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Anaerobic Microbial Dechlorination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Contaminated Kymijoki River Sediments

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Anaerobic Microbial Dechlorination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Contaminated Kymijoki River Sediments

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Academic dissertation

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Contents

ABSTRACT

Highly toxic polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) accumulate in the environment in sediments and soils. PCDD/Fs may pose considerable environmental and health risks because of their persistence in the environment. PCDD/Fs can be degraded slowly by microbial reductive dechlorination under anaerobic conditions which produces less chlorinated PCDD/Fs. Sediments of the Kymijoki River located in South-Eastern Finland are highly contaminated with PCDD/Fs and other chlorinated compounds. PCDD/Fs in sediments of the river mainly originate as unwanted byproducts from the production of the chlorophenol wood preservative Ky-5 between 1939 and 1984. The total volume of the most toxic 2,3,7,8-substituted PCDD/Fs contaminated sediments in the Kymijoki River, from Kuusankoski to the Baltic Sea, are estimated to be five million cubic meters of wet sediment. The Kymijoki River is the largest known source of PCDD/Fs to the Baltic Sea. The objective of this thesis was to gain knowledge on the sediment PCDD/Fs and the dechlorinating bacterial community at the Kymijoki River. We aimed to determine the potential for anaerobic microbial dechlorination of weathered PCDD/Fs in river sediments.

Sediment samples were collected from the Kymijoki River located at 0, 20, 30, and 60 km downstream from the main source of contamination, and at a reference site. An anaerobic microcosm study was conducted to determine dechlorination potential and the effect of temperature on the reductive dechlorination process for spiked 1,2,3,4-tetrachlorodibenzofuran (1,2,3,4-TeCDF). Concentrations of weathered PCDD/Fs, including a selection of non-2,3,7,8-substituted congeners, were determined in sediment samples. In addition, we determined the abundance and activity of the indigenous dechlorinating microbial community in sediments.

The measured mean total concentrations for 2,3,7,8-PCDD/Fs were high in sediments at all study sites of the river. At the most contaminated site, Kuusankoski, the concentrations for 2,3,7,8-PCDD/Fs were extremely high, at 1200 mg/kg dw. At all other study sites of the river the mean total concentrations for 2,3,7,8-PCDD/Fs varied between approximately 10 and 100 mg/kg dw. The predominant PCDD/F congeners were 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,8,9-HpCDF, and OCDF at all sites. The sediment PCDD/F congener profiles were similar to that of the Ky-5 wood preservative. Dechlorination of spiked 1,2,3,4-TeCDF to less chlorinated dibenzofurans was detected after 10 and 29 months in microcosms established with sediment from the all studied sites. The pathway of 1,2,3,4-TeCDF dechlorination was mainly via 1,3,4-trichlorodibenzofuran (TrCDF) to 1,3-dichlorodibenzofuran (DiCDF). The low temperature decreased the dechlorination rate of spiked TeCDF which suggests that dechlorination of weathered PCDD/Fs is limited at the *in situ* temperature of the river sediments. A native dechlorinating *Dehalococcoides*-like *Chloroflexi* community was present in sediment samples at all four sites of the river and at the reference site.

The dechlorination pathway of 1,2,3,4-TeCDF suggests that dechlorination of aged PCDD/F congeners could generate non-2,3,7,8-substituted and hence less toxic PCDD/Fs. However, concentrations of 2,3,7,8-substituted PCDD/Fs have remained at approximately the same levels over the last few decades at each sampling location of the Kymijoki River. Furthermore, no substantial changes in the relative abundances of weathered PCDD/Fs were observed over 2.5 years in laboratory microcosm studies,

indicating that anaerobic dechlorination of weathered PCDD/Fs was limited over the course of the experiment. Results of this study indicates that no or only minimal biodegradation of PCDD/F congeners has occurred in the river sediments over the last few decades since the contamination events. Therefore, concentrations of weathered PCDD/Fs in the sediments of the Kymijoki River are expected to remain at the same level for decades or centuries with further migration towards the Baltic Sea.

TIIVISTELMÄ

Polykloorattujen dibentso-*p*-dioksiinien ja dibentsofuraanien anaerobinen mikrobiologinen kloorinpoisto Kymijoen sedimenteissä

Polyklooratut dibentso-*p*-dioksiinit ja dibentsofuraanit (PCDD/F) ovat erittäin myrkyllisiä, sedimentteihin ja maaperään kertyviä yhdisteitä. PCDD/F-yhdisteet ovat merkittävä ympäristö- ja terveysriski, koska ne ovat pysyviä ympäristössä ja voivat kertyä eliöihin. PCDD/F-yhdisteiden on mahdollista hajota mikrobiologisella kloorinpoistolla anaerobisissa olosuhteissa hitaassa reaktiossa, jossa muodostuu klooria vähemmän sisältäviä yhdisteitä. Kymijoen pohjasedimenteissä esiintyy PCDD/F-yhdisteitä ja muita orgaanisia klooriyhdisteitä laajalla alueella korkeissa pitoisuuksissa. Suurimpana lähteenä joen PCDD/F-yhdisteille on ollut vuosina 1940-1984 puunsuoja-aineena käytetyn kloorifenolituote Ky-5:n valmistusprosessi, jossa nämä yhdisteet ovat syntyneet sivutuotteena. Haitallisimmilla 2,3,7,8-PCDD/F-yhdisteillä saastuneiden jokisedimenttien kokonaismäärän Kuusankosken ja Suomenlahden välillä on arvioitu olevan noin 5 000 000 m^3 sedimenttiä. Kymijoki on suurin yksittäinen PCDD/F-yhdisteiden päästölähde Itämereen. Työn tavoitteena oli lisätä tietoutta Kymijoen sedimenttien sisältämistä PCDD/F-yhdisteistä. Tarkoituksena oli tutkia joen sedimentteihin kertyneiden PCDD/F-yhdisteiden mahdollista hajoamista anaerobisella mikrobiologisella kloorinpoistoreaktiolla.

Sedimenttinäytteet kerättiin Kymijoelta 0 km:n, 20 km:n, 30 km:n ja 60 km:n etäisyydellä saastuneimmasta paikasta Kuusankoskelta sekä vertailupaikalta. Anaerobiset mikrokosmoskokeet suoritettiin kloorinpoistopotentiaalin sekä lämpötilan vaikutuksen tutkimiseksi malliaineena käytetyllä 1,2,3,4-tetraklooridibentsofuraanilla (1,2,3,4-TeCDF). Näytteistä määritettiin joen sedimentteihin kertyneiden 2,3,7,8-PCDD/F-yhdisteiden pitoisuudet sekä lisäksi sellaisten PCDD/F-yhdisteiden pitoisuuksia, joissa ei ole klooria kaikissa 2,3,7,8-asemissa. Lisäksi määritettiin klooriyhdisteitä hajottavien mikrobien esiintyvyyttä sedimenteissä.

Kymijoen kaikilta tutkimusalueilta määritetyissä näytteissä 2,3,7,8-PCDD/F-yhdisteitä esiintyi korkeissa keskiarvopitoisuuksissa. Kuusankoskella 2,3,7,8-PCDD/F pitoisuudet olivat noin 1200 mg/kg kuivaa sedimenttiä ja muilla tutkimuspaikoilla pitoisuudet olivat 10−100 mg/kg kuivaa sedimenttiä. Kaikilla tutkimusalueilla suurimpina pitoisuuksina PCDD/F-yhdisteistä esiintyivät 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,8,9-HpCDF ja OCDF. Joen sedimenteistä määritetty PCDD/F-yhdisteiden pitoisuusprofiili oli samanlainen kuin Ky-5-tuotteessa. Tutkimuksessa havaittiin 1,2,3,4-TeCDF-yhdisteen kloorinpoistoa sekä samanaikaisesti tri-, di- ja monokloorattujen furaanien muodostumista mikrokosmoksissa. 1,2,3,4-TeCDF:sta muodostui hajoamistuotteena eniten 1,3,4-TrCDF:a, josta muodostui edelleen 1,3-DiCDF:a. Huoneenlämpötilaa (21 °C) viileämpi lämpötila hidasti merkittävästi 1,2,3,4-TeCDF:n kloorinpoistoa, minkä perusteella voidaan arvioida PCDD/F-yhdisteiden hajoamisen hidastuvan joen sedimenttien *in situ* lämpötiloissa. Kloorinpoistoon kykeneviä *Dehalococcoides*-ryhmään kuuluvia *Chloroflexi* mikrobeja tunnistettiin kaikilta tutkimuspaikoilta.

Tutkimuksessa määritetyn 1,2,3,4-TeCDF:n hajoamisreitin perusteella voidaan päätellä, että mahdollinen kloorinpoistoreaktio tuottaa 2,3,7,8-yhdisteitä haitattomampia PCDD/F-yhdisteitä. Tulokset osoittivat, että joen sedimenttien 2,3,7,8-PCDD/F-yhdisteiden pitoisuudet ovat säilyneet samalla tasolla useiden vuosikymmenten ajan. Lisäksi

tutkimuksen aikana ei tapahtunut merkittäviä muutoksia PCDD/F-yhdisteiden suhteellisissa osuuksissa 2,5 vuoden aikana mikrokosmoksissa. Näiden tulosten perusteella tutkimuksessa ei havaittu sedimentteihin kertyneiden PCDD/F-yhdisteiden mikrobiologista hajoamista kloorinpoistoreaktiolla Kymijoen sedimenteissä. Pitoisuuksien voidaan olettaa säilyvän ennallaan sedimenteissä useiden vuosikymmenten ajan sekä mahdollisesti vähitellen kulkeutuvan sedimentin mukana Itämereen.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. S. Kuokka, A.-L. Rantalainen, and M.M. Häggblom, Anaerobic reductive dechlorination of 1,2,3,4-tetrachlorodibenzofuran in polychlorinated dibenzo-*p*-dioxin- and dibenzofuran-contaminated sediments of the Kymijoki River, Finland, Chemosphere, 2014 Mar; 98:58-65. [doi: 10.1016/j.chemosphere.2013.10.002]

II. S. Kuokka, A.-L. Rantalainen, M. Romantschuk, and M.M. Häggblom, Effect of temperature on the reductive dechlorination of 1,2,3,4-tetrachlorodibenzofuran in anaerobic PCDD/F-contaminated sediments, J Hazard Mater. 2014 Jun 15;274:72-8. [doi: 10.1016/j.jhazmat.2014.03.065]

III. Sanna Mäntynen, Anna-Lea Rantalainen, and Max M. Häggblom, Dechlorinating bacteria are abundant but anaerobic dechlorination of weathered polychlorinated dibenzo-*p*-dioxins and dibenzofurans in contaminated sediments is limited, Environmental Pollution, 2017; 231:560-568. [doi: 10.1016/j.envpol.2017.08.050]

THE AUTHOR'S CONTRIBUTION

I. Corresponding author. The study was planned by SM, ALR, and MH. SM performed all the experimental work. SM wrote the paper under supervision of ALR and MH.

II. Corresponding author. The study was planned by SM, ALR, and MH. SM performed all the experimental work. SM wrote the paper under supervision of MH. The co-authors revised the paper.

III. Corresponding author. The study was planned by SM, ALR, and MH. SM performed all the experimental work. SM wrote the paper under supervision of ALR and MH.

ABBREVIATIONS AND CONCEPTS

DGGE	denaturing gradient gel electrophoresis
DiCDF	dichlorodibenzofuran
DNA	deoxyribonucleic acid
dw	dry weight
ed	electron donor
GC-MS	gas chromatograph accompanied by the mass spectrometer
HxCDF	hexachlorodibenzofuran
HpCDD	heptachlorodibenzo-*p*-dioxin
HpCDF	heptachlorodibenzofuran
MoCDF	monochlorodibenzofuran
OCDD	octachlorodibenzo-*p*-dioxin
OCDF	octachlorodibenzofuran
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PCDD(s)	polychlorinated dibenzo-*p*-dioxin(s)
PCDD/F(s)	polychlorinated dibenzo-*p*-dioxin(s) and polychlorinated dibenzofuran(s)
PCDF(s)	polychlorinated dibenzofurans(s)
PeCDD	pentachlorodibenzo-*p*-dioxin
PeCDF	pentachlorodibenzofuran
qPCR	quantitative polymerase chain reaction
TeCDD	tetrachlorodibenzo-*p*-dioxin
TeCDF	tetrachlorodibenzofuran
TeCP	tetrachlorophenol
TrCDF	trichlorodibenzofuran
TEF	toxic equivalency factor; factor indicating the estimated toxic potency of an individual PCDD, PCDF or dioxin-like compound as compared to 2,3,7,8-TeCDD.
TEQ	toxic equivalent; concept developed to express the overall toxicity of a mixture of dioxins and dioxin-like compounds as a single value. The TEQ value is obtained by adding the product of the concentration or amount and the TEF for each toxic compound.
WHO	World Health Organization
WHO-TEF	toxic equivalency factor according to WHO; two sets issued, in 1998 and 2006
WHO-TEQ	toxic equivalents according to one of the WHO-TEF sets
2,3,7,8-PCDD/Fs	the 17 congeners with chlorines at position 2,3,7 and 8 (2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8- PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF)

1. INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) have extreme toxicity and high resistance to biodegradation. These halogenated organic contaminants exist globally in the environment and tend to accumulate in both soils and sediments. PCDD/F contaminated sites are a source of current and future environmental and health risks. Hence, information is needed to assess remediation strategies for sites contaminated with PCDD/Fs (Weber et al., 2008).

1.1 Properties, environmental behavior, and toxicity of PCDD/Fs

PCDD/Fs are a class of planar aromatic halogenated compounds which differ in their degree of chlorination, with 1-8 chlorines as substituents, and in their chlorination pattern. They can occur in the form of 75 PCDD congeners and 135 PCDF congeners. The general structures are given in Fig 1. The highest toxicity is considered to be for the 17 congeners of PCDD/Fs with chlorines at the lateral 2, 3, 7 and 8 positions. These 2,3,7,8-substituted PCDD/Fs are toxic to biota due to the capacity to bind to a cellular protein called the aryl hydrocarbon (Ah) receptor (Kutz et al., 1990). The toxicity of the different 2,3,7,8-substituted congeners varies with their different affinity for the Ah receptor (Poland and Knutson, 1982).

PCDD/F compounds have extremely low water solubilities, low vapor pressures, high octanol-water partition coefficients, and low Henry's law constants (Shiu et al., 1988; Mackay et al., 1992). Vapor pressure, water solubility and Henry's law constant decrease with higher chlorinated congeners while octanol-water partition coefficient increases (Shiu et al., 1988; Rordorf, 1986). All PCDD/Fs from mono- to octa-chlorinated congeners are considered to be highly hydrophobic (Shiu et al., 1988). Due to the physical and chemical properties, PCDD/Fs released to surface waters have a high tendency to associate with organic particles of sediment which increases with higher chlorination level (Govers and Krop, 1998). Thus sediments and soils have become ultimate sinks of PCDD/Fs in the environment (Eitzer and Hites, 1989; Rappe et al., 1987).

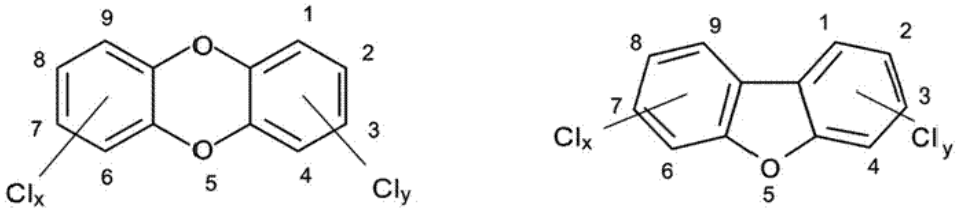

Figure 1. Molecular structure of polychlorinated dibenzo-*p*-dioxins (PCDDs) (a) and dibenzofurans (PCDFs) (b). x and y indicate the number of chlorine substitutions

The fate and persistence of PCDD/Fs in the environment is affected by a number of factors which include the physical and chemical properties of the compound, environmental factors such as temperature, pH, contaminant concentration, inhibitory elements, and types of microorganisms present in the environmental matrix, as well as the bioavailability of contaminants (Häggblom and Bossert, 2003). A variety of factors affect the bioavailability of organic compounds in complex environments such as in sediment including the extent of sorption of the compound to the sediment (Pignatello and Xing, 1996; Site, 2001), the residence time of the compound in the sediment in a process termed aging (Alexander, 1995), and the characteristics of the sediment i.e. organic carbon content and particle size (Lyytikäinen et al., 2003).

Toxic equivalency factors (TEFs) are assigned for the specific congeners of PCDDs, PCDFs and polychlorinated biphenyls (PCBs) to assess their toxicity (Van den Berg et al., 1998, 2006). TEFs are based on the toxic and biochemical effects of binding to the Ah receptor (Safe 1998; Van den Berg et al., 1998; Boening, 1998). TEF values relate the toxicity of each congener to the toxicity of the most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TeCDD). Non-2,3,7,8-substituted congeners are thought to have negligible toxicity (Van den Berg et al., 2006). TEF values are used to determine total toxic equivalency (TEQ) value of a mixture of dioxins and related compounds (Birnbaum and DeVito, 1995). The World health organization (WHO) assigned WHO-TEF values for human in 2005 (Van den Berg et al., 2006). Congener-specific bioaccumulation and biotransformation of PCDD/Fs may occur through the food chain (Opperhuizen and Sijm, 1990). Exposure to PCDD/Fs has been associated with a wide range of adverse health effects in humans including carcinogenic and immunotoxic effects (Leeuwen et al., 2000).

1.2 PCDD/Fs in the environment

PCDD/Fs are mainly discharged into the environment as unwanted byproducts of industrial processes. Anthropogenic sources are the principal routes for PCDD/Fs entering the environment (Fiedler et al., 1990, 1996; Esposito et al., 1980; Rappe 1993). PCDD/Fs can be formed in a wide range of processes which include chlorine bleaching of pulp and paper (LaFleur et al., 1990), metal refining industries (Anderson and Fisher, 2002), the manufacturing of herbicides and pesticides (Bertazzi et al., 1998), and waste incineration processes (Anderson et al., 1998; Cunliffe and Williams 2009). PCDD/F from anthropogenic sources are mainly formed in reactions of chlorophenols (Altarawneh et al., 2009).

Several studies have indicated that PCDD/Fs can also be emitted from natural processes (Gribble, 1994; Hashimoto et al. 1990), which include forest fires and other biogenic or geogenic processes (Kim et al., 2003). PCDD/Fs can be re-emitted also from secondary sources, i.e. deposition reservoirs (Zook and Rappe, 1994). A substantial fraction of PCDD/Fs originates from unidentified sources (Gaus et al., 2001; Wiberg et al., 2009).

High amounts of PCDD/Fs have been released to the environment, e.g. in an accident of chlorophenol manufacture in 1976 in Seveso, Italy (Bertazzi et al., 1998) and from the production and use of the herbicide Agent Orange during the Vietnam War (Schecter et al. 2015).

Some of the currently known PCDD/F contaminated sediment sites with their respective contamination sources are presented in Table 1. Industrialized urban regions usually have higher concentrations of PCDD/Fs than less industrialized rural regions (Wade et al., 1997). However, PCDD/Fs are detected even in clean environments because of long-range transport (Oehme, 1991; Srogi, 2008). PCDD/Fs can be transported through the atmosphere with aerosols and particulate material (e.g., fly ash) to soils and sediments (Eitzer and Hites, 1989) where they are found to be immobile and persistent. Concentrations of 2,3,7,8-PCDDs are usually greater than those of 2,3,7,8-PCDFs both in soils and sediments associated with chlorophenol contamination (Naile et al., 2011). Although the highest levels of PCDD/Fs are found in soils and sediments (Kulkarni et al., 2008), very low levels of these compounds have been detected in plants (Nunes et al., 2014), water (Castro-Jiménez et al., 2008) and air (Lohmann and Jones, 1998). PCDD/Fs are found in food especially in dairy products, meat, fish and other seafood (Domingo et al., 1999). PCDD/F compounds enter the humans almost exclusively from food and are stored in fatty tissues (Bernard et al., 2002; Malisch and Kotz, 2014).

Table 1. Global PCDD/F contamination in sediments of rivers and lakes

Location	PCDD/Fs (µg/kg d.w.)	Source	Reference
Wisconsin River, USA	30-200	Atmospheric deposition	Kuehl et al., 1987
Frierfjord, Norway	100-800	Production of metallic magnesium	Knutzen and Oehme, 1989
Passaic River sediment, USA	0.5-32	Manufacture of chlorinated compounds, 1970s	Wenning et al., 1992
Lake sediments, UK	4.5	Atmospheric deposition and local municipal activities	Rose et al., 1996
Ono River, Japan	0.4	Herbicide chlornitrophen (CNP)	Sakurai et al., 1996
Oder River and Akona Basin, Western Baltic sea	2.5-820	-	Dannenberger et al. 1997
Lake Grosser Arbersee sediment, Germany	2.3	Gaseous emission from mining and smelting or iron-ores and glass production, 1960s-1990s	Bruckmeier et al. 1997
Willamette River, Oregon USA	100-50000	-	Bonn et al., 1998
Kymijoki River sediment, Finland	120-190000; Up to 193000 (106 µg I-TEQ/kg dw)	Manufacture of chlorophenol fungicide Ky-5, 1940s-1980s	Verta et al., 1999; Salo et al., 2008
Spittelwasser River sediment, Germany	Up to 120	Magnesium and chlorine industry	Bunge et al. 2001
Hyeongsan River sediment, Korea	5-7600	Industrial waste stream	Koh et al., 2004
Lake Taihu sediment, China	1.3	-	Zhang et al., 2005
Nile River, Egypt	240-775	-	El-Kady et al.,2007
Elbe River, Pevestorf sediment, Germany	Up to 507	Metallurgical processes, 1950s	Götz et al., 2007
Lake Maggiore in Italy and Switzerland	n.d. to 400	-	Vives et al., 2007
Daliao River sediment, China	3.0	Organochlorine production since the 1980s	Zhang et al., 2008
Kentucky Lake, USA	n.d. to 4830	Variety of industries	Loganathan et al., 2008, Kannan et al., 2008

Lake sediment of Erie and Ontario, Canada	10	Industrial discharge, 1970s	Shen et al., 2008
East River sediment, China	2.1-4.8	Copper industry	Ren et al., 2009
Vaal River, South Africa	1.4-183	-	Nieuwoudt et al.2009
Lake Superior sediment, Canada	18	Atmospheric deposition	Shen et al. 2009
Lake Huron sediments, Canada	27	Atmospheric deposition	Shen et al. 2009
Liaohe River, China	0.24–27.49 ng WHO-TEQ kg−1	Combustion processes; coal burning, agricultural straw open burning, iron ore sintering, cement production	Zhang et al 2010
Polish rivers, Poland	146-1424	Atmospheric deposition	Niemirycz and Jankowska, 2011
Lake Awassa, Ethiopia	n.d. to 270	-	Urbaniak and Zalewski 2011
China -Tangshan -Qinhuangdao -Huludao -Panjin -Dalian -Dandong	<0.05		Naile et al, 2011
South Korea -Lake Shihwa -Asan -Sapgyo -Taean -Geum River -Yeongsan River	n.d. to 0.14		Naile et al., 2011
Lake Baikal, Russia	n.d. to 20	-	Ok et al.2013
Winam Gulf of Lake Victoria, Kenya	38-860	-	Omwoma et al. 2015

1.3 Microbial anaerobic reductive dechlorination

In anaerobic sediments the predominant biodegradation process for halogenated compounds including highly chlorinated PCDD/Fs and PCBs, hexachlorobenzene, tetrachloroethene, and pentachlorophenol is reductive dehalogenation (Häggblom and Bossert, 2003; Adrian and Löffler, 2016). In the process of reductive dechlorination chlorinated compounds are degraded to lower chlorinated byproducts by anaerobic bacteria (Bunge et al., 2001). Microbial reductive dechlorination under anaerobic conditions may potentially decrease the toxicity of PCDD/Fs through the removal of lateral chlorines (Bunge et al., 2001; Ballerstedt, 1997).

During the anaerobic reductive dechlorination process organohalide-respiring bacteria use the chlorinated compounds as terminal electron acceptors for respiration. The halogen substituent is removed from a molecule with concurrent addition of electrons to the molecule (Mohn and Tiedje, 1992). In reductive dehalogenation anaerobic organohalide-respiring bacteria gain energy from the process which can be used to support growth. Molecular hydrogen or other oxidizable compounds are used as the electron donor by most organohalide-respiring bacteria in the reaction where chlorine substituent is replaced with hydrogen (1).

(1) $R{-}Cl + H_2 \rightarrow R{-}H + H^+ + Cl^-$

Many microbial strains use halogenated compounds as electron acceptors during dehalorespiration under anaerobic processes (Bunge and Lechner, 2009; Ahn et al., 2005). Microbial molecular and phylogenetic analysis of various microbial consortia have indicated that bacteria in the phylum of *Chloroflexi,* with the genus *Dehalococcoides* in particular, are involved in the dechlorination of PCDD/Fs (Bunge et al., 2003). Previous studies have shown that e.g. *Dehalococcoides mccartyi* (formerly *D. ethenogenes*) strain 195 (Fennell et al. 2004, Ahn et al., 2008), *Dehalococcoides mccartyi* CBDB1 (Bunge et al., 2003) and *Dehalococcoides mccartyi* DCMB5 (Pöritz et al., 2015) in the phylum of *Chloroflexi* are capable of dechlorinating select PCDD/F congeners. Organohalide-respiring *Chloroflexi* are widely distributed and present in contaminated as well in uncontaminated ecosystems (Krzmarzick et al., 2012).

Several studies have demonstrated dechlorination of spiked TrCDD/F - OCDD/F resulting in lower chlorinated congeners in microcosms established with sediments from various sites, as summarized in Table 2. Studies of dechlorination of weathered PCDD/F are presented in Table 3. Ring cleavage of PCDDs under strictly anaerobic conditions has not been reported (Bunge and Lechner, 2009). Non or lower chlorinated products of reductive dechlorination are more likely to be released into the water column where they can be metabolized by aerobic degradation (Govers and Krop, 1998).

Microbial degradation of chlorinated organic compounds has an impact on the natural chlorine cycle in the environment. Environmental factors that may affect the reductive dechlorination rates of PCDD/Fs in anaerobic sediments include temperature, pH, electron acceptor and donor concentrations and their bioavailability, availability of nutrients, salinity, and organic matter concentrations (Bunge and Lechner, 2009). Some studies have shown dechlorination of PCDDs but there is limited information on dechlorination of PCDFs.

Table 2. Studies of dechlorination of spiked PCDD/Fs in sediment microcosms

Compound	Sediment Location	Reference
2,3,7,8-TeCDD	Hudson River, USA	Adriaens and Grbic-Galic, 1994
PeCDD/Fs - HpCDD/Fs	Hudson River, USA	Adriaens et al., 1995
1,2,3,4-TeCDD	Rhine River	Beurskens et al., 1995
OCDD	Passaic River, USA	Barkovskii and Adriaens, 1996
OCDD/F, PeCDD/F	Passaic River, USA	Adriaens et al.,1996
1,2,3,4-TeCDD, 1,2,3-TrCDD, 1,2,4-TrCDD	Saale River, Germany	Ballerstedt et al.,1997
PCDDs	Passaic River, USA	Barkovskii and Adriaens, 1998
2,3,7,8-TeCDD	Passaic River, USA	Albrecht et al.,1999
2,3,7,8-TeCDD	Taiwan	Kao et al., 2001
1,2,3,4-TeCDD	Arthur Kill Estuary, USA	Vargas et al., 2001
1,2,3,4-TeCDD, 1,2,3-TrCDD, 1,2,4-TrCDD	Spittelwasser River, Germany	Bunge et al. 2001
1,2,3,7,8-PeCDD	Spittelwasser River, Germany	Bunge et al 2003
1,2,4-TrCDD, 1,2,3-TrCDD	Saale River	Ballerstedt et al., 2004
1,2,3,4-TeCDD, 1,2,3,4-TeCDF	Paleta Creek, USA	Ahn et al., 2005
1,2,3,4,6,7,8-HpCDD	Passaic River, USA	Fu et al.2005
1,2,3,4-TeCDD	Kymijoki River, Finland; Gulf Island Pond, Maine; Lake Roosevelt, Washington	Ahn et al., 2008
1,2,3,4-TeCDD	Hackensack River, USA	Dam and Häggblom, 2017

Table 3. Dechlorination studies of weathered PCDD/Fs in sediments

Compound	Location of sediment	Study result	Reference
2,3,7,8-PCDD/Fs	Lake Ketelmeer, Rhine River, the Netherlands	Some PCDD/Fs showed disappearance in the anaerobic sediment, as compared differences between concentrations in the stored top-layer samples from 1972 and the concentrations in core layers deposited around 1970. For some PCDDs (1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD) and PCDFs (1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF), disappearance proved to be significant. 2,3,7,8-TCDD and 1,2,3,7,8,9-HxCDD seem to increase in the anaerobic lake sediment. Disappearance of the some PCDD/Fs may have been caused by microbial dechlorination reactions in the anaerobic lake sediment.	Beurskens et al., 1993
2,3,7,8-TeCDD	Passaic River, USA	Direct evidence of further lateral dechlorination of 2,3,7,8- TeCDD was obtained from the historically contaminated incubations; no isomer-specific identification of TrCDDs in spiked incubations was determined.	Barkovskii and Adriaens, 1996
2,3,7,8-TeCDD	Passaic River, USA	Production and dechlorination of 2,3,7,8-TeCDD was observed in anaerobic microcosm with site sediment.	Albrecht et al., 1999
PCDD/Fs	PCDD/F contaminated river sediments, Japan	All of the PCDD/F congeners detected were equally reduced without the accumulation of significant amounts of less-chlorinated congeners as the intermediate or end products. These results suggest that the apparent complete dechlorination of PCDD/Fs found in the microcosm was due to a combination of the dechlorinating activity of the "*Dehalococcoides*"-like organisms and the oxidative degradation of the dechlorinated products by aerobic bacteria with aromatic hydrocarbon dioxygenases.	Yoshida et al, 2005
PCDD/Fs	Kymijoki River, Finland	This study showed evidence for dechlorination of weathered PCDFs in Kymijoki River sediment mesocosms mediated by indigenous microorganisms.	Liu et al., 2013

1.4 Contamination of the Kymijoki River

Sediments of the Kymijoki River located in South-Eastern Finland are highly contaminated with PCDD/Fs (Verta et al., 2009). The Kymijoki River is the largest known source of PCDD/Fs to the Baltic Sea (Salo et al., 2008; Verta et al., 2007). Due to the high concentrations of PCDD/Fs, the Kymijoki River is one of the most contaminated sites in the world (Verta et al., 1999).

PCDD/Fs in the river mainly originate as unwanted byproducts from the production of the chlorophenol wood preservative Ky-5 between 1939 and 1984 (Verta et al., 1999). The predominating chlorophenols in Ky-5 were 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,4,6-trichlorophenol and pentachlorophenol (Kitunen et al., 1985). In addition to the chlorophenols, impurities in Ky-5 include PCDD/Fs, PCBs, polychlorinated diphenyl ethers (PCDE) and polychlorinated phenoxy anisoles (PCPA) (Humppi & Heinola, 1985; Vartiainen et al., 1995). The main fraction of PCDD/Fs in Ky-5 consisted of chlorinated furans (87 % of the total concentration and 95% of I-TEQ) and especially of heptachlorinated furans (72 %) (Vartiainen et al., 1995). The predominant PCDD/F congeners in Ky-5 were 1,2,3,4,6,7,8-heptachlorodibenzo-furan (HpCDF), 1,2,3,4,6,8,9-HpCDF, octachlorodibenzofuran (OCDF), 1,2,4,6,8,9 -hexachlorodibenzofuran (HxCDF), 1,2,3,4,6,8-HxCDF, and 1,2,4,6,7,8-HxCDF (Humppi & Heinola, 1985; Assmuth & Vartiainen, 1995). The pure Ky-5 product has been shown to consist of approximately 65 - 200 mg/kg of total PCDD/Fs (Vartiainen et al., 1995; Kitunen et al. 1985). Historical sediment concentrations of PCDD/Fs reflect the production rates of chlorinated compounds indicating poor degradability and extreme immobility in sediments of the Kymijoki River (Verta et al., 1999; Salo et al., 2008)

The release from production and use of Ky-5 into the Kymijoki River has resulted in extensive PCDD/F contamination in sediments at the Kuusankoski location, which has the highest concentrations of PCDD/Fs and decreases downstream from the site (Verta et al., 1999). The total volume of PCDD/F contaminated sediments in the Kymijoki River, from the most contaminated site at Kuusankoski to the Baltic Sea, are estimated to be five million cubic meters of wet sediment with a total mass of 5960 kg of the most toxic 2,3,7,8-substituted PCDD/F congeners (Salo et al., 2008). The mean sediment concentrations of total 2,3,7,8-substituted PCDD/Fs at different sites of the Kymijoki River determined by Salo et al. (2005) were 58.0 mg/kg dw at Kuusankoski, 9.5 mg/kg dw at Myllykoski, 7.2 mg/kg dw at Anjalankoski and 1.7 mg/kg dw at Lake Tammijärvi. The predominant PCDD/F congeners in Ky-5 are also the main congeners found in the river sediments (Verta et al., 1999). The sediments in Kymijoki River have also been highly contaminated with mercury, which have been released from the pulp mills (Salo et al., 2008).

Earlier studies indicate that sediments from the Kymijoki River contain active populations of native dechlorinating bacteria with potential for dechlorination of the PCDD/F contaminants (Ahn et al., 2008; Liu et al., 2013). Mesocosm studies have indicated dechlorination activity at the Myllykoski site in the Kymijoki River (Liu et al., 2013). A previous microcosm study also demonstrated the dechlorination of spiked pentachlorobenzene with the same samples as used in this study (Ylätupa, 2015). Therefore, microbial reductive dechlorination of PCDD/Fs in sediments of the Kymijoki River by indigenous bacteria may have the potential of

decreasing the toxicity of PCDD/Fs. Bioremediation of sediments *in situ* could avoid the redistribution of contaminants into the river system associated with dregning of sediments (Malve et al., 2003; Verta et al., 2009).

2. OBJECTIVES OF THE PRESENT STUDY

The overall objectives were to assess the potential for anaerobic microbial dechlorination of weathered PCDD/Fs in contaminated sediments of the Kymijoki River.

The more specific aims were to determine:

- the concentrations of PCDD/Fs in sediments at different sites of the river
- the PCDD/F dechlorination pathways and rates by congener-specific analysis in Kymijoki River sediment
- the potential for dechlorination of weathered PCDD/Fs in Kymijoki River sediment microcosms
- the preference for lateral vs. peri - dechlorination in the Kymijoki River sediment
- the effect of temperature on microbial dechlorination in sediments of the most contaminated site at Kuusankoski
- the abundance of the indigenous dechlorinating microbial *Dehalococcoides*-like *Chloroflexi* community in sediments at different sites of the Kymijoki River.

3. MATERIAL AND METHODS

3.1 Study sites

The Kymijoki River, located in South-Eastern Finland, runs to the Gulf of Finland in the Baltic Sea. Sediment samples were collected from Kuusankoski, Myllykoski, Anjalankoski, and Lake Tammijärvi that differ in their PCDD/F load. Relatively uncontaminated reference sediment samples were collected from Lake Urajärvi.

The most PCDD/F-contaminated study location is the Kuusankoski site, where a chlorophenol wood preservative (Ky-5) production plant operated in 1939-1984 (Verta et al., 1999). Myllykoski is located approximately 20 km, Anjalankoski 30 km, and Tammijärvi 60 km downstream of Kuusankoski. The sampling locations in Kuusankoski, Myllykoski and Anjalankoski were 200–500 m downstream from the pulp mills in sedimentation areas of suspended solids. Tammijärvi is located on the western end of two main branches of the Kymijoki River. Lake Urajärvi is an uncontaminated reference site 20 km to the west of Kuusankoski.

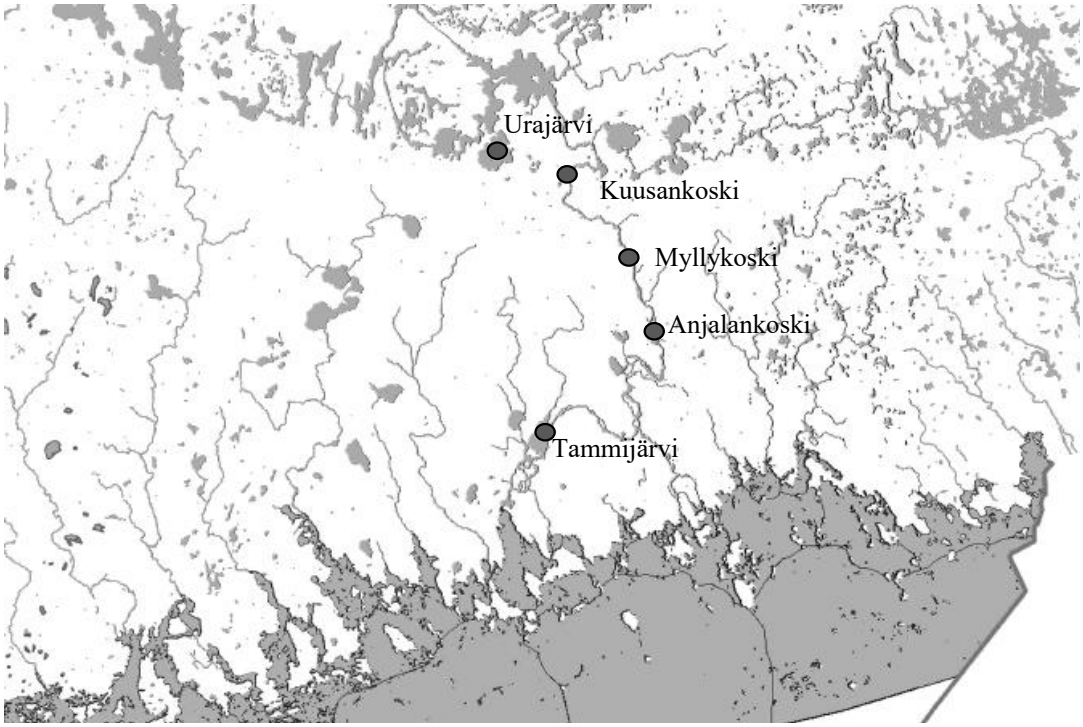

Figure 2. Study sites at the Kymijoki River: Kuusankoski, Myllykoski, Anjalankoski, and Lake Tammijärvi.

3.2 Sediment sampling

The sediment samples were collected during April and May 2009 according to Table 4. Two to three parallel sediment sample cores were collected at each study site. The sampled cores were divided into two or three sections according to depth. Subsamples were frozen for later analysis on the sampling day and the cores were stored at 4 °C under river water for establishment of microcosms.

3.3 Design of the experiments

In this study we examined the dechlorination rate and pathway of spiked 1,2,3,4-tetrachlorodibenzofuran (TeCDF) as a model compound in anaerobic sediment microcosms. We also studied the effect of temperature on 1,2,3,4-TeCDF dechlorination by indigenous sediment microorganisms at a range of incubation temperatures from 4 to 21 °C. Since chlorophenols were co-contaminants of the river sediments from the chlorophenolic Ky-5 fungicide, additional experiments were carried out with 1,2,3,4-TeCDF and co-amendment with 2,3,4,6-TeCP at a temperature of 21 °C. Experiments with spiked compounds were conducted in the presence of a combination of electron donors. Concentrations of weathered PCDD/Fs were determined in all 22 initial sediment core samples from different locations and in Kuusankoski and Tammijärvi microcosms after 30 months of incubation to obtain evidence for dechlorination of weathered PCDD/Fs. The abundance of the *Dehalococcoides*-like *Chloroflexi* community and total bacteria was determined in all 22 initial sediment core samples and in selected sediment microcosms after 24 months of incubation. The precise description of the experimental set-up and methods is provided in the original articles (I, II and III). The summary of the experimental set-up is presented in Table 4.

3.4 Chemical and molecular analysis

The chemical and molecular analyses are presented in Table 5. The samples for chemical analyses were quantified by a gas chromatograph-mass spectrometer (GC-MS, QP2010Ultra; Shimadzu, Kyoto, Japan) with temperature programs for the 30-m and 60-m columns (Zebron ZB-5MS) (Table 5).

DNA samples were amplified using DNA Engine Dyad (MJ Research, Inc., Waltham, MA) with *Chloroflexi*-specific primer sets and re-amplified using general bacterial PCR primers. *Dehalococcoides*-like *Chloroflexi* 16S rRNA genes and bacterial 16S rRNA genes were quantified for each DNA sample using qPCR. In Denaturing Gradient Gel Electrophoresis (DGGE) analysis, bands were excised, DNA eluted and amplified using general bacterial primers. The samples were sequenced at the Haartman Institute (University of Helsinki).

Table 4. Summary of the experimental set-up

Study Site	Depth of the sample	Microcosm experiment	Article
Kuusankoski (sample 1)	A 0-19cm	-1,2,3,4-TCDF+ed (+21°C)	I, II, III
		-live control + ed	I, II, III
		-live control	I, II, III
		-killed control	I, II, III
		-1,2,3,4-TCDF + 2,3,4,6-TeCP + ed	I, II, III
		-1,2,3,4-TCDF+ed +15°C	II
		-1,2,3,4-TCDF+ed +4°C	II
Kuusankoski (samples 2-3)	B 0-18cm + 18-34cm	-1,2,3,4-TCDF+ed	I, II, III
		-live control + ed	I, II, III
		-live control	I, II, III
		-killed control	I, II, III
		-1,2,3,4-TCDF + 2,3,4,6-TeCP + ed	I, II, III
		-1,2,3,4-TCDF+ed +15°C	II
		-1,2,3,4-TCDF+ed +4°C	II
Kuusankoski (samples 4-5)	C 0-13cm + 13-26cm	-1,2,3,4-TCDF+ed	I, II, III
		-live control + ed	I, II, III
		-live control	I, II, III
		-killed control	I, II, III
		-1,2,3,4-TCDF + 2,3,4,6-TeCP + ed	I, II, III
		-1,2,3,4-TCDF+ed +15°C	II
		-1,2,3,4-TCDF+ed +4°C	II
Myllykoski (samples 6-7)	A 0-15cm + 15-35cm	-1,2,3,4-TCDF+ed	I, III
		-live control + ed	I, III
		-live control	I, III
		-killed control	I, III
Myllykoski (samples 8-9)	B 0-15cm + 15-30cm	-1,2,3,4-TCDF+ed	I, III
Anjalankoski (samples 10-11)	A 0-15cm + 15-35cm	-1,2,3,4-TCDF+ed	I, III
		-live control + ed	I, III
		-live control	I, III
		-killed control	I, III
Anjalankoski (smples 12-13)	B 0-15cm + 15-30cm	-1,2,3,4-TCDF+ed	I, III
Tammijärvi (samples 14-16)	A 0-18cm + 18-36cm + 36-56cm	-1,2,3,4-TCDF+ed	I, III
		-live control + ed	I, III
		-live control	I, III
		-killed control	I, III
Tammijärvi (samples 17-18)	B 0-20cm + 20-40cm	-1,2,3,4-TCDF+ed	I, III
Urajärvi (samples 19-20)	A 0-15cm + 15-30cm	-1,2,3,4-TCDF+ed	I, III
		-live control + ed	I, III
		-live control	I, III
		-killed control	I, III
Urajärvi (samples 21-22)	B 0-15cm + 15-30cm	1,2,3,4-TCDF+ed	I, III

ed = electron donors

Table 5. Methods used in this thesis. More precise descriptions of methods are presented in I-III.

Analyses	Article	Reference/Manufacturer
Chemical analyses		
GC-MS analysis of 1,2,3,4-TeCDF and lower chlorinated congeners	I, II	70 ºC; 20 ºC min^{-1} to 230 ºC, then by 10 ºC min^{-1} to 250 ºC; 15 ºC min^{-1} to 300 ºC (30-m column, Zebron ZB-5MS) identified based on standards and Nakano and Weber (2001) and Hale et al. (1985)
GC-MS analysis of OCDF/OCDD to PeCDFs/ PeCDDs	I, III	80 ºC, 1 min; 20 ºC min^{-1} to 150 ºC, 3 ºC min^{-1} to 280 ºC (60-m column, Zebron ZB-5MS) (Nakano and Weber, 2001) identified based on standards and Lundgren et al., (2004) and Ryan et al., (1991)
Molecular analyses		
DNA-extraction	II, III	PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA)
PCR	II, III	Muyzer et al. 1993
qPCR	II, III	*Dehalococcoides*-like *Chloroflexi* 16S rRNA genes (Krzmarzick et al., 2012) bacterial 16S rRNA genes (Kanto Öqvist et al., 2008)
DGGE	II, III	Muyzer and coworkers (1993, 1998, 1999) DCode ™ System (Bio-Rad Inc., Hercules)
Sequencing and sequence analysis	II, III	sequencing at Haartman Institute, University of Helsinki; sequence analysis at the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST)

4. RESULTS AND DISCUSSION

4.1 PCDD/Fs in sediments of the Kymijoki River

The measured mean concentrations for 2,3,7,8-PCDD/Fs were extremely high at the most contaminated site Kuusankoski (III) where mean total concentrations for 2,3,7,8-PCDD/Fs were nearly 1200 mg/kg dw (Table 6). At all other study sites of the river the mean total concentrations for 2,3,7,8-PCDD/Fs varied between approximately 10 and 100 mg/kg dw. At all study sites the WHO-TEQ (mg/kg dw) for 2,3,7,8-PCDD/Fs were between 0,01 and 10 mg/kg dw (Table 6) which exceeded the higher guideline value for contaminated soils in Finland (0,0015 WHO-TEQ mg/kg dw). The concentrations of 2,3,7,8-PCDD/Fs were approximately at the same levels at each sampling location of the Kymijoki River compared to samples taken in 1998-2003 (Salo et al., 2008). However, the concentration of PCDD/Fs can vary greatly at the same sample location, even though samples were obtained from the highest concentration position according to coordinates of an earlier study (Verta et al., 2009). At all sites the predominant PCDD/F congeners were 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,8,9-HpCDF, and OCDF, corresponding to the PCDD/F profile in the fungicide Ky-5 where the same 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,8,9-HpCDF, and OCDF congeners were found at the highest concentrations (Kitunen et al., 1985; Humppi and Heinola, 1985). In this study the concentration of the 1,2,3,4,6,7,8-HpCDF in sediment was clearly higher than the OCDF concentration, in a ratio similar to the composition in Ky-5 as analysed by Vartiainen et al. (1995) and similar to the composition in Ky-5 contaminated sediment as analysed by Liu et al. (2013). These results indicate that no or only minimal biodegradation or transport of PCDD/F congeners has occurred in the river sediments during last few decades since the contamination events.

4.2 Dechlorination of spiked PCDF congener

4.2.1 Rate and extent of dechlorination

Anaerobic reductive dechlorination of spiked 1,2,3,4-TeCDF to TrCDF and DiCDF congeners varied widely between all study sites in the Kymijoki River after 10 and 29 months in sediment microcosms (I, Fig. 1). The highest dechlorination activity of 1,2,3,4-TeCDF was observed in the sediment microcosms of Kuusankoski, the most contaminated site, but dechlorination was also observed in the reference site, Lake Urajärvi (Table 6). The half-life of 1,2,3,4-TeCDF, calculated from first-order reaction rate constants, varied between 1.0 y and 14.1 y in the most active sediment core of each site at 21 °C. Dechlorination rates of 1,2,3,4-TeCDF in this study were similar or lower than in a previous microcosm study by Ahn et al. (2005) using sediments from San Diego Bay (CA, USA) and Tuckerton (NJ, USA) which may be due to sediment properties or other environmental factors.

4.2.2 Dechlorination pathways of 1,2,3,4-TeCDF

1,2,3,4-TeCDF appeared to be dechlorinated mainly via 1,3,4-TrCDF to 1,3-DiCDF (I, Fig. 2). In addition to this predominant route, 1,2,4-TrCDF, 2,4-DiCDF, 1,2-DiCDF, and 1,4-DiCDF were observed. 1,2,3-TrCDF and its dechlorination product with lateral chlorines, 2,3-DiCDF, were formed at very low concentrations in the Kymijoki River microcosms (Fig. 3). Low amounts of 3-MoCDF and 2-MoCDF were detected in microcosms over the 29-month incubation period. These results

Table 6. Concentrations of 2,3,7,8-PCDD/Fs (mg/kg dw), WHO-TEQ (mg/kg dw), abundance of *Chloroflexi* and total bacteria at time of sampling, organic matter (%) of dry weight, and pH at the studied sites of Kymijoki River determined in mean of 4-5 sediment core samples of each site. The extent of spiked 1,2,3,4-TeCF dechlorination (mol%) after 29 months calculated in means of three microcosms prepared with 4-5 sediment core samples.

	Kuusankoski	Myllykoski	Anjalankoski	Tammijärvi	Urajärvi
∑2,3,7,8-PCDD/Fs (mg/kg dw)	1175	96	43	9	2
WHO-TEQ (mg/kg dw) Van den Berg et al., 2006)	9.2	1.0	0.4	0.1	0.01
Extent of spiked 1,2,3,4-TeCF dechlorination (mol%)					
- 21 °C	46.2	6.1	9.4	18.3	18.1
- 15 °C	37.7				
- 4 °C	10.8				
Chloroflexi 16S rRNA genes/g dw sediment	1.47E+06	8.63E+05	6.97E+05	1.34E+06	4.97E+05
Total bacteria/g dw sediment	2.68E+09	5.80E+08	8.68E+08	2.72E+09	6.63E+08
Organic matter (%) of dry weight	51.7	25.3	34.3	15.3	12.8
pH	6.7	6.5	6.2	6.6	6.9

suggest that dechlorination was directed to certain positions of the dibenzofuran ring, with the first cleavage of chlorine occurring mainly in the lateral positions. The next dechlorination step was mainly from a peri position with an adjacent chlorine.

Previously, dechlorination of 1,2,3,4-TeCDF to TrCDFs-, DiCDFs, and MoCDFs was observed in laboratory studies, but the chlorine positions were not identified (Fennell et al., 2004; Ahn et al., 2005). In a study of 1,2,3,4,7,8-HxCDF by Liu and Fennell (2008), dechlorination occurred at the fully chlorinated ring in the same order as in the present experiment. However, in a previous study 1,2,3,4-TeCDD was dechlorinated to 2,3-DiCDD as the most abundant product (Wang et al., 2008). In this study, the initial dechlorination of 1,2,3,4-TeCDF occurred primarily at the lateral position. This suggests that dechlorination of aged PCDD/F congeners generates non-2,3,7,8-substituted and hence less toxic PCDD/Fs. Similarly, a recent study by Rodenburg et al. (2017) suggests that PCDD/Fs were reductively dechlorinated to non-2,3,7,8-substituted PCDD/Fs in sediments of the upper Hudson River.

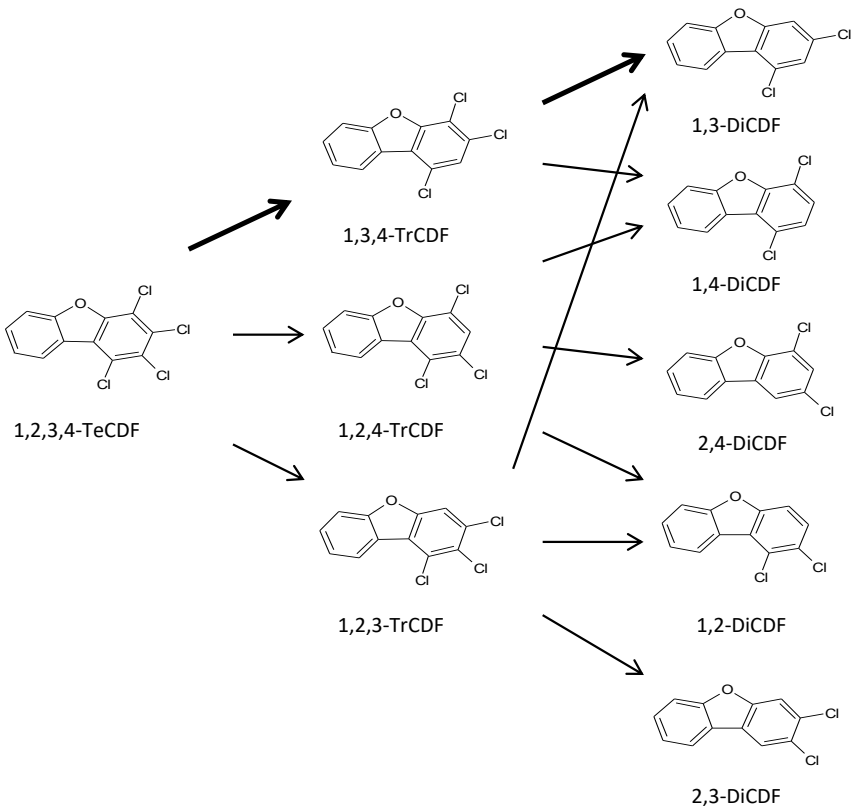

Figure 3. Dechlorination pathways observed for 1,2,3,4-TeCDF in microcosms of the Kymijoki River (I). Main pathway detected is marked with bold arrows.

4.2.3 Effect of temperature to the dechlorination

The effect of temperature on the extent of dechlorination of 1,2,3,4-TeCDF at incubation temperatures of 21 °C , 15 °C, and 4 °C was clearly observed during 29 months incubation in each of the five sediment cores with triplicate microcosm sets from the Kuusankoski site (II). Fig. 4 shows the dechlorination of 1,2,3,4-TeCDF to TrCDFs and DiCDFs in the most active sediment core in microcosms incubated at the three temperatures. The half-lives for spiked 1,2,3,4-TeCDF in all samples estimated from the first order reaction rate equations were 2.1 y at 21°C, 3.9 y at 15 °C, and 19.0 y at 4 °C. The abundance of *Dehalococcoides*-like *Chloroflexi* were approximately at the same level after 24 months of incubation at the different temperatures, suggesting no correlation with the rate of 1,2,3,4-TeCDF dechlorination. Based on our sediment measurements the temperature in sediments of the Kymijoki River was below 4 °C for half of the year and varied between 0 and 21 °C.

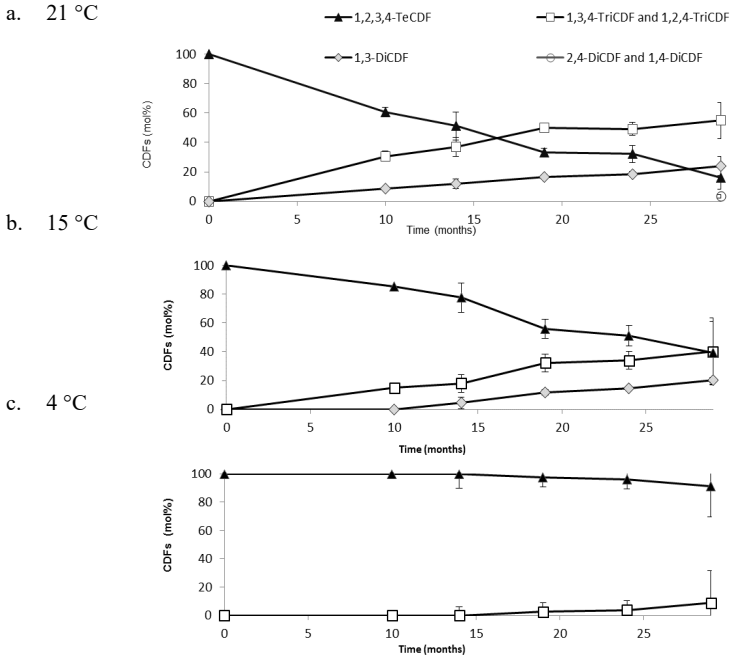

Figure 4. Time course of reductive dechlorination of 1,2,3,4-TeCDF and dechlorinated congeners at different temperatures in three parallel microcosms in sediment core A 0-19 cm from the Kuusankoski site of the Kymijoki River over 29 months of incubation. (II)

These results demonstrate that temperature significantly affects the rate of spiked TeCDF dechlorination in Kymijoki River sediments. The influence of temperature on dechlorination of chlorinated dibenzofurans has not been studied previously, but laboratory studies of other chlorinated compounds show decreased dechlorination rates at temperatures lower than 20-27 °C (Wu et al., 1997; Friis et al., 2007). The half-life for TeCDFs in sediments of the Baltic Proper, where the annual average temperature is about +7 °C, is estimated to be approximately 80 y (Kjeller and Rappe, 1995). The shorter half-lives observed for the Kymijoki sediment microcosms for TeCDF (also at 4 °C) is likely due to the higher dechlorination rate of spiked compounds, compared to aged PCDD/Fs that are more tightly bound to sediment particles. Results from our microcosm experiment showed a low dechlorination rate at 4 °C, which suggests the low dechlorination rate of aged PCDD/Fs at the typical temperature in the Kymijoki River sediments.

4.3 Dechlorination of weathered PCDD/Fs in Kymijoki River sediments

The extent of anaerobic reductive dechlorination of weathered PCDD/Fs was determined in microcosms by using sediment from Kuusankoski and Tammijärvi. Experiments were conducted by analyzing the concentrations of weathered PCDD/Fs at the beginning of the experiment and in triplicate microcosms after 30 months incubation (III). There were no obvious differences in the relative abundances of PCDD/Fs at time 0 and after 30 months of microcosm incubation with or without addition of electron donors or with additional spiked TeCDF congener and chlorinated phenol compound or in killed control microcosms (Fig. 5). The predominant congeners in all the analyzed Kymijoki River sediment samples were similar to the PCDD/F profile of the chlorophenol wood preservative Ky-5 (Vartiainen et al. 1995) and similar to the composition in Ky-5 contaminated sediment (Liu et al. 2013).

In sediment microcosms where reductive dechlorination of spiked 1,2,3,4-TeCDF was observed, the weathered PCDD/F congeners were nonetheless recalcitrant with no dechlorination or only minimal dechlorination of PeCDFs observed over 30 months. This may be due to the high tendency of the highly chlorinated PCDD/F congeners to sorb to organic matter in sediments and this binding is stronger for aged congener than for recently spiked congeners. Also, the dechlorination rate is expected to decrease due to decreased bioavailability with increasing number of chlorines (Kim et al. 2009), i.e. for the spiked TeCDF congener vs. PCDD/Fs.

In an earlier study, half-lives calculated for spiked PCDD/Fs were 1 – 4.1 years (Adriaens and Grbic-Galic, 1994) and it was estimated that actual half-lives in the environment may be orders of magnitude higher. Half-lives of 10 to 50 years are suggested for the PCDD/Fs in sediments based on biodegradation (Sinkkonen and Paasivirta 2000), but our study indicates that this can be longer. Our results differ also from the earlier study by Liu et al. (2013) which suggested that dechlorination of weathered octa-, hepta-, and hexa-CDFs occurred during the first two years of incubation in Kymijoki River sediment mesocosms.

In previous studies, dechlorination of weathered PCDD/F in sediments occurred to limited extent or not at all (Beurskens et al., 1993; Barkovskii and Adriaens, 1996; Albrecht et al., 1999; Yoshida et al, 2005; Liu et al., 2013). There are few studies of dechlorination of weathered PCBs obtained by comparing PCB profiles at different layers of sediments and different locations as well as to the congener profiles of commercial PCB mixtures (Aroclor products), for example in Hudson River sediment (Brown et al., 1984) in Woods Pond sediment (MA, USA) (Bedard and May, 1996), and in Lake Hartwell (SC, USA) (Magar et al., 2005).

In this study evidence of dechlorination was not obtained by comparing the PCDD/F profile of the Kymijoki sediments to the congener profile of the commercial chlorophenol wood preservative Ky-5. In addition, dechlorination was not observed by comparing the PCDD/F concentrations and congener profile to a previous study (Salo et al., 2008) and furthermore the relative percentages of PCDD/Fs did not change in microcosms during this study. Based on these combined results, half-lives for weathered PCDD/Fs can be estimated to be decades or centuries in the cold anaerobic sediments of the Kymijoki River.

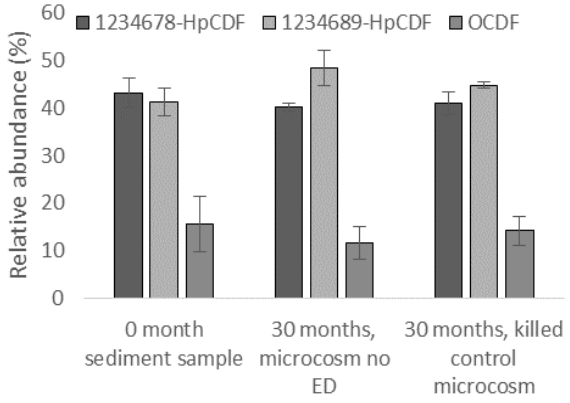

Figure 5. The relative percentages of OCDF, 1,2,3,4,6,8,9-HeptaCDF, and 1,2,3,4,6,7,8-HeptaCDF in Kuusankoski sediment samples at time 0 and in un-amended and in killed control microcosms after 30 months incubation. Values are means and error bars indicate standard deviation of two sediment samples at time 0 and three parallel microcosms after 30 months.

4.4 Dechlorinating communities in the Kymijoki River

A *Dehalococcoides*-like *Chloroflexi* community was present in the Kymijoki River sediments and also at the reference site (III). The abundance of *Dehalococcoides*-like *Chloroflexi* was approximately at the same level as observed for other contaminated sites reported in an earlier study using the same PCR primer sets (Krzmarzick et al., 2012). The abundance of *Dehalococcoides*-like *Chloroflexi* increased in the course of 24 months incubation in all microcosms. No correlation was observed between the total sediment concentration of PCDD/Fs and the abundance of *Dehalococcoides*-like *Chloroflexi* at time 0 for the 22 sediment samples. The abundance of *Dehalococcoides*-like *Chloroflexi* in sediment may have grown because of other more bioavailable chlorinated compounds such as chlorophenols. Furthermore, no correlation was observed between the extent of spiked 1,2,3,4-TeCDF dechlorination and the abundance of *Dehalococcoides*-like *Chloroflexi*. This result suggests that *Dehalococcoides*-like *Chloroflexi* present in the sediment were not able to dechlorinate aged PCDD/Fs because of limiting factors in the environment. Dechlorination of other weathered compounds were not determined during the study. In an earlier study by Liu et al. (2014) the *Chloroflexi* community identified to the *Dehalococcoides mccartyi* Pinellas subgroup increased over 6 months study in anaerobic enrichment cultures derived from Kymijoki River sediments. The addition of halogenated co-substrates has been shown to stimulate growth and activity of *Dehalococcoides*-like *Chloroflexi* (Ahn et al., 2005, 2008) and this might be a means for stimulating the dechlorination of PCDD/Fs.

The DGGE profiles (using nested PCR with *Chloroflexi*-specific primers) and sequences of dominant bands indicated that dechlorinating *Dehalococcoides*-like *Chloroflexi* were present at each study site. A predominant band corresponding to *Dehalococcoides mccartyi* strain CBDB1 (and other strains of the "Pinellas subgroup") was present in all sediment samples. In addition, other DGGE bands/sequences suggested that Kymijoki River sediments harbor a diverse community of *Dehalococcoides*–related *Chloroflexi*. In a recent study by Dam et al. (2017) a *Dehalococcoides mccartyi* strain was identified in enrichment cultures established from Kymijoki River and the study suggested that this *D. mccartyi* strain were responsible for reductive dechlorination of 1,2,3,4-TeCDD.

5. CONCLUSIONS AND FUTURE RESEARCH NEEDS

In this thesis we gained information of the potential for microbial dechlorination of weathered PCDD/Fs in anaerobic sediments of the Kymijoki River. It is important to have more knowledge on dechlorination of weathered PCDD/Fs due to their environmental and health risks.

Degradation of chlorinated contaminants in sediments is generally assumed to largely depend on the presence of native microorganisms capable of natural degradation processes. In this study, native dehalogenating bacteria belonging to *Dehalococcoides* spp. were detected in the Kymijoki River sediments and these were capable of dechlorinating a spiked PCDF congener. Nonetheless, dechlorination of weathered PCDD/Fs was not observed in the river sediment over the course of this study.

Based on the results of this thesis, limited or no degradation or transport of weathered PCDD/Fs in sediments of the Kymijoki River appears to have occurred over the last two decades. The high concentrations of weathered PCDD/Fs in the sediments of the Kymijoki River can be estimated to remain at the same level for decades or centuries with further migration towards the Baltic Sea**.**

Results of this study will be useful when planning the Kymijoki River restoration or in plans to remediate contaminated sediments by using microbial dechlorination of aged PCDD/Fs. These results should be considered in predicting the environmental fate of PCDD/Fs in sediments. High concentrations in these sediments will not decrease by native microorganisms during several decades. Thus, new *in situ* remediation technologies need to be developed or alternatively removal or capping of the most contaminated sediments from the river should be considered.

Research in the future should determine the factors that limit dechlorination of weathered PCDD/Fs, such as strong sorption to sediment and limited bioavailability to microorganisms. In future, research is needed on remediation techniques to increase bioavailability or degradation of weathered PCDD/Fs in sediments. Factors affecting degradation and behavior of PCDD/Fs in the environment has to be taken into account in environmental and health risk assessment.

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