This is to certify that the
thesis entitled

ANAEROBIC TRANSFORMATION OF DDT IN PINE RIVER SEDIMENTS

presented by

TOMEKA KENYATTA PRIOLEAU

has been accepted towards fulfillment
of the requirements for the

MASTER OF SCIENCE degree in CROP AND SOIL SCIENCES

Major Professor's Signature

12/05/03

Date

ANAEROBIC TRANSFORMATIONS OF DDT IN PINE RIVER SEDIMENTS

By

Tomeka Kenyatta Prioleau

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop & Soil Sciences

2003

ABSTRACT

ANEROBIC TRANSFORMATIONS OF DDT IN PINE RIVER SEDIMENTS

By

Tomeka Kenyatta Prioleau

This study examined the fate of DDT in anaerobic sediments taken from two locations within the Pine River Superfund site in St. Louis, Michigan. Pine River sediment microcosms were analyzed for their ability to dechlorinate DDT and its metabolites DDD, and DDE under anaerobic conditions. Dechlorination of ^{14}C-DDT to ^{14}C -DDD occurred in both abiotic (autoclaved) and biologically active sediment slurries. No transformations of DDD or DDE were observed. Based on recovery of added DDT as DDD there was a greater amount of dechlorination occurring in bottom (10-20cm) layer sediments than in the top (0-10 cm) layer. Similar amounts of DDT were recovered as DDD in sediments from the two locations with different levels of historical contamination by DDT and co-contaminants. Anaerobic sediment slurry systems known to dechlorinate PCBs and/or PBBs were tested for their ability to transform DDT. These consisted of Red Cedar River sediments inoculated with microorganisms eluted from Pine River, Hudson River, and Silver Lake sediments. All systems supported the dechlorination of DDT to DDD by both abiotic and biotic processes, with abiotic transformations accounting for the majority (63-95%) DDT transformation. $FeSO_4$ amendments were also utilized in an attempt to enhance reductive dechlorination of DDT. The $FeSO_4$ amendment enhanced DDT dechlorination in Hudson River microcosms, however no further transformations beyond DDD were observed.

DEDICATION

This work is dedicated to my family who has always encouraged and supported me, and especially to my Mother -thank you for having faith in me.

ACKNOWLEDGEMENTS

First, I would like to thank a few faculty members who were very important to me here at Michigan State University. I would like to thank Dr. Stephen Boyd for his patience and guidance and for allowing me the opportunity to work with him. Dr. John Quensen for his guidance in many aspects of my research but especially the laboratory portion. I would like to thank Dr. James Tiedje and Dr. Karen Chou for serving on my guidance committee; your insight is greatly appreciated. I would like to thank Dr. James Jay for his unending guidance and support in my educational endeavors.

Secondly, I would like to thank all of my classmates and labmates who helped and encouraged me through the years. Many lifelong friendships were made here and these friendships I will always hold dear.

Lastly, I would like to thank my family for always encouraging me to go on and be successful at whatever I seek to accomplish. Thank you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

History of DDT

1,1,1-Trichloro-2, 2 bis (p-chlorophenyl) ethane (DDT) was one of the first chlorinated organic insecticides discovered. Synthesis of DDT was reported in 1874 but its effectiveness as an insecticide was not discovered until 1939 (World Health Organization, 1979). In 1874 a young chemistry student, Othmar Zeidler at the University of Strassburg, Germany developed DDT (Zimmerman and Lavine, 1946). Zeidler was not seeking to develop an insecticide when he discovered DDT during his studies. However, much later, a team of research scientists at a large chemical company was working to develop more potent insecticides. In the autumn of 1939, Dr. Paul Muller, working in the laboratories of J.R. Geigy, A.C., Basle Switzerland, synthesized the same compound that had been developed by Zeidler years before (Zimmerman and Lavine, 1946; West and Campbell, 1952; Brooks, 1974). Soon, he and his colleagues, Dr. Paul Lauger and Dr. Robert Weisman, discovered the insecticidal value of DDT (West and Campbell, 1952, Monclova, 1946). In 1942, the Geigy Company in New York received its first shipment of DDT from Geigy, Switzerland and then submitted DDT to the United States Department of Agriculture for testing. Soon its value to the U.S Armed Forces for controlling insect borne diseases (e.g. malaria) would be demonstrated. By 1943, DDT was in commercial production at the Cincinnati Chemical Works, and in early 1944, DuPont, Merck, and Hercules Powder Company also went into commercial production of DDT (Zimmerman and Lavine, 1946).

DDT was used extensively during the Second World War among allied troops and proved effective in controlling diseases such as malaria and typhus, which are spread by insects (ATSDR, 2001). Upon use by the military in 1944, the general public began using DDT extensively to control pests. As a result, DDT was sprayed directly into homes including onto the floors, walls, and beds, and also dusted directly onto the body. This very effective insecticide was widely used because insects not only transmit disease but also cause discomfort and destroy property and agricultural crops.

From 1945 until it was banned, DDT was one of the most widely used pesticides for the control of insects on agricultural crops (ATSDR, 2001). As a result of increased insect resistance to DDT, and growing environmental concerns, the use of this chemical in agriculture in the United States began to decline by 1959. In 1946, evidence of toxicity of DDT to humans was reported (Zimmerman and Lavine, 1946). It began to be evident through ecological studies that fish and birds of prey suffered most from the effects of DDT. Based on ecological considerations the United States Environmental Protection Agency (EPA) banned its use as an insecticide in 1972 (WHO, 1979). Most other industrialized countries, including West Germany, also banned the use of DDT in the early 1970's. In the former German Democratic Republic (GDR) however, DDT was still produced and used until the end of the 1980's (Voldner and Li, 1995; Mitra and Raghu, 1998). Ultimately, a number of other developed countries restricted the use of DDT except when it was needed for the protection of health (WHO, 1979); however it remains in use for controlling mosquito-borne malaria in many nations in Africa, Asia and Latin America. DDT has been recognized as among the best man made compounds for use as an insecticide. Many environmental groups have advocated that DDT be

phased out of use worldwide by 2007 because of its toxicity and environmental effects (Roberts, 2001).

DDD (1,1-Bis (4-chlorophenyl)-2,2-dichloroethane) and DDE (2,2-Bis (4-chlorophenyl)-1,1-dichloroethene) are transformation products of DDT formed in the environment (Table 1.1). DDD was used as an insecticide for contact control of leaf rollers and other insects on vegetables and tobacco, but its use has been banned (Agrochemicals Desk Reference, 1993). DDE has no commercial use but its presence as a persistent environmental transformation product of DDT is a major concern (ATSDR, 2000). DDT and its metabolites DDD and DDE are still found in various proportions in soils and sediments and have been reported at 3,422 of 22,000 sites identified as posing a danger to human and animal life by the EPA National Sediment Quality Survey (Quensen et al, 1998).

Environmental Effects

Environmental contamination of land and water from DDT has occurred due to past production and disposal processes, and agricultural application. The Pine River reservoir adjacent to the former Velsicol Chemical Corporation in St. Louis, Michigan is heavily contaminated with DDT and has been designated a Superfund site by EPA. Due to its inherent structural stability, and strong adsorption to soil and sediment solids, DDT is particularly recalcitrant in the environment (EPA, 1986). Some investigations have reported that DDT will remain present in the soil for 2 years while others have found that the process of degradation can take more than 15 years (Alexander, 1994).

Table 1.1. Chemical names and acronyms of DDT and its derivatives used in the text.

DDT= 1,1,1 trichloro-2,2-bis (p-chlorophenyl) ethane

DDD= 1,1, dichloro-2, 2-bis (p-chlorophenyl) ethane

DDE= 1,1, dichloro-2, 2-bis (p-chlorophenyl) ethylene

DDMU=1-chloro-2, 2-bis (p-chlorophenyl) ethene

DDMS= 1-chloro-2, 2-bis (p-chlorophenyl) ethane

DDNU= 1,1-bis (p-chlorophenyl) ethylene

DDOH= 2,2-bis (p-chlorophenyl) ethanol

DDCN= (bis (p-chlorophenyl)-acetonitrile)

DDA= bis (p-chlorophenyl) acetate

DBP= 4,4'- dichlorobenzophenone

Because of its long half-life, DDT and its derivatives (e.g. DDE, DDD) will continue to be found in the environment in various proportions several decades after introduction.

A major concern about exposure to DDT and its derivatives is bioaccumulation which is defined as an increase in the concentration of a chemical in an organism over time compared to the chemical's concentration in the environment (EXTOXNET, 2000). This accumulation occurs mainly through prolonged exposure of invertebrates, earthworms, fish, and aquatic vegetation to contaminated soil, sediment and water. Because of its lipophilic nature DDT accumulates in the fatty tissues of organisms causing bioaccumulation in the food chain.

Bioaccumulation in the food chain can also lead to biomagnification. Biomagnification is a process that results in an increased concentration of a chemical in an organism higher than the levels found in its food (EXTOXNET, 1999). For example DDT levels in soil of 10 ppm manifested concentrations of 141 ppm in earthworms and 444 ppm in robins (EXTOXNET, 1999). Biomagnification may lead to concentrations high enough to cause adverse effects on reproduction and even death in animals at the top of the food chain. Consequently even low levels in soil, water, and air may be an endangerment to certain species.

Studies have shown that DDT is toxic to birds and aquatic organisms. Birds are exposed to DDT primarily through the food web when they ingest DDT-contaminated organisms such as fish (ATSDR, 2001). One of the major concerns with chronic exposure of birds to DDT is its effects on reproduction, especially eggshell thinning and embryo mortality (WHO, 1979). DDT derivatives (primarily DDE) have similar toxic effects and are responsible for the thinning of eggshells of birds and impaired

reproduction in wildlife; predator birds such as the bald eagle are most sensitive to these effects (WHO, 1979; Karte, 1992; Wiemeyer et. al., 1993). It has been further suggested that DDE causes additional adverse effects including increased embryo mortality in birds (Heath et al., 1969).

Degradation Mechanisms of DDT- Dechlorination and Dehydrochlorination

There are several degradation pathways of DDT that suggest sequential dechlorination of the molecule through two types of reactions: reductive dechlorination and dehydrochlorination. Also, through oxidative reactions, the carbon skeleton of DDT can be degraded. Reductive dechlorination is the only known biodegradation mechanism for certain significant pollutants including highly chlorinated polychlorinated biphenyls (PCBs), hexachlorobenzene, tetrachloroethene and pentachlorophenol (Mohn and Tiedje, 1992). Reductive dehalogenation of DDT has been observed to be the primary degradation pathway in anaerobic soil, sediment, and sewage waste (Hill and McCarty, 1967, Guenzi and Beard, 1967, Burge, 1971; Jensen et al, 1972; Zoro et al. 1974).

The process of anaerobic dechlorination requires an electron donor (reductant) and proceeds by the removal of a chlorine atom directly from the biphenyl ring, with the simultaneous addition of a hydrogen atom to the molecule (Hill and McCarty, 1967; Morrill et al., 1982). The halogen atoms are then released as halide anions. In an anaerobic community, the availability of electron acceptors is frequently the limiting resource and a major determinant of species composition. After depletion of more common natural electron acceptors (e.g. NO_3^-, SO_4^{2-}) certain chlorinated aromatic compounds may be used in a process called halorespiration. Therefore, the population of

reducing organisms depends on the availability of electron acceptors. In principle, reductive dechlorination can occur by either nucleophilic or free radical substitution but evidence from natural or model anaerobic systems supports a free radical mechanism (March, 1985). Reductive dechlorination generally occurs under anaerobic conditions but is also involved in aerobic degradation of certain highly halogenated compounds (Mohn and Tiedje, 1992). The transfer of electrons to DDT is the essential process for its transformation to less chlorinated derivatives which may then be subject to aerobic metabolism. Dehalogenation generally makes xenobiotic compounds less toxic and more readily biodegradable (Mohn and Tiedje, 1992). Hence, this mechanism is very important because of its involvement in the environmental fate of pesticides and industrial chemicals, and its application to bioremediation of pollutants and hazardous wastes (Mohn and Tiedje, 1992).

Dehydrochlorination involves the removal of hydrogen and chlorine from organochlorine insecticides forming a double bond. This reaction typically takes place between the saturated chlorinated carbon and hydrogen on the neighboring carbon leaving a carbon-carbon double bond on the substrate (Lal and Saxena, 1982). A number of studies have shown that under aerobic conditions dehydrochlorination is the dominant reaction (Lal and Saxena, 1982). A very familiar example of this reaction is the formation of DDE from DDT.

Chlorine substituents on molecules such as DDT contribute to their environmental persistence; DDT for example is recalcitrant, whereas its non-chlorinated analogue diphenylmethane is readily biodegradable (Alexander, 1977). Evidence was presented by Sternersen, (1965) and Wedemeyer (1966) concerning the capability of microorganisms

to dechlorinate DDT. Both recognized that the degradation of DDT by bacteria was inversely related to the availability of oxygen in the environment. DDT can be biodegraded to a series of metabolites such as DDD (1,1-dichloro-2, 2-bis (p-chlorophenyl) ethane, DDE (1,1-dichloro-2, 2 bis (p-chlorophenyl) ethylene, and DDMU (1-chloro-2, 2-bis (p-chlorophenyl) ethene. Experiments with marine sediments and radiolabeled DDT showed that biodegradation could transform DDT into DDD, DDE, DDOH (2,2-bis (p-chlorophenyl) ethanol and DDNS (2,2-bis (p-chlorophenyl) ethane (Patil et al., 1972) (Figure 1.1). The next sections will further discuss the individual transformation pathways of DDT.

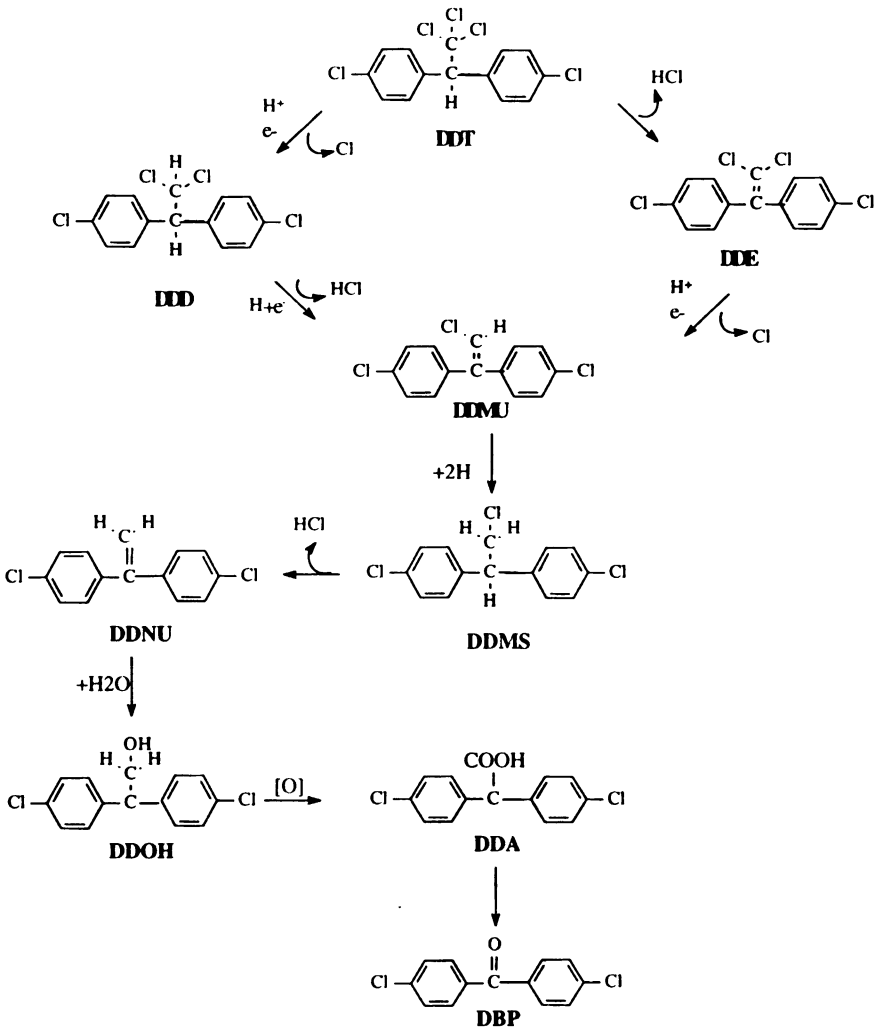
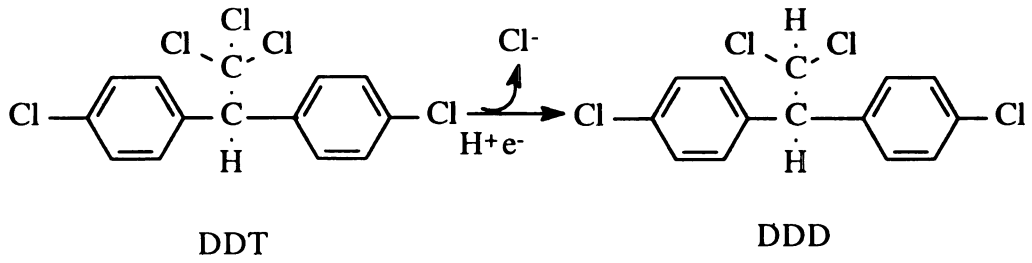


Figure 1.1. Transformation pathways of DDT by Aerobacter aerogenes (Wedemeyer, 1967).

DDT to DDD

Reductive dechlorination of DDT to DDD is a common first step in the environmental transformation of DDT (Figure 1.2). The transfer of electrons to DDT is the essential process for its degradation to form less chlorinated derivatives (Esaac and Matsumura, 1980) In the environment, dechlorination of DDT is carried out extensively by microorganisms, but may also occur abiotically. There are several reports of the conversion of DDT to DDD under anaerobic conditions (Johnson et al., 1967; Kallman and Andrews, 1963; Wedermeyer, 1966; Guenzi and Beard, 1967; Ko and Lockwood, 1968; Patil et al., 1970).



DDT DDD

Figure 1.2. Reductive dechlorination of DDT to DDD under anaerobic conditions (Rockhind and Blackburn, 1986).

Researchers have isolated microorganisms that carry out the conversion of DDT to DDD under anaerobic conditions. Johnson and Goodman (1967), reported that of 27 microorganisms examined, 23 bacterial species could convert p,p' DDT to p,p' DDD under anaerobic conditions. *Escheria coli* and *Aerobacter aerogenes* isolated from the gastrointestinal tract of rats were capable of degrading DDT to DDD (Mendel, 1966). Wedemeyer (1967) reported that extracts of *Aerobacter aerogenes* catalyze the

degradation of DDT to DDD, DDE, DDMU, DDMS, DDNU, DDA, and DBP. By a technique of sequential analysis the metabolic pathway was postulated as DDT→DDD→DDMU→DDMS→DDNU→DDA→DBP, or DDT→DDE (see Figure 1.1).

Several studies have been conducted to investigate the conditions under which dechlorination of DDT takes place. DDT is reductively converted to DDD in sewage sludge under anaerobic conditions. (Hill and McCarty, 1967; Jensen et al., 1972; Albone et al., 1972; Zoro et al., 1974.) Zoro (1974) concluded that direct microbial metabolism does not account for the reductive dechlorination of DDT in treated sewage sludge. His results showed that the conversion of DDT to DDD in the environment is mediated by reduced iron porphyrins. Guenzi et.al (1971) published a wide-ranging study that showed flooded soils favor the degradation of DDT. Under anaerobic conditions in soil, conversion of DDT to DDD was more rapid than in moist soils where aerobic conditions presumably exist. In a study by Guenzi and Beard, (1968), DDT was converted to DDD by microorganisms in an anaerobic soil system.

Because DDD is a highly toxic compound, the conversion of DDT to DDD in soil cannot be considered as a detoxification step. Moreover, DDD is more stable than DDT itself in soil. Therefore, this conversion may contribute to the persistence of DDT residues in soil and water (Ko and Lockwood, 1968).

Abiotic systems

In addition to microbially mediated reactions, the addition of some chemical agents in anaerobic systems may accelerate the transformation of DDT to DDD (Quirke et al., 1979). These chemical agents serve as catalysts for reductive dechlorination of organochlorine insecticides under anaerobic conditions. Studies on such catalytic systems was reported by Castro (1964), Miskus et al., (1965), and Zoro et al. (1974). DDT was effectively converted to DDD in the presence of an anhydrous, anaerobic solution of ferrous deuteroporphyrin, and by hemoglobin or hematin in the presence of excess sodium dithionite under anaerobic conditions (Castro, 1964; Miskus 1965). Addition of a detergent (Tween 80) or ethanol to the incubation media containing hematin, ferrous sulfate, and DDT increased degradation (Zoro et al., (1974). It has been shown that DDD formation is related to the levels of ferrous iron present in the anaerobic environment (Glass, 1972). Furthermore, DDT dechlorination increases as redox potential decreases (Burge, 1971; Guenzi et al., 1971; Glass, 1972; Parr and Smith, 1974,).

Biological reductive systems

Reductive degradation of insecticides in soil is believed to be mediated by microbial activity based on two experimental observations: sterile soil is often devoid of reductive activity, and organic matter amendments to non-sterile soil stimulates the reductive processes (Esaac and Matsumura, 1980). Studies by Guenzi and Beard (1967, 1968) established that microbial processes were responsible for the dechlorination of DDT. This was because there was no detection of DDT metabolism in sterile soil after anaerobic incubation for 2 and 4 weeks with ^{14}C DDT added to soil. Likewise, Pfaender

and Alexander (1973) demonstrated the presence of DDT-metabolizing organisms in fresh water, sewage, and marine environments. The rate of anaerobic conversion of DDT to DDD was increased with the addition of alfalfa. One possible reason for this was that alfalfa provided favorable nutrients for the microorganisms stimulating the transformation and increasing the rate of conversion (Guenzi and Beard, 1968). Similarly, Burge (1971) found DDD to be the only product after incubation of DDT in soil amended with alfalfa or alfalfa distillate. Johnsen (1971) reported that after incubation with or without addition of cattle manure, DDT was degraded readily in soil, principally to DDD. Conversion of DDT to DDD was more rapid in waterlogged soil than in moist soil where aerobic conditions presumably existed (Ko and Lockwood, 1968). In addition, Miskus et al (1965) reported that bovine rumen fluid degrades DDT to DDD. Matsumura et al. (1971) examined the fate of DDT and reported that the majority of microbial isolates from water and bottom silt of Lake Michigan reductively dechlorinated DDT to DDD. Likewise, studies of isolated bacteria showed DDD to be the main metabolic product in lab cultures (Barker et al., 1965; Sternersen, 1965; Subba Rao and Alexander, 1985; Wedemeyer, 1967).

DDT to DDE

DDE can be formed from DDT via dehydrochlorination, an oxidative process (Mohn and Tiedje, (1992) (Figure 1.3). Aerobic dehydrochlorination of DDT simultaneously removes the hydrogen and chlorine from the aliphatic portion of the molecule resulting in the formation of a carbon-carbon double bond. Patil et al., (1970) found that in aerobic environments such as ocean water, DDE was the major

biodegradation product of DDT. A number of studies have demonstrated the metabolism of DDT to DDE in soils. Guenzi and Beard, (1968) completed a study that suggests DDT is probably degraded first to DDE in aerobic soil treatments.

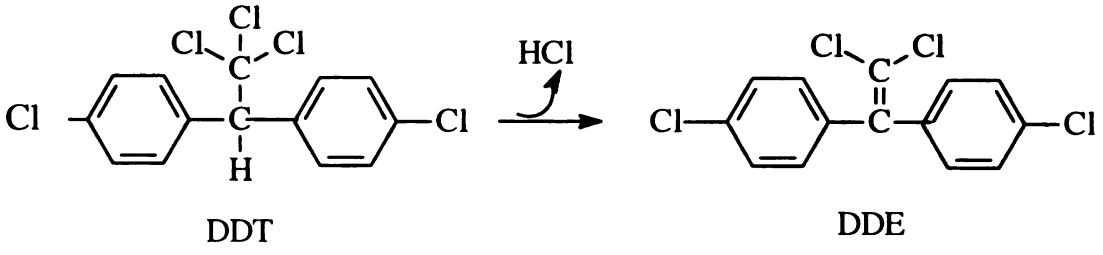


Figure 1.3. Aerobic dehydrochlorination of DDT to DDE (Rockhind and Blackburn, 1986)

Dehydrochlorination of DDT to DDE has been observed in aerobic and anaerobic environments, and in model systems containing reduced poryphins (Guenzi and Beard, 1967; Burge, 1971; Marei et al., 1978; Quirke et al., 1979). Wedemeyer (1967) confirmed the formation of DDE after dehydrochlorination of DDT by whole cells or cell-free extracts of *Aerobacter aerogenes*. Similarly, studies by Hitch and Day (1992) and Spencer et al., (1996) indicated aerobic soil metabolism of DDT to DDE. Hitch and Day (1992) concluded that certain conditions (e.g., metals content, moisture content) of the soil environment affect the degradation of DDT to DDE.

DDD TO DDMU

The transformation of DDD to DDMU has been proposed in DDT metabolism studies (Quirke et. al., 1979, Quensen et. al., 1998). This conversion occurs by reductive dechlorination to DDD followed by dehydrochlorination to DDMU in aerobic and anaerobic environments. This, and the subsequent conversion of DDMU to DDA and DBP, was observed by Quirke et. al. (1979). Quensen et al., (1998) observed a trace amount of DDMU formed from the transformation of DDD in Palos Verdes sediments. Wedemeyer (1967) observed the metabolic pathway of DDT to be DDT→ DDD→ DDMU→ DDMS→ DDNU→ DDA→ DBP. These dechlorination reactions occurred under aerobic and anaerobic conditions and were carried out by microbial enzyme systems with very specific environmental requirements to support these reactions.

DDE to DDMU

Typically, DDE has been viewed as a dead end product in the metabolism of DDT (Hay and Focht, 1998). DDE is a metabolite of DDT that is very persistent in the environment. It was believed for a long time that DDE was not microbially transformed but studies have proven reductive dechlorination under anaerobic conditions. Quensen et al. (1998) reported the formation of DDMU from the anaerobic microbial degradation of DDE in the Palos Verdes sediments. There were significant differences in the rates and extents of DDE dechlorination among the sediments from the three sites investigated. Furthermore, another investigation of the factors controlling the rate of DDE dechlorination to DDMU in the sediments showed that sediment depth and temperature affect the dechlorination rate of DDE (Quensen et.al., 2001).

Further Transformations

In studies on the transformation of DDT, the presence of intermediate metabolites, aside from the ones previously discussed have been observed. DBP, DDA, and DDCN are some of these metabolites formed from the degradation of DDT in anaerobic systems. Microorganisms in pure cultures have shown transformation of DDT to be: DDT→DDD→DDMU→DDMS→DDNU→DDA→DBP or DDT→DDE (Wedemeyer, 1967). Marei et. al., (1978) found that DDCN was formed during the incubation of DDT with sewage sludge. These results are very similar to those observed by Jensen et. al., (1972) which showed the transformation to DDD, DDMU, DBP, and DDCN in anaerobic sewage sludge.

REFERENCES

Agrochemicals Desk Reference: Environmental Data. 1993. Lewis Publishers. Chelsea, Michigan.

Aislabie, J.M. 1997. Microbial degradation of DDT and its residues-a review. New Zealand Journal of Agricultural Research. 40: 269-282.

Albone, E. S., Eglington, G. N., Evans, C. and Rhead. M.M. 1972. Formation of bis-(p-chlorophenyl) acetonitrile (p,p'-DDCN) from p, p-DDT in anaerobic sewage sludge. Nature (London). 240:420-421

Alexander, M. 1977. Introduction to soil microbiology. 2nd ed. New York, John Wiley.

Alexander, M. 1994. Recalcitrant molecules. In: Biodegradation and Bioremediation. Academic Press, New York.

Agency for Toxic Substances and Disease Registry (ATSDR). http://www.atsdr.cdc.gov/.

Barker, P.S., Morrison, F. O., and Whitaker, R. S. 1965. Conversion of DDT to DDD by Proteus vulgaris. A bacterium isolated from the intestinal flora of a mouse. Nature (London). 205:621-622.

Brooks, G.T. 1974. Chlorinated Insecticides: Volume I Technology and Application. CRC Press, Inc. Cleveland, Ohio.

Burge, W. D. 1971. Anaerobic decomposition of DDT in soil. Acceleration by volatile components of alfalfa. J. Agric. Food Chem. 19:375-378

Castro, C.E. 1964. J. Am. Chem. Soc. 86:2310.

Esaac, E. G., and Matsumuura, F. 1980. Metabolism of insecticides by reductive systems. Pharmac. Ther. 9:1-26

EXTOXNET Extension Toxicology Network: Pesticide Information Profiles. 2000. http://ace.orst.edu/cgi-bin/mfs/01/pips/ddt.htm?6.

Glass, B. L. 1972. Relation between the degradation of DDT and the iron redox system in soils. J. Agric. Fd. Chem. 20:324

Guenzi, W. D., and Beard, W. E. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science. 156; 1116-1117.

Guenzi, W. D., and Beard, W. E. 1968. Anaerobic conversion of DDT to DDD and Aerobic Stability of DDT in Soil. Soil. Soil Sci. Soc. Amer. Proc. 32: 522-524.

Guenzi, W. D., Beard, W.E., and Viets Jr., F.G. 1971. Influence of soil treatment on persistence of six chlorinated hydrocarbon insecticides in the field. Soil Sci. Soc. Am., Proc. 35: 910-913.

Hay, A. G., and Focht, D. D. 1998. Cometabolism of 1,1-Dichloro-2, 2Bis (4-Chlorophenyl) Ethylene by Pseudomonas acidovorans M3GY Grown on Biphenyl. Appl. Environ. Microbiol. 64:2141-2146.

Heath, R., J. Spann and J. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies. Nature 224: 47-48.

Hill D. W., and McCarty, P. L. 1967. Anaerobic degradation of selected chlorinated hydrocarbon pesticides. J. Water Pollut. Control Fed. 39: 1259-1277.

Hitch R.K., and Day H.R. 1992. Unusual Persistence of DDT in some western USA soils. Bull. Environ. Contam. Toxicol. 48: 259-264.

Jensen, S.; Gothe, R.; Kindertedt, M.O. 1972. Bis-(p-Chlorophenyl)-Acetonitrile (DDN), a new DDT derivative formed in anaerobic digested sewage sludge and Lake Sediment. Nature. 240:421-422.

Johnson, B.T., Goodman, R.N. 1967. Conversion of DDT to DDD by Pathogenic and Saprophytic Bacteria Associated with Plants. Science. 157: 560-561.

Kallman, B.J., and A.K. Andrews, 1963. Reductive dechlorination of DDT to DDD by yeast. Science 141: 1050-1051.

Korte, F., Ed. 1992. Textbook of ecological chemistry. Basics and concepts for the ecological assessment of chemicals. Thieme-Verlag: Stuttgart.

Ko W.H., Lockwood J. L. 1968. Conversion of DDT to DDD in soil and the effects of these compounds on soil microorganisms. Canadian Journal of Microbiology. 14:1069-1073.

Lal, R., and Saxena, M. D. 1982. Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. 46:95-127

March, J. 1985. Advanced Organic Chemistry, Wiley, New York, N.Y., 3rd ed., 1346 pp.

Marei, A. S. M., J. M. E., Rinaldi, G.; and Zoro, J. A. and Eglinton, G. 1978. The Environmental Fate of DDT. Chemosphere. 12: 993-998.

Matsumura, F., Patil, K. C., and Boush, G.M. 1971. DDT metabolized by microorganisms from Lake Michigan. Nature (London). 230:325-326.

Mendel, J.L., and Walton. M.S. 1966. Conversion of p,p'-DDT to p,p'-DDD by intestinal flora of rat. Science. 151:1527-1528.

Miskus, R. P., Blair, D. P., and Casida, J.E. 1965. J. Agric. Fd. Chem. 13:481.

Mohn W.W., Tiedje J. M. 1992. Microbial reductive dehalogenation. Microbiol Rev. 56:482-507.

Morrill, L. G., Mahilum, B.C., and Mohioddin, S.H. 1982. Organic Compounds in Soils: Sorption, Degradation and Persistence. Ann Arbor Science Publishers, Ann Arbor, MI., 326 pp.

Parr, J. F., and Smith, S. 1974. Transformation of DDT in an Everglades muck as affected by lime, ferrous iron, anaerobiosis. Soil Science. 118:45-52.

Parr J.F, Willis, G. H., and Smith, S. 1970. Soil anaerobiosis: II. Effect of selected environments and energy sources on the degradation of DDT. Soil Science. 110:306-312.

Patil K.C., Matsumura F., Boush G.M. 1970. Degradation of Endrin, Aldrin, and DDT by Soil Microorganisms. Applied Microbiology. 19: 879-881.

Patil, K.C., F. Matsumura, and G.M. Boush. 1972. Metabolic transformation of DDT, dieldrin, aldrin, and endrin by marine microorganisms. Environ. Sci, Technol. 21: 397-399.

Pfaender, F.K., and Alexander, M. (1973). Effect of Nutrient Additions on the Apparent Cometabolism of DDT. J. Agr. Food Chem. 21: 397-399.

Quensen J.F., Tiedje J.M., Jain M.K., and Mueller, S. A. 2001. Factors controlling the rate of DDE dechlorination to DDMU in Palos Verdes margin Sediments under anaerobic conditions. Environ. Sci. Technol. 35; 286-291.

Quensen, J. F., III, Mueller, S. A.; Jain, M. K.; Tiedje, J. M. 1998. Science. 280: 722-724.

Quirke, J.M.E.; Marei, A. S. M.; Eglinton, G. 1979. The Degradation of DDT and its degradative products by reduced iron (III) poryphyrins and ammonia. 3:151-155.

Rochind, M. L., and Blackburn. 1986. Microbial decomposition of chlorinated aromatic compounds. EPA/600/2-86/090 p. 138-145.

Spencer, W.F., Singh, G.C., Taylor, D., LeMert, R. A. M., Cliath, M.; and Farmer, W.J. 1996. DDT persistence and volatility as affected by management practices after 23 years. J. Environ. Qual. 25:815-821.

Sternersen, J. H. V. 1965. DDT metabolism in resistant and susceptible stable flies and in bacteria. Nature (London). 207:660-661.

Subba Rao, R. V., Alexander Martin. 1985. Bacterial and Fungal Cometabolism of 1,1,1-Trichloro-2,2 bis (p-chlorophenyl)Ethane (DDT) and its Breakdown Products. Applied and Environmental Microbiology. 49:509-516.

Voldner, E.C.; Li, Y.F. 1995. Sci. Total Environ. 160/161, 201-210.

Mitra, J.; Raghu, K. 1998. Bull. Environ. Contam. Toxicol. 60: 585-591.

Wedemeyer, G. 1967. Dechlorination of 1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane by Aerobacter aerogenes. Appl. Micorbiol. 15:569-574.

Wedemeyer, G. 1966. Dechlorinationof DDT by Aerobacter aerogenes. Science. 152:647.

West, T. F. and Campbell, G. A. 1950. DDT and Newer Persistent Insecticides, revised 2nd ed., Chapman and Hall, London.

Wiemeyer, S., C. Bunck and C. Stafford. 1993. Environmental contaminants in bald eagle eggs- 1980-1984 and further interpretations of relationships to productivity and shell thickness. Archives of Environmental Contamination and Toxicology 24: 213-227.

World Health Organization, 1979. Environmental Health Criteria 9: DDT and its Derivatives, Geneva.

Zimmerman, O.T., Lavine, I. 1946. DDT- Killer of Killers, Industrial Research Service, Dover, New Hampshire.

Zoro, J. A., Hunter, J. M., and Eglington, G. 1974. Degradation of p, p'-DDT in Reducing Environments. Nature. 247:235-237.

CHAPTER 2

SITE HISTORY AND METHODOLOGY

Site History

The Velsicol Chemical Corporation plant site formerly known as Michigan Chemical is located on the Pine River in St. Louis, Michigan (Figure 2.1). The chemical plant operated between 1936 and 1978 and manufactured a wide array of chemicals including 1,1,1- trichloro-2, 2 bis (p-chlorophenyl) ethane (DDT), polybrominated biphenyls (PBB), hexabromobenzene (HBB), and tris (2,3-dibromopropyl) phosphate (TRIS). In 1974, the facility became the subject of intense scrutiny after bags of the white powder PBB were mistakenly shipped instead of the feed additive magnesium oxide to operators throughout Michigan resulting in feed for dairy cattle that was contaminated with PBB. This action led to investigations by the Michigan Department of Natural Resources (DNR) and the U.S. Environmental Protection Agency (EPA) to determine if there were other negligent activities by Velsicol that presented a threat to the environment. They discovered that poor waste management practices such as process waste discharges directly into the Pine River had led to widespread contamination of the plant site and the adjoining Pine River Impoundment. There was considerable contamination of site soils and Pine River sediments with DDT, PBB, HBB and Tris. There were also elevated levels of DDT and other contaminants in fish (Table 2.1). DDT levels in the Pine River sediments adjacent to the plant as high as 44,000 ppm have resulted in DDT levels in fish that greatly exceed the maximum safe level of 5 ppm as set by the Food and Drug Administration (FDA). Because of the widespread contamination the site was deemed to pose a threat to human health and the environment.

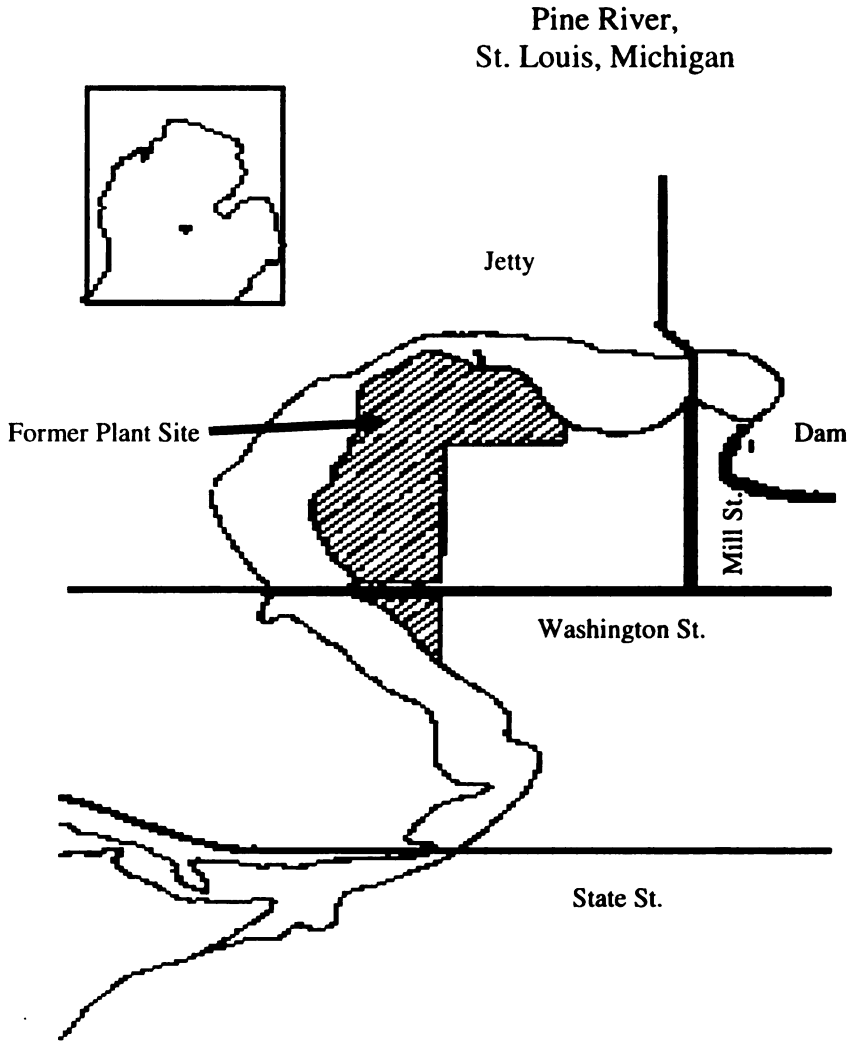


Figure 2.1. The Velsicol Chemical Corporation plant site located on the Pine River in St. Louis, Michigan. Adapted from Morris et. al., (1993).

As a result of the investigations the State of Michigan and the U.S. EPA closed the facility in 1976 and remedial measures for the contaminated site began in October, 1978.

Location	Collection Year	Avg. Conc. (ppm)	Max. Conc. (ppm)	Min. Conc. (ppm)
Dam	1983*	0.08	0.22	0.05
Dam	1985	9.66	18.66	5.27
Dam	1994	23.79	47.31	1.65
Dam	1997	26.82	72.56	10.61
Impoundment	1989	10.48	39.76	0.06
Impoundment	1995	16.15	43.27	0.51
Impoundment	1997	34.57	89.92	2.47

Table 2.1. Concentrations of total DDT in skin-off carp fillets from below the St. Louis Dam and in the St. Louis Impoundment. *Fish collected in 1983 were not analyzed for DDE and DDD, only DDT (EPA, 1998).

The initial remedial measures stopped discharges from the plant into the Pine River and subsequently the buildings and structures on the site were demolished. Agreements made between EPA, the State of Michigan and Velsicol included a plan to stop off-site migration of DDT and the other contaminants. This plan included excavation of the contaminated on-site soil, isolation of the site with a low-permeability slurry wall and clay cap, and other measures to control and monitor the boundaries of the site. After many hearings, a consent judgement-agreement was made between the Michigan DNR, EPA and Velsicol in 1982. The judgment did not require Velsicol to treat or remove contaminated sediments from the Pine River/ St. Louis Impoundment (the so-called no-action alternative) but they did pay restitution to the State for studies at the site. To deal with the issue of fish contamination in the River, the State of Michigan issued a no consumption advisory for all species of fish in the river. Regulators were

convinced that with time new sediment deposition would isolate the contaminated sediments and levels of DDT in fish would decline.

The Velsicol Chemical Plant Site has been under continuous investigation to monitor the effectiveness of the slurry wall and cap, as well as the contaminant levels in fish. The agencies collected sediment and fish data throughout the 1980's and 1990's. The studies revealed that contaminant levels in sediments had not decreased over time, and that there were still elevated or perhaps increasing levels of DDT in fish well above the FDA tolerance level of 5 ppm (Table 2.1).

As a result of these findings, the Pine River reservoir was designated a Superfund site in 1986, and U.S. EPA began emergency remedial action at the site in 1998. The plan was to immediately remove the most contaminated sediments, and then continue removal of lesser-contaminated adjacent sediments. The cleanup goal for this project is 5ppm DDT in sediments. This concentration would result in the removal of the majority of the contamination and therefore result in acceptable risk levels. At the 5 ppm concentration level the reduction of DDT concentrations in carp was reduced from ~42.5 ppm to ~1.7 ppm, making the fish clean enough to eat (EPA, 1998). The remedial plan chosen to carry this out would remove 533,000 pounds of DDT from the St. Louis impoundment. This involved physically isolating certain sections of the River with temporary coffer dams (sheet piling) to reduce the amount of resuspension of contaminated sediments during the dredging operations and to enhance dewatering. Accordingly, the plan involved removal of water from the impounded area and treatment using sand filters and activated carbon columns; treated water was then pumped back into the River. To ensure discharge requirements were being met, periodic sampling was

performed. The exposed sediments would be solidified by treating with a strengthening agent such as kiln dust, excavated and disposed of at on offsite RCRA subtitle C landfill. The proposed date of completion for this work was the end of 2001, but this date has now been extended.

Introduction

Degradation pathways of DDT in the environment have been studied for decades. These studies have indicated several possible chemical and biological transformations that lead to the degradation of DDT in the environment. Studies have shown degradation under aerobic and anaerobic conditions. Microorganisms in soils, sediments, sewage sludges as well as iron porphyrins have proven effective in degrading DDT. Laboratory studies have used radiolabeled compounds to help identify the specific degradation pathways. These studies have provided important insights but questions still remain regarding which specific mechanisms are operative under different environmental conditions, whether these reactions are chemical or biological in nature, and the exact sequence of intermediates produced from these reactions.

Objective

In this first study we examined the fate of DDT, DDD, and DDE in anaerobic Pine River sediments contaminated with DDT. The extent of degradation was monitored over a 32 week time period. The objectives were to determine the pathways of DDT degradation and determine the abiotic and biotic contributions to the anaerobic transformation of DDT. An additional objective was to determine if sediment depth influenced DDT degradation in the Pine River. A second study sought to evaluate the dechlorination of DDT by microbial communities with demonstrated capability to dechlorinate PCBs, as well as to determine if $FeSO_4$ amendments would stimulate the transformation of DDT.

MATERIALS AND METHODS

Materials

The chemicals DDT (99% purity), 1,1-dichloro-2, 2 bis (p-chlorophenyl) ethane DDD (99%purity), 2,2-bis (p-chlorophenyl) 1,1-dichloroethylene DDE (99.9% purity) were obtained from Ultra Scientific, Co., North Kingstown, RI. DDMU (100% purity) was obtained from AccuStandard Inc., New Haven, CT. Ring labeled ^{14}C-DDT (97 % radiochemical purity) ^{14}C-DDD (3.6mCi/mmol, 100 % radiochemical purity) ^{14}C-DDE (13mCi/mmol, 98% radiochemical purity) were obtained from the Sigma Chemical Co. St. Louis, MO.

Sediment Sampling and Analyses

Pine River Sediments

Sediments were collected from two different sites on the Pine River at the Velsicol Chemical Corporation Superfund Site in St. Louis, Michigan (Figure 2.2). Sediment samples were collected by inserting 5 cm x 90 cm polyvinyl chloride (PVC) pipes into the sediment as deep as possible. Holed rubber stoppers with an attached rubber flap were placed at one end of the pipe to release water and gas as the PVC pipes were inserted. As the pipes were withdrawn the rubber flap sealed to prevent loss of sediment and overlying water. After withdrawal, the pipes were capped, tightly sealed with tape to minimize oxygen exposure and transported to the laboratory. The samples were stored at 4°C until used.

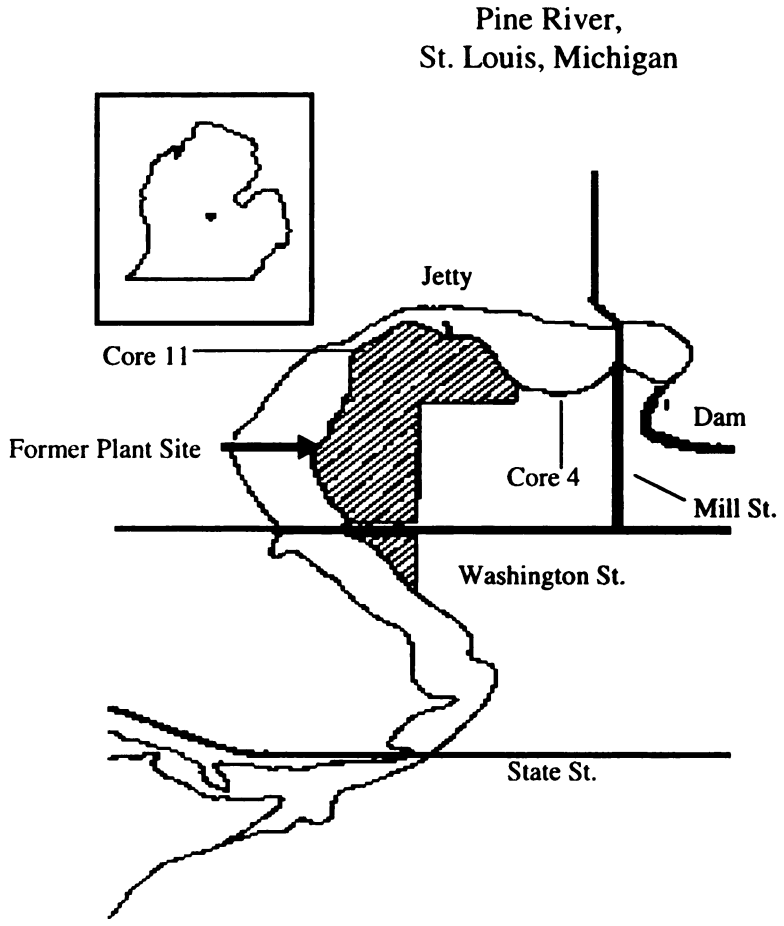


Figure 2.2- The Velsicol Chemical Corporation plant site located on the Pine River in St. Louis, Michigan and sampling points for core 4 and core 11. Adapted from Morris et. al., (1993).

Two sediment cores were selected for study in this experiment. Samples containing trace levels (~0-5 ppm) of contaminants were collected slightly downstream (core 11) of the region of highest DDT contamination and samples containing a greater DDT concentration (~2000-4000 ppm) were obtained slightly upstream of this region (core 4) (EPA, 1986). Anaerobic sediment slurries were prepared in a 1:1 ratio of sediment to Reduced Anaerobic Mineral Media (RAMM) (Shelton and Tiedje, 1984) as described by Quensen et al. (1998). The sediments were removed from the PVC pipes and separated into two sections (top and bottom sections), each approximately 10 cm

long. They were immediately placed into oxygen-free [N_2/CO_2 (80:20, vol/vol)] flasks, which were continuously flushed using a Hungate gassing apparatus (Hungate, 1968). The flasks contained an approximately equal volume of RAMM (600ml) and sediment which were slurried using a magnetic stirrer.

Pine River Sediment Microcosms

Microcosms were setup using core 11 and core 4 sediments amended with ^{14}C radiolabelled- DDT, -DDD or -DDE as substrates for the assays. A mixture of each compound was made containing 6.9 mg of ^{14}C-DDT, and 141.3 mg of unlabelled DDT; 8.9 mg of ^{14}C-DDD and 51 mg of unlabelled DDD; and 2.5 mg of ^{14}C-DDE and 56.8 mg of unlabelled DDE diluted with acetone to 5.2, 2.1, and 2.1 mL of DDT, DDD, and DDE respectively. This gave a solution concentration of 28.5 mg/mL for DDT, 28.5 mg/mL for DDD, and 28.2 mg/mL for DDE and an activity of approximately 1.07X 10^5 dpm/mL as verified by liquid scintillation counting (Beckman LS6500). A volume of 7 mL of sediment slurry (containing about 2 g of sediment on a dry weight basis) was transferred to N_2/CO_2 purged 20 ml glass vials and spiked with 7µl of ^{14}C-labelled-DDT, -DDD, or-DDE. This gave an activity of 7.5 X 10^5 dpm/microcosm. The microcosms were mixed thoroughly after addition of the DDT, DDD, or DDE, capped with Teflon stoppers, sealed with aluminum crimp caps, and stored in the dark at room temperature (22° to 25° C).

Samples for the determination of biological (live) as well as non-biological (sterile) transformations occurring in the sediments were set up for this study. Four replicate samples were sacrificed at intervals of 0, 4, 8, 12, 16, 24, and 32 weeks for live,

and 0, 8, 16, and 32 weeks for the sterile samples, for each compound (DDT, DDD, DDE) and each section of sediment. The sterile microcosms were autoclaved three consecutive days for 1 hour before the addition of ^{14}C-labeled compounds to the vials. Upon completion of the specified incubation period, samples were immediately frozen.

Microcosms amended with Pine River and Silver Lake Inocula

Additional microcosms were setup using inoculated sediment slurries previously shown to dechlorinate PCBs. Non-PCB-contaminated Red Cedar River sediments were pre-incubated, sterilized, then inoculated with microorganisms eluted from downstream Pine River and Silver Lake sediments. Specifically, samples of air-dried Red Cedar River sediments (2 grams) were weighed into 20 mL glass vials. The vials were evacuated and refilled with N_2 in an anaerobic chamber then sealed with butyl stoppers. For the pre-incubation step, 1 L of RAMM was prepared with the addition of 1 mL of ethanol and 25 mL of inocula eluted from the specified sediment slurry as described by Quensen et. al., (1988; 1990). The microcosms were first tested for their ability to maintain strict anaerobic conditions. For this, 6 mL of inoculated RAMM were added to each sample vial with a sterile syringe through the stopper and incubated in the dark for one week at 37°C. After 7 days, headspace gas was analyzed for methane production then autoclaved one hour for two consecutive days. The sample vials were then re-inoculated to determine the DDX dechlorination activity. Using sterile anaerobic technique the butyl stoppers were removed from the vials. The vials were then flushed with oxygen-free N_2/CO_2 and 1mL of eluted inoculum and 7 µL of either ^{14}C-DDT, -DDD, or -DDE were added. Some of the Silver Lake inoculated microcosms were also amended with 1 mL of a 10 mM $FeSO_4$ solution. All vials were resealed with Teflon stoppers and aluminum crimp caps and stored in the dark at room temperature for the predetermined incubation period. For the sterile (abiotic) samples, the microcosm setup was the same with the exclusion of inoculum addition.

Red Cedar Unamended Microcosms.

Red Cedar sediments were tested for their ability to dechlorinate DDT. Sediments were collected from the Red Cedar River, placed in glass jars, capped and transported to the laboratory and stored at 4°C. Subsequently, sediments were removed from the jars and placed into flasks which were being flushed with oxygen-free N_2/CO_2 (80:20, vol/vol) using a Hungate gassing apparatus (Hungate, 1968), and which contained an equal volume (600 ml) of RAMM. The sediment-RAMM mixtures were slurried using a magnetic stirrer. Red Cedar sediment slurry (7 mL) was transferred into continuously flushed vials, spiked with 7 µl of either ^{14}C radiolabelled- DDT, -DDD or -DDE, capped with Teflon stoppers, sealed with aluminum crimp caps, and then stored for their incubation period.

Microcosms amended with Hudson River Inocula and $FeSO_4$

Red Cedar sediment microcosms were setup as described above, inoculated with microorganisms eluted from Hudson River sediments, then amended with $FeSO_4$ to evaluate the potential stimulatory effects of $FeSO_4$ on dechlorination (Zwiernick et al, 1999). Sediment microcosms containing Red Cedar sediment slurried in RAMM were autoclaved for one hour two consecutive days. The samples were re-opened and flushed with a continuous stream of N_2/CO_2 while adding 1mL of eluted Hudson River inoculum along with 7µl of either ^{14}C radiolabelled -DDT, -DDD or -DDE. Some of the Hudson River microcosms were also amended with 1mL of a 10mM $FeSO_4$ solution. The vials were resealed with Teflon stoppers and aluminum crimp caps and stored in the dark at room temperature for the predetermined incubation period. Abiotic microcosms were

setup in the same fashion, with the exclusion of inoculation, then autoclaved for one hour on three consecutive days before the addition of ^{14}C-labeled compounds to the vials. Four replicate samples were sacrificed at intervals of 0, 8, 16, 24, and 32 weeks for live, and 0, 8, 16, and 32 weeks for the autoclaved controls. Upon completion of the specified incubation period, samples were frozen until subsequent analysis.

Methane Analysis

After each incubation period, methane analysis was used to demonstrate strict anaerobic conditions (production of methane), as well as to show the biological activity in each microcosm. Headspace gas (20 µl) of the microcosms withdrawn was analyzed for methane using a gas chromatograph equipped with a flame ionization detector.

Extraction and TLC Analysis

According to the methods of Quensen et.al (1998), frozen samples were thawed and extracted three times by shaking for 10 minutes with 7 ml of petroleum ether and acetone (5:2,vol:vol). Solvent phases were combined and evaporated to a volume of ~500 µl under a stream of dry nitrogen gas. Sample extracts (20 µl) were spotted on activated silica gel TLC plates. The plates were developed to 15 cm with a (5:95,vol: vol) of petroleum ether and hexane in a lined TLC chamber at room temperature. Autoradiography was used to determine locations of the parent compound and metabolites on the TLC plates. Kodak Scientific Imaging Film (X-OMAT AR) was exposed to the TLC plates for 7 days at negative 20°C before developing.

Autoradiography films and TLC plates were placed side by side on a light box where the parent compound and metabolite zones were visualized and marked for scraping. Liquid scintillation counting was used to determine the ^{14}C activity in the scrapings.

REFERENCES

Forba, R.W. U.S. EPA Pine River Contamination Survey, St. Louis, MI, EPA-330/2-80-031, 1980.

Forba, R.W. U.S. EPA Summary of Pine River Reservoir Sediment Sampling Survey, St. Louis, MI, EPA-330/2-82-001, 1982.

Hungate, R.E. 1968. Adv. Microbiol. 3B,117.

Morris, P.J., Quensen, J.F III, Tiedje J.M., and Boyd, S.A. 1993. An assessment of the Reductive Debromination of Polybrominated Biphenyls in the Pine River reservoir.

Environmental Science and Technology. 27: 1580-1586.

Quensen, J.F III; Tiedje, J.M.; and Boyd, S.A. 1988. Reductive dechlorination of Polychlorinated Biphenyls by anaerobic microorganisms from sediments.

Quensen, J.F III; Boyd S.A.; and Tiedje J.M. 1990. Dechlorination of Four commercial Polychlorinated Biphenyl Mixtures (Aroclors) by Anaerobic Microorganisms from Sediments. Appl and Environ. Microbiol. 56: 2360-2369.

Quensen, J.F. III, Mueller, S.A., Jain, K.M. 1998. Reductive dechlorination of DDE to DDMU in marine sediment microcosms. Science. 280: 722-724.

Shelton, D.R.; Tiedje,J.M. 1984. Appl. Environ. Microbiol. 47 850.

U.S. EPA Region 5. 1998. National Remedy Review Board, National Remedy Selection Briefing: Velsicol Chemical Site, St. Louis, MI.

U.S.EPA Region 5. 1998. Draft Streamlined Remediation Investigation Report: Velsicol Chemical Site, St. Louis, MI.

Zwiernick, M.J., Quensen J.F. III, Boyd, S.A. 1999. FeSO4 amendments stimulate extensive anaerobic PCB dechlorination.

CHAPTER 3

RESULTS AND DISCUSSION

DDT Transformations

DDT, PBBs, HBBs, and heavy metals are some of the compounds that heavily contaminate sediments at the Pine River Reservoir Superfund Site in St. Louis, Michigan. Studies on the Pine River Reservoir indicated the presence of DDT in concentrations as high as 4% by weight of the sediment (Forba et al. 1980; 1982). Levels of total DDT in fish sampled between 1985 and 1997 have been alarmingly high and may be increasing. The average DDX (Σ DDT+DDD+DDE) concentrations in fish taken from below the Pine River Dam between 1985 and 1997 increased from 10ppm to 27 ppm, and between 1989 and 1997 the concentrations below the impoundment increased from 10ppm to 35 ppm (EPA, 1998). The continuing high levels of DDT in Pine River sediments and in fish taken from the Pine River in proximity to the Velsicol Superfund site led to investigations focusing on potential environmental transformation of these compounds in the sediments. Morris et. al. (1993) investigated the degradation of PBBs in the Pine River sediments and found evidence of only very limited in situ anaerobic biobromination of PBBs. Microorganisms capable of PBB debromination were found in Pine River sediments, however, high concentrations of co-contaminants were believed to inhibit in situ debromination. Quensen et al. (1998) discovered the presence of DDMU originating from the dechlorination of DDE in Palos Verdes sediments. These findings led to studies by Roberts (2001) to determine if the Pine River could support the dechlorination of DDT and its metabolites DDD and DDE. Sediment analysis identified the DDT metabolites DDD, DDE, and DDMU. Roberts (2001) sought to identify the

origin of DDMU detected in the sediments and evaluate the transformations of DDT, DDD and DDE under strict anaerobic conditions in controlled laboratory experiments. In these investigations the transformation of DDT to DDD was observed, but no transformations of DDD or DDE occurred. The present study was undertaken to further investigate the transformation of DDT, DDD, and DDE in Pine River sediments under anaerobic conditions and to evaluate the dechlorination of DDT by microbial communities with demonstrated capability to dechlorinate PCBs. The Pine River sediments did support the anaerobic dechlorination of DDT to DDD by both biotic and abiotic transformations, however it did not support transformations of DDD or DDE. Similar results were obtained in previously non-DDT contaminated Red Cedar sediments inoculated with microorganisms from Pine River, Hudson River and Silver Lake.

Transformations in biologically active microcosms

Pine River Sediments incubated under anaerobic (methanogenic) conditions supported dechlorination of DDT to DDD in both sediment cores studied, top and bottom sections. In core 4 top and bottom sections there was dechlorination of DDT to DDD (Figure 3.1). The greatest amount of dechlorination was observed at the termination of the incubation (32 weeks) for both top and bottom sections. The average recovery for DDT was 45% (of ^{14}C DDT added) for 16 and 24 weeks for the top section and 39% and 32% for 16 and 24 weeks, respectively, for the bottom section. The average DDT recovery at 32 weeks for the top section was 32% and 19% for the bottom section. Thus, based on loss of DDT, the greatest amount of dechlorination occurred in the bottom section of sediment.

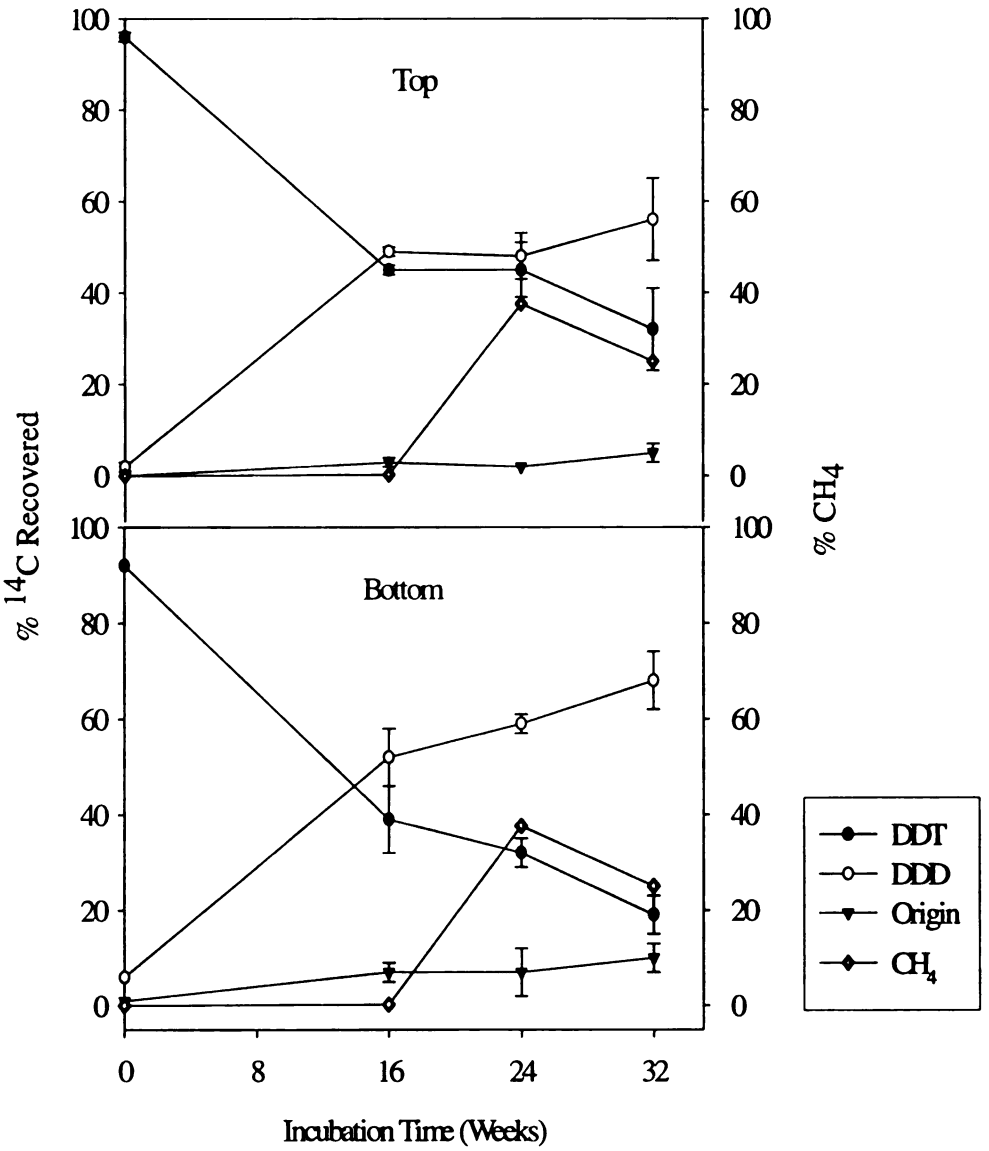


Figure 3.1. Transformation of DDT to DDD in Core 4 top and bottom layer anaerobic sediment microcosms during a 32 week incubation period.

In core 11 sediments there was also dechlorination of DDT to DDD (Figure 3.2). Transformation of DDT occurred for the top and bottom sections of sediments, with the greatest amounts occurring in the bottom section of sediment. The average recovery of DDT at 16 and 24 weeks was 23 and 30%, respectively, for the top section and 11 and 9% for the bottom section of sediment. The greatest amount of dechlorination occurred at 32 weeks for the top and bottom sections of sediment. The amount of DDT recovered at 32 weeks (termination of experiment) was 18 and 5% for the top and bottom sections of sediment, respectively (Figure 3.2).

As the amount of DDT recovered decreased over time, the amount of DDD recovered increased (Figures 3.1, 3.2, 3.3). For core 4 from 16 to 32 weeks the amount of DDD recovered ranged from 48 to 56% for the top section of sediment and 52 to 68% for the bottom section of sediment. For core 11 between 16 and 32 weeks, the recovery range for the top section of sediments ranged from 58 to 66% and 67 to 72% for the bottom section of sediment. A Student's t-test was performed to assess the statistical significance of differences in DDD for the top and bottom sections of core 4 and core 11. When comparing the statistical significance ($P<0.05$) between the differences in recoveries from the top and bottom sections and between the two cores, there was no significant difference between the top and bottom sections in core 4, or in the top and bottom sections of core 11.

Overall a slightly greater amount of dechlorination occurred in core 11 where 72% of the DDT had been transformed to DDD, as compared to 68% in core 4 (based on recoveries). However when compared statistically there was no significant difference between the top section of core 4 and the top section of core 11, or between the bottom

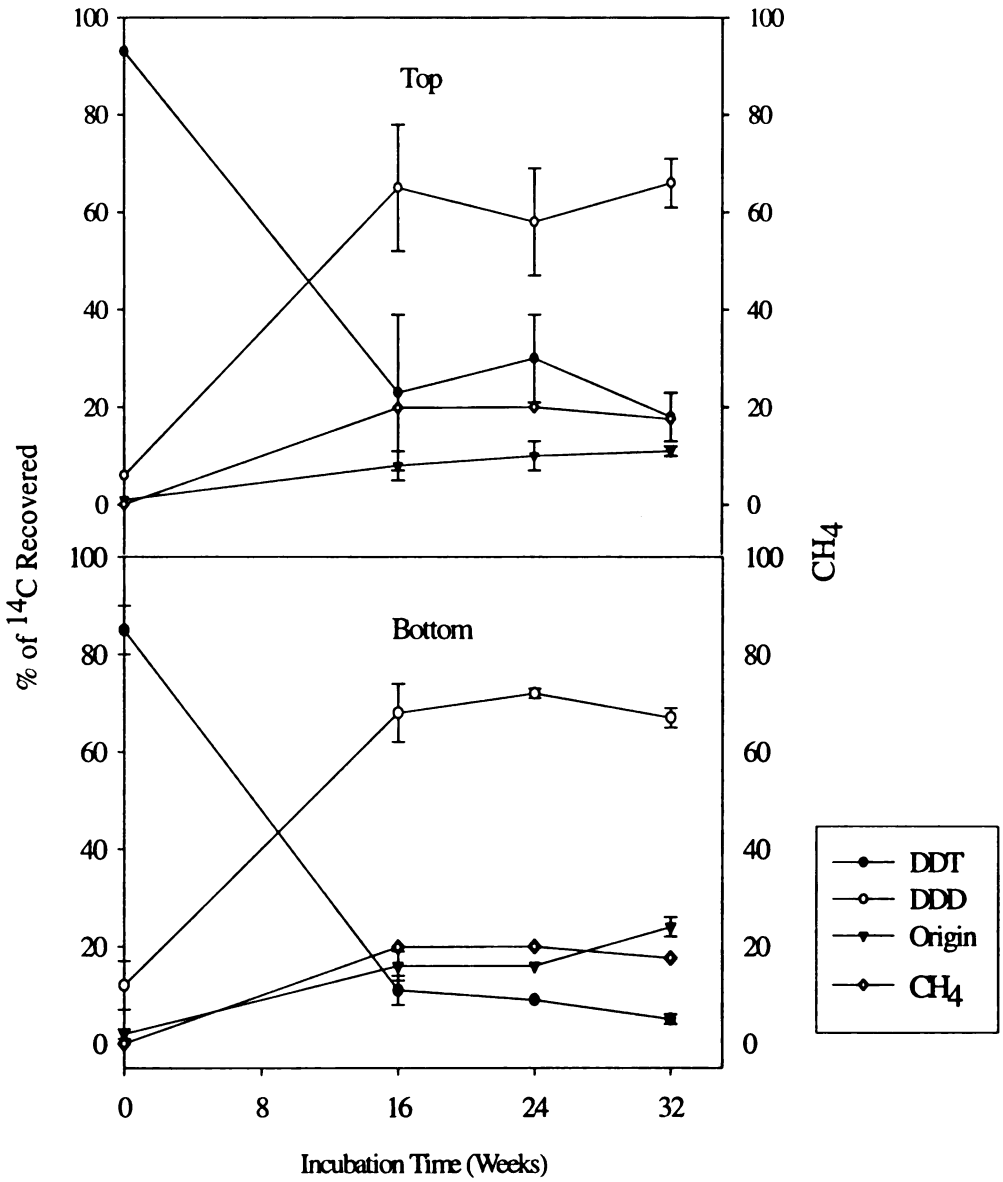


Figure 3.2. Anaerobic dechlorination of DDT to DDD in Core 11 top and bottom layer anaerobic sediment microcosms over a 32 week incubation period.

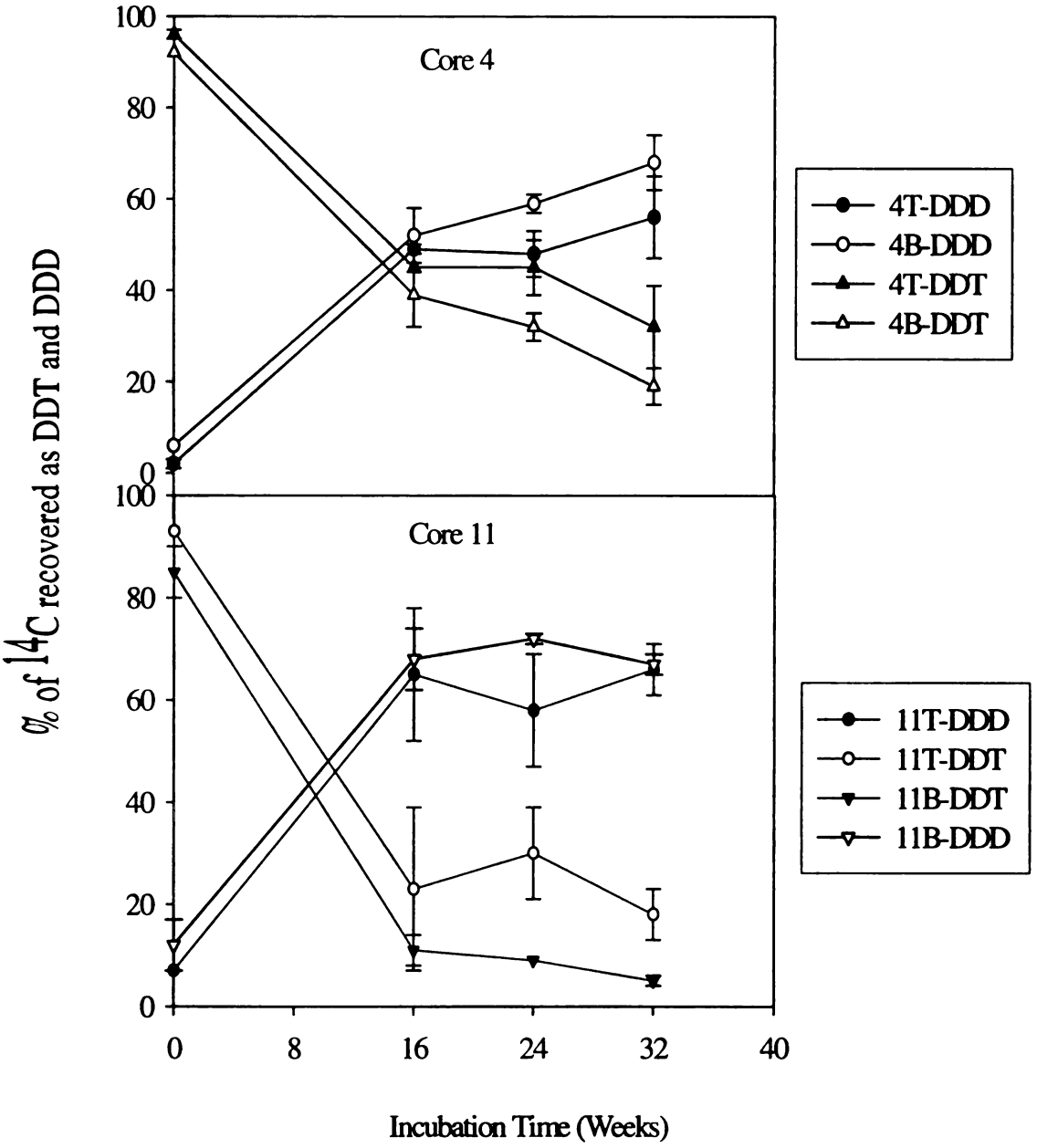


Figure 3.3. Comparison of anaerobic dechlorination of DDT in Core 4 and 11 top (T) and bottom (B) sections over a 32 week incubation period.

section of core 4 and the bottom section of core 11. Core 11 was taken upstream from the region of highest contamination and core 4 that was taken downstream from this region. Based on previous sediment analyses (EPA, 1998) DDX levels in the proximity of core 11 were estimated as between ~0 and 5 ppm, and between ~2000 to 4000 ppm for sediments in proximity to core 4. It is also likely that sediments in the area that core 4 was taken were heavily co-contaminated with the products from the outfall, including PBB, HBB and TRIS. Despite these differences in background levels of DDX and co-contaminants, the transformation of DDT to DDD was very similar in core 4 and core 11 sediments.

Other metabolites were observed on the TLC plates but not analyzed because they were present in all samples in trace amounts (Figure 3.4). Some dechlorination of DDT was observed in the time 0 samples. This could have been caused by abiotic reactions taking place in the freezer or transformations occurring before the samples were frozen. At 0 weeks DDD was observed in the range of 2-12% (% of DDT added) for all sediments. (Table 3.1).

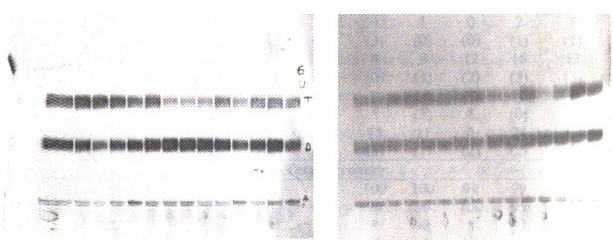


Figure 3.4. Autoradiograms of parent compounds and metabolite zones on TLC plates for transformations of DDT.

Table 3.1. Recovery of ^{14}C DDT and transformation products in Pine River sediments based on TLC Analysis. Sample microcosms were established using the top (T) and bottom (B) halves of sediment cores from two different locations (cores 4 and 11). The standard deviation is reported in parenthesis. *24 week Autoclaved samples were not setup.

Incubation Time	Core 4T		Core 4B		Core 11T		Core 11B	
Weeks	Live	Auto	Live	Auto	Live	Auto	Live	Auto
			% Recovered as DDT					
0	96	96	92	89	93	94	85	88
	(1)	(2)	(0)	(4)	(0)	(2)	(5)	(3)
16	45	75	39	74	23	48	11	47
	(1)	(6)	(7)	(6)	(16)	(5)	(3)	(6)
24	45	*	32	*	30	*	9	*
	(6)	*	(3)	*	(9)	*	(0)	*
32	32	63	19	57	18	59	5	73
	(9)	(7)	(4)	(9)	(5)	(9)	(1)	(5)
			% Recovered as DDD					
0	2	2	6	7	6	5	12	9
	(1)	(0)	(0)	(1)	(0)	(1)	(5)	(2)
16	49	21	52	21	65	40	68	40
	(1)	(5)	(6)	(5)	(13)	(5)	(6)	(4)
24	48	*	59	*	58	*	72	*
	(5)	*	(2)	*	(11)	*	(1)	*
32	56	28	68	26	66	34	67	18
	(9)	(2)	(6)	(8)	(5)	(7)	(2)	(2)
			% Recovered as Polar Metabolites					
0	0	1	1	4	1	0	2	1
	(0)	(0)	(0)	(3)	(0)	(0)	(1)	(1)
16	3	4	7	4	8	12	16	13
	(1)	(0)	(2)	(0)	(3)	(2)	(3)	(3)
24	3	*	7	*	10	*	16	*
	(0)	*	(5)	*	(3)	*	(0)	*
32	7	8	10	15	11	6	24	6
	(2)	(8)	(3)	(3)	(1)	(2)	(2)	(3)
			% Total Recovery					
0	98	99	99	100	100	99	99	98
16	97	100	98	99	96	100	95	100
24	96	*	98	*	98	*	97	*
32	95	99	97	98	95	99	96	98

Transformations in autoclaved microcosms

Transformation of DDT to DDD was observed in the autoclaved treatments (Figures 3.5, 3.6, 3.7). The amount of DDT transformed in the sediments was less in the autoclaved sediments than for the live treatments. The amount of DDT recovered in the Core 4 top layer sediments ranged from about 63% to 96% with the greatest amount of dechlorination occurring at 32 weeks (63% of added DDT recovered). In the core 4 bottom layer sediments, the DDT recovered ranged between 57-89% with the greatest amount of dechlorination (lowest DDT recovery) occurring at 32 weeks (57% of the added DDT recovered) (Figure 3.5). In the core 11 top layer sediments, the amount of DDT recovered ranged from 48% to 94% over the 32 week time period. In the bottom layer sediments, the DDT recovered ranged between 47% to 88% with the greatest amount of dechlorination occurring at 16 weeks (47% of added DDT recovered) (Figure 3.6).

There was a considerable amount of transformation activity occurring in both cores, but there was a larger percentage of DDT dechlorination occurring in the Core 11 sediments based on recoveries. T-tests were also used to compare autoclaved microcosms at 32 weeks. When compared statistically, at a 95% confidence level there was a significant difference between the bottom section of core 4 and the bottom section of core 11. There was also a significant difference observed between the top and bottom sections of core 11. In both sections of core 11 the greatest amount of dechlorination occurred at 16 weeks whereas in core 4 the greatest amount of dechlorination occurred at 32 weeks. Furthermore there was also the increase in DDD as the DDT was transformed.

As the percentage of ^{14}C recovered as DDT decreased, the percent recovery of DDD increased over the 32 week time period (Figure 3.5, 3.6, 3.7).

For the abiotic and live microcosms methane production in the headspace of microcosms was used as an indicator of biological activity in sediment microcosms. The live microcosms show headspace methane production up to 37% over the 32-weeks, indicating that there was biological activity in the sediments. The methane production in autoclaved sediments was less than 0.01 % or undetectable, therefore giving no indication of biological activity in these sediments. The observation of DDD production in the autoclaved sediments indicates that DDT dechlorination was occurring by abiotic reactions as well as biologically.

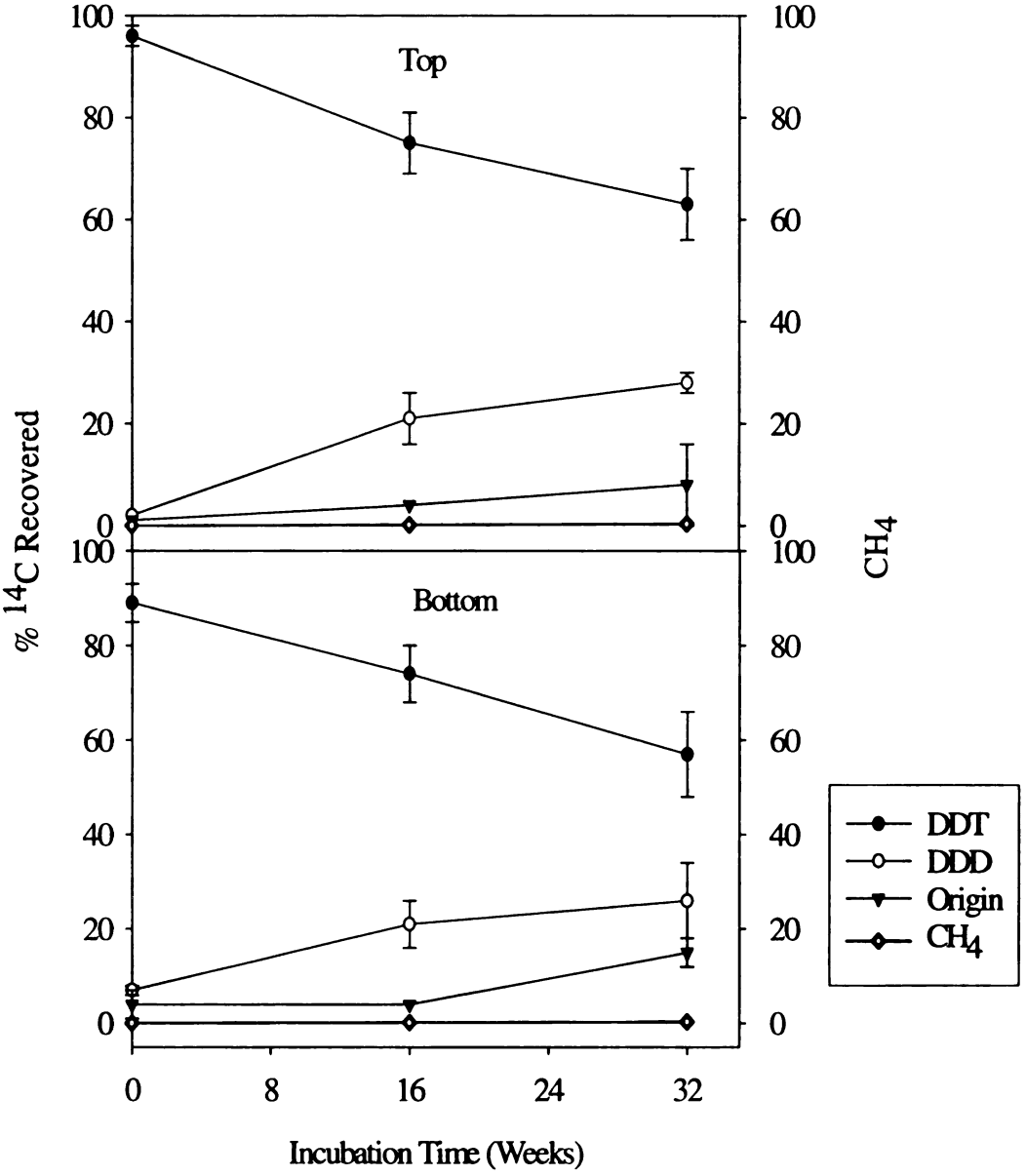


Figure 3.5. Transformation of DDT to DDD in Core 4 (top and bottom sections) abiotic sediment microcosms during a 32 week incubation period.

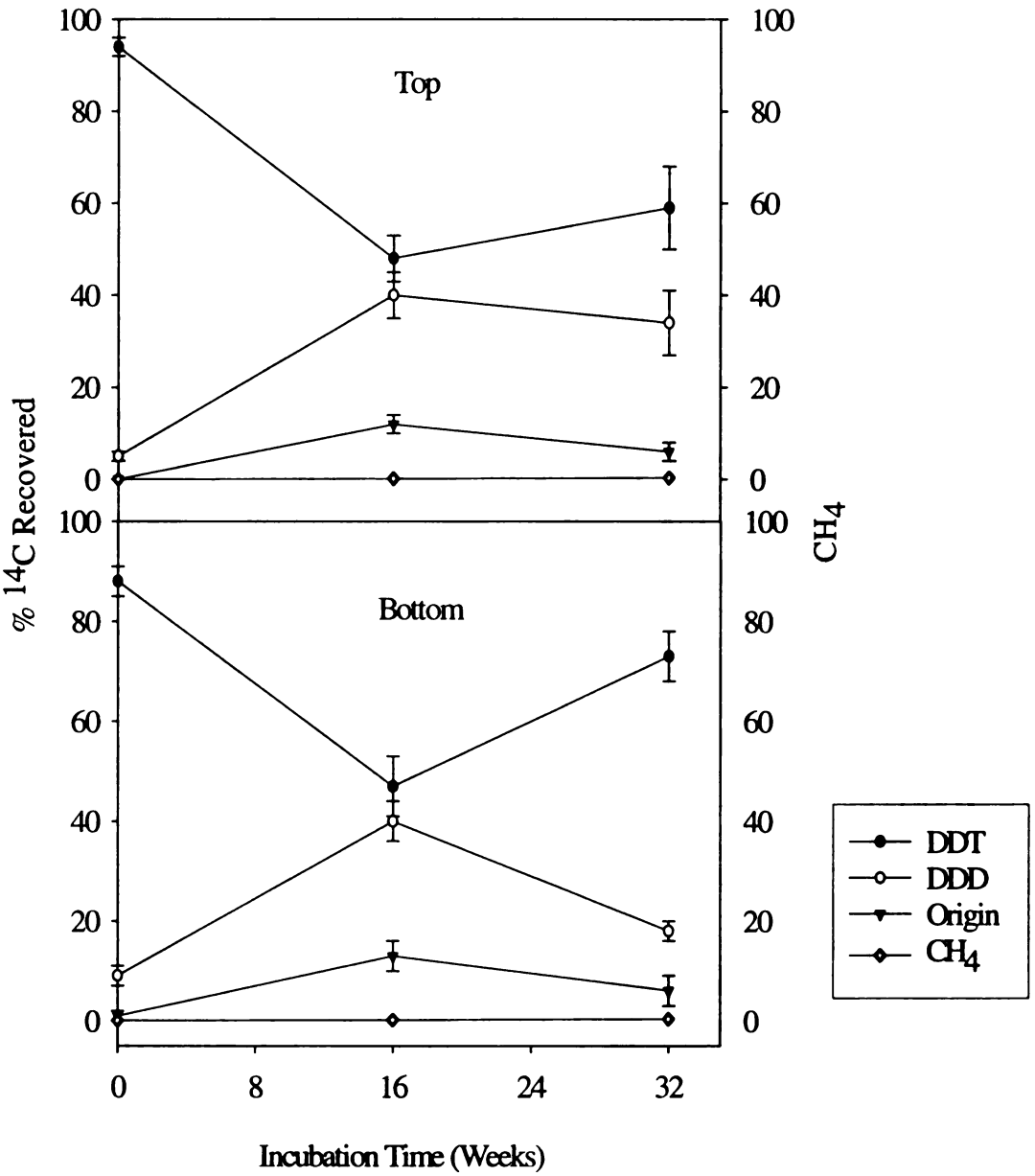


Figure 3.6. Transformation of DDT to DDD in Core 11 (top and bottom sections) abiotic sediment microcosms during a 32 week incubation period.

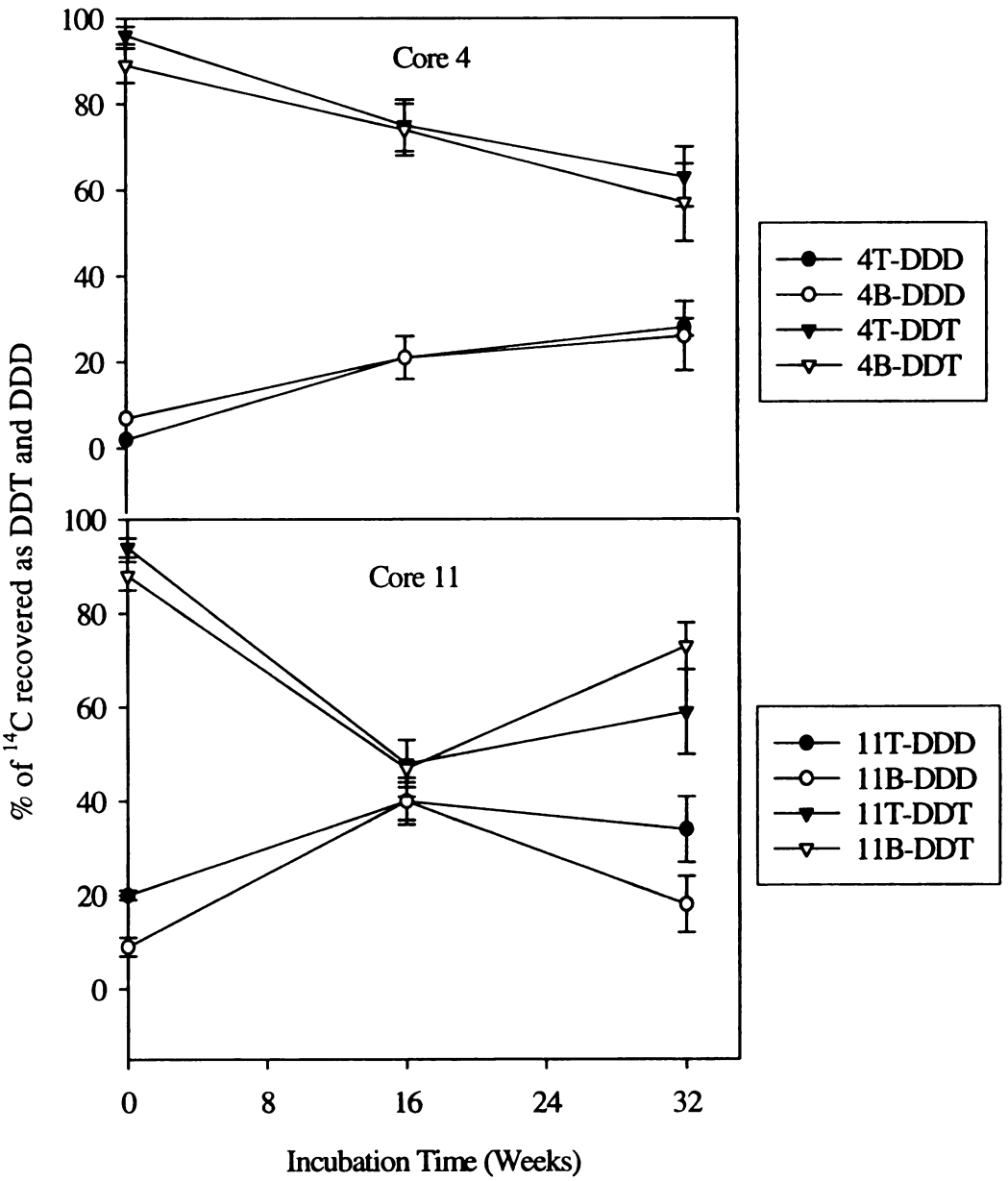


Figure 3.7. Comparison of anaerobic dechlorination of DDT in abiotic Core 4 and 11 top (T) and bottom (B) sections during a 32 week incubation period.

Abiotic and Biotic Transformations

It was evident that transformations other than biotic were also occurring in the sediment microcosms. Transformations of DDT to DDD occurred in both autoclaved and non-autoclaved sediments. The contributions of each process were calculated for the 16 and 32 week samples from each core (Figure 3.8 and Table 3.2). The biotic transformation contributions were calculated based on the difference in recoveries between live and autoclaved treatments divided by the DDD recovered in the live microcosms. The abiotic transformations were calculated by dividing the DDD formed in the autoclaved sediment microcosms by the DDD formed in the live sediment microcosms. At 16 and 32- week time points both abiotic and biotic processes contributed significantly to the transformation of DDT to DDD in the Pine River sediments. These contributions differed with each core and each section of sediment.

At 16 weeks, 43 and 40% of DDD formed in core 4 top and bottom sections was due to abiotic transformation, and 57 and 60% was formed by biotic transformations, respectively. In core 11 top and bottom sections 62 and 59% of DDD was formed by abiotic transformations and 38 and 41% by biotic transformations, respectively. Thus after 16 weeks of incubation biotic processes had a greater contribution in the formation of DDD in the core 4 sediments, in contrast to core 11 sediments where there was a greater abiotic contribution to the transformation of DDT to DDD. At 32 weeks the abiotic contribution to DDD formation was 50 and 38% for core 4 top and bottom sections, and 52 and 27% core 11 top and bottom sections, respectively. The biotic contributions were 50 and 62% for core 4 top and bottom sections, and 48 and 73% for core 11 top and bottom sections, respectively. T-tests were also used to compare

significant differences between live and autoclaved samples at 16 and 32 weeks. At a 95% confidence level, all samples were statistically different when compared to corresponding live samples at 16 and 32 weeks.

Incubation Time (wk)	Core 4T		Core 4B		Core 11T		Core 11B	
	BI	ABI	BI	ABI	BI	ABI	BI	ABI
	DDD formation (% of total)							
16	57	43	60	40	38	62	41	59
32	50	50	62	38	48	52	73	27

Table 3.2. DDD formation due to biotic and abiotic transformations of DDT in Pine River Sediment microcosms constructed using top (T) and bottom (B) sections of sediment cores 4 and 11. The biotic (BI) fraction was determined by dividing the difference in DDD recovered in live treatments by the DDD recovered in autoclaved treatments and dividing by the DDD recovered in the live treatment (X 100). The abiotic (ABI) fraction was determined by dividing the DDD recovered in the autoclaved sediments by the DDD recovered in the live sediment (X 100).

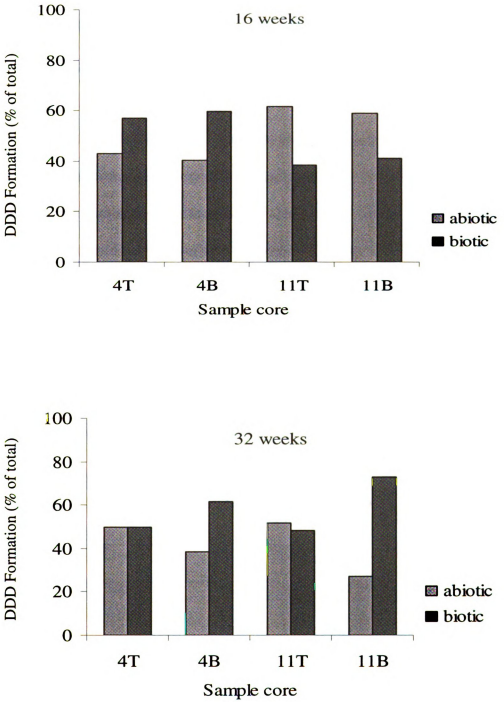


Figure 3.8. DDD recovered from the abiotic and biotic transformations of DDT in Pine River sediment microcosms constructed using the top (T) and bottom (B) sections of sediment cores 4 and 11. The biotic fraction was determined by dividing the difference of the live and autoclaved treatments by the live treatment. The abiotic fraction was determined by dividing the DDD recovered in the autoclaved treatments by the DDD recovered in live treatments.

^{14}C Recovery of DDT

The ^{14}C recovery gives the amount of DDX (=DDT+ DDD+ origin) recovered from the aqueous phase and solvent extractions of the sediment microcosms. Transformations of DDT (to DDD) occurred in all live and autoclaved sediment microcosms. At time zero total ^{14}C recovery in live sample microcosms ranged from 91-99%, with an average recovery of 95%, and from 87-95% in autoclaved sediment microcosms with an average recovery of 92%. After 16 weeks incubation, the recovery totals were considerably lower with an average recovery of 77% in live microcosms and 82% in the autoclaved microcosms. There was not a considerable decrease thereafter. In the 32 week samples the average recovery ranged from 72-98% in live samples and 77-82% in autoclaved microcosms. There was some unexplained loss of radioactivity in these sample microcosms. This loss could be from reasons such as volatilization or loss during the extraction process, but there is no definite explanation for this loss (Table 3.3).

	% ^{14}C Recovery of DDX (DDT+DDD+Origin)							
Incubation Time (wks)	Core 4T		Core 4B		Core 11T		Core 11B	
	Live	Auto	Live	Auto	Live	Auto	Live	Auto
	96	95	99	93	93	92	91	87
0	(5)	(4)	(1)	(2)	(3)	(3)	(4)	(2)
	84	81	99	87	78	79	71	79
16	(4)	(1)	(3)	(8)	(2)	(8)	(1)	(2)
	79	*	99	*	82	*	73	*
24	(1)		(1)		(7)		(0)	
	80	77	98	82	82	78	72	77
32	(4)	(5)	(3)	(9)	(1)	(3)	(0)	(0)

Table 3.3. ^{14}C Recovery in Pine River sediment microcosms over 32 weeks. Recoveries represent the amount of DDX (DDT+DDD+Origin) recovered from aqueous and solvent extractions of sediment microcosms for top (T) and bottom (B) sections of cores 4 and 11. The standard deviation is reported in parenthesis. *24 week autoclaved samples were not setup.

Transformations of DDD and DDE in sediment microcosms

DDD and DDE were not degraded significantly in sediment microcosms after 32 weeks of incubation in sterile and non-sterile microcosms (Table 3.4, Figure 3.9). The average recovery of ^{14}C-DDD over the 32 -week incubation period in live treatments was 97% of the added DDD with a range of 90-100%. The recovery range for the sterile microcosms ranged from 88-99% DDD with an average recovery of 97%. The recovery of DDE in the sediment microcosms was similar to that in the DDD treated sediments. Over the 32- week incubation period, the ^{14}C-recovery ranged from 87-96% for non-sterile microcosms and 78-97% for sterile microcosms. These results show that after 32 weeks incubation there were no significant transformations of DDD and DDE in the Pine River sediment microcosms.

Incubation Time (weeks)	Core 4T		Core 4B		Core 11T		Core 11B	
	Live	Auto	Live	Auto	Live	Auto	Live	Auto
				% Recovered as DDD				
0	100	97	99	99	*	99	97	99
	(0)	(0)	(0)	(0)	*	(0)	(0)	(0)
16	99	98	99	97	99	97	97	94
	(0)	(0)	(0)	(1)	(0)	(1)	(1)	(0)
24	99	*	95	*	94	*	95	*
	(0)	*	(6)	*	(2)	*	(0)	*
32	96	99	97	99	97	97	90	88
	(0)	(0)	(2)	(1)	(0)	(1)	(0)	(9)
				% Recovered as DDE				
0	96	94	92	86	94	93	96	78
	(1)	(2)	(8)	(12)	(0)	(7)	(1)	(11)
16	93	97	93	95	95	95	95	95
	(2)	(1)	(4)	(3)	(2)	(2)	(0)	(5)
24	94	*	89	*	92	*	91	*
	(1)	*	(9)	*	(1)	*	(1)	*
32	91	95	92	92	87	88	87	96
	(1)	(5)	(1)	(5)	(7)	(6)	(7)	(2)

Table 3.4. Recovery of added ^{14}C-DDD and -DDE in Pine River sediment microcosms. Sample microcosms were established using the top (T) and bottom (B) halves of sediment cores from two different locations (cores 4 and 11). The standard deviation is reported in parenthesis. *24 week autoclaved samples were not analyzed

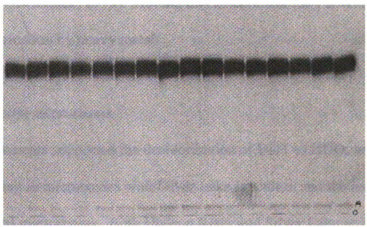


Figure 3.9. Autoradiogram of parent compound on TLC plate for transformation of DDD.

Red Cedar Sediment Microcosms

Different sediments and potential sources of dechlorinating inocula were studied in combination to see if they would support the degradation of DDT, DDD, or DDE. Air-dried sediments from the Red Cedar River (no known previous exposure to DDT or PCBs above normal background) were slurried with RAMM and treated with inocula eluted from various sediments, and in some cases $FeSO_4$ amendment. The inocula were from several locations that had previously shown the ability to dechlorinate PCBs or PBBs (Morris et. al., 1993). These locations are the Red Cedar River (MI), Hudson River (NY), Pine River (MI), and Silver Lake (MA). In a study conducted by Zwiernick et. al., $FeSO_4$ was used as an amendment to stimulate dechlorination. Its stimulatory effects were attributed to two factors: (1) provision of sulfate as an electron acceptor, which stimulates growth of sulfate-reducing bacteria responsible for dechlorination activity, and (2) provision of Fe2+ which precipitates sulfide formed during sulfate reduction, hence reducing sulfide toxicity. Accordingly, once sulfate is consumed, an increased number of sulfate reducers utilize PCBs as an alternate electron acceptor, leading to extensive dechlorination (Zwiernick, 1999). Adding this amendment may also alleviate inhibition of PCB dechlorination by heavy metals.

Biologically active microcosms

All treatments supported the dechlorination of DDT to DDD, with the greatest amount occurring in microcosms with Silver Lake inoculum and the least in the Hudson River inoculated microcosms. Both Hudson River and Silver Lake sediments are contaminated with PCBs and have a demonstrated ability to dechlorinate PCBs (Quensen

et. al., 1988; Quensen et.al., 1990). DDD formation was directly related to loss of DDT. The dechlorination of DDT to DDD was the only significant transformation observed in the live microcosms. The DDT recovery range for Red Cedar sediment microcosms was between 18 and 55%. The greatest amount of DDT recovered (least dechlorination) occurred in the Hudson River inoculated sediments where 55% of the DDT added was recovered after 32 weeks of incubation; and the least DDT recovered was observed in the Silver Lake inoculated sediments with 19% of DDT recovered (Figure 3.10).

Hudson River and Silver Lake inoculated sediments were also treated with $FeSO_4$ in an attempt to enhance the dechlorination of DDT. There was about 15% more DDD recovered in the Silver Lake inoculated microcosms without $FeSO_4$ added (73%) than in Silver Lake microcosms with $FeSO_4$ added (59%). However, in the Hudson River inoculated microcosms, greater DDD recovery was observed in sediments with $FeSO_4$ added (48%with, 36% without). The greatest amount of DDD formed was observed in Silver Lake inoculated microcosms where 73% of DDT added was recovered as DDD, and the least occurred in the Hudson River inoculated sediments with 36% recovered as DDD. Dechlorination of DDT by Pine River and Silver Lake inocula was greater than that by indigenous Red Cedar River microorganisms. Zwiernick (1999) observed enhanced dechlorination of PCBs by Hudson River microorganisms with the addition of $FeSO_4$. This is consistent with results presented here for DDT as indicated by a decrease in DDT recovery and an increased amount of DDD formed in the Hudson River inoculated microcosms amended with $FeSO_4$ as compared to the corresponding non-$FeSO_4$ amended sediments.

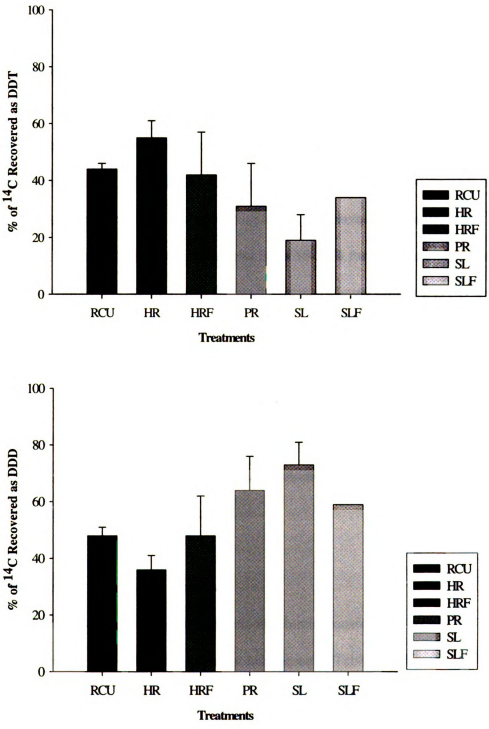


Figure 3.10. Recovery of DDT and DDD under strict anaerobic conditions from Red Cedar Unamended (RCU), Hudson River (HR), Hudson River with $FeSO_4$ (HRF), Pine River (PR), Silver Lake (SL), and Silver Lake with $FeSO_4$ (SLF) inocula after 32 weeks.

Abiotic microcosms

Transformation of DDT to DDD was observed in both live and autoclaved sediments, however it was greater in the live sediments than in the autoclaved sediments for the Red Cedar, Hudson River, and Pine River inoculated sediments as shown in Table 3.5. Overall, the Silver Lake inoculated Red Cedar sediments showed the greatest transformation of DDT to DDD. For the Silver Lake inoculated sediments there was slightly greater transformation occurring in the autoclaved sediments. After 32 weeks of incubation, the recovery of DDT ranged from 15-58% for autoclaved samples. The DDD recovery range was 30-79% after 32 weeks for autoclaved samples. DDD was formed from DDT by both abiotic and biotic transformations.

As in the Pine River sediment microcosms, methane production was used as an indicator of biological activity in sediment microcosms. The live microcosms revealed headspace gas methane production up to 48% over 32 weeks, indicating that there was biological activity in the sediments. The methane production in autoclaved sediments was less than 0.01 % or undetectable.

Abiotic and Biotic transformations

Relative contributions of abiotic and biotic transformations of DDT to DDD were calculated for the Red Cedar sediment microcosms (Table 3.6, Figure 3.11). Biotic transformation contributions were calculated based on the difference between the DDD recovered in live microcosms and DDD recovered in autoclaved microcosms divided by the DDD formed in the live microcosms. Abiotic transformations were calculated by dividing the DDD recovered in the autoclaved sediments, by the DDD recovered in the

% Recovered as DDT												
	RCU		HR		HRF		PR		SL		SLF	
Wks	L	A	L	A	L	A	L	A	L	A	L	A
24	55		45		31		27		18		41	
	(10)		(5)		(5)		(4)		(10)		(4)	
32	44	58	55	58	42	58	31	32	19	15	34	15
	(2)	(8)	(6)	(8)	(15)	(8)	(15)	(0)	(9)	(1)	(0)	(1)
% Recovered as DDD												
	RCU		HR		HRF		PR		SL		SLF	
Wks	L	A	L	A	L	A	L	A	L	A	L	A
24	39		45		58		67		76		55	
	(9)		(6)		(5)		(4)		(9)		(3)	
32	48	30	36	30	48	30	64	61	73	79	59	79
	(3)	(5)	(5)	(5)	(14)	(5)	(12)	(0)	(8)	(1)	(0)	(1)

Table 3.5. Anaerobic transformation of DDT in sterilized Red Cedar (RC) River sediment microcosms inoculated with microorganisms eluted from Hudson River (HR), Pine River (PR) or Silver Lake (SL). Some microcosms were also amended with $FeSO_4$ (F). Uninoculated (U) Red Cedar River sediment was also incubated before (live-L) and after (auto-A) autoclaving. Standard deviations of the means are given in parenthesis.

live sediments. The results showed that abiotic processes were responsible for the majority of dechlorination occurring in these treatments but there is substantial biotic dechlorination occurring. The abiotic contributions were 63, 83, 63, and 95% for Red Cedar unamended, Hudson River, Hudson River with $FeSO_4$ and Pine River, respectively, and the biotic contributions were 38, 17, 38, and 5% for Red Cedar unamended, Hudson River, Hudson River with $FeSO_4$ and Pine River treatments respectively. Silver Lake abiotic and biotic contributions were 108 and -8%, and Silver Lake with $FeSO_4$ abiotic and biotic contributions were 134 and -34% respectively. These values are much greater than those exhibited by the other treatments because the value for autoclaved DDD recovered was greater than non-autoclaved DDD recovered, thus giving

a value greater than 100 using the established calculations. In all of the inoculated Red Cedar sediment microcosms the majority of DDT transformation occurred by abiotic processes as opposed to the Pine River sediment microcosms in which the abiotic/ biotic contributions varied across all sites.

% of added DDT recovered as DDD		
Inoculum	abiotic	biotic
RC	63	38
HR	83	17
HRF	63	38
PR	95	5
SL	108	-8
SLF	134	-34

Table 3.6. DDD formation due to biotic and abiotic transformations of DDT at 32 weeks. Microcosms were set up using uninoculated Red Cedar River (RCU) sediment, as well as autoclaved RC sediment slurries which were inoculated with microorganisms eluted from RC, Hudson River (HR), Pine River (PR) or Silver Lake (SL), and in some instances amended with $FeSO_4$ (F). Both autoclaved (abiotic) and non-autoclaved (biotic) microcosms were then incubated under anaerobic conditions for 32 weeks. The biotic fraction was determined by dividing the difference between the DDD recovered in live and DDD recovered in autoclaved treatments by the DDD recovered in the live treatment (X100). The abiotic fraction was determined by dividing the DDD recovered in the autoclaved microcosms by the DDD recovered in the live microcosms (X100).

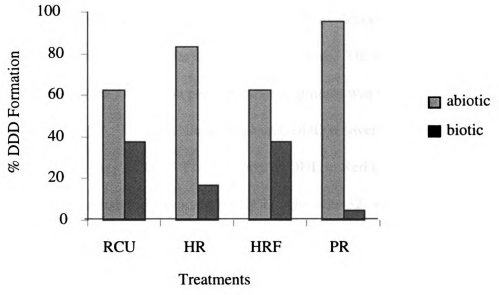


Figure 3.11. The fraction of DDD recovered resulting from biotic and abiotic transformations of DDT at 32 weeks. Microcosms were set up using uninoculated Red Cedar River (RCU) sediment, and autoclaved RC sediment slurries which were inoculated with microorganisms eluted from RC, Hudson River (HR), Pine River (PR) or Silver Lake (SL) and in some instances amended with $FeSO_4$ (F). Both autoclaved (abiotic) and non-autoclaved (biotic) microcosms were then incubated under anaerobic conditions for 32 weeks. The abiotic fraction was determined by dividing the DDD recovered in the autoclaved microcosms by the DDD recovered in the live treatments (X 100%). The biotic fraction was determined by dividing the difference of the DDD recovered in the live microcosms and the DDD recovered in the autoclaved microcosms by the DDD recovered in the live treatment microcosms (X 100%).

Transformations of DDD and DDE in sediment microcosms

After a 32-week incubation period, there was no evidence of DDD and DDE degradation in any of the Red Cedar sediment microcosms. The average recovery of ^{14}C DDD after the 32 -week incubation period in live treatments was 97% of the added DDD with a range of 87-99%. In the sterile microcosms, DDD recovery ranged from 96-99% with an average recovery of 98%. The recovery of DDE in Red Cedar sediment microcosms was less than that observed for DDD. Over the 32- week incubation period, the recovery of DDE ranged from 81-92% of added DDE for live microcosms, and 82-89% for sterile microcosms. These results give evidence that after 32 weeks incubation Red Cedar sediments did not support the transformations of DDD and DDE in laboratory microcosms, including those inoculated with known dehalogenating populations.

SUMMARY

This study was conducted to examine the fate of DDT, DDD, and DDE in the Pine River sediments. The transformation of DDT to DDD did occur in the Pine River sediments under anaerobic conditions by both abiotic and biotic processes. Biotic transformations accounted for the majority of DDT dechlorination in the core 4 sediments, and abiotic transformations accounted for the majority in the core 11 sediments. Based on recovery totals there appeared to be a greater amount of dechlorination occurring in the bottom layer sediments in both cores. However, statistically there was no significant difference ($P<0.05$) between the cores or sections. Similar amounts of DDD were recovered in core 4 and 11 microcosms despite the greater amount of co-contaminants present at the location of core 4. Our examination did not establish any other pathways in the Pine River sediment microcosms. Transformations of DDD or DDE were not observed.

Sediment slurry systems known to dechlorinate PCBs and/or PBBs were tested for their ability to transform DDT. These consisted of Red Cedar River sediments, and autoclaved Red Cedar River sediments inoculated with microorganisms eluted from Hudson River, Silver Lake and Pine River. All treatments supported the dechlorination of DDT to DDD by both abiotic and biotic processes, with the majority (63%-95%) occurring by abiotic processes. There was no observation of DDD or DDE transformation. Hudson River and Silver Lake microcosms were amended with $FeSO_4$ to test its ability to stimulate reductive dechlorination of DDT. The $FeSO_4$ amendment did enhance DDT dechlorination in the Hudson River microcosms consistent with its

stimulatory effects on PCB dechlorination. However, no further transformations beyond DDD were observed in these sediment microcosms.

These studies suggest that the predominant transformation of DDT in anaerobic sediments is dechlorination to DDD, and that these processes occur both biologically and abiotically. These findings will be beneficial in establishing the processes and conditions under which DDX transformations take place in the environment.

REFERENCES

Forba, R.W. U.S. EPA Pine River Contamination Survey, St. Louis, MI, EPA-330/2-80-031, 1980.

Forba, R.W. U.S. EPA Summary of Pine River Reservoir Sediment Sampling Survey, St. Louis, MI, EPA-330/2-82-001, 1982.

Morris, P.J., Quensen, J.F III, Tiedje J.M., and Boyd, S.A. 1993. An assessment of the Reductive Debromination of Polybrominated Biphenyls in the Pine River reservoir. Environmental Science and Technology. 27: 1580-1586.

Quensen, J.F III; Tiedje, J.M.; and Boyd, S.A. 1988. Reductive dechlorination of Polychlorinated Biphenyls by anaerobic microorganisms from sediments.

Quensen, J.F III; Boyd S.A.; and Tiedje J.M. 1990. Dechlorination of Four commercial Polychlorinated Biphenyl Mixtures (Aroclors) by Anaerobic Microorganisms from Sediments. Appl and Environ. Microbiol. 56: 2360-2369.

Quensen, J.F. III, Mueller, S.A., Jain, K.M. 1998. Reductive dechlorination of DDE to DDMU in marine sediment microcosms. Science. 280: 722-724.

Roberts, M.G. 2001. Microbial transformation of DDT in Pine River Sediments. Thesis. Michigan State University.

U.S.EPA Region 5. 1998. Draft Streamlined Remediation Investigation Report: Velsicol Chemical Site, St. Louis, MI.

Zwiernick, M.J., Quensen J.F. III, Boyd, S.A. 1999. FeSO4 amendments stimulate extensive anaerobic PCB dechlorination