018. The pH was measured using 1g of soil dispensed in 5 mL of distilled water, according to ISO 10390:2021. Metals were analysed by ALS Scandinavia, by using ICP-SFMS according to SS-EN ISO 172942:2016, after digestion of the soil samples in heating blocks with either aqua regia according to SS-EN ISO 54321:2021 (for analysis of Sb and Mo) or 7M HNO_3 (all other compounds). The pesticides (other than DDX) were analysed by Eurofins according to method described in Rashid et al. (2010), except for analyses of permethrin, for which Eurofins in house method nbr 210 was used. The water holding capacity was determined following the protocol of ISO 11268-1:2012.

In addition to the basic soil characterisation described above (reported in Table 2), metals and other pesticides than DDX were also analysed (Table S8).

Table S7 Soil sampling sites: name, location, current land use/land management.

Location (name, county, country)	Current land use/land management
Deje Syd, plantskola, Värmland, Sweden	Unused grassland, regularly cut
Jakobsbyn plantskola, Västra Götaland, Sweden	Spruce tree nursery
Ljungaskog plantskola, Västra Götaland, Sweden	Horse grazing
Kolleberga plantskola, Skåne, Sweden	Unused grassland, regularly cut
Deje Nord plantskola, Värmland, Sweden	Unused grassland, regularly cut
Sya plantskola, Östergötland, Sweden	Agricultural land for growing grains
Stakheden plantskola, Dalarna, Sweden	Unused grassland, regularly cut
Klockatorp plantskola, Östergötland, Sweden	Clear cut forest land
Åby plantskola, Småland, Sweden	Unused land, trees and shrubs regrowth for at least 20 years

Table S8 Concentrations of inorganic contaminants and other pesticides than DDX (mg/kg DW).

Site	Deje Syd	Jakobs-byn	Ljunga-skog	Kolle-berga	Deje Nord	Sya	Stak-heden	Klocka-torp	Åby
As	1.36	0.792	1.52	0.810	1.12	2.88	1.03	0.862	0.895
Ba	61.8	14.2	13.2	8.90	65.5	20.7	30.4	12.2	22.1
Cd	<0.1	<0.1	0.10	<0.1	<0.1	<0.1	0.11	<0.1	0.11
Co	2.65	0.823	3.37	1.60	2.49	2.23	2.14	0.571	1.98
Cr	6.06	4.04	7.05	3.84	6.42	7.42	6.79	2.76	4.78
Cu	5.76	3.41	4.54	3.34	4.49	12.80	3.81	8.95	6.28
Hg	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Ni	4.13	1.27	4.25	2.59	3.80	5.13	2.79	1.04	2.83
Pb	10.6	6.63	7.74	8.38	10.90	11.60	9.52	11.90	8.70
V	19.2	7.29	18.9	25.0	20.4	12.8	25.3	7.74	13.1
Zn	39.4	12.0	27.9	19.5	31.6	30.4	31.5	9.47	35.3
Ag	<0.05	<0.05	<0.05	<0.05	<0.05	0.25	<0.05	<0.05	<0.05
Mo	0.330	0.313	0.484	0.204	0.349	0.921	0.517	0.288	0.327
Sb	0.119	0.082	0.144	0.110	0.650	0.165	0.276	0.119	0.114
Sn	1.560	0.889	0.934	0.546	1.71	1.70	2.15	1.32	1.01
DW (metal analysis) (%)	80.3	90.4	90.2	92.9	80.4	94.8	85.7	76.2	92.5
pentachloroaniline	<LOQ	<LOQ	<LOQ	0.035	<LOQ	0.020	<LOQ	<LOQ	0.058
pentachloroaniline/Quintozene	<LOQ	<LOQ	<LOQ	0.036	<LOQ	0.023	<LOQ	<LOQ	0.061
pentachlorobenzene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.012
β-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.012	<LOQ	<LOQ	<LOQ
γ-HCH (lindane)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.023	<LOQ
3,4-dichloroaniline	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.025
hexachlorobenzene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.019
permethrin	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	19
DW (permethrin analysis) (%)	80.5	89.3	90	93.4	80.9	93.4	84.9	76.9	93

DW, dry weight

Extraction and clean-up of soils.

Lab A. *Chemicals*: The same chemicals were used as in Part 1. *Method*: 1 g of wet soil was homogenised with 5 g of anhydrous Na_2SO_4 with a mortar and added to 34-mL stainless steel extraction cells. Each cell was filled with a cellulose filter in the bottom, 4 g of active silica, and the soil homogenate. ^{13}C-Labelled IS (Table S3) was added and cells filled to the top with anhydrous Na_2SO_4. Another cellulose filter was fitted prior to capping of the cell. A procedure blank containing anhydrous Na_2SO_4 and active silica was included in every batch of samples extracted. The extraction was performed using the ASE 350 Accelerated Solvent Extraction system (Thermo Scientific Dionex, Stockholm, Sweden) based on the method in Kim *et al*. (2019). The extraction was performed in one static cycle (5 min) with DCM:acetone (9:1). Extraction temperature was 100°C, rinse volume 60% and the purge time 100 s. The sample extract was evaporated to 5 mL (rotary evaporation), transferred to 8 mL glass vial, further evaporated under a gentle stream of nitrogen and solvent exchanged to toluene with a final volume of 0.4 mL. RS (Table S3) was added to GC vials after transferring of the extracts.

Lab B. *Chemicals*: Dichloromethane (DCM, suprasolv), *n*-hexane (suprasolv) and isooctane (suprasolv) were purchased from Merck (Darmstadt, Germany). Copper (ACS reagent, granular Cu, 10-40 mesh, ≥99.9%) and glass wool came from Sigma–Aldrich (Steinheim, Germany). Sodium sulfate anhydrous (Na_2SO_4, AnalaR NORMAPUR) were purchased from VWR International (Leuven, Belgium). Hydrochloric acid (HCl, 30%) came from Merck (Darmstadt, Germany). Silica (SiO_2, 0.063–0.200 mm) and Alumina oxide (Al_2O_3) were purchased from Merck (Darmstadt, Germany). *Method*: Freeze dried soil (0.5–1 g) was placed into a pre-cleaned Soxhlet thimble and ^{13}C-labelled IS (Table S3) was added. After Soxhlet extraction (24 h) using 200 mL of DCM (Huang et al. 2018), the extract was rotary evaporated to 5 mL and then transferred to a test tube. The sample was further evaporated under a gentle stream of nitrogen gas and the solvent exchanged to *n*-hexane, with a final volume of 1 mL. A procedural solvent blank was

included in every batch of samples analysed. After extraction, activated granular copper (1 g) was added to the sample and mixed vigorously to remove sulphur. The sample was then further cleaned up by alumina silica column (10-mm diameter) composed of Al_2O_3:SiO_2 (1:2 v/v, 6.1 g) and Na_2SO_4 (3 g) similar to Huang et al. (2018). Target compounds were eluted with 45 mL of DCM:*n*-hexane (2:3 v/v). The extract was then reduced to 5 mL (rotary evaporation), transferred to a test tube, further evaporated under a gentle stream of nitrogen and finally solvent exchanged to isooctane, with a final volume of 2 mL. RS (PCB 97 and 188; Table S3) was added and the sample was vortexed (5 min) before an aliquot was transferred to a GC vial for analysis.

S1.3.2 Determination of $C_{W,free}$ using the POM method

In brief, 76-µm POM strips (CS Hyde, Lake Villa, IL, US) were cut into 4- × 5-cm pieces (weights given in Table S9) and washed by subsequent end-over-end agitated 2 × 24-h 1:1 *n*-hexane/acetone extraction (v/v), 2 × 24-h methanol extraction and finally 1 × 24-h Milli-Q water washing in a 500-mL Pyrex bottle with PTFE-lined screw cap. Solvents were exchanged in each washing step after 24 h and the cleaned POM-strips were stored cold (+ 4°C) in the 500-mL Pyrex bottle in fresh Milli-Q water until use. The strips were then put in amber glass bottles with PTFE-lined screw caps together with 25 g (corresponding DW) of homogenised soil and then mixed with 93 mL of water containing 0.001 mol/L of $CaCl_2$ and 0.015 mol/L of NaN_3 (with 1-mL headspace remaining), i.e., as in part 1 but with $CaCl_2$ instead of PO_4-buffer to harmonise with the requirements of the leaching test standard series ISO-EN ISO 21268. Vials were tumbled end-over-end for 28 days in the dark. Thereafter, the POM was removed, rinsed with ultrapure water and wiped dry with a tissue before being placed in a clean scintillation vial and frozen (−20°C) until extraction.

Instrumental analysis.

Lab A analysed soils and POM extracts using GC-MS as described in part 1.

Lab B The analysis of soil and POM-extracts was done using GC-MS/MS with a GC (Agilent Technologies, 7890 A) coupled to a triple quadrupole mass spectrometer (Agilent Technologies, 7010, S/MS Triple Quad). The injector (held at 275ºC) was operated in splitless mode, a Siltek-deactivated goose neck liner (Restek Corporation, USA) and an injection volume of 2 µL was used. The analytes were separated on a DB-5 capillary column (60 m × 250 µm i.d. × 0.25 µm, Agilent Technologies). The temperature program for the column oven was: 100ºC (held for 1 min), 15ºC/min to 250ºC, then 10ºC/min to 280ºC (held for 12 min), then 20ºC/min to 300ºC (held for 3 min). Helium (He) was used as carrier gas at a constant flow (2 mL/min) throughout the run. The MS/MS was operated in electron ionisation (EI) mode at 70 eV. The ion source temperature was 300ºC and the transfer line temperature 310ºC. Nitrogen (N_2) was used as collision gas and He as quench gas in the collision cell. The analysis was carried out using multiple reaction monitoring (MRM) mode. Identification and quantification were performed using authentic reference standards. More details are given in Table S3. For data evaluation the software Agilent MassHunter Quantitative Analysis (for QQQ) was used.

Table S9 Weights (g) of POM-strips used in part 2 (application of the POM method to DDX contaminated sites)

Weights registered at Lab A (g)		Weights registered at Lab B (g)		Calculated weights for "II"-halves	
Deje SydA:a	0.22380	Deje SydB:a	0.22923		
Deje SydA:b	0.21386	Deje SydB:b	0.21361		
Deje SydA:c	0.21625	Deje SydB:c	0.24384		
JakbobsbynA:a	0.21607	JakobsbynB:a	0.22236		
JakbobsbynA:b	0.21901	JakobsbynB:b	0.22395		
JakbobsbynA:c	0.21692	JakobsbynB:c	0.23995		
LjungaskogA:a	0.21666	LjungaskogA:a-I	0.11184	LjungaskogA:a-II	0.10482[B]
LjungaskogA:b	0.21254	LjungaskogA:b-I	0.10978	LjungaskogA:b-II	0.10276[B]
LjungaskogA:c	0.21436	LjungaskogA:c-I	0.10189	LjungaskogA:c-II	0.11247[B]
		LjungaskogB:a-I	0.11390	LjungaskogB:a-II	0.117[C]
		LjungaskogB:b-I	0.12556	LjungaskogB:b-II	0.106[C]
		LjungaskogB:c-I	0.11535	LjungaskogB:c-II	0.116[C]
KollebergaA:a	0.21075	KollebergaB:a	0.23763		
KollebergaA:b	0.21430	KollebergaB:b	0.23940		
KollebergaA:c	0.21477	KollebergaB:c	0.24187		
Deje NordA:a	0.20966	Deje NordB:a	0.23155		
Deje NordA:b	0.21771	Deje NordB:b	0.23490		
Deje NordA:c	0.20690	Deje NordB:c	0.21052		
SyaA:a	0.20176	SyaB:a	0.21810		
SyaA:b	0.21866	SyaB:b	0.23342		
SyaA:c	0.21268	SyaB:c	0.23505		
StakhedenA:a	0.21493	StakhedenB:a	0.23995		
StakhedenA:b	0.21105	StakhedenB:b	0.23826		
StakhedenA:c	0.21559	StakhedenB:c	0.24135		

Weights registered at Lab A (g)		Weights registered at Lab B (g)		Calculated weights for "II"-halves	
KlockatorpA:a	0.21471	KlockatorpA:a-I	0.10656	KlockatorpA:a-II	0.10815[B]
KlockatorpA:b	0.21485	KlockatorpA:b-I	0.11388	KlockatorpA:b-II	0.10097[B]
KlockatorpA:c	0.21472	KlockatorpA:c-I	0.10823	KlockatorpA:c-II	0.10649[B]
		KlockatorpB:a-I	0.11275	KlockatorpB:a-II	0.119[C]
		KlockatorpB:b-I	0.12166	KlockatorpB:b-II	0.110[C]
		KlockatorpB:c-I	NA[A]	KlockatorpB:c-II	NA[A]
ÅbyA:a	0.21590	ÅbyB:a	0.23301		
ÅbyA:b	0.21739	ÅbyB:b	0.22226		
ÅbyA:c	0.20970	ÅbyB:c	0.22632		
Mean weight of full POM-strips	0.214		0.231		
s.d.	0.0042		0.010		
r.s.d.%	2%		4%		

The letter "A" or "B" after the name of the site indicate which Lab (Lab A or Lab B) that performed the tumbling of the POM-soil-water slurries. The letters "a", "b" and "c" indicates the n=3 replicates and the suffix "-I" or "-II" indicates the two halves of a POM.

[A]NA, not available due to missing data

[B]Difference between the weight of the full POM-strip and the weight of the "I"-half.

[C]Difference between the mean weight of full POM strips shaken by Lab B and the weight of the "I"-half.

S1.3.3 Control of depletion of DDX from the soil during the POM-test

The percentage depletion of DDX from the soils (Table S10) was calculated as the fraction extracted from the initial amount in the soils (Eqn S1):

$$\text{DDX (\%) extracted} = 100 \times (1 - [m_{soil,eq} \div m_{soil,init}]) \qquad \text{(S1)}$$

where $m_{soil,init}$ is the initial (analysed) amount of DDX in the soil and $m_{soil,eq}$ is the amount of DDX in soil at equilibrium (at the end of the test), which is derived from mass balance calculation (Eqn S2):

$$m_{soil,eq} = m_{soil,init} - m_{POM,eq} - m_{W,eq} \qquad \text{(S2)}$$

where $m_{POM,eq}$ and $m_{W,eq}$ is the amount of DDX found in the POM (analysed) and the water phase (calculated from the POM-concentration and the K_{POM}) at equilibrium (at the end of the test).

Table S10 Depletion of DDX (mean values of n=3) from the soils during the test (%).

Mean depletion (n=3)		DDT, *p,p'*-	DDT, *o,p'*-	DDE, *p,p'*-	DDE, *o,p'*-	DDD, *p,p'*-	DDD, *o,p'*-	Dicofol	DBP, *p,p'*-
Lab A	Deje Syd	13%	12%	23%	24%	6%	5%	19%	3%
	Jakbobsbyn	4%	4%	7%	8%	3%	2%	10%	1%
	Ljungaskog Lab A	10%	9%	19%	21%	6%	5%	31%	3%
	Kolleberga	7%	7%	14%	16%	4%	3%	12%	1%
	Deje Nord	10%	11%	24%	23%	5%	7%	85%	10%
	Sya	13%	13%	24%	27%	6%	9%	110%	21%
	Stakheden	13%	14%	27%	31%	5%	6%	129%	21%
	Klockatorp Lab A	5%	4%	7%	7%	3%	3%	32%	3%
	Åby	8%	14%	21%	27%	13%	3%	160%	16%
	Max.	13%	14%	27%	31%	13%	9%	160%	21%
Lab B	Deje Syd	5%	10%	16%	NA	11%	6%	15%	0%
	Jakbobsbyn	2%	4%	5%	NA	3%	2%	2%	0%
	Ljungaskog Lab B	8%	15%	12%	36%	6%	5%	13%	0%
	Kolleberga	3%	6%	9%	21%	5%	3%	1%	0%
	Deje Nord	6%	10%	15%	19%	10%	7%	11%	1%
	Sya	10%	15%	21%	27%	19%	15%	17%	2%
	Stakheden	6%	13%	18%	20%	8%	8%	16%	1%
	Klockatorp Lab B	3%	3%	4%	3%	3%	3%	3%	0%
	Åby	7%	9%	4%	1%	19%	6%	13%	0%
	Max.	10%	15%	21%	36%	19%	15%	17%	2%

S1.4 Verification of POM as a biomimetic method (Part 3)

S1.4.1 Earthworm mortality and reproduction tests

Ecotoxicity tests on *Eisenia fetida* following the protocol of ISO 11268-1:2012 (mortality) and ISO 11268-2:2023 (reproduction) were performed on sub-samples for the nine soils from the field sites, and one ISO control soil (in single samples) and the earth worm's accumulation of DDX was analysed. The ISO control soil was made, following the description in ISO 11268-1, from sand (SiO_2; 50-70 mesh particle size), kaolinite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$) both from Sigma–Aldrich, peat natural from Weibulls, Åby, Sweden and calcium carbonate (99%) from Fisher Scientific.

Glass containers were filled with 600 g of soil (dry mass). Each soil was wetted with milli-Q water to reach a water content corresponding to 40% of the maximum water holding capacity (WHC) of the soil. Adult worms of *Eisenia fetida* with a clitellum and a wet weight of 300–600 mg were selected for the test. The worms were rinsed with deionised water and gently dried with moist tissue before weighing and 12 worms were added to each glass container of soil. Containers were covered with parafilm with small holes to allow air exchange and stored at room temperature (20 ± 2°C) with controlled light/dark cycles (16 h light/8 h dark). Worms were fed with horse manure and the water content of the soil was checked once a week.

After 28 days of exposure, surviving worms were removed from the soils, counted, rinsed with deionised water, weighed and checked for malformations. The containers of soil were left for a further 28 days. The water content was kept to 40% of the WHC of the soil and 5 g of horse manure was added at the first week. The number of offspring was counted by placing the glass containers in a water bath at 40°C, which was then raised to 60°C.

S1.4.2 DDX accumulation in earthworms

The rinsed worms were placed in Petri dishes with moistened filter papers for 48 h to empty their digestive tracts. Petri dishes were covered with parafilm with small air holes, and the moistened filter papers were replaced with new filter papers twice. After 48 h, the worms were rinsed with deionised water, weighed and frozen in glass jars at -20°C prior to extraction.

Extraction of worm tissues was conducted based on the method by Henriksson et al. (2017) with minor modifications. Frozen worms were homogenised with anhydrous Na_2SO_4 at a ratio of seven times their wet weight, using a pestle and mortar and addition of liquid nitrogen during the homogenisation. Each homogenate was packed into glass columns and IS was added to the top. Lipids and analytes were extracted by using four times the homogenate column height of *n*-hexane and DCM (1:1, v/v). After evaporation of the *n*-hexane:DCM mixture, the extracts were weighed to determine the lipid content of the earthworms.

Before clean-up of the worm extracts, multilayer silica columns, including potassium hydroxide treated and sulfuric acid treated silica gel, were tested as clean-up method. Recoveries of spiked concentrations of native 4,4-DBP and dicofol were less than 5%. Therefore, clean-up using columns packed with 5 g of 10% deactivated silica were tested. Fat was successfully removed and recoveries for all 10 native DDX ranged between 85-110%. Deactivated silica was therefore used for clean-up in this study.

The fat was dissolved in 0.5 mL of *n*-hexane prior to clean-up using 10% deactivated silica gel. The silica gel was pre-washed with 50 mL of *n*-hexane before the extracts were transferred to the columns. Analytes were eluted with 5 mL of *n*-hexane, 15 mL L of 3:1 *n*-hexane:DCM, and 20 mL of DCM. The extracts were evaporated, and solvent exchanged to 0.4 mL of toluene in GC vials. ^{13}C-PCB 81 (12 ng) was added as RS to all vials. The extracts were stored in a freezer (-20° C) until GC-MS analysis. In addition to the nine worm samples, three procedure blanks containing only anhydrous Na_2SO_4 were included and followed all procedure steps. GC-MS analysis was carried out as described in part 1.

S2 Results and discussion

S2.1 Water Concentrations, C_W (Part 1)

Table S11. Water concentrations (C_W).

C_W (pg/L)	*p,p'*-DDM	*p,p'*-DBP	*p,p'*-DDMU	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT	Dicofol
C1(28d;pH7)A	995	2 540	2 160	472	430	639	378	659	481	<LOD
C1(28d;pH7)B	1 000	2 050	24 000	513	427	657	375	680	478	<LOD
C1(28d;pH7)C	860	2 100	1 300	863	385	629	385	766	487	<LOD
C2(3d;pH7)A	6 450	32 200	5 300	1 000	844	2 090	1 230	1 270	1 430	<LOD
C2(3d;pH7)B	6 580	32 000	67 000	1 100	949	1 970	1 240	1 450	1 410	<LOD
C2(3d;pH7)C	6 870	35 800	27 500	982	889	1 820	1 150	1 380	1 420	<LOD
C2(7d;pH7)A	4 710	25 100	71 600	875	750	1 650	852	1 431	989	<LOD
C2(7d;pH7)B	4 930	23 800	13 400	698	624	1 520	717	1 085	726	<LOD
C2(7d;pH7)C	4 740	24 000	30 900	696	702	1 470	669	1 270	863	<LOD
C2(14d;pH7)A	4 350	21 900	2 940	847	506	1 263	614	1 110	921	<LOD
C2(14d;pH7)B	3 800	22 200	6 410	841	618	1 308	584	1 109	919	<LOD
C2(14d;pH7)C	4 400	20 000	8 770	689	667	1 121	576	1 136	932	<LOD
C2(28d;pH7)A	4 730	21 600	3 770	597	513	1 033	614	938	741	<LOD
C2(28d;pH7)B	4 850	23 100	2 890	604	552	1 081	568	930	889	<LOD
C2(28d;pH7)C	4 950	22 100	22 140	447	556	1 095	517	801	827	<LOD
C2(56d;pH7)A	6 970	25 200	34 100	492	486	899	380	929	779	<LOD
C2(56d;pH7)B	-	-	-	-	-	-	-	-	-	-
C2(56d;pH7)C	4 300	20 900	2 100	471	407	808	454	859	622	<LOD
C2(28d;pH4.1)A	5 060	24 900	2 080	694	602	993	627	907	948	<LOD
C2(28d;pH4.1)B	5 060	22 100	2 010	680	472	1 086	556	886	812	<LOD
C2(28d;pH4.1)C	4 960	24 800	2 120	963	484	994	519	863	881	<LOD
C2(28d;pH8.4)A	4 680	22 900	14 900	1 010	496	908	605	918	660	<LOD
C2(28d;pH8.4)B	4 980	24 800	2 120	492	455	936	554	901	674	<LOD
C2(28d;pH8.4)C	4 910	23 400	263 100	688	482	915	704	956	613	<LOD
C3(28d;pH7)A	39 400	182 692	51 800	4 130	3 320	8 420	4 030	6 270	3 580	7 360
C3(28d;pH7)B	36 500	215 792	13 600	3 710	2 690	8 930	4 170	6 380	3 650	6 990
C3(28d;pH7)C	27 800	210 628	9 430	3 640	2 690	9 040	4 170	6 460	2 880	8 200
C4(28d;pH7)A	383 000	3 061 000	196 000	357 000	697 000	239 000	694 000	185 000	603 000	71 619
C4(28d;pH7)B	337 000	2 810 000	64 400	54 000	50 000	127 000	112 000	104 000	79 100	76 278
C4(28d;pH7)C	376 000	2 410 000	58 700	48 300	32 600	130 000	84 000	111 000	69 000	71 291

Red values are data that were excluded from further evaluation, due to blank/sample-ratio >10% (see Table S5 and S6). Blue values indicate outliers.

S2.2 POM Concentrations, C_{POM} (Part 1)

Table S12 POM concentrations, C_{POM}.

C_{POM} (µg/kg POM)	*p,p'*-DDM	*p,p'*-DBP	*p,p'*-DDMU	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT	Dicofol
C1(28d;pH7)A	9.78	5.07	19.1	67.7	65.3	69.3	79	76.9	115	5.35
C1(28d;pH7)B	14.2	10.6	22.7	73.1	75.0	76.1	90	77.0	119	3.19
C1(28d;pH7)C	13.2	7.41	22.6	72.8	73.9	78.5	92	80.2	120	5.81
C2(3d;pH7)A	540	433	995	209	191	185	153	195	165	205
C2(3d;pH7)B	689	468	867	200	177	162	150	162	166	176
C2(3d;pH7)C	634	522	867	216	226	184	152	183	172	205
C2(7d;pH7)A	555	376	907	200	194	139	135	140	118	186
C2(7d;pH7)B	574	349	852	190	192	167	133	153	112	195
C2(7d;pH7)C	553	389	909	193	189	142	124	164	113	198
C2(14d;pH7)A	559	409	1 020	206	187	186	132	160	131	192
C2(14d;pH7)B	536	360	862	187	180	178	134	164	155	164
C2(14d;pH7)C	558	377	902	188	176	175	125	159	133	147
C2(28d;pH7)A	553	438	995	202	184	164	138	151	131	139
C2(28d;pH7)B	570	428	972	201	192	167	139	152	144	172
C2(28d;pH7)C	549	421	885	193	193	163	137	153	140	161
C2(56d;pH7)A	555	445	998	222	199	163	146	151	115	-
C2(56d;pH7)B	592	447	1 050	222	207	173	147	144	117	175
C2(56d;pH7)C	560	399	917	190	186	154	123	147	84	161
C2(28d;pH4.1)A	632	479	1 040	194	196	194	136	118	122	121
C2(28d;pH4.1)B	685	455	1 140	222	189	174	144	127	121	120
C2(28d;pH4.1)C	703	475	1 010	230	216	204	147	125	121	134
C2(28d;pH8.4)A	685	529	1 166	242	227	172	151	123	137	54.7
C2(28d;pH8.4)B	630	452	1 014	226	197	162	144	128	133	63.1
C2(28d;pH8.4)C	641	512	1 072	248	204	181	158	134	143	12.4
C3(28d;pH7)A	3 350	4 500	3 080	2 970	2 760	1 180	915	1 150	1 050	2 180
C3(28d;pH7)B	3 570	4 200	2 960	3 030	2 900	1 400	1 079	1 160	1 210	2 080
C3(28d;pH7)C	3 270	4 200	2 920	3 250	2 780	1 160	915	1 150	1 130	2 010
C4(28d;pH7)A	26 300	20 000	20 100	31 100	31 600	28 400	18 600	20 600	13 400	25 400
C4(28d;pH7)B	21 700	19 000	16 800	24 900	26 500	26 100	16 600	17 900	10 600	22 600
C4(28d;pH7)C	29 400	19 000	19 300	26 700	30 200	24 900	16 100	18 600	11 100	25 900

Red values are data that were excluded from further evaluation, due to blank/sample-ratio >10% (see Table S5).

S2.3 Partitioning Coefficients, K_{POM} (Part 1)

S2.3.1 Calculated K_{POM}

Table S13 log POM-water partitioning coefficients, log K_{POM} for the studied DDX in the different tests (pH- time- and concentration tests).

	p,p'-DDM	*p,p'*-DBP	*p,p'*-DDMU	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT	Dicofol
C1(28d;pH7)A	3.99	**3.30**	3.95	5.16[A]	5.18[A]	5.04[A]	5.32	5.07[A]	5.38	-[B]
C1(28d;pH7)B	4.15	**3.71**	**2.98**	5.15[A]	5.24[A]	5.06[A]	5.38	5.05[A]	5.40	-[B]
C1(28d;pH7)C	4.19	**3.55**	4.24	4.93[A]	5.28[A]	5.10[A]	5.38	5.02[A]	5.39	-[B]
C2(3d;pH7)A	4.92	4.13	5.27	5.32	5.35	4.95	5.09	5.18	5.06	-[B]
C2(3d;pH7)B	5.02	4.17	4.11	5.27	5.27	4.91	5.08	5.05	5.07	-[B]
C2(3d;pH7)C	4.96	**4.16**	4.50	5.34	**5.41**	5.01	5.12	5.12	5.08	-[B]
C2(7d;pH7)A	5.07	4.17	4.10	5.36	5.41	4.93	5.20	4.99	**5.07**	-[B]
C2(7d;pH7)B	5.07	4.17	4.80	5.44	5.49	5.04	5.27	5.15	**5.19**	-[B]
C2(7d;pH7)C	5.07	4.21	4.47	5.44	5.43	4.99	5.27	5.11	**5.12**	-[B]
C2(14d;pH7)A	5.11	4.27	5.54	5.39	5.57	5.17	5.33	5.16	**5.15**	-[B]
C2(14d;pH7)B	5.15	4.21	5.13	5.35	5.46	5.13	5.36	5.17	5.23	-[B]
C2(14d;pH7)C	5.10	4.28	5.01	5.44	5.42	5.19	5.34	5.15	5.16	-[B]
C2(28d;pH7)A	5.07	4.31	5.42	5.53	5.56	5.20	5.35	5.21	5.25	-[B]
C2(28d;pH7)B	5.07	4.27	5.53	5.52	5.54	5.19	5.39	5.21	5.21	-[B]
C2(28d;pH7)C	5.05	4.28	4.60	5.64	5.54	5.17	5.42	5.28	5.23	-[B]
C2(56d;pH7)A	4.90	4.25	4.47	5.65	5.61	5.26	5.58	5.21	**5.17**	-[B]
C2(56d;pH7)B	-[C]	-[C]	-[C]	-[C]	-[C]	-[C]	-[C]	-[C]	-[C]	-[C]
C2(56d;pH7)C	5.11	4.28	5.64	5.60	5.66	5.28	5.43	5.23	**5.13**	-[B]
C2(28d;pH4.1)A	5.10	4.28	5.70	5.45	5.51	5.29	5.33	5.12	**5.11**	-[B]
C2(28d;pH4.1)B	5.13	4.31	5.75	5.51	5.60	5.21	5.41	5.16	**5.18**	-[B]
C2(28d;pH4.1)C	5.15	4.28	5.68	5.38	5.65	5.31	5.45	5.16	**5.14**	-[B]
C2(28d;pH8.4)A	5.17	**4.36**	4.89	5.38	5.66	5.28	5.40	5.13	5.32	-[B]
C2(28d;pH8.4)B	5.10	4.26	5.68	5.66	5.64	5.24	5.41	5.15	**5.30**	-[B]
C2(28d;pH8.4)C	5.12	4.34	3.61	5.56	5.63	5.30	5.35	5.15	5.37	-[B]
C3(28d;pH7)A	4.93	4.39	4.77	5.86	5.92	**5.15**	**5.36**	**5.26**	**5.47**	5.47
C3(28d;pH7)B	4.99	4.29	5.34	5.91	6.03	**5.19**	**5.41**	**5.26**	**5.52**	5.47
C3(28d;pH7)C	5.07	4.30	5.49	5.95	6.01	**5.11**	**5.34**	**5.25**	**5.59**	5.39
C4(28d;pH7)A	4.84[A]	**3.81**	**5.01**	4.94[A]	4.66[A]	5.08[A]	**4.43**	5.05[A]	**4.35**	5.55[A]
C4(28d;pH7)B	4.81	**3.83**	**5.42**	5.66	5.72	5.31	**5.17**	**5.24**	**5.13**	5.47
C4(28d;pH7)C	4.89	**3.90**	**5.52**	5.74	5.97	5.28	**5.29**	**5.22**	**5.20**	5.56

Red values are log K_{POM}-values that were excluded from evaluation, due to either blank/sample-ratio >10% (see Table S5), or a recovery that did not meet the chosen limits (red bold values), see Table S6. Blue values are outliers.

[A]Values excluded from final K_{POM} determination, (in addition to the values that did not meet the quality criteria (red values) and the values for experiments with tumbling times <28 days), due to either possible overestimation of C_W (applies to the C1-level) or outliers (applies to the replicate C4(28d;pH7)A, where the majority of the compounds had a C_W that differed significantly from the other two replicates and thus all data from this sample was excluded from final K_{POM} determination.

[B]Not available due to C_W < LOD.

[C]Not available due to mistake during sample preparation.

S2.3.2 Effect of pH on K_{POM}

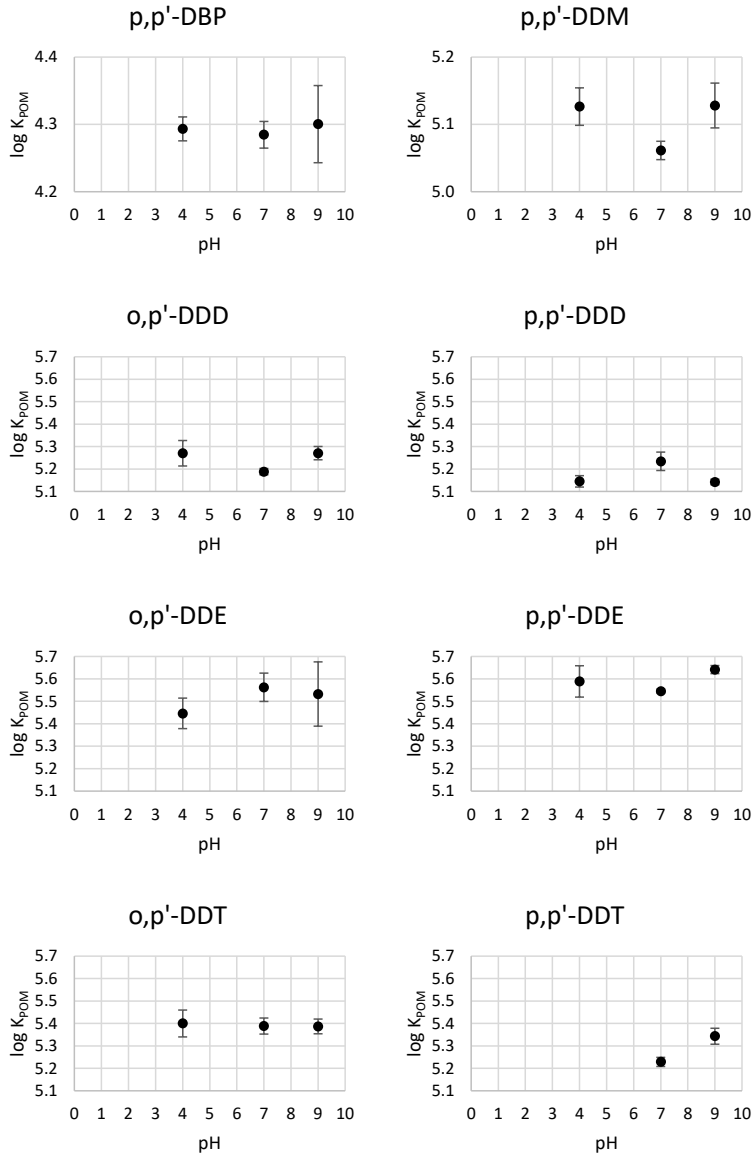


Figure S1. Mean log K_{POM} for the DDX as a function of pH. Error bars represent the standard deviation determined from n=3 POM/water-partitioning tests (data from 28 days of tumbling using the C2-level). p,p′-DDMU and dicofol excluded due to lack of data.

Table S14. Statistics from JMP one-way ANOVA and comparisons for all pairs using Tukey–Kramer HSD.

	p,p'-DDM	*p,p'*-DBP	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT
One-way ANOVA Prob>*F*	0.0353*	0.8908	0.3786	0.0811	0.0444*	0.9516	0.0118*	0.0170*
8.4	A	A	A	A	A	A	A	A
4.1	A B	A	A	A	A	A	B	-
7.0	B	A	A	A	A	A	B	B

The pH-effect was not evaluated for *p,p'*-DDMU and dicofol due to lack of data (blank/sample-ratios >10% and C_W < LOD respectively).

S2.3.3 Time to reach equilibrium

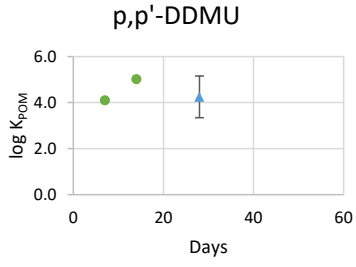


Figure S2. log K_{POM} as a function of tumbling time (days) for *p,p'*-DDMU derived for the C2-concentration level. Single values (green circles) and mean of n=2 (blue triangle). Error bar represent the standard deviation. Green circles and blue triangle indicate the pH of the test; 7 and 8.4 respectively.

Table S15. Evaluation of the kinetic experiment (time to reach equlibrium).

	p,p′-DBP	*p,p′*-DDM	*o,p′*-DDD	*p,p′*-DDD	*p,p′*-DDMU	*o,p′*-DDT	*o,p′*-DDE	*p,p′*-DDT	*p,p′*-DDE
log *K*ow (SPARC 2024)[A]	4.34	5.34	5.98	6.11	6.16	6.68	6.82	6,84	6.89
Mean_7d	4.18	5.07	4.98	5.08	4.10	5.24	5.41	NA	5.44
Mean_14d	4.25	NA	5.16	5.16	5.01	5.34	5.39	5.19	5.48
Mean_28d	4.29	5.11	5.24	5.17	4.25	5.39	5.51	5.27	5.59
Mean_56	4.26	5.01	5.27	5.22	NA	5.51	5.63	NA	5.64
s.d._7d	0.024	0.003	0.057	0.082	NA	0.040	0.046	NA	0.039
s.d._14d	0.036	NA	0.030	0.012	NA	0.015	0.044	0.051	0.076
s.d._28d	0.027	0.040	0.053	0.052	0.907	0.039	0.100	0.067	0.055
s.d._56d	0.024	0.151	0.017	0.015	NA	0.106	0.034	NA	0.034
n_7d	3	3	3	3	1	3	3	0	3
n_14d	3	0	3	3	1	3	3	2	3
n_28d	8	9	9	9	2	9	9	5	9
n_56d	2	2	2	2	0	2	2	0	2
assuming homoscedasticity									
P-value (*t*-test[A]) 28d:7d	0.005	0.45	0.0039	0.047	-	0.010	0.030	-	0.012
P-value (*t*-test[A]) 28d:14d	0.079	-	0.037	0.648	-	0.066	0.069	0.182	0.022
P-value (*t*-test[A]) 28d:56d	0.210	0.080	0.518	0.245	-	0.017	0.156	-	0.326

Compounds arranged in hydrophobicity order (increasing K_{OW}-values from left to right).

[A]Obtained with SPARC 2024-02-28 logD (25°C, ionic strength 0).

S2.3.4 Effect of concentration on K_{POM}

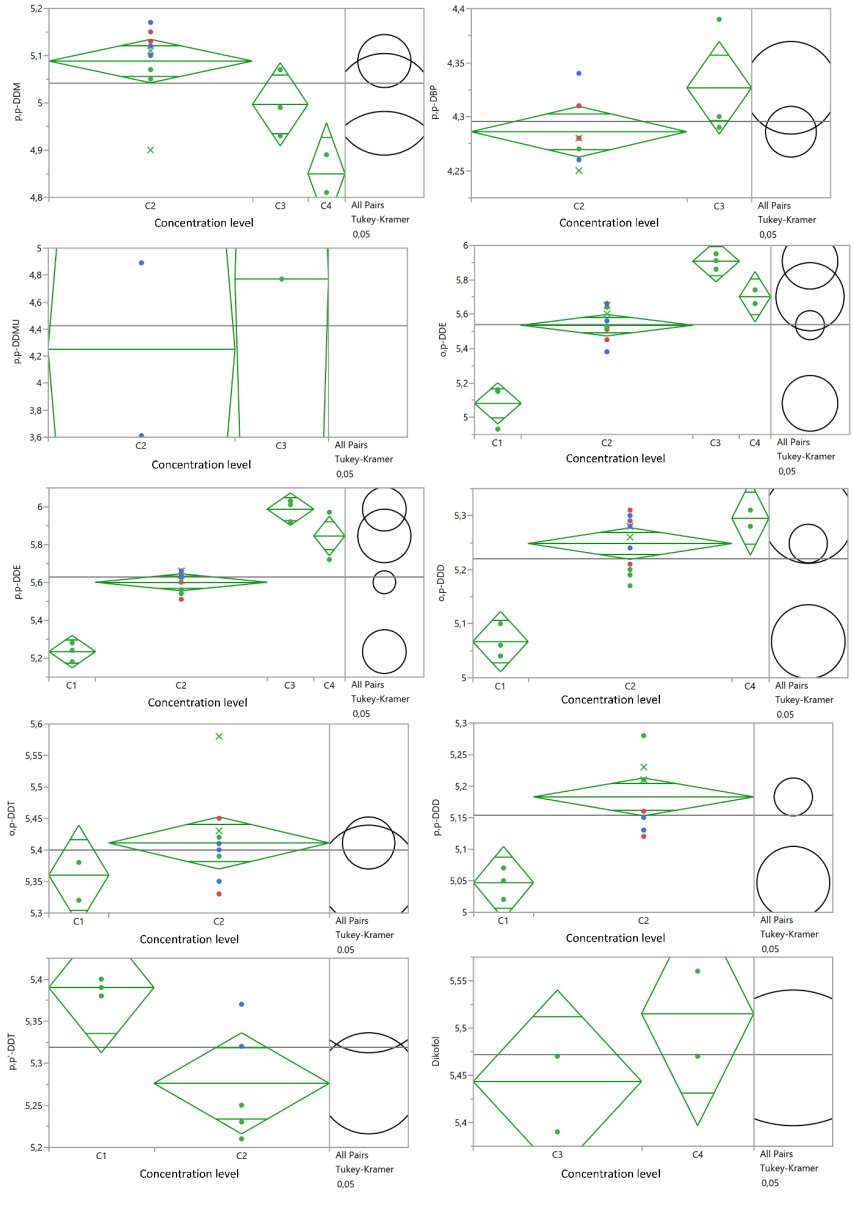


Figure S3. log K_{POM} calculated at the different concentration levels (C1, C2, C3 and C4) at 28 d equilibration time (circles) and 56d (crosses) L. Green colour indicate pH 7.0, red pH 4.1 and blue pH 8.4. log K_{POM}-values based on C_W or C_{POM} that did not meet the quality criteria (or was obvious outliers) excluded (see Table S13).

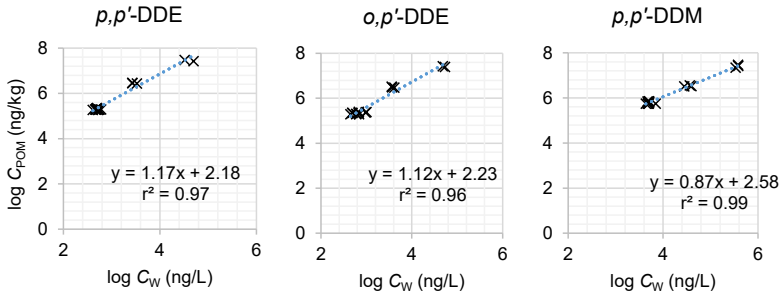


Figure S4 Logarithmic isotherms for *p,p′*-DDE, *o,p′*-DDE and *p,p′*-DDM i.e. for compounds with valid data from three concentration (C)-levels.

Table S16. Evaluation of the concentration experiment.

log K_{POM}		*p,p′*-DDM	*p,p′*-DBP	*p,p′*-DDMU	*o,p′*-DDE	*p,p′*-DDE	*o,p′*-DDD	*o,p′*-DDT	*p,p′*-DDD	*p,p′*-DDT	dikofol
Mean_C1					5.08	5.24	5.07	5.36	5.05	5.39	
Mean_C2		5.09	4.29	4.25	5.53	5.60	5.25	5.41	5.18	5.27	
Mean_C3		5.00	4.33	4.77	5.91	5.99					5.45
Mean_C4		4.85			5.70	5.85	5.30				5.52
s.d._C1					0.132	0.051	0.031	0.034	0.024	0.0092	
s.d._C2		0.072	0.028	0.907	0.101	0.054	0.049	0.067	0.050	0.067	0.072
s.d._C3		0.071	0.055		0.047	0.061					0.048
s.d._C4		0.060			0.056	0.171	0.022				0.063
n_C1		0	0	0	3	3	3	3	3	3	0
n_C2		11	10	2	11	11	11	11	11	5	0
n_C3		3	3	1	3	3	0	0	0	0	3
n_C4		2	0	0	2	2	2	0	0	0	2
Oneway anova Prob>*F*	C1	-	-	-	C	C	B	0.24	0.0006*	0.030*	-
	C2	A	0.094	0.72	B	B	A	0.24	0.0006*	0.030*	-
	C3	A B	0.094	0.72	A	A	-	-	-	-	0.23
	C4	B	-	-	A B	A	A	-	-	-	0.23

Mean values of log K_{POM} and standard deviations (s.d.). Statistics from JMP one-way ANOVA comparing the means derived at the different concentration levels, and comparisons for all pairs using Tukey–Kramer HSD (letters) or pooled *t*-test (values). Concentration-levels not connected by same letter are significantly different (the alphabetical order of the letters shows the order of magnitude of the K_{POM} (where A represents the highest value, B the second highest and so on). Values marked with asterisks (*) indicate significant difference $P < 0.05$.

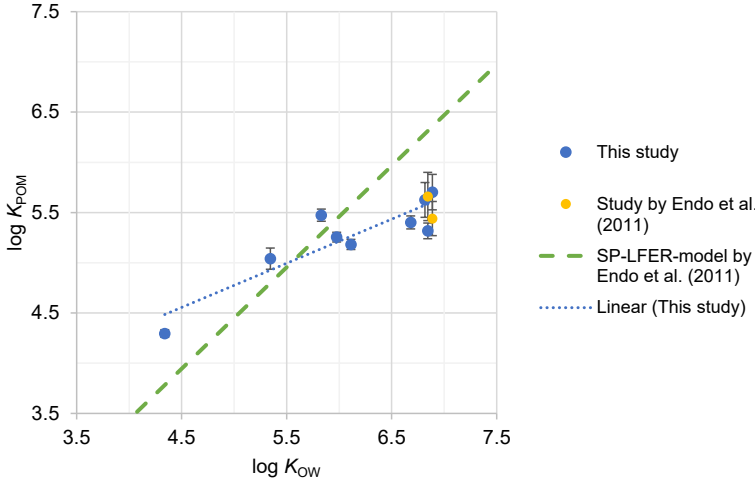


Figure S5 log K_{POM} for DDX from this study and the study of Endo et al. (2011) as a function of log K_{OW} (Table S1). Error bars show standard deviations (see Table 3). The green dotted line represents the SP-LFER-model (log K_{POM} = 1.01 × log K_{OW} -0.60) derived by Endo et al., (2011). The blue dotted line is the linear regression (log K_{POM} = 0.44×log K_{OW} + 2.6; with an explanatory power of r^2=0.81) of our values (DDMU excluded).

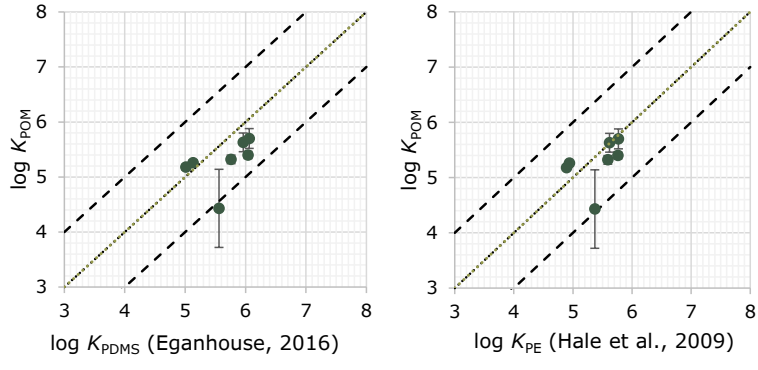


Figure S6 log K_{POM} for DDX from this study *v.* (a) log K_{PDMS} derived by Eganhouse (2016) for PDMS of 10-µm thickness and (b) log K_{PE} derived by Hale et al. (2009), for PE of 51-µm thickness.

S2.4 Soil Concentrations, C_{soil} (Part 2)

Table S17 Concentrations of DDX in soils collected from nine plant nurseries.

	Csoil (mg/kg DW)	Deje Syd	Jakobsbyn	Ljungaskog	Kolleberga	Deje Nord	Sya	Stakheden	Klockatorp	Åby
Lab A	*p,p'*-DDT	2.42	4.25	3.83	4.62	4.94	6.38	6.42	21.2	86.7
	o,p'-DDT	0.530	0.979	0.892	1.11	1.20	1.45	1.43	5.43	21.0
	p,p'-DDE	0.860	0.634	1.18	0.720	1.45	1.82	1.92	1.70	2.81
	o,p'-DDE	0.0144	0.0136	0.0232	0.0195	0.0484	0.0295	0.0644	0.0484	0.0916
	p,p'-DDD	0.412	0.535	0.599	0.674	0.867	0.856	0.872	3.27	15.7
	o,p'-DDD	0.107	0.146	0.168	0.183	0.192	0.361	0.280	0.788	4.52
	dicofol	0.135	0.0948	0.167	0.129	0.678	1.005	0.776	1.05	3.19
	p,p'-DBP	0.171	0.211	0.211	0.237	0.286	0.176	0.236	0.238	0.397
	p,p'-DDMU	0.0025	0.0024	0.0046	0.0044	0.0062	0.0125	0.0066	0.0316	0.0305
	p,p'-DDM	0.00100	0.00125	0.00197	0.00105	0.00134	0.00104	0.0006	0.0037	0.0068
	SUM DDX	4.65	6.87	7.09	7.70	9.66	12.1	12.0	33.8	134.4
Lab B	*p,p'*-DDT	3.40	5.53	5.12	5.45	6.14 ± 0.684 (11%)	7.21	6.87	18.4	48.9
	o,p'-DDT	0.484	0.797	0.764	0.931	1.03 ± 0.0546 (5.3%)	1.12	1.10	4.76	19.3
	p,p'-DDE	0.902	0.709	1.21	0.887	1.61 ± 0.0686 (4.3%)	1.77	2.00	2.41	11.6
	o,p'-DDE	<LOQ	<LOQ	0.00870	0.00927	0.0406 ± 0.00438 (11%)	0.0248	0.0664	0.0772	1.68
	p,p'-DDD	0.101	0.162	0.173	0.154	0.240 ± 0.0142 (5.9%)	0.265	0.253	1.63	2.92
	o,p'-DDD	0.0433	0.0581	0.0556	0.0598	0.098 ± 0.00611 (6.3%)	0.201	0.096	0.506	1.14
	dicofol	0.0647	0.0621	0.121	0.140	0.273 ± 0.0140 (5.1%)	0.405	0.429	0.350	0.672
	p,p'-DBP	0.158	0.137	0.179	0.187	0.233 ± 0.00514 (2.2%)	0.294	0.344	0.354	0.876
	p,p'-DDMU	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	p,p'-DDM	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	SUM DDX	5.16	7.45	7.63	7.82	9.66	11.3	11.2	28.5	87.0
	Mean r.s.d.% of triplicate					6.4%				

Analyses of subsamples (single analysis) performed by Lab A and Lab B. Three analyses were performed on the subsample Deje Nord, by Lab B for which mean values, standard deviations and relative standard deviations (r.s.d.) are given. The concentrations of *p,p'*-DDMU and *p,p'*-DDM was close to, or below, the limit of quantification (LOQ) in all studied soils (for both Lab A and B).

Table S18 Mean soil concentrations of DDX and standard deviations (n=2); mean values of analyses performed at Lab A and Lab B.

Mean Csoil (mg/kg DW)	Deje Syd	Jakobsbyn	Ljungaskog	Kolleberga	Deje Nord	Sya	Stakheden	Klockatorp	Åby
p,p'-DDT	2.9	4.9	4.5	5.0	5.5	6.8	6.6	20	68
o,p'-DDT	0.51	0.89	0.83	1.0	1.1	1.3	1.3	5.1	20
p,p'-DDE	0.88	0.67	1.2	0.80	1.5	1.8	2.0	2.1	7.2
o,p'-DDE	0.014	0.01	0.016	0.014	0.044	0.027	0.065	0.063	0.89
p,p'-DDD	0.26	0.35	0.39	0.41	0.55	0.56	0.56	2.5	9.3
o,p'-DDD	0.08	0.10	0.11	0.12	0.15	0.28	0.19	0.65	2.8
dicofol	0.10	0.08	0.14	0.13	0.48	0.71	0.60	0.70	1.9
p,p'-DBP	0.16	0.17	0.20	0.21	0.26	0.23	0.29	0.30	0.64
SUM DDX	4.9	7.2	7.4	7.8	9.7	12	12	31	110
s.d.									
p,p'-DDT	0.69	0.90	0.91	0.59	0.85	0.59	0.32	2.0	27
o,p'-DDT	0.032	0.13	0.090	0.13	0.12	0.24	0.23	0.48	1.2
p,p'-DDE	0.029	0.053	0.021	0.12	0.11	0.037	0.05	0.50	6.2
o,p'-DDE	NA	NA	0.010	0.0072	0.0055	0.0033	0.0014	0.020	1.1
p,p'-DDD	0.22	0.26	0.30	0.37	0.44	0.42	0.44	1.2	9.1
o,p'-DDD	0.045	0.062	0.080	0.087	0.067	0.11	0.13	0.20	2.4
dicofol	0.049	0.023	0.032	0.0080	0.29	0.42	0.25	0.49	1.8
p,p'-DBP	0.009	0.052	0.023	0.035	0.037	0.083	0.076	0.082	0.34
r.s.d.%									
p,p'-DDT	24%	19%	20%	12%	15%	9%	5%	10%	39%
o,p'-DDT	6%	14%	11%	13%	11%	19%	18%	9%	6%
p,p'-DDE	3%	8%	2%	15%	8%	2%	3%	24%	86%
o,p'-DDE	NA	NA	64%	50%	12%	12%	2%	32%	127%
p,p'-DDD	86%	76%	78%	89%	80%	75%	78%	47%	97%
o,p'-DDD	60%	61%	71%	72%	46%	40%	69%	31%	85%
dicofol	50%	30%	23%	6%	60%	60%	41%	71%	92%
p,p'-DBP	6%	30%	12%	17%	14%	35%	26%	28%	53%

Relative standard deviation (r.s.d.%; s.d. ÷ mean × 100) above 50% highlighted in red. NA, not available due to <LOQ-values in Lab B's analyses.

Table S19. Composition of DDX in soils as mean values of analyses from Lab A and Lab B.

	Composition in soils:	Deje Syd	Jakobsbyn	Ljungaskog	Kolleberga	Deje Nord	Sya	Stakheden	Klockatorp	Åby
Mean of n=2 (Lab A and Lab B)	*p,p'*-DDT	59%	68%	61%	65%	57%	58%	57%	64%	60%
	o,p'-DDT	10%	12%	11%	13%	12%	11%	11%	16%	19%
	p,p'-DDE	18%	9%	16%	10%	16%	15%	17%	7%	8%
	o,p'-DDE	0%	0%	0%	0%	0%	0%	1%	0%	1%
	p,p'-DDD	5%	5%	5%	5%	6%	5%	5%	8%	8%
	o,p'-DDD	2%	1%	2%	2%	2%	2%	2%	2%	2%
	dicofol	2%	1%	2%	2%	5%	6%	5%	2%	2%
	p,p'-DBP	3%	2%	3%	3%	3%	2%	3%	1%	1%
Lab A n=1	*p,p'*-DDT	52%	62%	54%	60%	51%	53%	53%	63%	64%
	o,p'-DDT	11%	14%	13%	14%	12%	12%	12%	16%	16%
	p,p'-DDE	18%	9%	17%	9%	15%	15%	16%	5%	2%
	o,p'-DDE	0%	0%	0%	0%	1%	0%	1%	0%	0%
	p,p'-DDD	9%	8%	8%	9%	9%	7%	7%	10%	12%
	o,p'-DDD	2%	2%	2%	2%	2%	3%	2%	2%	3%
	dicofol	3%	1%	2%	2%	7%	8%	6%	3%	2%
	p,p'-DBP	4%	3%	3%	3%	3%	1%	2%	1%	0%
Lab B n=1	*p,p'*-DDT	66%	74%	67%	70%	64%	64%	62%	65%	56%
	o,p'-DDT	9%	11%	10%	12%	11%	10%	10%	17%	22%
	p,p'-DDE	17%	10%	16%	11%	17%	16%	18%	8%	13%
	o,p'-DDE	0%	0%	0%	0%	0%	0%	1%	0%	2%
	p,p'-DDD	2%	2%	2%	2%	2%	2%	2%	6%	3%
	o,p'-DDD	1%	1%	1%	1%	1%	2%	1%	2%	1%
	dicofol	1%	1%	2%	2%	3%	4%	4%	1%	1%
	p,p'-DBP	3%	2%	2%	2%	2%	3%	3%	1%	1%

Table S20 Ratio between soil concentration (C_{soil}) analysed by Lab A and Lab B.

$C_{soil,LabA}/C_{soil,LabB}$	$\Sigma p,p'$-DDT/D	$\Sigma o,p'$-DDT/D	p,p'-DDT	o,p'-DDT	p,p'-DDE	o,p'-DDE	p,p'-DDD	o,p'-DDD	dicofol	p,p'-DBP
Deje Syd	0.81	1.2	0.71	1.1	1.0	NA	4.1	2.5	2.1	1.1
Jakobsbyn	0.84	1.3	0.77	1.2	0.89	4.6	3.3	2.5	1.5	1.5
Ljungaskog	0.84	1.3	0.75	1.2	1.0	2.7	3.5	3.0	1.4	1.2
Kolleberga	0.94	1.3	0.85	1.2	0.81	2.1	4.4	3.1	0.9	1.3
Deje Nord	0.91	1.2	0.80	1.2	0.90	1.2	3.6	2.0	2.5	1.2
Sya	1.0	1.4	0.88	1.3	1.0	1.2	3.2	1.8	2.5	0.60
Stakheden	1.0	1.4	0.93	1.3	1.0	1.0	3.4	2.9	1.8	0.69
Klockatorp	1.2	1.2	1.2	1.1	0.71	0.63	2.0	1.6	3.0	0.67
Åby	2.0	1.2	1.8	1.1	0.24	0.05	5.4	4.0	4.8	0.45
Min.	0.81	1.2	0.7	1.1	0.2	0.05	2.0	1.6	0.9	0.5
Max.	2.0	1.4	1.8	1.3	1.0	2.7	5.4	4.0	4.8	1.5
Mean	1.1	1.3	0.96	1.19	0.83	1.26	3.65	2.59	2.27	0.97

NA, not available due to C_{soil} <LOQ.

S2.5 POM Concentrations, C_{POM} (Part 2)

Table S21 Concentration of DDX in POM (µg/g POM) analysed by Lab A and Lab B.

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Lab A								
C_{POM} µg/g POM								
Deje Syd:a	34.3	7.21	23.0	0.387	2.52	0.629	2.77	0.573
Deje Syd:b	31.9	7.23	23.3	0.390	2.48	0.657	3.06	0.519
Deje Syd:c	37.8	8.11	22.8	0.423	2.82	0.655	2.98	0.630
Jakbobsbyn:a	17.9	3.83	5.04	0.116	1.74	0.385	1.03	0.273
Jakbobsbyn:b	19.1	4.06	5.10	0.123	1.67	0.394	1.19	0.276
Jakbobsbyn:c	20.0	4.24	5.29	0.119	1.69	0.382	0.99	0.277
LjungaskogA:a-II	46.6	9.95	27.1	0.573	4.14	0.964	6.02	0.735
LjungaskogA:b-II	49.7	9.97	27.7	0.600	4.17	0.961	6.39	0.874
LjungaskogA:c-II	42.8	9.45	24.7	0.530	3.73	0.881	5.84	0.678
Kolleberga:a	36.8	9.07	12.1	0.365	3.05	0.615	1.92	0.357
Kolleberga:b	39.7	9.07	12.0	0.361	2.95	0.591	1.72	0.350
Kolleberg:c	40.5	9.02	12.5	0.348	3.05	0.606	1.67	0.355
Deje Nord:a	60.5	15.5	40.7	1.31	4.88	1.67	80.7[B]	3.20
Deje Nord:b	58.9	14.3	38.2	1.25	5.04	1.58	61.5[B]	3.39
Deje Nord:c	63.4	16.3	43.1	1.32	4.88	1.61	62.6[B]	3.47
Sya:a	102	22.9	57.2	0.972	6.29	3.87	182[B]	4.46
Sya:b	97.0	25.2	49.9	0.921	6.31	3.87	117[B]	4.27
Sya:c	95.0	21.2	48.9	0.901	6.48	3.86	95.8[B]	4.26
Stakheden:a	103	25.6	61.9	2.30	5.25	2.09	99.5[B]	6.03
Stakheden:b	102	22.9	60.1	2.35	5.35	2.18	134[B]	5.81
Stakheden:c	95.7	23.2	58.0	2.30	5.24	2.01	119[B]	4.99
KlockatorpA:a-II	123	27.5	14.9	0.400	11.15	3.18	40.3	0.910
KlockatorpA:b-II	122	28.1	15.8	0.439	12.08	3.36	43.3	0.953
KlockatorpA:c-II	117	27.5	14.3	0.405	10.92	3.23	36.1	0.912
Åby:a	849	391	72.4	2.98	254	14.3	1389[B]	8.09
Åby:b	836	318	66.8	2.82	215	13.1	1250[B]	8.03
Åby:c	849	322	69.5	2.85	218	14.4	913[B]	6.15
LjungaskogB:a-II	29.0	6.39	17.1	0.356	2.59	0.578	3.64	0.469
LjungaskogB:b-II	36.7	7.11	20.8	0.382	3.16	0.709	4.96	0.470
LjungaskogB:c-II	32.9	7.14	18.1	0.388	2.84	0.640	4.53	0.497
KlockatorpB:a-II	70.7	13.9	8.88	0.223	7.51	1.84	17.0	0.513
KlockatorpB:b-II	71.7	14.2	10.2	0.228	7.90	1.98	18.2	0.579
KlockatorpB:c-II	NA	NA	NA	NA	NA	NA	NA	NA
Mean concentration of DDX in POM (µg/g POM) analysed by Lab A								
Lab A								
Mean C_{POM} µg/g POM								
Deje Syd	34.7	7.52	23.1	0.400	2.61	0.647	2.94	0.574
Jakbobsbyn	19.0	4.04	5.15	0.119	1.70	0.387	1.07	0.275
LjungaskogA	46.4	9.79	26.5	0.568	4.01	0.935	6.08	0.762
LjungaskogB	32.9	6.88	18.7	0.375	2.86	0.642	4.38	0.479
Kolleberga	39.0	9.05	12.2	0.358	3.02	0.604	1.77	0.354
Deje Nord	60.9	15.4	40.7	1.29	4.94	1.62	68.3[B]	3.36
Sya	97.9	23.1	52.0	0.931	6.36	3.87	132[B]	4.33
Stakheden	100	23.9	60.0	2.32	5.28	2.09	117[B]	5.61
KlockatorpA	121	27.7	15.0	0.414	11.38	3.25	39.9	0.925
KlockatorpB[A]	71.2	14.1	9.52	0.226	7.70	1.91	17.6	0.546
Åby	845	344	69.6	2.88	229	13.93	1184[B]	7.42

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Lab B								
C_{POM} µg/g POM								
Deje Syd:a	20.0	5.29	15.9	0.255	1.13	0.285	0.949	0.051
Deje Syd:b	21.6	5.71	16.3	0.268	1.22	0.292	1.03	0.102
Deje Syd:c	18.5	5.06	15.5	0.241	1.15	0.274	1.10	0.032
Jakobsbyn:a	11.6	3.27	4.04	0.057	0.466	0.141	0.118	0.0128
Jakobsbyn:b	13.3	3.74	4.27	0.066	0.569	0.155	0.117	0.0198
Jakobsbyn:c	11.5	3.21	3.79	0.058	0.571	0.137	0.110	0.0234
LjungaskogB:a-I	NA	NA	19.3	0.336	1.11	0.310	2.02	0.0875
LjungaskogB:b-I	22.3	6.59	9.71	0.333	1.28	0.305	1.09	0.0538
LjungaskogB:c-I	24.8	7.50	17.4	0.311	1.02	0.313	1.99	0.0517
Kolleberga:a	18.3	5.38	8.06	0.199	0.879	0.196	0.111	0.0311
Kolleberga:b	18.5	5.67	8.29	0.208	0.961	0.217	0.110	0.0219
Kolleberga:c	18.3	5.7	8.63	0.211	0.639	0.182	0.109	0.0190
Deje Nord:a	40.9	11.3	26.1	0.828	2.65	0.729	3.10	0.487
Deje Nord:b	40.0	12.1	25.0	0.853	2.81	0.736	2.63	0.345
Deje Nord:c	44.7	12.4	29.6	0.921	2.38	0.845	4.02	0.271
Sya:a	92.6	19.8	41.8	0.748	4.99	3.524	8.78	0.869
Sya:b	64.1	17.7	39.5	0.735	8.60	3.137	6.92	0.516
Sya:c	69.1	16.7	38.7	0.724	3.08	3.389	7.50	0.371
Stakheden:a	43.5	14.5	38.4	1.40	2.44	0.840	7.17	0.211
Stakheden:b	42.6	14.1	39.3	1.46	1.78	0.821	7.10	0.272
Stakheden:c	46.8	14.8	36.4	1.27	2.21	0.835	6.67	0.282
KlockatorpB:a-I	52.5	14.4	9.29	0.210	5.81	1.407	0.233	0.0795
KlockatorpB:b-I	52.5	14.9	9.11	0.221	3.79	1.318	1.48	0.0831
KlockatorpB:c-I	NA	NA	NA	NA	NA	NA	NA	NA
Åby:a	430	178	49.6	2.24	NA	7.66	9.34	0.375
Åby:b	396	180	50.5	2.21	72.4	6.79	10.05	0.337
Åby:c	380	183	52.1	2.23	52.9	7.12	9.00	0.648
LjungaskogA:a-I	32.1	10.6	26.4	0.551	1.34	0.405	0.235	0.202
LjungaskogA:b-I	34.5	10.9	28.0	0.540	1.37	0.396	0.239	0.161
LjungaskogA:c-I	34.1	11.0	27.0	0.519	1.75	0.444	0.258	0.156
KlockatorpA:a-I	113	25.9	14.9	0.489	7.15	2.67	0.243	0.920
KlockatorpA:b-I	114	28.4	16.3	0.517	9.69	2.73	0.231	0.868
KlockatorpA:c-I	129	30.1	16.1	0.553	11.3	3.09	0.247	1.50
Mean concentration of DDX in POM (µg/g POM) analysed by Lab B.								
Lab B								
Mean C_{POM} µg/g POM								
Deje Syd	20.0	5.35	15.9	0.255	1.17	0.284	1.03	0.0617
Jakbobsbyn	12.2	3.41	4.03	0.060	0.535	0.144	0.115	0.0187
LjungaskogA	33.6	10.8	27.1	0.537	1.49	0.415	0.244	0.173
LjungaskogB	23.6[A]	7.04[A]	15.5	0.327	1.14	0.309	1.70	0.0643
Kolleberga	18.4	5.59	8.33	0.206	0.826	0.198	0.110	0.0240
Deje Nord	41.9	11.9	26.9	0.867	2.61	0.770	3.25	0.368
Sya	75.3	18.1	40.0	0.736	5.56	3.35	7.73	0.585
Stakheden	44.3	14.5	38.1	1.38	2.14	0.832	6.98	0.255
KlockatorpA	119	28.2	15.8	0.520	9.39	2.83	0.240	1.10
KlockatorpB[A]	52.	14.7	9.20	0.215	4.80	1.36	0.855	0.0813
Åby	402	180	50.7	2.22	62.6[A]	7.19	9.46	0.453
Standard deviation of mean concentrations of DDX in POM (µg/g POM). *n*=3 unless otherwise given.								
Lab A								
s.d. C_{POM} (µg/g)								
Deje Syd	3.0	0.52	0.26	0.020	0.18	0.016	0.15	0.055
Jakbobsbyn	1.1	0.21	0.13	0.004	0.032	0.006	0.10	0.0019
LjungaskogA	3.4	0.29	1.6	0.036	0.25	0.047	0.28	0.10
LjungaskogB	3.8	0.42	1.9	0.017	0.28	0.065	0.68	0.016
Kolleberga	2.0	0.03	0.24	0.009	0.059	0.012	0.13	0.0038
Deje Nord	2.3	1.0	2.5	0.038	0.092	0.046	11[D]	0.14
Sya	3.4	2.0	4.5	0.037	0.103	0.007	45[D]	0.11
Stakheden	4.1	1.5	2.0	0.026	0.062	0.088	17[D]	0.55
KlockatorpA	3.7	0.34	0.76	0.021	0.62	0.093	3.6	0.024
KlockatorpB[C]	0.71	0.21	0.90	0.0031	0.28	0.10	0.81	0.047
Åby	7.2	41	2.8	0.088	22	0.74	245[D]	1.1
Lab B								
s.d. C_{POM} µg/g								
Deje Syd	1.6	0.33	0.43	0.013	0.046	0.0093	0.075	0.036
Jakbobsbyn	0.99	0.29	0.24	0.0050	0.060	0.0096	0.0048	0.0053
LjungaskogA	1.3	0.19	0.81	0.016	0.23	0.026	0.012	0.025
LjungaskogB	1.8[C]	0.65[C]	5.1	0.014	0.13	0.0038	0.53	0.020
Kolleberga	0.12	0.19	0.28	0.0064	0.17	0.018	0.0010	0.0063
Deje Nord	2.5	0.58	2.4	0.048	0.22	0.065	0.71	0.11
Sya	15	1.6	1.6	0.012	2.8	0.20	0.95	0.26

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Stakheden	2.2	0.34	1.5	0.095	0.33	0.010	0.27	0.039
KlockatorpA	8.6	2.1	0.72	0.032	2.1	0.23	0.0083	0.35
KlockatorpB[C]	0.034	0.39	0.13	0.0074	1.4	0.063	0.88	0.0025
Åby	26	2.3	1.3	0.017	14	0.44	0.54	0.17
Relative standard deviation (r.s.d. %) r.s.d. >25% highlighted in red. *n*=3 unless otherwise is given								
Lab A								
r.s.d.%								
Deje Syd	9%	7%	1%	5%	7%	2%	5%	10%
Jakbobsbyn	6%	5%	3%	3%	2%	2%	10%	1%
LjungaskogA	7%	3%	6%	6%	6%	5%	5%	13%
LjungaskogB	12%	6%	10%	5%	10%	10%	15%	3%
Kolleberga	5%	0%	2%	2%	2%	2%	7%	1%
Deje Nord	4%	7%	6%	3%	2%	3%	16%[D]	4%
Sya	3%	9%	9%	4%	2%	0%	34%[D]	3%
Stakheden	4%	6%	3%	1%	1%	4%	15%[D]	10%
KlockatorpA	3%	1%	5%	5%	5%	3%	9%	3%
KlockatorpB[C]	1%	1%	9%	1%	4%	5%	5%	9%
Åby	1%	12%	4%	3%	9%	5%	21%[D]	15%
Mean RDS%	9%	10%	9%	8%	9%	8%	16%	11%
Mean r.s.d.% all data	**5.1%**							
Lab B								
r.s.d.%								
Deje Syd	8%	6%	3%	5%	4%	3%	7%	59%
Jakbobsbyn	8%	9%	6%	8%	11%	7%	4%	29%
LjungaskogA	4%	2%	3%	3%	15%	6%	5%	14%
LjungaskogB	8%[C]	9%[C]	33%	4%	12%	1%	31%	31%
Kolleberga	1%	3%	3%	3%	20%	9%	1%	26%
Deje Nord	6%	5%	9%	6%	8%	8%	22%[D]	30%
Sya	20%	9%	4%	2%	50%	6%	12%[D]	44%
Stakheden	5%	2%	4%	7%	15%	1%	4%[D]	15%
KlockatorpA	7%	7%	5%	6%	22%	8%	3%	32%
KlockatorpB[C]	0%	3%	1%	3%	30%	5%	103%	3%
Åby	6%	1%	2%	1%	22%	6%	6%[D]	37%
Mean r.s.d.%	8%	7%	8%	6%	21%	7%	22%	30%
Mean r.s.d.% all data	**11%**							

NA, not available (data missing). Ljungaskog and Klockatorp site names are follow by the lab in which the tumbling of the POM-soil-water slurry was performed. The letters “a”, “b” and “c” indicates the n=3 replicates.

[A] *n*=2 (missing data for one POM replicate)

[B] Depletion >80% therefore removed from further calculation and analysis

[C] *n*=2 (missing data for one replicate)

[D] Depletion >80% therefore removed from further calculation and analysis

Table S22 Ratio between POM concentration (C_{POM}) analysed by Lab A and Lab B.

Lab A/Lab B (mean C_{POM})	Σ*p,p'*-DDT/D	Σ*o,p'*-DDT/D	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Deje Syd	1.8	1.4	1.7	1.4	1.5	1.6	2.2	2.3	2.9	9.3
Jakbobsbyn	1.6	1.2	1.6	1.2	1.3	2.0	3.2	2.7	9.3	15
LjungaskogA	1.4	1.0	1.4	0.91	1.0	1.1	2.7	2.3	25	4.4
LjungaskogB	1.4	1.0	1.4[A]	1.0[A]	1.4	1.2	2.5	2.1	2.8	9.1
Kolleberga	2.2	1.7	2.1	1.6	1.5	1.7	3.6	3.0	16	15
Deje Nord	1.5	1.3	1.5	1.3	1.5	1.5	1.9	2.1	NA	9.1
Sya	1.3	1.3	1.3	1.3	1.3	1.3	1.1	1.2	NA	7.4
Stakheden	2.3	1.7	2.3	1.6	1.6	1.7	2.5	2.5	NA	22
KlockatorpA	1.0	1.0	1.0	1.0	1.0	0.8	1.2	1.1	166	0.8
KlockatorpB[A]	1.0	1.0	1.4	1.0	1.0	1.0	1.6	1.4	21	6.7
Åby	2.4	1.9	2.1	1.9	1.4	1.3	3.7[A]	1.9	NA	16
Mean			1.6	1.3	1.3	1.4	2.4	2.1	35	10

Ratios >2.5 highlighted in red. Mean C_{POM} based on n=3 unless otherwise given. NA, ratio not analysed/calculated due to depletion >80% for POM-soil tests at Lab A.
[A]n=2 (missing data for one replicate).

Table S23 Relative standard deviation (r.s.d.), in percentage for C_{POM} analysed by Lab A and Lab B.

r.s.d. (%)	Klockatorp				Ljungaskog			
Extraction lab	A		B		A		B	
Analysis lab	A (n=3)	B (n=3)	A (n=2)	B (n=2)	A (n=3)	B (n=3)	A (n=3)	B (n=3)
p,p'-DDT/D	2.5	6.6	0.9	19	6.0	3.2	9.4	4.6
o,p'-DDT/D	1.1	6.1	1.4	29	2.6	1.5	5.2	6.3
p,p'-DDE	4.1	3.7	6.7	17	4.9	2.4	8.3	28
o,p'-DDE	4.2	5.0	1.0	16	5.1	2.5	3.7	3.5
dicofol	7.4	2.8	3.3	6.2	3.8	4.1	13	29
p,p'-DBP	2.2	26	6.0	24	11	12	2.7	2.0
Mean	3.6	8.4	3.2	18	5.6	4.3	7.1	12
Mean r.s.d. Lab A, all data: 4.9%								
Mean r.s.d. Lab B, all data: 11%								

POM strip shaken at its own lab and the other lab.

S2.6 Water Concentrations, $C_{W,free}$ (Part 2)

Table S24 Freely dissolved pore water concentrations ($C_{W,free}$ ng/L) of individual DDX determined with the passive sampler, POM by Lab A and Lab B.

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Lab A $C_{W,free}$ (ng/L)								
Deje Syd:a	165	28.6	45.6	0.916	16.6	3.50	9.32	29.0
Deje Syd:b	153	28.7	46.2	0.924	16.3	3.65	10.3	26.3
Deje Syd:c	182	32.2	45.2	1.00	18.5	3.64	10.0	31.9
Jakobsbyn:a	86.1	15.2	10.0	0.27	11.4	2.14	3.46	13.8
Jakobsbyn:b	92.1	16.1	10.1	0.291	11.0	2.19	3.98	14.0
Jakobsbyn:c	96.5	16.8	10.5	0.281	11.1	2.12	3.34	14.0
LjungaskogB:a-II	224	39.5	53.7	1.36	27.2	5.35	20.2	37.2
LjungaskogB:b-II	239	39.5	54.9	1.42	27.4	5.34	21.5	44.2
LjungaskogB:c-II	206	37.5	49.0	1.25	24.5	4.90	19.6	34.3
Kolleberga:a	177	36.0	24.1	0.866	20.1	3.42	6.44	18.1
Kolleberga:b	191	36.0	23.8	0.854	19.4	3.28	5.79	17.7
Kolleberga:c	195	35.8	24.7	0.825	20.0	3.37	5.61	18.0
Deje Nord:a	291	61.6	80.6	3.11	32.1	9.29	271[B]	162
Deje Nord:b	283	56.7	75.6	2.96	33.1	8.81	206[B]	171
Deje Nord:c	305	64.7	85.4	3.12	32.1	8.93	210[B]	176
Sya:a	489	90.7	113	2.30	41.4	21.5	612[B]	226
Sya:b	467	99.9	98.8	2.18	41.4	21.5	393[B]	216
Sya:c	457	84.1	96.8	2.13	42.6	21.4	322[B]	216
Stakheden:a	498	101	123	5.45	34.5	11.6	334[B]	305
Stakheden:b	491	90.7	119	5.56	35.2	12.1	449[B]	294
Stakheden:c	461	91.9	115	5.46	34.4	11.1	399[B]	252
KlockatorpB:a-II	594	109	29.5	0.947	73.2	17.7	135	46.1
KlockatorpB:b-II	589	112	31.2	1.04	79.4	18.7	146	48.2
KlockatorpB:c-II[A]	NA	NA	NA	NA	NA	NA	NA	NA
Åby:a	4088	1551	143	7.07	1670	79.3	4665[B]	409
Åby:b	4027	1260	132	6.67	1413	72.7	4198[B]	406

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Åby:c	4085	1278	138	6.74	1434	80.2	3065 [B]	311
LjungaskogA:a-II	140	25.4	33.8	0.842	17.0	3.21	12.2	23.8
LjungaskogA:b-II	177	28.2	41.1	0.905	20.7	3.94	16.7	23.8
LjungaskogA:c-II	158	28.3	35.9	0.918	18.6	3.56	15.2	25.2
KlockatorpA:a-II	340	55.3	17.6	0.529	49.3	10.2	57.1	26.0
KlockatorpA:b-II	345	56.5	20.1	0.539	51.9	11.0	61.0	29.3
KlockatorpA:c-II	798	143	38.5	1.32	131	26.1	139	69.3
Lab B $C_{W,free}$ (ng/L)								
Deje Syd:a	96.5	21.0	31.5	0.604	7.41	1.58	3.19	2.60
Deje Syd:b	104	22.6	32.3	0.634	7.99	1.62	3.47	5.17
Deje Syd:c	88.8	20.1	30.6	0.571	7.57	1.52	3.69	1.60
Jakobsbyn:a	56.0	13.0	7.99	0.135	3.06	0.78	0.397	0.650
Jakobsbyn:b	64.0	14.8	8.45	0.156	3.74	0.863	0.394	1.00
Jakobsbyn:c	55.6	12.7	7.49	0.136	3.75	0.762	0.368	1.18
LjungaskogB:a-I	388[C]	88.4[C]	38.2	0.796	7.28	1.72	6.79	4.43
LjungaskogB:b-I	107	26.1	19.2	0.789	8.42	1.70	3.67	2.72
LjungaskogB:c-I	120	29.7	34.5	0.737	6.68	1.74	6.70	2.62
Kolleberga:a	88.3	21.3	16.0	0.471	5.78	1.09	0.372	1.57
Kolleberga:b	89.2	22.5	16.4	0.493	6.31	1.21	0.369	1.11
Kolleberga:c	88.2	22.8	17.1	0.499	4.20	1.01	0.365	0.963
Deje Nord:a	197	44.7	51.7	1.96	17.4	4.05	10.4	24.6
Deje Nord:b	193	48.1	49.5	2.02	18.5	4.09	8.82	17.5
Deje Nord:c	215	49.0	58.6	2.18	15.6	4.69	13.5	13.7
Sya:a	446	78.5	82.8	1.77	32.8	19.6	29.5	44.0
Sya:b	309	70.3	78.2	1.74	56.5	17.4	23.2	26.1
Sya:c	333	66.3	76.6	1.71	20.2	18.8	25.2	18.8
Stakheden:a	209	57.5	76.1	3.32	16.0	4.67	24.1	10.7
Stakheden:b	205	56.1	77.8	3.46	11.7	4.56	23.8	13.8
Stakheden:c	226	58.8	72.2	3.02	14.5	4.64	22.4	14.3
KlockatorpB:a-I	253	57.1	18.4	0.498	38.2	7.82	0.783	4.02
KlockatorpB:b-I	253	59.3	18.0	0.523	24.9	7.33	4.96	4.20
KlockatorpB:c-I	NA	NA	NA	NA	NA	NA	NA	NA
Åby:a	2071	707	98.1	5.30	NA[E]	42.5	31.4	19.0
Åby:b	1907	716	100	5.22	476	37.8	33.8	17.1
Åby:c	1827	725	103	5.28	348	39.6	30.2	32.8
LjungaskogA:a-I	155	42.0	52.3	1.30	8.83	2.25	0.789	10.2
LjungaskogA:b-I	166	43.1	55.5	1.28	8.97	2.20	0.804	8.16
LjungaskogA:c-I	164	43.5	53.4	1.23	11.5	2.47	0.866	7.91
KlockatorpA:a-I	546	103	29.6	1.16	47.0	14.8	0.816	46.5
KlockatorpA:b-I	551	113	32.2	1.22	63.7	15.2	0.775	43.9
KlockatorpA:c-I	620	120	31.9	1.31	74.4	17.2	0.828	76.1
Means of the freely dissolved water concentrations (C_W ng/L) of individual DDX determined with the passive sampler, POM by 1) Lab A and 2) Lab B.								
Lab A Mean $C_{W,free}$ (n=3)								
Deje Syd	167	29.8	45.7	0.947	17.1	3.60	9.87	29.0
Jakbobsbyn	91.5	16.0	10.2	0.282	11.2	2.15	3.59	13.9
LjungaskogA	223	38.8	52.5	1.34	26.4	5.20	20.4	38.6
LjungaskogB	158	27.3	37.0	0.889	18.8	3.57	14.7	24.2
Kolleberga	188	35.9	24.2	0.848	19.8	3.36	5.95	17.9
Deje Nord	293	61.0	80.5	3.06	32.4	9.01	N/A	170
Sya	471	91.6	103	2.21	41.8	21.5	N/A	219
Stakheden	483	94.7	119	5.49	34.7	11.6	N/A	284
KlockatorpA	581	110	29.7	0.982	74.8	18.1	134	46.8
KlockatorpB n=2	343	55.9	18.8	0.534	50.6	10.6	59.0	27.6
Åby	4066	1363	138	6.83	1506	77.4	N/A	376
Lab B Mean $C_{W,free}$ ng/L (n=3)								
Deje Syd	96.4	21.2	31.5	0.603	7.66	1.58	3.45	3.12
Jakbobsbyn	58.5	13.5	7.98	0.142	3.52	0.802	0.386	0.945
LjungaskogA	162	42.9	53.7	1.27	9.76	2.31	0.820	8.76
LjungaskogB[F]	113[F]	27.9[F]	30.6	0.774	7.46	1.72	5.72	3.26
Kolleberga	88.5	22.2	16.5	0.488	5.43	1.10	0.368	1.22
Deje Nord	202	47.3	53.3	2.05	17.2	4.28	10.9	18.6
Sya	362	71.7	79.2	1.74	36.5	18.6	26.0	29.6
Stakheden	213	57.5	75.3	3.27	14.1	4.62	23.4	12.9
KlockatorpA	572	112	31.2	1.23	61.7	15.7	0.806	55.5
KlockatorpB n=2[G]	253	58.2	18.2	0.510	31.5	7.57	2.87	4.11
Åby	1935	716	100	5.27	412[H]	40.0	31.8	22.9
Standard deviations of mean water concentrations of DDX (ng/L) analysed by 1) Lab A and 2) Lab B.								
Lab A s.d. $C_{W,free}$ (n=3)								
Deje Syd	14	2.1	0.52	0.048	1.2	0.087	0.50	2.8
Jakbobsbyn	5.2	0.82	0.26	0.0085	0.21	0.034	0.34	0.10

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
LjungaskogA	17	1.2	3.2	0.084	1.6	0.26	0.96	5.1
LjungaskogB	18	1.7	3.7	0.041	1.9	0.36	2.3	0.80
Kolleberga	9.5	0.13	0.48	0.021	0.39	0.068	0.43	0.19
Deje Nord	11	4.0	4.9	0.090	0.60	0.25	N/A	7.0
Sya	16	7.9	9.0	0.087	0.68	0.042	N/A	5.6
Stakheden	20	5.9	3.9	0.062	0.41	0.49	N/A	28
KlockatorpA	18	1.4	1.5	0.051	4.0	0.52	12	1.2
KlockatorpB[I]	3.4[I]	0.83[I]	1.8[I]	0.0074[I]	1.8[I]	0.55[I]	2.7[I]	2.4[I]
Åby	34	163	5.5	0.21	143	4.1	N/A	56
Lab B s.d. $C_{W,free}$ (n=3)								
Deje Syd	7.5	1.3	0.85	0.031	0.30	0.052	0.25	1.8
Jakbobsbyn	4.8	1.1	0.48	0.012	0.39	0.054	0.016	0.27
LjungaskogA	6.1	0.76	1.6	0.039	1.5	0.14	0.041	1.3
LjungaskogB	8.6[I]	2.6[I]	10	0.033	0.88	0.021	1.8	1.0
Kolleberga	0.55	0.76	0.56	0.015	1.1	0.10	0.0033	0.32
Deje Nord	12	2.3	4.7	0.11	1.4	0.36	2.4	5.5
Sya	73	6.2	3.2	0.028	18	1.1	3.2	13
Stakheden	11	1.4	2.9	0.23	2.2	0.056	0.92	2.0
KlockatorpA	41	8.3	1.4	0.076	14	1.3	0.028	18
KlockatorpB[I]	0[I]	1.6[I]	0.25[I]	0.018[I]	9.4[I]	0.35[I]	3.0[I]	0.13[I]
Åby	124	9.3	2.5	0.040	91[I]	2.4	1.8	8.6
Relative standard deviations of mean water concentrations of DDX (ng/L) analysed by 1) Lab A and 2) Lab B.								
Lab A r.s.d. $C_{W,free}$ (n=3)								
Deje Syd	7.0	5.6	0.9	4.1	5.8	2.0	4.1	7.9
Jakbobsbyn	4.7	4.2	2.1	2.5	1.6	1.3	7.8	0.6
LjungaskogA	6.1	2.4	4.9	5.1	5.1	4.1	3.8	10.8
LjungaskogB	9.5	5.0	8.3	3.7	8.1	8.3	12.6	2.7
Kolleberga	4.1	0.3	1.6	2.0	1.6	1.6	6.0	0.9
Deje Nord	3.1	5.4	5.0	2.4	1.5	2.3		3.4
Sya	2.8	7.1	7.1	3.2	1.3	0.2		2.1
Stakheden	3.3	5.1	2.7	0.9	1.0	3..4		8.0
KlockatorpA	2.5	1.0	4.1	4.2	4..4	2.3	7.4	2.2
KlockatorpB[J]	0.7	9.8	3.3	2.5	7.7	4.3		12.1
Åby								
Lab B r.s.d. $C_{W,free}$ (n=3)								
Deje Syd	6.4	5.0	2.2	4.3	3.2	2.7	6.0	48.2
Jakbobsbyn	6.7	6.9	4.9	6.8	9.1	5.5	3.4	23.4
LjungaskogA	3.1	1.4	2.4	2.5	12.6	5.1	4.1	11.7
LjungaskogB	5.3	6.5	28.4	3.5	11.5	1.2	29.2	2.0
Kolleberga	0.5	2.8	2.8	2.5	16.5	7.4	0.7	21.4
Deje Nord	4.9	4.0	7.3	4.5	6.8	6.9		24.3
Sya	16.5	7.1	3.3	1.3	41.2	4.8		35.7
Stakheden	4.1	1.9	3.1	5.6	12.7	1.0		12.4
KlockatorpA	5.9	6.1	3.7	5.0	18.3	6.6	2.8	26.3
KlockatorpB[J]	5.2	1.1	2.0	0.6	9.9	12.0		30.6
Åby								

[A]NA, not analysed (missing data for this replicate)
[B] Depletion >80% therefore removed from further calculation and analysis
[C]Ljungaskog1: p,p-DDT and o,p-DDT excluded due to outliers.
[D]NA, not analysed (missing data for this replicate)
[E]Åby1 missing data for p,p-DDD
[F]*n*=2: p,p-DDT and o,p-DDT in Ljungaskog1 excluded due to outliers.
[G]*n*=2 (missing data for one replicate)
[H]*n*=2; Åby1 missing data for p,p-DDD
[I]*n*=2 (missing data for one replicate)
[J]*n*=2

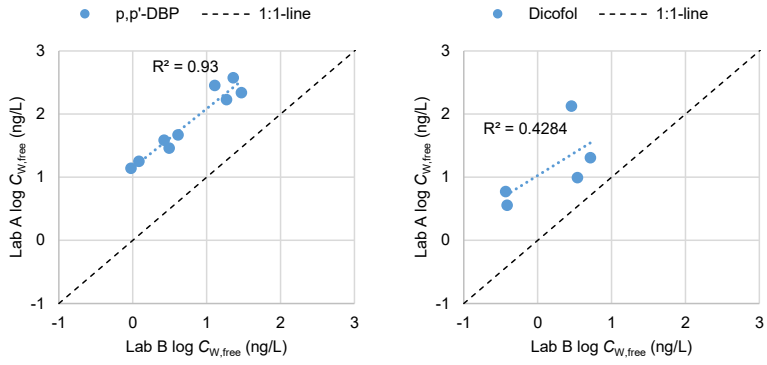


Figure S5: Comparison of pore water concentrations (log values) of *p,p'*-DBP and dicofol compounds from 9 sampling sites analysed by two labs. Dicofol data were excluded at 4 sampling sites due to high depletion.

S2.7 Worm Concentrations and Ecotoxicity tests

Table S25 Worm (*Eisenia fetida*) concentrations (ng/g WW) of DDXs and the fat (lipid) content of the worms (%).

C_{worm} (ng/g WW)	*p,p′*-DBP	*p,p′*-DDM	dicofol	*o,p′*-DDD	*p,p′*-DDD	*p,p′*-DDMU	*o,p′*-DDT	*o,p′*-DDE	*p,p′*-DDT	*p,p′*-DDE	Lipid (fat) content (%)
Deje Syd	15.9	1.20	193	167	392	12.3	584	28.5	2275	1534	2.03
Jakobsbyn	20.0	1.22	99.5	151	489	17.6	932	17.6	2329	664	2.46
Ljungaskog	31.0	1.45	243	186	645	17.6	1269	42.6	4268	2129	2.51
Kolleberga	11.1	0.94	100	185	483	9.24	1017	21.7	2749	810	1.97
Deje Nord	34.6	2.34	1186	249	696	23.4	1404	86.6	4488	2550	3.45[A]
Sya	182	5.08	6790	973	2418	37.0	3345	95.5	14273	6561	2.61
Stakheden	129	12.3	1213	511	1553	52.5	2338	150	6905	3935	2.29
Klockatorp	70.8	4.27	890	526	1857	37.7	2978	30	12143	1077	1.97
Åby	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ISO control worms	0.0	0.17	0	0	0	0	0	0	0	0	1.75

The sample from Åby was not analysed (NA), due to no surviving worms after 28 days of exposure.

[A]Overestimated value (some Na_2SO_4 was found in the extract which added extra weight).

Table S26 Individual DDX and sums of DDX lipid normalised worm (*Eisenia fetida*) concentrations expressed as internal residues (µmol/kg lipid).

C_{worm_lipid} (µmol/kg lipid)	*p,p′*-DBP	*p,p′*-DDM	dicofol	*o,p′*-DDD	*p,p′*-DDD	*p,p′*-DDMU	*o,p′*-DDT	*o,p′*-DDE	*p,p′*-DDT	*p,p′*-DDE	Σ*p,p′*-DDT/D	Σ*o,p′*-DDT/D	Σ8-DDX	Σ10-DDX
Deje Syd	3.13	0.25	25.8	25.7	60.4	2.1	81.2	4.42	317	238	377	107	755	758
Jakobsbyn	3.24	0.21	10.9	19.1	62.0	2.5	107	2.24	267	84.7	329	126	555	558
Ljungaskog	4.92	0.24	26.2	23.2	80.4	2.5	143	5.35	480	267	561	166	1 030	1 033
Kolleberga	2.24	0.20	13.7	29.3	76.4	1.7	145	3.46	393	129	469	175	792	794
Deje Nord[A]	>4.00	>0.29	>92.8	>22.6	>63.1	>2.4	>115	>7.89	>367	>232	>430	>137	>905	>907
Sya	27.8	0.82	702	116	289	5.0	361	11.5	1541	790	1 831	478	3 839	3 844
Stakheden	22.3	2.27	143	69.6	212	8.1	288	20.6	850	540	1 062	357	2 145	2155
Klockatorp	14.3	0.91	122	83.2	294	6.7	426	4.74	1736	172	2 031	509	2 852	2 860
Åby	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ISO control worms	0.0	0.04	0	0.0	0	0.0	0	0.01	0	0	0.03	0.02	0.06	0.10

[A] C_{worm_lipid} for the sample Deje Nord is underestimated due to an overestimated fat content of worms for this sample.

Table S27 log–log correlation coefficients (r^2) of lipid concentrations in *Eisenia fetida v.* various chemical measurements and slope and intercepts of log–log linear (y = kx + m) regressions (n = 7; the samples Åby and Deje Nord excluded, due to 100% mortality of worms and incorrect weight of fat content).

r^2	$\log C_{worm\_lipid}$ *v.* $\log C_{POM}$	$\log C_{worm\_lipid}$ *v.* $\log C_{S}$	$\log C_{worm\_lipid}$ *v.* $\log C_{TOC}$
p,p′-DBP	0.84	0.00012	0.24
dicofol[A]	0.97	0.93	0.97
o,p′-DDD	0.93	0.69	0.93
p,p′-DDD	0.82	0.61	0.89
o,p′-DDT	0.85	0.72	0.92
o,p′-DDE	0.95	0.60	0.89
p,p′-DDT	0.90	0.67	0.90
p,p′-DDE	0.93	0.65	0.93
Σ*p,p′*-DDT/D	0.90	0.67	0.92
Σ*o,p′*-DDT/D	0.89	0.71	0.93
Σ10-DDX	0.90	0.65	0.91
Slope values (k):			
p,p′-DBP	0.81	0.08	2.47
dicofol	0.66	0.95	1.13
o,p′-DDD	0.77	0.89	1.03
p,p′-DDD	1.05	0.84	1.13
o,p′-DDT	0.81	0.75	0.93
o,p′-DDE	0.80	0.98	1.12
p,p′-DDT	1.05	0.91	1.15
p,p′-DDE	0.88	1.36	1.28
Σ*p,p′*-DDT/D	1.05	0.90	1.16
Σ*o,p′*-DDT/D	0.82	0.76	0.95
Σ10-DDX	0.92	0.88	1.10
Intercept values (m):			
p,p′-DBP	0.37	0.86	-3.25
dicofol	0.72	1.68	-0.18
o,p′-DDD	1.18	1.74	-0.03
p,p′-DDD	0.93	1.75	-0.35
o,p′-DDT	1.02	1.85	0.12
o,p′-DDE	0.60	1.82	0.02
p,p′-DDT	0.50	1.71	-0.59
p,p′-DDE	0.78	1.63	-0.56
Σ*p,p′*-DDT/D	0.54	1.73	-0.60
Σ*o,p′*-DDT/D	1.06	1.87	0.10
Σ10-DDX	0.84	1.83	-0.41

The concentration of *p,p′*-DDM and *p,p′*-DDMU was close to LOQ in the soil and thus not included in the evaluation.

[A]n=5 (data for Sya and Stakheden excluded due to >100% depletion in the POM-tests.

S2.8 Calculation of K_{TOC}

Concentrations of DDX in the soil and pore water at equilibrium, $C_{soil,eq}$ and $C_{w,free}$ were used to calculate K_{TOC} (L/kg TOC) according to Eq S3 for the here studied soils:

$$K_{TOC} = \frac{K_d}{f_{TOC}} = \frac{C_{soil,eq}}{C_{W,free} * f_{TOC}} \quad \text{(S3)}$$

where f_{TOC} is the fraction of TOC in the soils (Table 2). The soil concentration at equilibrium, $C_{soil,eq}$, was calculated as the remaining amount in the soil at the end of the POM test ($m_{soil,eq}$, see Eqn S1 and S2 in S1.3.3), divided by the amount of soil in the tests (kg DW).

Table S28 log soil–porewater partitioning coefficients normalised to total organic carbon content, log K_{TOC} (L/kg TOC) and associated statistics (means; standard deviations, s.d.; and relative standard deviations, r.s.d. %).

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Lab A log K_{TOC}								
Deje Syd:a	5.89	6	5.94	5.86	6.16	6.25	5.86	5.54
Deje Syd:b	5.93	6	5.94	5.86	6.17	6.23	5.81	5.59
Deje Syd:c	5.85	5.94	5.95	5.82	6.11	6.23	5.82	5.5
Jakbobsbyn:a	6.37	6.49	6.46	6.35	6.35	6.52	6.09	5.87
Jakbobsbyn:b	6.34	6.46	6.46	6.32	6.37	6.51	6.02	5.87
Jakbobsbyn:c	6.32	6.44	6.44	6.34	6.36	6.52	6.1	5.86
LjungaskogA:a-II	5.89	6.02	5.96	5.84	6.02	6.18	5.47	5.45
LjungaskogA:b-II	5.86	6.02	5.95	5.81	6.02	6.18	5.43	5.37
LjungaskogA:c-II	5.93	6.04	6	5.87	6.07	6.22	5.47	5.48
Kolleberga:a	6.11	6.18	6.13	6	6.23	6.43	5.96	5.83
Kolleberga:b	6.07	6.18	6.13	6	6.24	6.45	6.01	5.84
Kolleberg:c	6.06	6.18	6.11	6.02	6.23	6.44	6.03	5.83
Deje Nord:a	5.9	5.95	5.85	5.79	6.12	6	-[B]	4.92
Deje Nord:b	5.91	5.99	5.88	5.82	6.11	6.02	-[B]	4.89
Deje Nord:c	5.87	5.93	5.82	5.79	6.13	6.02	-[B]	4.88
Sya:a	6.03	6.12	6.05	5.95	6.26	6.16	-[B]	4.76
Sya:b	6.04	6.06	6.12	5.96	6.26	6.15	-[B]	4.78
Sya:c	6.06	6.15	6.13	5.98	6.25	6.16	-[B]	4.78
Stakheden:a	5.81	5.84	5.82	5.67	6.14	6.11	-[B]	4.54
Stakheden:b	5.82	5.9	5.84	5.67	6.13	6.09	-[B]	4.56
Stakheden:c	5.85	5.89	5.85	5.67	6.14	6.13	-[B]	4.64
KlockatorpA:a-II	6.11	6.25	6.3	6.25	6.21	6.21	5.29	5.28
KlockatorpA:b-II	6.11	6.25	6.28	6.21	6.18	6.19	5.26	5.26
KlockatorpA:c-II	6.13	6.25	6.32	6.25	6.22	6.2	5.36	5.28
Åby:a	6.09	5.86	5.99	5.77	5.71	6.55	-[B]	4.7
Åby:b	6.1	5.96	6.03	5.81	5.79	6.59	-[B]	4.71
Åby:c	6.09	5.96	6.01	5.8	5.79	6.54	-[B]	4.85
LjungaskogB:a-II	6.11	6.22	6.19	6.08	6.23	6.41	5.74	5.65
LjungaskogB:b-II	6.01	6.18	6.09	6.05	6.15	6.32	5.58	5.65
LjungaskogB:c-II	6.05	6.17	6.16	6.03	6.19	6.37	5.62	5.62
KlockatorpB:a-II	6.36	6.56	6.54	6.52	6.39	6.45	5.77	5.53
KlockatorpB:b-II	6.35	6.55	6.48	6.51	6.37	6.42	5.74	5.48
Lab B log K_{TOC}								
Deje Syd:a	6.31	6.1	6.17		5.88	6.2	6.03	6.57
Deje Syd:b	6.28	6.07	6.16		5.84	6.19	5.99	6.27
Deje Syd:c	6.35	6.12	6.18		5.86	6.21	5.95	6.78
Jakobsbyn:a	6.68	6.46	6.62		6.4	6.55	6.88	7.01
Jakobsbyn:b	6.62	6.4	6.59		6.32	6.51	6.88	6.83
Jakobsbyn:c	6.68	6.47	6.64		6.31	6.56	6.91	6.75
LjungaskogB:a-I	5.76	5.51	6.14	5.56	6.06	6.19	5.88	6.31
LjungaskogB:b-I	6.36	6.13	6.47	5.54	5.99	6.2	6.18	6.52
LjungaskogB:c-I	6.32	6.07	6.19	5.6	6.1	6.19	5.89	6.54
Kolleberga:a	6.5	6.33	6.42	5.91	6.12	6.44	7.29	6.79
Kolleberga:b	6.49	6.31	6.41	5.89	6.08	6.4	7.3	6.95
Kolleberga:c	6.5	6.3	6.39	5.88	6.27	6.48	7.3	7.01
Deje Nord:a	6.18	6.03	6.14	5.94	5.81	6.07	6.09	5.68
Deje Nord:b	6.19	5.99	6.16	5.92	5.78	6.06	6.16	5.83
Deje Nord:c	6.14	5.99	6.08	5.89	5.86	6	5.96	5.94
Sya:a	6.13	6.05	6.2	5.99	5.8	5.91	6.02	5.78
Sya:b	6.3	6.1	6.22	5.99	5.49	5.96	6.14	6.02
Sya:c	6.27	6.13	6.23	5.99	6.04	5.92	6.1	6.16
Stakheden:a	6.25	5.99	6.09	5.96	5.92	6.04	5.94	6.27
Stakheden:b	6.26	6	6.08	5.94	6.06	6.05	5.94	6.16
Stakheden:c	6.22	5.98	6.12	6.02	5.96	6.04	5.97	6.14

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
KlockatorpB:a-I	6.43	6.49	6.68	6.76	6.19	6.38	7.23	6.52
KlockatorpB:b-I	6.43	6.47	6.69	6.73	6.38	6.41	6.41	6.5
Åby:a	6.14	6.2	6.86	7.3		6.2	6.07	6.47
Åby:b	6.18	6.19	6.85	7.31	5.48	6.26	6.04	6.51
Åby:c	6.2	6.19	6.84	7.3	5.65	6.24	6.09	6.23
LjungaskogA:a-I	6.2	5.91	5.98	5.17	5.97	6.07	6.88	5.95
LjungaskogA:b-I	6.17	5.9	5.95	5.2	5.96	6.08	6.88	6.04
LjungaskogA:c-I	6.17	5.9	5.98	5.27	5.85	6.03	6.84	6.06
KlockatorpA:a-I	6.08	6.22	6.47	6.38	6.1	6.09	7.21	5.45
KlockatorpA:b-I	6.08	6.18	6.42	6.35	5.96	6.08	7.23	5.47
KlockatorpA:c-I	6.02	6.15	6.43	6.32	5.89	6.02	7.2	5.23
Lab A Mean log K_{TOC}								
Deje Syd	5.89	5.98	5.95	5.85	6.14	6.24	5.83	5.55
Jakbobsbyn	6.34	6.46	6.45	6.34	6.36	6.51	6.07	5.87
LjungaskogA	5.89	6.02	5.97	5.84	6.04	6.2	5.46	5.43
Kolleberga	6.08	6.18	6.13	6.01	6.23	6.44	6	5.83
Deje Nord	5.89	5.96	5.85	5.8	6.12	6.01	3.56	4.89
Sya	6.04	6.11	6.1	5.96	6.25	6.16	4.74	4.77
Stakheden	5.82	5.87	5.84	5.67	6.14	6.11	NA	4.58
KlockatorpA	6.12	6.25	6.3	6.24	6.21	6.2	5.3	5.27
Åby	6.09	5.93	6.01	5.79	5.76	6.56	NA	4.75
Mean of sites with <10% depletion:	6.16	6.23	6.38	6.29	6.19	6.27	6.07	5.59
s.d.	0.12	0.18	0.11	0.07	0.1	0.19	NA	0.26
N	4	4	2	2	8	9	1	5
Lab B Mean log K_{TOC}								
Deje Syd	6.31	6.1	6.17	NA	5.86	6.2	5.99	6.54
Jakbobsbyn	6.66	6.45	6.62	NA	6.34	6.54	6.89	6.87
LjungaskogB	6.15	5.91	6.27	5.57	6.05	6.19	5.99	6.46
Kolleberga	6.49	6.32	6.41	5.89	6.16	6.44	7.3	6.92
Deje Nord	6.17	6	6.12	5.92	5.82	6.04	6.07	5.82
Sya	6.23	6.1	6.22	5.99	5.78	5.93	6.08	5.99
Stakheden	6.24	5.99	6.1	5.97	5.98	6.04	5.95	6.19
KlockatorpB[A]	6.43	6.48	6.68	6.75	6.29	6.39	6.82	6.51
Åby	6.17	6.19	6.85	7.3	5.57	6.23	6.07	6.4
Mean of sites with <10% depletion:	6.34	6.31	6.61	7.02	6.11	6.26	6.67	6.41
s.d.	0.16	0.16	0.17	0.39	0.18	0.18	0.49	0.37
N	9	5	5	2	6	8	5	9
Lab A s.d. log K_{TOC}								
Deje Syd	0.042	0.033	0.006	0.028	0.032	0.011	0.025	0.043
Jakbobsbyn	0.026	0.023	0.012	0.014	0.008	0.007	0.045	0.003
LjungaskogA	0.034	0.012	0.028	0.029	0.028	0.022	0.022	0.058
LjungaskogB	0.053	0.028	0.047	0.022	0.044	0.045	0.085	0.015
Kolleberga	0.024	0.002	0.01	0.012	0.009	0.009	0.035	0.005
Deje Nord	0.017	0.031	0.031	0.013	0.009	0.013	1.76	0.02
Sya	0.015	0.045	0.044	0.018	0.008	0.002		0.01
Stakheden	0.02	0.032	0.019	0.005	0.005	0.019		0.054
KlockatorpA	0.014	0.005	0.023	0.023	0.023	0.012	0.054	0.011
KlockatorpB[A]	0.004	0.006	0.042	0.005	0.015	0.023	0.019	0.037
Åby	0.004	0.059	0.022	0.019	0.046	0.024		0.082
Lab B s.d. log K_{TOC}								
Deje Syd	0.034	0.026	0.008		0.017	0.013	0.04	0.257
Jakbobsbyn	0.035	0.037	0.027		0.052	0.029	0.018	0.134
LjungaskogA	0.017	0.006	0.017	0.052	0.068	0.027	0.021	0.061
LjungaskogB	0.017	0.006	0.017	0.052	0.068	0.027	0.021	0.061
Kolleberga	0.003	0.016	0.017	0.018	0.098	0.04	0.004	0.11
Deje Nord	0.026	0.022	0.04	0.024	0.044	0.037	0.101	0.13
Sya	0.092	0.041	0.017	0.004	0.278	0.028	0.06	0.19
Stakheden	0.023	0.012	0.02	0.037	0.075	0.006	0.02	0.069
KlockatorpA	0.032	0.035	0.022	0.029	0.107	0.036	0.015	0.135
KlockatorpB[A]	0	0.013	0.005	0.016	0.135	0.02	0.578	0.014
Åby	0.031	0.006	0.011	0.003	0.118	0.028	0.027	0.153
Lab A r.s.d.% log K_{TOC} (s.d./mean)								
Deje Syd	0.70%	0.60%	0.10%	0.50%	0.50%	0.20%	0.40%	0.80%
Jakbobsbyn	0.40%	0.40%	0.20%	0.20%	0.10%	0.10%	0.70%	0.10%
LjungaskogA	0.60%	0.20%	0.50%	0.50%	0.50%	0.40%	0.40%	1.10%
LjungaskogB	0.60%	0.20%	0.50%	0.50%	0.40%	0.40%	0.40%	1.00%
Kolleberga	0.40%	0.00%	0.20%	0.20%	0.10%	0.10%	0.60%	0.10%
Deje Nord	0.30%	0.50%	0.50%	0.20%	0.10%	0.20%	49%	0.40%
Sya	0.30%	0.70%	0.70%	0.30%	0.10%	0.00%		0.20%
Stakheden	0.30%	0.50%	0.30%	0.10%	0.10%	0.30%		1.20%
KlockatorpA	0.20%	0.10%	0.40%	0.40%	0.40%	0.20%	1.00%	0.20%

	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
KlockatorpB[A]	0.10%	0.10%	0.60%	0.10%	0.20%	0.40%	0.30%	0.70%
Åby	0.10%	1.00%	0.40%	0.30%	0.80%	0.40%		1.70%
Min.	0%	0%	0%	0%	0%	0%	0.30%	0%
Max.	3%	4%	3%	4%	4%	4%	49%	4%
Lab B r.s.d.% log K_{TOC} (s.d./mean)								
Deje Syd	0.50%	0.40%	0.10%		0.30%	0.20%	0.70%	3.90%
Jakbobsbyn	0.50%	0.60%	0.40%		0.80%	0.40%	0.30%	2.00%
LjungaskogA	0.30%	0.10%	0.30%	1.00%	1.10%	0.40%	0.30%	1.00%
LjungaskogB	0.30%	0.10%	0.30%	0.90%	1.10%	0.40%	0.40%	0.90%
Kolleberga	0.00%	0.30%	0.30%	0.30%	1.60%	0.60%	0.10%	1.60%
Deje Nord	0.40%	0.40%	0.70%	0.40%	0.70%	0.60%	1.70%	2.20%
Sya	1.50%	0.70%	0.30%	0.10%	4.80%	0.50%	1.00%	3.20%
Stakheden	0.40%	0.20%	0.30%	0.60%	1.30%	0.10%	0.30%	1.10%
KlockatorpA	0.50%	0.60%	0.30%	0.40%	1.80%	0.60%	0.20%	2.50%
KlockatorpB[A]	0.00%	0.20%	0.10%	0.20%	2.10%	0.30%	8.50%	0.20%
Åby	0.50%	0.10%	0.20%	0.00%	2.10%	0.50%	0.50%	2.40%
Min.	0%	0%	0%		0%	0%	0%	0%
Max.	2%	2%	2%		5%	2%	8%	4%

Values highlighted in red indicate depletion >10% (see S1.3.3 Control of depletion of DDX).

[A]$n = 2$, otherwise $n = 3$.

[B]Not calculated due to depletion >80% for POM-soil tests at Lab A.

Table S29 Ratio between log K_{TOC} derived by Lab A and Lab B.

Ratio log K_{TOC}/log K_{TOC} LabA/LabB	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	dicofol	*p,p'*-DBP
Deje Syd						1.01		0.85
Jakbobsbyn	0.95	1.00	0.98		1.00	1.00	0.88	0.85
Ljungaskog		0.99			1.00	1.00		0.84
Kolleberga	0.94	0.98			1.01	1.00		0.84
Deje Nord					1.04	0.99		
Sya								
Stakheden					1.03	1.01		
Klockatorp	0.95	0.97	0.94	0.92	0.99	0.97		0.81
Åby	0.99					1.05		

Data from tests with >10% depletion (see Table S10) was excluded.

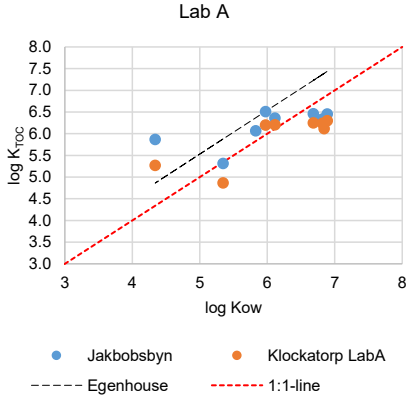


Figure S7: log K_{TOC} values for two of the studied soils (Jakobsbyn and Klockatorp) versus log K_{OW}. The relationship between log K_{TOC} and log K_{OW} (y=1.01×log K_{OW} +0.48) found by Eganhouse et al., (2018) for DDX in sediments has been included for comparison. Our log K_{TOC} values for the studied soils did not show particularly good linear correlation with log K_{OW} (Pearson $r = 0.69$ and 0.80). This is probably due to degradation of the thermolabile compounds (and formation of degradation products) during the GC-analysis leading to incorrect K_{TOC} values. For example, log K_{TOC} of *p,p'*-DBP (log K_{OW} = 4.3) seem to have been overestimated, while the K_{TOC} of the DDTs (K_{OW} of 6.68 and 6.84) may have been underestimated in the given examples.

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Enhanced DDT degradation in aged soils via fungal treatment and surfactant application

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is a legacy pesticide that, despite decades of discontinued use, remains in soils worldwide, posing long-term risks to ecosystems and human health. This study investigated the mycoremediation potential of 5 fungal species in historically contaminated forest nursery soils from Sweden, focusing on the degradation of DDT and its transformation products (collectively referred to as DDX). Aged, contaminated soils were incubated with fungi and a lignocellulosic substrate (straw) for 30 days in petri dishes. *Pholiota adiposa, Trametes versicolor* and *Pleurotus ostreatus* achieved the DDX degradation at 80 ± 3%, 72 ± 8%, and 69 ± 3% respectively, while *Hypholoma fasciculare* achieved lower degradation (45 ± 15%), and *Agaricus bisporus* showed no significant degradation. A complementary experiment, where the same soils were exposed to a liquid solution of enzymes from *P. ostreatus* for 8 days demonstrated that the addition of the surfactant Tween 80 mobilized soil-bound DDX into aqueous solution, where degradation by *P. ostreatus* enzymes occurred. Tween 80 also enhanced ligninolytic enzyme activity, suggesting synergistic effects on DDX degradation. This is the first study to compare bioremediation performance of multiple white-rot fungi of DDT in historically contaminated field soil using up-scalable and environmentally relevant conditions. Its findings support the use of selected ligninolytic fungi and amendment with the surfactant Tween 80, and therefore provides a basis for future environmental remediation.

1. Introduction

Dichlorodiphenyltrichloroethane (DDT) is a toxic, hydrophobic and persistent organochlorine compound that has been used as a pesticide across the world. It is now banned in many countries and is known for its harmful environmental and health effects (Stockholm [1]). The environmental degradation rate of DDT is slow, and the half-life in soils can be over 30 years [2]. In addition, during the partial degradation of DDT, persistent and toxic transformation products, such as dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD) and dicofol, are formed [3,4]. In environmental assessments, DDT and its break-down products DDE and DDD are commonly grouped as the primary toxic transformation products, and collectively termed DDTs, as they will hereafter be referred to in this paper.

Lignin-degrading fungi, particularly those with a "white-rot" degradation strategy, have been studied in laboratory settings for their capacity to degrade DDT and its transformation products using oxidative lignin-degrading mechanisms [3]. White-rot fungi are typically saprotrophs within the mushroom-forming class *Agaricomycetes* [5]. To decay lignin, they rely on an extracellular oxidative process involving enzymes such as class II peroxidases, laccases, and dye decolorizing peroxidases [6]. During plant cell wall decomposition, wood and recalcitrant litter decomposers must overcome lignin, which hinders access to cellulose and hemicellulose. Degradation requires enzymes or oxidative agents capable of acting on the complex, non-hydrolysable lignin structures. Additionally, the white-rot enzyme system may help scavenge complex-bound nitrogen in nutrient-poor environments [7]. The relevance of these oxidative systems for remediation is that ligninolytic enzymes are versatile enough to act on chlorinated aromatic pollutants like DDT and its residues [8]. Since DDT can degrade into several transformation products, DDT and all its degradation residues are hereafter referred to as DDX.

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Ligninolytic enzymes are exuded extracellularly, allowing them and the intermediary oxidative compounds they produce to permeate their environment. One example is manganese-dependent peroxidases (Mn-peroxidases), class II peroxidases that oxidizes compounds at long-range using high-redox, low molecular weight intermediates [6]. These peroxidases use Mn^{2+}, H^{+}, and H_2O_2 as substrates, producing Mn^{3+} and H_2O, and require continuous H_2O_2 release by the fungi to remain active. Similar mechanisms apply to other peroxidases, while laccases cleave phenolic rings using O_2 as an electron acceptor [9]. Thus, lignin-degrading enzymes can act on many phenolic soil substances. However, due to the short half-life of isolated enzymes, live fungi must be introduced to non-sterile, contaminated soils to ensure continuous enzyme production and oxidative activity [8].

Bioremediation with lignin-degrading fungi should therefore optimize fungal growth and production of enzymes and oxidative agents, especially H_2O_2. However, fungal DDT degradation by oxidative enzymes is complex, producing toxic, less hydrophobic transformation products. This reduces sorption of DDTs to soil organic matter and increases their bioavailability to fungi and other organisms. Lower hydrophobicity also raises vertical mobility, risking groundwater or surface water contamination [10]. It is therefore essential to analyze both primary and later-stage DDT transformation products to assess risks of fungal treatment.

The most comprehensive fungal DDT degradation pathway has been outlined by Mohapatra et al. [8]. DDT first undergoes reductive dechlorination to form toxic DDE and DDD [3,4], followed by oxidative dechlorination that increases solubility via carboxyl group addition and removal of the other two aliphatic chlorines (Fig. 1). This enables broader microbial degradation, including by bacteria. Dicofol, for example, is transformed to the less harmful DBP through reduction and hydroxylation [11], with further oxidation and hydroxylation reactions leading to ring cleavage and mineralization to CO_2 [12].

The brown rot fungi *Fomitopsis pinicola* and *Gloeophyllum trabeum* degrade DDX using Fenton chemistry [13–15], while *Pleurotus ostreatus* appears to use Mn-peroxidase, lignin-peroxidase, and cytochrome P450 [14]. Co-cultivating *G. trabeum* with the surfactant-producing bacterium *Ralstonia picketti* increased DDT degradation from 54% to 75% in 7 days [15], and 62% degradation was achieved using purified laccase [16]. More recently, *Aspergillus niger* was shown to degrade up to 99.7% of DDTs, although in liquid culture [17]. This underscores a key challenge in mycoremediation; while many fungi and bacteria can degrade DDX, applying these methods to aged, contaminated soils and at large scale in situ remains difficult [18].

Surfactants are widely used in soil remediation to reduce the sorption of hydrophobic pollutants to organic matter, enhancing their mobility [19,20]. In soil washing, their amphiphilic nature enables transfer of hydrophobic contaminants into the aqueous phase. Hydrophobic tails bind pollutants, while hydrophilic heads interact with water, reducing surface tension and improving infiltration into soil pores. This mechanism encapsulates DDX in hydrophobic micelle cores, increasing bioavailability [20]. However, surfactants pose challenges such as microbial toxicity, high costs, and persistent emulsions. Tween 80, a biodegradable, polyoxyethylene, low-toxicity non-ionic surfactant, shows promise. It is minimally toxic to fungi and roots, and unlike ionic surfactants, does not strongly adsorb to soil minerals [20], allowing continued contaminant mobilization and microbial degradation [21]. It also performs well in organic-rich soils where hydrophobic pollutants are tightly sorbed.

In boreal countries, the forest industry used DDT against pine weevil (*Hylobius abietis*) infestations until 1974 [4,22]. Consequently, over 50 old forest nurseries in Sweden are classified as heavily polluted with DDT [23], and efficient and sustainable remediation methods are searched for. In the present study, soil from one of these polluted fields was treated with different fungi, and the impacts on DDX were studied. Additionally, *Pleurotus ostreatus* (oyster mushroom) was used to produce a water suspension of ligninolytic enzymes, which was studied for its capacity to degrade DDX in the liquid phase, with the use of Tween 80 to enhance solved concentrations.

Although white-rot fungi have been shown to transform DDT under laboratory conditions, it remains uncertain whether this capacity translates to historically contaminated field soils, where long-term sorption and reduced bioavailability constrain degradation. Many previous studies have relied on liquid cultures [24,25] or artificially spiked soils [26], but no one has yet evaluated fungal DDX degradation in field soils contaminated many decades ago. A fungus-to-soil ratio compatible with potential scale-up was applied, and the fungi were inoculated using grain spawn, a commercially available material. Effective mycoremediation requires both the ability to transform DDX and the capacity

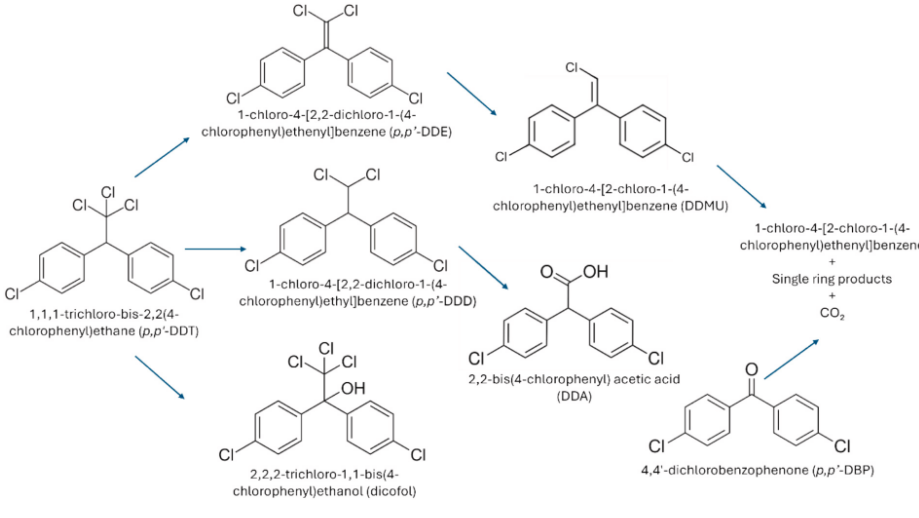

Fig. 1. Proposed pathway of DDT transformation by white-rot fungi adapted from Mohapatra et al. [8].

to colonize and express degradative enzymes in the polluted soil; therefore, multiple fungal species were compared under controlled conditions. It was hypothesised that Tween 80 would enhance DDX accessibility and the influence of Tween 80 was assessed in a slurry-based enzyme suspension. Additionally, it was expected that strong wood colonisers would perform well due to the high expression of ligninolytic enzymes. However, as soil-adapted fungi are favoured by the environment, a comparable activity of this type of fungi cannot be ruled out. While fungal transformation of DDT and the use of Tween 80 as a surfactant are not in themselves novel techniques, this study aimed to bridge the gap between laboratory research and real-world application by evaluating these approaches under more environmentally relevant and scalable conditions. The overall goal was to develop a method that could be progressively scaled up for on-site remediation of persistent organic pollutants.

2. Methods

2.1. Microorganisms and spawn production

Agar cultures of *Pleurotus ostreatus* M2191, *Hypholoma fasciculare* MUCL 047611, *Agaricus bisporus* MUCL 031615 and *Trametes versicolor* M9911 were obtained from Mycelia BVBA, Belgium. The species *Pholiota adiposa* M4200 was obtained from Ecofungi, Sweden. The cultures were maintained on Modified Melin-Norkrans (MMN) medium, prepared according to the recipe from Islam & Ohga [27], with a final pH of 5.6. The medium contained 10 g L^{-1} glucose, 3 g L^{-1} malt extract, 0.25 g L^{-1} $(NH_4)_2HPO_4$, 0.025 g L^{-1} NaCl, 0.5 g L^{-1} KH_2PO_4, 0.05 g L^{-1} $CaCl_2$, 0.15 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.012 g L^{-1} $FeCl_3{\cdot}6H_2O$, 0.003 g L^{-1} thiamine, and 15 g L^{-1} agar. For production of spawn, the fungal cultures were propagated until full colonization on sterile rye grain amended with 4% $CaCO_3$ (weight (w)/w of dry mass) at 22 °C.

2.2. Soil sampling and treatment material characterization

A soil sample was obtained from the Kolleberga forest nursery (Klippan municipality, Sweden, co-ordinates: 56.070803, 13.265375) in early autumn 2023 as a core from the surface layer to a depth of 20 cm. Average temperatures that month were 21 °C during the day and 15 °C at night, with ~25 mm of precipitation [28]. The collected soil was sieved through a 2 mm mesh and stored at 4 °C in the dark for approximately 8 weeks prior to use, in order to limit microbial activity and prevent plant (flora) growth. Before the start of the experiment, the soil was analyzed for its content of DDT and its transformation products (2.3.2). In addition, pH was determined in a 1 g wet weight (ww) soil to 10 mL deionized water slurry using a pH meter (S20 SevenEasy pH, Mettler Toledo AB, Stockholm, Sweden). Total nitrogen and total carbon were determined by dry combustion (1350 °C) of 0.5 g material and elemental analysis using a TruMac instrument (LECO Corporation, MI, USA). Water content was measured gravimetrically by drying 0.5 g material at 105 °C (24 h). Organic matter content was measured by combustion at 505 °C for 4 h. Inorganic nitrogen was extracted from the treatments using 2.5 mL of 2 M KCl per 1 g of soil and shaking overnight. Samples were then centrifuged at 3000 rpm for 10 min and passed through a 0.2 μm syringe filter (Product number: 15141499, Fisherbrand™, Gothenburg, Sweden) and analyzed for ammonium and nitrate by an AA3 AutoAnalyzer (SEAL Analytical, Mequon, WI, USA). Soil oxidative capacity was measured with an enzymatic assay of ligninolytic enzymes as described in section 2.5. The described analyses were also performed for all treatment material samples taken at the start (day 1) and end (day 30) of the experiment.

2.3. Microcosm experiments

2.3.1. Experimental set-up in petri dishes

To analyze the potential of different fungal strains for DDT degradation, the experiment was set up with five different fungal species (*P. ostreatus, H. fasciculare, A. bisporus, Ph. adiposa* and *T. versicolor*) plus one control. The control was composed of heat-killed (autoclaved) spawn instead of live grain spawn. The experiment was performed with four replicates, forming a total of 24 microcosms (petri dishes). Sampling was done on day 1 and after 30 days of incubation.

For each fungal treatment, 132 g dry weight (dw) of the sieved Kolleberga soil was mixed by hand in a pre-cleaned (burned at 400 °C) glass beaker with 44 g ww of fungal grain spawn. Dehydrated straw pellets (Granngården, Sweden, product number: 1165829) were then mixed with deionized water at a 1:4 ratio. An amount of 44 g of the hydrated straw pellets was then added to the beaker as additional substrate for fungal growth. The control treatment was inoculated with 44 g (ww) heat-killed (autoclaved) spawn of *P. ostreatus*. The material in the beakers (~220 g/treatment or control) was carefully mixed.

To create replicates ($n = 4$) for each treatment ($n = 5$) and the control, 4 subsampled aliquots (55 g ww) of the prepared mixture were added to glass petri dishes (per treatment/control).

Each replicate was then gently mixed once more. For chemical analysis for the initial state (day 1), 5 g (ww) of each dish was sampled for DDX, organic matter, water content, inorganic nitrogen and pH analysis as described in section 2.2. Thus the experiment was made up by 6 × 4 petri dishes each consisting of 50 g of the soil/fungal grain spawn/straw mixture. The petri dishes were kept at 20 °C for 30 days, and the subsamples for chemical analyses were stored at −20 °C in amber glass jars with PFTE lids until analysis.

On day 30, each petri-dish was weighed and the contents homogenized by grinding with pestle and mortar. A sample of 10 g (ww) was taken from each petri-dish for DDX analysis. Samples of 5 g were also taken from the petri-dishes for measurements of organic matter, water content, and inorganic nitrogen and pH. All samples were kept and stored as for day 1.

In the creation of the different treatments, the addition of fungal spawn was by wet weight. As the water content of each fungal spawn varied slightly, the total water of the petri-dish also varied. Consequently, during subsampling by wet weight, the soil fraction could be underrepresented in samples from dishes where water content was highest. This effect would be most pronounced for the control, where autoclaved spawn (highest water content) was used.

2.3.2. Experimental set-up in respiration tests

The aim of this test was to determine the ability of *P. ostreatus* to survive and propagate in DDX contaminated soil and to observe the activity of the native microorganisms in response to heat-killed spawn addition.

Respiration in the soil alone, soil with addition of heat-killed spawn, and soil with addition of grain spawn of *P. ostreatus* were measured in triplicate. This experiment was performed in boxes suited for mushroom production (filter #10, Sac O2, Nevele, Belgium). Each box contained 500 g of soil and 50 g of spawn. Respiration was measured using carbon dioxide loggers (Extech CO210, Nashua, USA), which were placed directly above the gas exchange filter on the box. Each box was enclosed in a plastic cone with height 45 cm, a closed base, and an open top with diameter 25 cm. Carbon dioxide emissions were measured once every hour for 14 days.

2.3.3. Experimental set-up in water suspension

In this experiment, the impact of a surfactant, Tween 80, on release of organic matter bound DDX into the liquid phase and subsequent DDX degradation by fungal ligninolytic enzymes was studied.

Soil collected from the Kolleberga plant school, as previously described in section 2.2, was used. Spawn of *P. ostreatus* was produced as described above, section 2.1; however, wheat grains were used instead of rye grains. Heat-treated straw pellets were used to induce the production of ligninolytic enzymes. The surfactant Tween 80 (Product number P1754, Merck KGaA, Darmstadt, Germany) was applied at 1 g

L^{-1}, according to Guo et al. [20].

To create a water suspension that promoted production of ligninolytic enzymes, heat-treated straw pellets were shaken in de-ionised water overnight (10 g L^{-1}). The straw was filtered out using 6 μm filter paper (Whatman™ qualitative filter paper, Grade 3, Product number 28413989, Merck KGaA, Darmstadt, Germany) and the solution was sterilized. Aliquots (50 mL) of the solution was transferred to autoclaved 300 mL Erlenmeyer flasks, and spawn of *P. ostreatus* was added (40 g L^{-1}). The inoculated samples were then cultivated on a shaker at 200 rpm for 5 days for enzyme production. After this, the spawn and produced mycelium were filtered out, again using 6 μm filter paper. Meanwhile, un-inoculated straw solution was stored at 4 °C. Contaminated soil was added to each flask at a concentration of 100 g L^{-1} (5 g of soil), and Tween 80 was added at 1 g L^{-1} to a subset of the samples. The composition of treatments and the control are described in Table 1. All samples were incubated on a shaker at 150 rpm for 8 days. Each day, 1 mL aliquots were taken from each flask and frozen at −20 °C for analysis of ligninolytic enzyme activity. After 8 days, the samples were centrifuged at 3000 rpm for 3 min, and the supernatant was collected and stored at 4 °C until analysis.

2.4. Quantification of DDX

Grade and vendors of all chemicals and materials used for chemical analysis are listed in Table S1 in the supporting information.

2.4.1. Extraction and cleanup of DDX in soil

The extraction and sample clean-up followed the procedure described by Enell et al. [29] (Part 2 - Extraction and clean-up of soils; Lab B). In short, after treatment, the soil samples were freeze dried, and a subsample of the dried soil (0.5–1 g) was placed into a pre-cleaned Soxhlet thimble, to which 10 ng of 7 isotope-(^{13}C)-labelled internal standards (10 μL of 1 ng μL^{-1} of each IS in an isooctane solution) (Table S2) was added. The sample was Soxhlet extracted for 24 h using 200 mL DCM similar to Huang et al. [30], rotary evaporated until 5 mL remained and then transferred to a 15 mL glass test tube. The sample was further evaporated under a gentle stream of nitrogen gas and the solvent was exchanged to *n*-hexane with a final volume of 1 mL. A procedural solvent blank was included in every batch of samples analyzed.

Activated granular copper (1 g) was added to the extract, which was mixed vigorously to remove any sulfur present. The extract was then further cleaned by transferring it onto an alumina silica column (10 mm diameter) composed of (from bottom to top) Al_2O_3:SiO_2 (1:2 *v*/v, 6.1 g) and sodium sulfate (Na_2SO_4, 3 g) similar to Huang et al. [30]. Target compounds were eluted with 45 mL DCM:*n*-hexane (2:3 *v*/v). The extract was then rotary evaporated to 5 mL, transferred to a 15 mL glass test tube, and further evaporated under a gentle stream of nitrogen gas, after which the solvent was exchanged to isooctane with a final volume of 2 mL. After addition of the recovery standard (Table S2), the extract was vortexed (5 min), before a 0.5 mL aliquot was transferred to a GC vial for instrumental analysis.

Table 1
Treatments and control composition in the experiment performed in water suspension ($n = 4$, except for Treatment 2; $n = 3$ due to mould contamination).

	Tween 80	*P. ostreatus* enzyme suspension	Sterilized straw solution	DDX contaminated soils
Control (Control)	−	−	+	+
Treatment 1 (T80)	+	−	+	+
Treatment 2 (*P. ostreatus*)	−	+	−	+
Treatment 3 (T80 + *P. ostreatus*)	+	+	−	+

T80: Tween 80.

2.4.2. Extraction of DDX in water suspension

The apparently dissolved concentration of DDX in the liquid fraction was measured using liquid-liquid-extraction. A portion (30 mL) of the (supernatant) was combined with DCM at a ratio of 1:1 (v/v) in a separation funnel. Isotope-(^{13}C)-labelled internal standard was added (10 ng per IS; Table S2). The mixture was shaken for 1 min, then left undisturbed for 5 min until 2 distinct layers formed. The solvent layer was drained into a pre-cleaned (burned at 400 °C and rinsed with DCM) conical flask, and the extraction was repeated once. Any remaining water content of the extract was removed by addition of Na_2SO_4, and the organic solvent was then poured into a round bottom flask through filter paper (Whatman™ Grade 3). The sample was rotary evaporated until ~3 mL remained and transferred to a glass test tube. The sample was further evaporated under a gentle stream of nitrogen gas and the solvent exchanged to isooctane, with a final volume of 1 mL. Recovery standard was then added (10 ng; Table S2). Instrumental analysis by GC–MS/MS was conducted in the same way as described in section *2.4.3.*

2.4.3. DDX analysis by GC–MS/MS

Gas chromatography-tandem mass spectrometry (GC–MS/MS) analysis was also performed following the procedure described by Enell et al. [29] (Lab B); using an Agilent 7890 A gas chromatograph coupled to an Agilent 7010 triple quadrupole mass spectrometer (GC–MS/MS Triple Quad) equipped with an Agilent 7693 autosampler. Instrumental conditions (GC–MS/MS injector, oven program, column, and source parameters) were identical to those described [29]. The analysis was performed using multiple reaction monitoring (MRM) mode. Data processing and quantification were performed using Agilent MassHunter Quantitative Analysis (for QQQ) software. The limit of detection (LOD) was set to a signal-to-noise ratio of 3, and the limit of quantification (LOQ) as the lowest calibration point reliably quantified by the instrument.

A *p,p*'-DDT quality control solution was prepared (Table S2) to monitor degradation of DDT to DDD in the GC–MS injector, with a limit of between 10% and 20% degradation deemed acceptable to report DDT and DDD separately [31,32]. As the degradation of DDT within this study never exceeded 10%, compounds could be reported separately.

In the petri-dishes experiment, the total amount of DDX (μg) post treatment (day 30) in each petri dish treatment was calculated by multiplying the DDX concentration (μg g^{-1} dw) in the treatment material by the total amount of dry material in the petri-dish. Thus, samples from day 1 and day 30 were characterized and compared. Total amount was presented rather than concentrations, to mitigate up-concentration effects as organic matter degraded, decreasing the mass in the dish while the DDX amount remained unchanged.

2.5. Ligninolytic enzyme assay

Ligninolytic enzyme assays were performed on subsamples of each liquid culture using the colorimetric MBTH-DMAB method [33] as described in Kyaschenko et al. [34]. A SpectraMax Plus 384 microplate reader (Molecular Devices, San Jose, CA, USA) was used to measure absorbance at 590 nm, caused by the oxidative binding of MBTH to DMAB in the presence of Mn^{2+}, at intervals of 15 s over a 10-min period. The bonding of MBTH to DMAB can be catalyzed by Mn-peroxidase, lignin-peroxidase and laccase, all of which are, according to literature, capable of degrading DDX. Here, the assay results are reported as the added activity of all these potential activities (i.e. Mn-independent and H_2O_2 independent activities were not subtracted). The initial rate of increase in absorbance was converted to U oxidative activity per μL of culture medium of using a standard curve created with purified Mn-peroxidase (Sigma-Aldrich product no: 803057; CAS: 114995–15-2).

2.6. Statistical analysis

Changes in organic matter percentage (% OM) over time and across treatments were assessed using a linear mixed-effects model. The model included Treatment (Control, *P. ostreatus*, etc.), Time Point (day 1, day 30), and their interaction (Treatment × Time Point) as fixed effects. Petri dish was included as a random effect to account for repeated measures of the same dish. The model was fitted using the lmer function in R from the package lme4 (version 1.1–37). The same was done for inorganic nitrogen content, but with log transformation due to skewed data. Differences in DDX total amounts before and after treatment were also analyzed using linear mixed model effects, with the same fixed effects as listed above. Post-hoc testing was performed using R package emmeans to conduct Tukey's HSD analysis for pairwise differences between treatments. PERMANOVA was used to assess whether the relative composition of compounds changed between time points (adonis2 function; vegan package; R), using the Euclidean distance as dissimilarity measure, with Petri dish number (1–24) as a stratification factor, and 999 permutations. Significance threshold for all analyses was set to $\alpha = 0.05$. Area under curve (AUC) was calculated to compare the CO_2 outputs in the respiration tests, with ANOVA and Tukey HSD post-hoc comparison.

3. Results

3.1. DDX degradation in soil microcosms by white rot fungi

Soil concentrations of DDT and its transformation products before and after treatment are shown in Fig. 2 and in Table S3. The total amounts of DDX (∑DDX amounts) in the systems were significantly affected by treatment, time and their interaction. Post-hoc tests indicated that ∑DDX decreased significantly for *P. ostreatus, T. versicolor, Ph. adiposa,* and *H. fasciculare*, but not for *A. bisporus* and the control with heat-killed inoculum. The ∑DDX amounts were decreased most significantly by *Ph. adiposa* at 80 ± 3%, *T. versicolor* (72 ± 8%), *P. ostreatus* (69 ± 3%), and *H. fasciculare* (45 ± 15%). In pair-wise post-hoc testing (Tukey HSD; $\alpha = 0.05$) *Ph. adiposa* showed significantly higher degradation than all other species except *T. versicolor,* while *P. ostreatus* showed statistically indistinguishable degradation to *T. versicolor* (Table 2). Degradation by *H. fasciculare* was significant, but lower than all other species except for *A. bisporus*.

There was some variation in starting amounts of DDX between the treatments (Fig. 2), which likely can be related to individual variation in spawn properties, as described in section 2.3.1. Despite this variation, a clear trend was observed with a major reduction in DDX amounts for treatments based on *P. ostreatus, T. versicolor* and *Ph. adiposa*. The chemical analysis of DDX proved to be robust as mean relative standard deviation in total DDX amount among replicates was 9.9 ± 7.1% including the control.

The proportion of transformation products further down the metabolic chain of degradation (*p,p'*-DBP, DDMU, DDM) did not change significantly, as the ∑DDX levels decreased during the experiment ($p = 0.63$). This indicates that the degradation products of DDT's transformation were also degraded, and at a similar rate.

3.2. Effects of treatments on soil properties

In addition to DDX amounts, water content, organic matter (OM, % of soil on a wet weight basis), inorganic nitrogen ($\sum NH_4^+, NO_3^-$) and pH were also recorded for the original soil and all samples at the start (day 1) and end (day 30) of the petri dish experiment (Table 2). The original starting soil had a ∑DDX concentration of 7.8 mg kg^{-1} dw, and its physiochemical properties before addition of inocula are given in Table S4.

A significant overall increase in inorganic nitrogen from day 1 to day 30 was observed across all samples ($p < 0.05$). The interaction between time and treatment was not significant ($p > 0.5$), suggesting this increase was consistent across treatments. However, *T. versicolor* and *Ph. adiposa* alone showed significant increases in available inorganic nitrogen when tested separately using estimated marginal means (EMM) with Bonferroni correction ($p < 0.05$).

A linear mixed-effects model of OM (%) indicated a significant decrease over time ($\beta = -18.5$, $p < 0.0001$). A significant Treatment × Time interaction ($p < 0.01$) showed that the extent of this decrease differed significantly between treatments. Because the spawn was added by wet weight rather than dry weight (and water content varied between

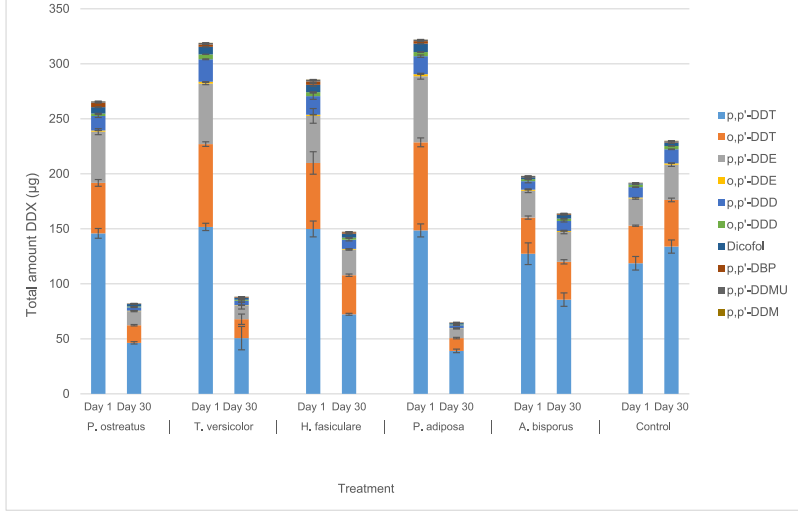

Fig. 2. The average total amount of DDX pre- (day 1) and post treatment (day 30) in each petri dish treatment. Data shown are mean ± std. ($n = 4$).

Table 2
Characteristics of the treated soil and control before and after incubation. Subsamples were taken on day 1 and day 30. Data is given as mean ± std. ($n = 4$). OM (%): fraction of organic matter in the soil on a wet weight basis (%); Mass reduction: weight loss between day 1 and day 30; *indicates significant change between day 1 and day 30.

Treatment	Water content (%)	OM (%)	Mass reduction (%)	pH	Relative reduction ΣDDX (%)	Inorganic N (mg g^{-1} dw)
Control (day 1)	28 ± 4	13 ± 5		6.0		0.07 ± 0.01
Control (day 30)	30 ± 8	10 ± 0.5	9.0 ± 3*	6.2	−20 ± 8	0.16 ± 0.1
P. ostreatus (day 1)	24 ± 0.6	29 ± 6		6.0		0.09 ± 0.02
P. ostreatus (day 30)	16 ± 7	16 ± 1*	8.8 ± 1*	5.5	69 ± 3*	0.11 ± 0.03
T. versicolor (day 1)	30 ± 1	20 ± 0.4		6.5		0.43 ± 0.1
T. versicolor (day 30)	32 ± 5	9 ± 1*	16 ± 3*	6.4	72 ± 8*	1.13 ± 0.2
H. fasciculare (day 1)	30 ± 0.6	22 ± 0.4		5.2		0.06 ± 0.01
H. fasciculare (day 30)	27 ± 3	10 ± 0.8*	9.7 ± 3*	4.6	45 ± 15*	0.07 ± 0.03
Ph. adiposa (day 1)	31 ± 4	22 ± 8.0		5.8		0.26 ± 0.3
Ph. adiposa (day 30)	35 ± 4	9 ± 0.5*	6.6 ± 4	5.7	80 ± 3*	0.5 ± 0.3
A. bisporus (day 1)	31 ± 0.4	16 ± 1		6.0		0.1 ± 0.00
A. bisporus (day 30)	29 ± 8	10 ± 1*	7.9 ± 6	6.1	17 ± 16	0.24 ± 0.03

species), the initial OM% at day 1 also differed significantly between treatments. Thus, the fungi were active in all treatments, degrading organic matter, though the extent of their activity depended on the species. In the control, a decrease in OM% was also observed, presumably due to degradation by native soil microorganisms, but non-significantly.

3.3. Respiration tests

Visual observation confirmed vivid fungal growth, indicating that colonization of soil by *P. ostreatus* could be successful at a scale 10 times higher than in petri-dish 30-day incubation test (Fig. S1). The baseline CO_2 output for the untreated soil remained stable between 400 and 440 ppm h^{-1} over the course of the 2-week trial, and the standard deviation remained low (Fig. 3). The addition of organic matter in the form of spawn with live mycelium resulted in a fast increase of respiration. In the control, in which autoclaved (dead) spawn was added, respiration increased within 2 days to a similar level as when spawn with live *P. ostreatus* was added, indicating that the native soil community responded to the input of resources as much as the added fungus. Respiration decreased at approximately the same time for live *P. ostreatus* and the control, returning to a baseline of between 400 and 500 ppm h^{-1} CO_2 production after ~14 days, indicating that microorganisms (added or native) rapidly utilized the available portion of the added organic substrate. The AUC for each treatment was analyzed and compared using ANOVA with Tukey HSD post-hoc testing and revealed that no significant difference was present between the control and the *P. ostreatus* treatment ($p > 0.05$), but both were significantly higher than the blank ($p < 0.05$).

3.4. DDX degradation in water suspension

Addition of the surfactant Tween 80 resulted in release of 6.6 μg (± 0.9) of ∑DDX into the liquid fraction. This corresponds to ~17% of the calculated 39 μg ∑DDX added to the system via the soil, raising the dissolved concentration significantly compared to the control, where only 0.5% was released. Addition of *P. ostreatus* enzyme suspension alone had no significant effect (Fig. 4), but the addition of both Tween 80 and enzyme suspension resulted in a reduction of ∑DDX concentrations in the liquid phase by 64% compared to addition of Tween 80 only. Consequently, it can be inferred that Tween 80 releases DDX from the soils, making it available for degradation by the enzymes produced by *P. ostreatus*. In total, the combined treatment with Tween 80 and *P. ostreatus* enzyme suspension resulted in that 6.1% of the ∑DDX in the

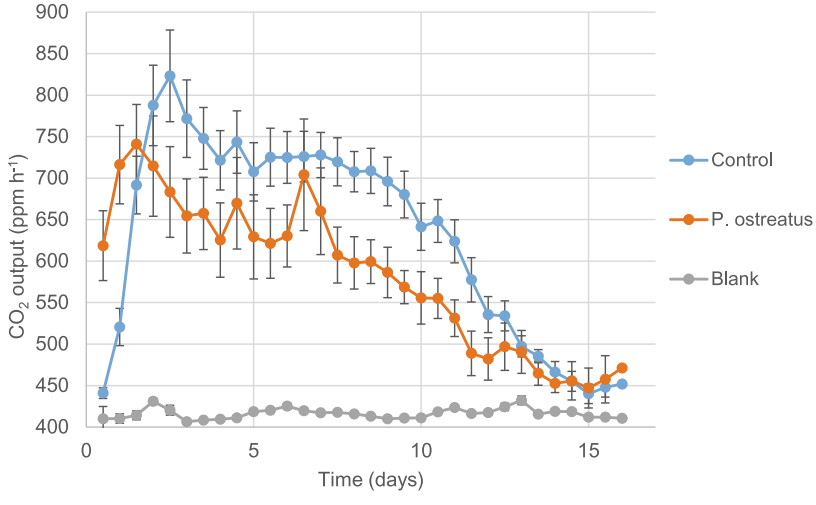

Fig. 3. Respiration over time in untreated soil (blank), soil amended with autoclaved spawn (control) and soil amended with *P. ostreatus*. Data shown are mean ± std. ($n = 3$) of hourly measurements, averaged over 12 h.

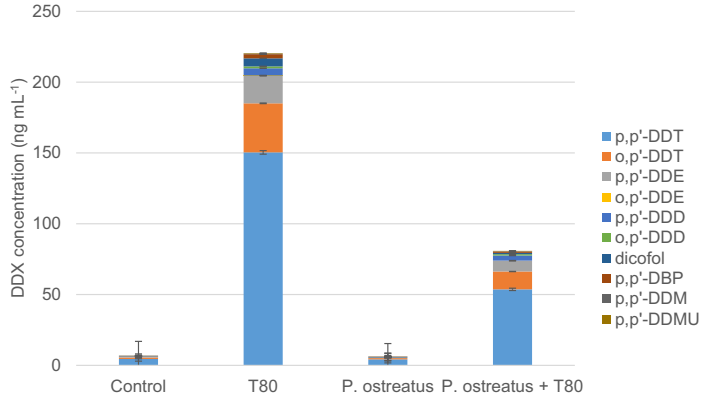

Fig. 4. Concentration (ng mL^{-1}) of DDX in the liquid phase of a slurry from aged, contaminated soil untreated (Control), treated with Tween 80 (Treatment 1: T80), enzyme suspension of *P. ostreatus* (Treatment 2: *P. ostreatus*) and a combination of both (Treatment 3: *P. ostreatus* + T80) for 8 days. Data is given as mean ± std. (n = 4), except for Treatment 2 (n = 3).

system was degraded during a period of 8 days (Fig. 4).

When enzyme suspension of *P. ostreatus* was used, all 3 replicates (one excluded due to mould growth) showed clear activity of oxidative enzymes, which decreased to base level after 3 days (Fig. 5).

4. Discussion

4.1. DDX degradation in soil microcosms

The results from the soil microcosm experiments (Fig. 2, Table S3) are consistent with a study by Fan et al. [26], which investigated the degradation potential of the white rot fungus *Flammulina velutipes*, in spiked soils, and observed 66% degradation of DDTs after a 30-day. In the present study, we had a similar duration but double amount of soil, and DDX concentrations were 3–5 times higher. Zhao et al. [16] also employed a similar incubation time (25 days) and achieved 62% degradation but only used purified laccase. Their DDX concentrations were approximately the same as in our study, though their soil volume was half of what was used in this study, and was spiked with DDT only 4 weeks before incubation. This consistency between studies is promising, but the current knowledge gap in mycoremedation of DDT contaminated soils is how to effectively upscale an effective process. Currently, while effective in spiked soils and in liquid systems, there has been no effective large-scale treatment. This study's design was aimed to be easily upscalable to treatment plant or field remediation trials. Mechanical mixing of contaminated soil, substrate and white-rot fungi is a realistic scenario using commercially produced spawn and inexpensive substrate amendments. This approach aligns most closely with biopiling ex-situ treatment, where contaminated soil is mixed with bulking agents and periodically aerated. Here, fungal inoculation functions as a composting-style treatment, but targeting DDT. These results serve as proof of concept for fungal bioremediation in aged field soils where DDX is strongly sorbed, where indigenous soil communities alone do not achieve measurable degradation, meeting the primary objective of this study.

The comparative screening of five white-rot fungi in this study provides valuable insight into species-specific degradation capacities and can be used to inform future bioremediation strategies. Although

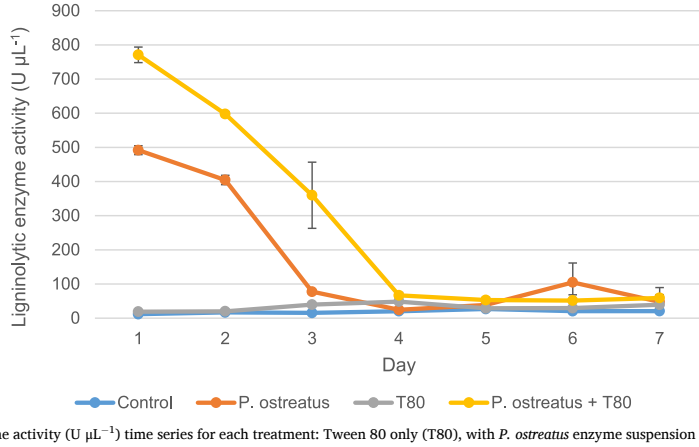

Fig. 5. Ligninolytic enzyme activity (U μL^{-1}) time series for each treatment: Tween 80 only (T80), with *P. ostreatus* enzyme suspension only (*P. ostreatus*), with both (*P. ostreatus* + T80) and with neither (Control). Data is given as mean ± std. (n = 4), except *P. ostreatus* (n = 3).

P. ostreatus and *T. versicolor* are among the most frequently documented DDT-degrading fungi in the literature [8,14,35,36], their performance was matched by *Ph. adiposa*. *P. ostreatus* and *T. versicolor* both colonize wood as their natural habitat, but exclusively above ground while *Ph. adiposa* grows at the soil-wood interface. Both *A. bisporus* and *H. fasciculare* are classified as secondary decomposers with slower growth rates and narrower substrate preferences, which may limit their ability to cope with complex, aged DDT-contaminated soils. The result of this study suggests that ecological niche and fungal life history traits, such as substrate preference and soil growth behavior, are as important as enzymatic capability in determining bioremediation potential.

White-rot fungi possess genes encoding ligninolytic enzymes, including laccases, manganese peroxidases (MnPs), and versatile peroxidases (VPs) in different amounts and proportions. These enzymes are associated with auxiliary activity families AA1 and AA2 in the CAZy database [37], with AA1 encompassing laccases and laccase-like multicopper oxidases, and AA2 including class II peroxidases, such as MnPs and VPs. Two of the three most successful species in this study *P. ostreatus* and *T. versicolor,* (Fig. 2) possess 12 and 9 AA1 genes, and 10 and 26 AA2 genes respectively [37]. *Ph. adiposa* is less well-characterized at the genomic level, but its close relatives *Ph. conissans*, *Ph. hylandensis*, and *Ph. molesta* possess between 11 and 17 AA1-type genes and 15 to 16 AA2-type genes. Enzymatic studies in literature of *Ph. adiposa* in particular show the secretion of laccase, manganese peroxidase, and lignin peroxidase under ligninolytic conditions [38]. A higher number of AA1 and AA2 genes in a genome suggests a broader enzymatic repertoire and potential for expression.

In contrast, *A. bisporus* was less effective in degrading DDT, likely due to its limited ligninolytic enzyme repertoire and reduced oxidative capacity. *A. bisporus* is known to encode only 2 AA2 enzymes, and 13 AA1-domain-containing proteins [37]. In a study on the life-cycle dependent ligninolytic enzyme activity of *A. bisporus*, two laccase and one MnP genes were expressed but only during the spawning phase, which may explain the lack of degradation of DDX in the petri dishes, as fungi were in vegetative growth phase [39].

While genomic data for *H. fasciculare* remain limited and somewhat inconsistent, its close relative *Hypholoma sublateritium* has been sequenced and shown to contain three multicopper oxidases (a group including laccases) and 22 class II peroxidase genes, according to JGI MycoCosm [40]. This enzyme profile suggests a strong potential for DDT degradation; however, the modest performance by *H. fasciculare* in this study highlight that genomic data alone are insufficient to predict bioremediation performance.

Importantly, actual enzyme activity is strongly influenced by environmental and substrate-specific cues. Environmental triggers such as the presence of lignin, veratryl alcohol, manganese addition, nitrogen, or amendment with woody biomass can enhance the expression and activity of ligninolytic enzymes [41–44]. Other organic options for stimulation of ligninolytic enzyme activity are oxalic, citric, malic, and succinic acids. These have been reported to enhance desorption of organic contaminants in soil [45], and can act as mediators for laccase and Mn-peroxidase activity. A strategy involving amendments with these stimulants may also be effective in optimizing fungal degradation of persistent pollutants in soil.

P. ostreatus was chosen for the respiration experiment (Fig. 3), which was slightly upscaled compared to the petri dish experiment. *P. ostreatus* was selected due to its rapid growth rate, high ligninolytic enzyme production, widespread commercial availability, low substrate preference specificity, and highly documented success in bioremediation [46]. The successful colonization of the soil indicates high potential for upscaling. However, the drop in activity after two weeks indicates that repeated amendments with substrate or inoculum may be necessary for sustained treatment. The respiration level in the control using autoclaved *P. ostreatus* spawn was comparable to that of the *P. ostreatus* spawn. The visual difference observed in the data was not statistically relevant, and many factors such as temperature, relative humidity, and the added moisture in the autoclaving step may well contribute to differences both within and between treatments. Stimulation of the native soil community by a large input of organic matter has been shown to instigate DDT degradation [14]. Additionally, indigenous microbial populations were found to be capable of mineralizing DDTs by up to 67% in 7 weeks in liquid cultures with 5 g of aged, contaminated soils [24]. Betancur-Corredor et al. [47] also found that soil amendment with phosphate and urea stimulated the indigenous microbial community to degrade 94% of the DDTs in soil over 8 weeks. However, the lack of DDX degradation in the control of the soil mesocosm experiment (Fig. 2) (with heat-killed inoculum) indicated that this phenomenon may not be ubiquitous, and in our context, the addition of a white-rot fungus was essential, or at least beneficial, for remediation. This suggests that in this soil, DDX degradation was not primarily controlled by bacterial mineralization capacity, but by access to fungal-driven degradation processes.

From a remediation perspective, the key significance of these findings is that substantial DDX reduction can be achieved in historically contaminated soils under low-technology conditions (soil mixing, lignocellulosic amendment, fungal inoculation). This supports the feasibility of fungal-based treatment as a realistic supplement to existing soil remediation approaches and demonstrates species-specific differences in DDX removal. Such a system could potentially replace excavation and landfill or heating, be an alternative to immobilization with activated carbon, or could complement soil washing.

4.2. DDX degradation in water suspension

The effectiveness of the surfactant Tween 80 in DDT remediation has been previously demonstrated across various concentrations. High concentrations, up to 1 g L^{-1}, over 60 times the critical micelle concentration (0.012 mM or 0.016 g L^{-1}), have been shown to increase solubilization of DDTs following a second-order curve, whereby co-inoculation with white-rot fungi and biosurfactant-producing bacteria was suggested as a potential avenue for further research [20]. In our study, 17% of the 39 µg of DDX introduced into the system were degraded when combined treatment with Tween 80 and *P. ostreatus* enzyme were tested. With 5 g of soil added, this degradation reduced the concentration from 7.8 mg kg^{-1} to 6.4 mg kg^{-1}. The results also demonstrate the potential of multiple washing steps and continuous input of ligninolytic enzymes via a live fungal liquid culture, rather than relying solely on enzyme extracts. A key aim of the study was to assess enzyme longevity in a non-sterile liquid culture. Enzymatic activity ceased within four days, suggesting that future approaches should involve a live fungus to sustain degradation. Repeated additions of stock enzyme extract every four days would be unrealistically costly and labour intensive as a management practice to apply on large scale. This time scale concurs with studies indicating that laccase and Mn-peroxidase enzymes have an expected half-life of around 1 to 4 days [48,49]. When Tween 80 was added, activity was higher, indicating a stimulating effect of the surfactant on oxidative activity within the system. Technologically, this points toward a two-stage slurry bioreactor remediation design of a surfactant-assisted soil wash to transfer a fraction of DDX into the aqueous phase, followed by a bioreactor-based oxidative treatment step using live fungal cultures or repeated enzyme application to degrade mobilized residues.

This experiment indicates a high potential for soil washing in line with previous work on similar scale by Rios et al. [50]. In future, the surfactant exposure procedure may be optimized to release a higher proportion of the DDX, and fungal growth medium could be replenished frequently to sustain degradation. With a live fungus in the system, Mn-peroxidase and laccase activities would theoretically be maintained as long as sufficient oxygenation and resources remain within the system to keep the fungus active. In this study, the fungal mycelium was separated to avoid sorption of DDX to the hyphae, complicating measurement of the dissolved (bioavailable) fraction, and hyphal adhesion remains to be studied if this type of treatment system is developed further.

Tween 80 has been found to enhance the production and activity of ligninolytic enzymes in various fungal species, potentially through increased fungal growth and the release of enzyme mediators. Studies have demonstrated that Tween 80 does not exhibit toxicity to fungi, and in fact, it has been found to boost enzyme production. For example, it significantly increased the production of Mn-peroxidase in *Phanerochaete chrysosporium* [51] and promoted laccase activity in *Pleurotus sajor-caju*, while in *Pleurotus eryngii*, it contributed to increased fungal biomass and therefore mycoremediation [52,53]. Additionally, in a system with *Stereum ostrea*, the presence of Tween 80 was linked to enhanced ligninolytic enzyme production, likely due to the growth stimulation and possible release of phenolic compounds or other mediators [54]. These findings collectively suggest a potential of Tween 80 to promote fungal growth and enzyme production in a remediation context.

The biodegradability of Tween 80 also plays a key role in its long-term feasibility for application in environmental remediation. Lee et al. [55] found that 33% of Tween 80 was degraded over a 21-day incubation period, primarily through microbial metabolism of its fatty acid tails. This suggests that, while Tween 80 facilitates contaminant mobilization, its surfactant properties may diminish over time, and this could then reduce its emulsification effects in soil. Additionally, Ahn et al. [56] demonstrated that activated carbon could efficiently recover applied surfactants, offering a method to mitigate residual surfactant accumulation. These factors collectively indicate that Tween 80 is not only effective in mobilizing DDT in aged soil but also has potential to be acceptable considering long-term environmental impact.

Importantly, the contribution of this study lies not in the discovery of new techniques, but in demonstrating the feasibility of applying established biodegradation strategies under realistic and scalable conditions. Together, the soil microcosm and Tween 80 experiments support the hypothesis that select WRF can degrade DDX in aged, contaminated field soils, and that surfactant assisted mobilization can increase access to otherwise strongly sorbed, unavailable DDX residues. By using historically contaminated, aged field soils rather than freshly spiked samples, and by implementing a liquid-phase soil washing system derived from a practical, commercially available inoculum (*P. ostreatus*), the study addresses key barriers to real-world application. Although the current setup remains small in scale, the integration of soil washing and pollutant degradation within a single treatment solution highlights the potential for development into a larger-scale, closed-system remediation approach.

5. Conclusions

In this study, we found that the fungal species *P. ostreatus, T. versicolor, Ph. adiposa* and *H. fasciculare* were all capable of degrading DDX to between 45% (*H. fasciculare*) and 80% (*Ph. adiposa*) of the initial amount within a 30-day experiment. For further upscaling toward a feasible remediation technique, there are optimization processes to be explored, such as of soil:spawn ratio, carbon and nitrogen sources, and maintenance of soil aeration and structure at larger volumes. Additionally, the present system was performed using a closed system. The effect of treatment on leaching could therefore not be monitored and should be included in follow-up studies. In an upscaled experiment, it was shown that respiration by the DDX degrading fungus *P. ostreatus* decreased over a two-week period when inoculated in soil, indicating a need for renewed input of organic matter, and/or re-inoculation of spawn.

Also, a soil washing process was explored and Tween 80 was shown to mobilize DDX into the liquid phase of a soil slurry, thus increasing the bioavailability and degradation rates of the DDX. Treatment with Tween 80 and *P. ostreatus* enzymes resulted in a 64% degradation of the released DDX. Though promising, leaching of DDX into ground water, or creation of anaerobic conditions unsuitable for white rot fungi, are potential risks in a combined in-situ treatment with Tween 80 and white-rot fungi. This indicates potential for a two-step soil washing followed by degradation of DDX in liquid culture process by white-rot fungi.

Overall, this study shows the effectiveness of white-rot fungi to degrade DDX in aged contaminated soils. Both in-situ inoculation of the fungus and a soil washing process can be considered feasible approaches.

CRediT authorship contribution statement

S. Casey: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **K. Wiberg:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **A.-K. Dahlberg:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **B.D. Lindahl:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **M. Hultberg:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used ChatGTP in order to debug code in RStudio. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stephanie Casey reports financial support was provided by Geological Survey of Sweden. Stephanie Casey reports financial support was provided by Sveaskog AB. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cejgas.2026.100051.

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Supplemental Information

Title: Enhanced DDT degradation in aged soils via fungal treatment and surfactant application

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Contents

1. Supplementary methods

1.1

Table S1. Grade and vendors of chemicals and materials used for chemical analysis

Product	Chemical name	Vendor	Product number	CAS number
Calcium carbonate	$CaCO_3$	Sigma-Aldrich	C4830	471-34-1
Potassium chloride	KCl	Sigma-Aldrich	P5405	7447-40-7
Whatman® cellulose extraction thimbles	-	Merck	WHA10350243	-
Dichloromethane	CH_2Cl_2	VWR International	1.06054.2500	75-09-2
n-hexane	$CH_3(CH_2)_4CH_3$	VWR International	1.04371.2500	110-54-3
Activated granular copper	Cu	Merck	311405	7440-50-8
Aluminum oxide	Al_2O_3	Merck	544833	1344-28-1
Silicon Dioxide	SiO_2	Merck	922587	7631-86-9
Sodium sulphate	Na_2SO_4	Merck	238597	7757-82-6
Isooctane	$(CH_3)_2CHCH_2C(CH_3)_3$	Merck	1.15440	540-84-1
3-Methyl-2-benzothiazolinone-hydrazonehydrochloride (MBTH)	$C_8H_{10}ClN_3S$	Merck	1.04527	4338-98-1
4-(Dimethylamino)-benzaldehyde (DMAB)	$C_{20}H_{27}NO_3$	Merck	D2004	172611-73-3
Manganese-dependant lignin peroxidase (MnP)	-	Merck	803057	114995-15-2
Ethylenediaminetetraacetic acid (EDTA)	$(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$	Merck	E6758	60-00-4
Hydrogen peroxide solution (30%)	H_2O_2	Merck	31642-M	7722-84-1
Sodium acetate	$C_2H_3NaO_2$	Merck	241245	127-09-3
Sodium succinate dibasic hexahydrate	$NaOOCCH_2CH_2COONa \cdot 6H_2O$	Merck	S2378	6106-21-4
Sodium DL lactate solution	$CH_3CH(OH)COONa$	Merck	L4263	72-17-3

1.2

Table S2. Target compounds, ^{13}C-labelled internal standards (IS) (final concentration 1 ng μL^{-1}), and ^{13}C-labelled recovery standard (RS), along with vendors, purities, and precursor and product ions.

Compounds	Vendor	Chemical purity (%)	Precursor ion	Product ion	Internal standard (IS)
Target compounds					
o,p′-DDT	Cambridge Isotope Laboratories, Inc., Massachusetts, USA.	99.2	235	165	^{13}*C-o,p′*-DDT
p,p′-DDT		99.9	235	165	^{13}*C-p,p′*-DDT
o,p′-DDD		97.5	235	165	^{13}*C-o,p′*-DDD
p,p′-DDD		99.2	235	165	^{13}*C-p,p′*-DDD
o,p′-DDE		99.7	246	176	^{13}*C-o,p′*-DDE
p,p′-DDE		99.5	246	176	^{13}*C-p,p′*-DDE
p,p′-DDMU	Sigma-Aldrich	98.9	212	176	^{13}*C-o,p′*-DDE
p,p′-DDM	LGC Standards, Guilford, UK	99.5	201	165	^{13}*C-o,p′*-DDE
p,p′-DBP	Sigma-Aldrich	98.7	139	111	^{13}*C-o,p′*-DDE
Dicofol	Cambridge Isotope Laboratories	99.48	251	139	^{13}*C*-Dicofol
Internal standards (IS)					
7 DDX solution					
^{13}*C-o,p′*-DDT	Cambridge Isotope Laboratories, Inc., Massachusetts, USA.	99.5	247	177	
^{13}*C-p,p′*-DDT		>98	247	177	
^{13}*C-o,p′*-DDD		96.2	247	177	
^{13}*C-p,p′*-DDD		98.2	247	177	
^{13}*C-o,p′*-DDE		99.6	258	188	
^{13}*C-p,p′*-DDE		>98	258	188	
^{13}*C-dicofol*		99.5	263	145	
Recovery standard (RS)					
^{13}C-PCB 97	Cambridge Isotope Laboratories, Inc., Guelp, Onatorio, Canada.	99%	340	268	
^{13}C-PCB 188		99%	406	336	

2. Supplementary results

2.1

Table S3. Soil amounts of DDT and its transformation products in each Petri dish before (day 1) and after (day 30) treatment

Treatment	*p,p'*-DDT (ng)	*o,p'*-DDT (ng)	*p,p'*-DDE (ng)	*o,p'*-DDE (ng)	*p,p'*-DDD (ng)	*o,p'*-DDD (ng)	Dicofol (ng)	*p,p'*-DBP (ng)	*p,p'*-DDMU (ng)	*p,p'*-DDM (ng)
P. ostreatus 1 (day 1)	150000	53000	55000	1200	18000	2900	6500	4900	1600	150
P. ostreatus 2 (day 1)	140000	43000	43000	1000	12000	2100	5200	3600	1100	100
P. ostreatus 3 (day 1)	140000	37000	40000	880	12000	2000	5000	3500	1100	120
P. ostreatus 4 (day 1)	160000	51000	48000	1100	10000	2000	6400	4400	910	80
P. ostreatus 1 (day 30)	48000	18000	14000	430	3300	850	2400	210	420	27
P. ostreatus 2 (day 30)	49000	17000	14000	440	3300	860	1800	190	370	26
P. ostreatus 3 (day 30)	44000	15000	12000	400	3100	780	1900	190	370	25
P. ostreatus 4 (day 30)	44000	15000	11000	350	2400	640	2600	150	290	20
T. versicolor 1 (day 1)	140000	70000	52000	1600	20000	4400	6900	1900	1400	200
T. versicolor 2 (day 1)	150000	73000	55000	1600	20000	4500	6600	2100	1400	190
T. versicolor 3 (day 1)	160000	80000	57000	1800	22000	4700	7500	2200	1300	210
T. versicolor 4 (day 1)	160000	78000	56000	1700	20000	4200	6500	2100	1300	190
T. versicolor 1 (day 30)	87000	12000	8700	280	2600	660	1200	160	260	25
T. versicolor 2 (day 30)	38000	12000	8800	250	2700	670	1100	230	260	27
T. versicolor 3 (day 30)	38000	12000	8900	270	2400	620	1100	150	220	22
T. versicolor 4 (day 30)	40000	33000	23000	580	8300	1800	3500	2100	470	71
H. fasciculare 1 (day 1)	130000	25000	20000	610	7000	2300	2400	460	3000	54
H. fasciculare 2 (day 1)	160000	76000	54000	1300	21000	4100	7700	4300	1200	190
H. fasciculare 3 (day 1)	150000	70000	49000	1300	21000	4100	8200	4000	1200	210
H. fasciculare 4 (day 1)	160000	69000	48000	1200	18000	3900	8700	3200	1100	160
H. fasciculare 1 (day 30)	75000	39000	25000	710	4800	1200	4700	1300	360	53
H. fasciculare 2 (day 30)	70000	35000	23000	660	8100	1800	3300	1300	430	83
H. fasciculare 3 (day 30)	72000	34000	22000	640	10000	2000	3300	1000	470	110
H. fasciculare 4 (day 30)	72000	34000	23000	660	10000	2100	3500	1400	560	110
P. adiposa 1 (day 1)	140000	73000	57000	1800	15000	3500	7300	2200	1300	140
P. adiposa 2 (day 1)	140000	77000	58000	1800	14000	3300	7500	2200	1300	140
P. adiposa 3 (day 1)	150000	77000	56000	1800	16000	3600	7400	2100	1200	160
P. adiposa 4 (day 1)	170000	94000	69000	2000	20000	4300	8600	2600	1500	190
P. adiposa 1 (day 30)	44000	14000	10000	300	3000	720	1900	260	350	38
P. adiposa 2 (day 30)	39000	12000	8500	240	3000	690	1200	370	290	31
P. adiposa 3 (day 30)	36000	9700	7500	220	2400	580	1000	220	260	27
P. adiposa 4 (day 30)	37000	12000	8700	270	2600	630	1100	310	290	28
A. bisporus 1 (day 1)	150000	33000	26000	770	5500	1900	2800	410	1600	40
A. bisporus 2 (day 1)	140000	28000	20000	560	4800	1500	2400	380	980	28

A. bisporus 3 (day 1)	110000	35000	29000	1800	12000	2200	650	63	490	96
A. bisporus 4 (day 1)	110000	35000	23000	840	8500	2000	1000	64	510	78
A. bisporus 1 (day 30)	71000	35000	30000	1000	9100	2100	3800	720	560	68
A. bisporus 2 (day 30)	78000	28000	22000	720	6200	1500	2800	450	460	51
A. bisporus 3 (day 30)	91000	35000	27000	940	11000	2700	3700	770	570	100
A. bisporus 4 (day 30)	100000	39000	30000	1000	9900	2400	3400	730	650	85
Control 1 (day 1)	140000	33000	27000	1000	8900	2500	830	59	880	60
Control 2 (day 1)	110000	34000	23000	870	9500	2300	710	53	710	76
Control 3 (day 1)	120000	35000	25000	760	10000	2300	1300	84	480	76
Control 4 (day 1)	100000	35000	24000	790	9300	2200	610	81	570	87
Control 1 (day 30)	130000	47000	37000	1300	13000	3200	4900	1100	760	140
Control 2 (day 30)	150000	41000	30000	1000	13000	3000	4000	900	660	150
Control 3 (day 30)	140000	38000	29000	1000	12000	2900	3400	720	700	140
Control 4 (day 30)	120000	44000	33000	1700	13000	3000	470	110	710	120

Average	***p,p'*-DDT (ng)**	***o,p'*-DDT (ng)**	***p,p'*-DDE (ng)**	***o,p'*-DDE (ng)**	***p,p'*-DDD (ng)**	***o,p'*-DDD (ng)**	**Dicofol (ng)**	***p,p'*-DBP (ng)**	***p,p'*-DDMU (ng)**	***p,p'*-DDM (ng)**
P. ostreatus (day 1)	150000	46000	47000	1000	13000	2200	5800	4100	1200	110
P. ostreatus (day 30)	46000	16000	13000	410	3000	780	2200	190	360	25
T. versicolor (day 1)	150000	75000	55000	1700	20000	4500	6900	2100	1400	200
T. versicolor (day 30)	51000	17000	12000	350	4000	940	1800	650	300	36
H. fasciculare (day 1)	150000	60000	43000	1100	17000	3600	6700	3000	1700	150
H. fasciculare (day 30)	72000	36000	23000	670	8400	1800	3700	1300	450	88
P. adiposa (day 1)	150000	80000	60000	1900	16000	3700	7700	2300	1300	160
P. adiposa (day 30)	39000	12000	8700	260	2800	660	1300	290	300	31
A. bisporus (day 1)	130000	33000	24000	1000	7700	1900	1700	230	910	61
A. bisporus (day 30)	86000	34000	27000	930	9000	2200	3400	670	560	76
Control (day 1)	120000	34000	25000	860	9500	2300	870	69	660	75
Control (day 30)	130000	42000	32000	1300	13000	3000	3200	700	710	140

Standard Deviation	***p,p'*-DDT (ng)**	***o,p'*-DDT (ng)**	***p,p'*-DDE (ng)**	***o,p'*-DDE (ng)**	***p,p'*-DDD (ng)**	***o,p'*-DDD (ng)**	**Dicofol (ng)**	***p,p'*-DBP (ng)**	***p,p'*-DDMU (ng)**	***p,p'*-DDM (ng)**
P. ostreatus (day 1)	8900	6100	5500	110	2700	390	680	590	240	24
P. ostreatus (day 30)	2100	1300	1100	36	390	88	340	22	47	3
T. versicolor (day 1)	6600	4200	2000	95	770	170	400	110	35	8
T. versicolor (day 30)	21000	9400	6300	140	2500	510	1000	820	99	20
H. fasciculare (day 1)	14000	20000	13000	290	5800	750	2600	1500	780	59
H. fasciculare (day 30)	1700	2100	1200	28	2200	340	610	140	74	22
P. adiposa (day 1)	12000	8100	5300	110	2100	400	530	170	110	22
P. adiposa (day 30)	3200	1400	870	30	240	53	360	58	34	4
A. bisporus (day 1)	20000	2900	3300	500	2800	260	900	170	470	28
A. bisporus (day 30)	12000	3900	3100	130	1800	440	370	130	70	18
Control (day 1)	12000	1100	1400	110	420	110	270	13	150	10
Control (day 30)	12000	3300	3100	280	620	130	1600	360	37	9

2.2

Table S4. Characteristics of the experimental soil (Kolleberga, Klippan, Sweden)

Water content (%)	OM (%)	pH	C_s ∑DDX (mg kg^{-1} dw)	C_w ∑DDX (ng L^{-1})	Tot-N (%)	Tot-C (%)	Mn (mg kg^{-1} dw)	Cu (mg kg^{-1} dw)
2.0	1.91	6.32	7.8	170	0.08	1.92	128	4.47

OM (%): fraction of organic matter in the soil; C_w: freely dissolved concentration in the pore water; C_s: soil concentration

2.3

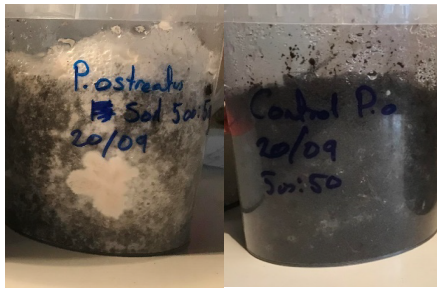

Figure S1. Visual growth of mesocosms containing soil mixed with *P. ostreatus* inoculated straw (left) or autoclaved spawn (Control) (right).

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2026:31

Dichlorodiphenyltrichloroethane (DDT) is a persistent organic pollutant that remains in many soils decades after its ban, posing long-term environmental risks. This thesis investigates the potential of white-rot fungi to degrade DDT and its transformation products (DDX) in aged soils from former Swedish forest nurseries. Using fungus-inoculated substrates and surfactants to enhance pollutant availability, degradation was tested across experimental scales. Partitioning coefficients for passive sampling were also utilised to measure potentially bioavailable DDX. The results demonstrate fungal degradation potential while highlighting challenges for field-scale remediation

Stephanie Casey received her doctoral education at the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences. She holds a Master's degree in General Biology from Lund University, and a Bachelor's degree in Biochemistry from the University of Sussex.

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