Ecotoxicology and Biogeochemical Functioning of Bleached Pulp Mill Recipient and Non-recipient Lake Sediments

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Academic Dissertation in Microbiology

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Front cover: Forest lake Matolampi in Kittilä, Lappland, Finland. Photograph: Kimmo Suominen.

Kyllä te vietätte elämää! Korjaatte, puuhaatte ja hypitte aamusta iltaan. Mokoma hosuminen voi olla jopa vaarallista. Johan sitä masentuu kun vain ajatteleekin kaikkia niitä, jotka tekevät työtä ja raatavat, ja mitä hyötyä siitä muka on. Eräs sukulaiseni luki trigonometriaa tuntokarvansa lerpalleen, ja kun hän oli oppinut kaiken, tuli Mörkö ja söi hänet suuhunsa. Joopa joo, Mörön vatsassa hän sitten lojui niin erinomaisen viisaana!

Tove Jansson, *Muumipapan urotyöt*

What a life! No end of changing and building up and pulling down again and jumping about. Such a lot of work may turn out to be really harmful. Oh, I'm dejected just to think of all the people who work and buzz and bumble about, and what it all leads to. I had a cousin once who studied trigonometry until his whiskers drooped, and when he had learnt it all a Groke came and ate him up. Yes, so wise he was while lying in the Groke's stomach!

Tove Jansson, *The Exploits of Moominpappa Described by Himself*

CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following articles, referred in the text by roman numerals:

I. **Suominen**, **K.P.**, Jaakkola, T., Elomaa, E., Hakulinen, R. and Salkinoja-Salonen, M.S., 1997. Sediment accumulation of organic halogens in pristine forest lakes - Tracking of a single defined discharge of pulp bleaching wastewater. *Environmental Science and Pollution Research* 4(1):21-30.

II. **Suominen, K.P.**, Wittmann, C., Kähkönen, M.A. and Salkinoja-Salonen, M.S. 1998. Organic halogen, heavy metals and biological activities in pristine and pulp mill recipient lake sediments. *Water Science and Technology* 37(6/7):79-86.

III. Kähkönen, M. **Suominen**, **K.,** Manninen, P. and Salkinoja-Salonen, M.S. 1998. 100 years of sediment deposition history of organic halogens and heavy metals in pristine forest lakes and pulp mill recipient area in Finland. *Environmental Science and Technology* 32(11):1741-1746.

IV. **Suominen, K.**, Wittmann, C., Liukkonen, M., Kähkönen, M. and Salkinoja-Salonen, M.S. 1999. Toxicologic assessment of a recipient lake sediment of a kraft pulping discharge. *Environmental Toxicology and Chemistry.* In press.

THE AUTHOR'S CONTRIBUTION

Paper I:
Kimmo Suominen has written the paper and is the corresponding author. He has done the field work, interpreting of the results and all experimental work on sediment analysis, and TOC and AOX analysis for the water.

Paper II:
Kimmo Suominen is the correspondent author of the paper. He has done the field work, and the experimental work, except toxicity tests and enzyme activity measurements. He has participated in interpretation the results, and the preparation of the manuscript.

Paper III:
Kimmo Suominen has done the field work. He was responsible for the sediment dating (^{210}Pb and ^{137}Cs). He has done the EOX-analysis and part of carbon analysis. Kimmo Suominen has participated in interpretation of the results and in writing work. He has prepared all figures, except the Figure 3, and par of the Figure 1.

Paper IV:
Kimmo Suominen has written the paper and is the corresponding author. He has done the field work and the interpretation of the results. Kimmo Suominen has done the experimental work, except toxicity tests and enzyme activity measurements. Kimmo Suominen has prepared all figures in the paper (Figure 1 only partly).

ABBREVIATIONS

AOX	Adsorbable organic halogen
ATP	Adenosine triphosphate
BOX	Bound organic halogen
BOD	Biological oxygen demand
COD_{Cr}	Chemical oxygen demand (Cr^{6+} as oxidant)
d.m.	Dry matter
d.w.	Dry weight
EC	Enzyme Commission
EC_{50}	Effective concentration causing an effect in 50% of the exposed R
ECF	Elemental chlorine free bleaching
EOS	Extractable organic sulphur
EOX	Extractable organic halogen
EROD	7-ethoxyresorufin-*o*-deethylase
ISO	International Organisation for Standardisation
K_{ow}	*n*-octanol-water partition coefficient
MMO	Methane monooxygenase
MUF	Methyl umbelliferyl
PAH	Polyaromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDD/DF	Polychlorinated dibenzo-*p*-dioxin(s) and -furan(s)
pMMO	Particulate (membrane bound) methane monooxygenase
s.c.e.	Standard calomel electrode
sMMO	Soluble methane monooxygenase
TCF	Total chlorine free pulping
TOC	Total organic carbon
TOX	Total organic halogen

Pulp bleaching steps

C	Cl_2 bleaching step
D	ClO_2 bleaching step
E	Alkaline extraction stage
Eo	Oxygen-reinforced alkaline extraction stage
Eop	Peroxide-reinforced alkaline extraction stage
H	Hypochlorite bleaching stage
O	Oxygen delignification stage

1. INTRODUCTION

1.1 History of pulp production and bleaching in Finland

The first pulp mill in Finland started in 1876 (Kuisma, 1993), and in 1998 there were 19 pulp mills in Finland (Karessuo, 1999). Figure 1a displays the pulp production in Finland between 1918 and 1998. Production of sulphite pulp has ceased, and the production of kraft pulp and mechanical pulp have increased continuously since 1945. In 1998, 6.7 million tons of chemical pulp was produced, 90% of which was bleached (Karessuo, 1999) (Figure 1a). In addition to chemical pulp, 4.6 million tons of mechanical mass was produced in 1998 (Karessuo, 1999).

Chlorine chemicals are efficient in oxidising double bonds in organic matter, such as lignin. Chlorine chemicals have been used for pulp bleaching in Finland since early 20th century and chlorine gas since 1927 (Hoving, 1949). When chlorine gas was used for pulp bleaching, 6 to 10% of the chlorine was converted into organic form (Hise, 1996). This organic bound chlorine, often measured as activated carbon adsorbable organic halogen (AOX, see chapter 1.3), consisted of medium sized chlorinated compounds (200 to 800 g mol^{-1}), such as chlorinated lignin (Jokela, 1997), and small molecular size components, like chlorophenols chlorinated catechols and guaiacols (Salkinoja-Salonen *et al.*, 1984).

Organic halogen from pulp mills have accumulated into recipient sediments (*e.g.* Maatela, *et al.*, 1990; Pellinen and Soimasuo, 1993; Palm and Lammi, 1995; Saski *et al.*, 1997; Kankaanpää *et al.*, 1997) and aquatic organisms (Suntio, *et al.*, 1988; Hayer and Pihan, 1996). These organic halogens may be toxic (Engwall *et al.*, 1997) or genotoxic (Holmbom, 1990; Nylund *et al.*, 1994). The use of chlorine gas for pulp bleaching in Finland was abandoned for environmental reasons in 1993 (Karessuo, 1994). Pulp is now bleached with chlorine dioxide and oxygenating chemicals. Quantities of bleaching chemicals in used Finland from 1930 to 1993 are shown in Figure 1b. In modern pulp mills, using chlorine dioxide and non-chlorine chemicals for bleaching, and equipped with secondary treatment plants for waste waters, 2% to 4% of organic chlorine used for bleaching ends up to recipient water ecosystem as adsorbable organic halogen (AOX), calculated from the data of Salkinoja-Salonen *et al.* (1998).

This paper continues the series of thesises on the forest industry related waste water and biodegradation of organic halogens (Apajalahti, 1987; Pellinen, 1986; Häggblom, 1988; Kitunen, 1990; Puhakka, 1990; Valo, 1990; Uotila, 1993; Briglia, 1995; Jokela, 1997; Saski, 1998; Kostyál, 1998; Laine, 1998; Nohynek, 1999).

1.2 Organic halogen in lake sediments

Sediments are an important sink for organic halogens in the environment. Organic halogen in the sediment can originate from local sources such as pulp mills (*e.g.* Salkinoja-Salonen *et al.*, 1981; Jokela, 1997; Salkinoja-Salonen *et al.*, 1998) or from atmospheric fallout (Manninen, 1990; Jokela *et al.*, 1992). Organic halogens are also formed in nature (Siuda and DeBernardis, 1973; Neidleman and Geigert, 1986; Asplund and Grimvall, 1991; Asplund, 1992; Gribble, 1992, 1994; van Pée, 1996; Hjelm, 1996; Johansson; 1996; Hoekstra *et al.*, 1999). Natural production of organic halogen may exceed that from anthropogenic sources (Asplund and Grimvall, 1991; Asplund, 1992; Kankaanpää *et al.*, 1997; Hoekstra *et al.*, 1999). Asplund (1992) estimated anthropogenic emissions of organic halogen in Sweden in late 1987 (30×10^3 tons a^{-1}) to contribute only 0.4% of the

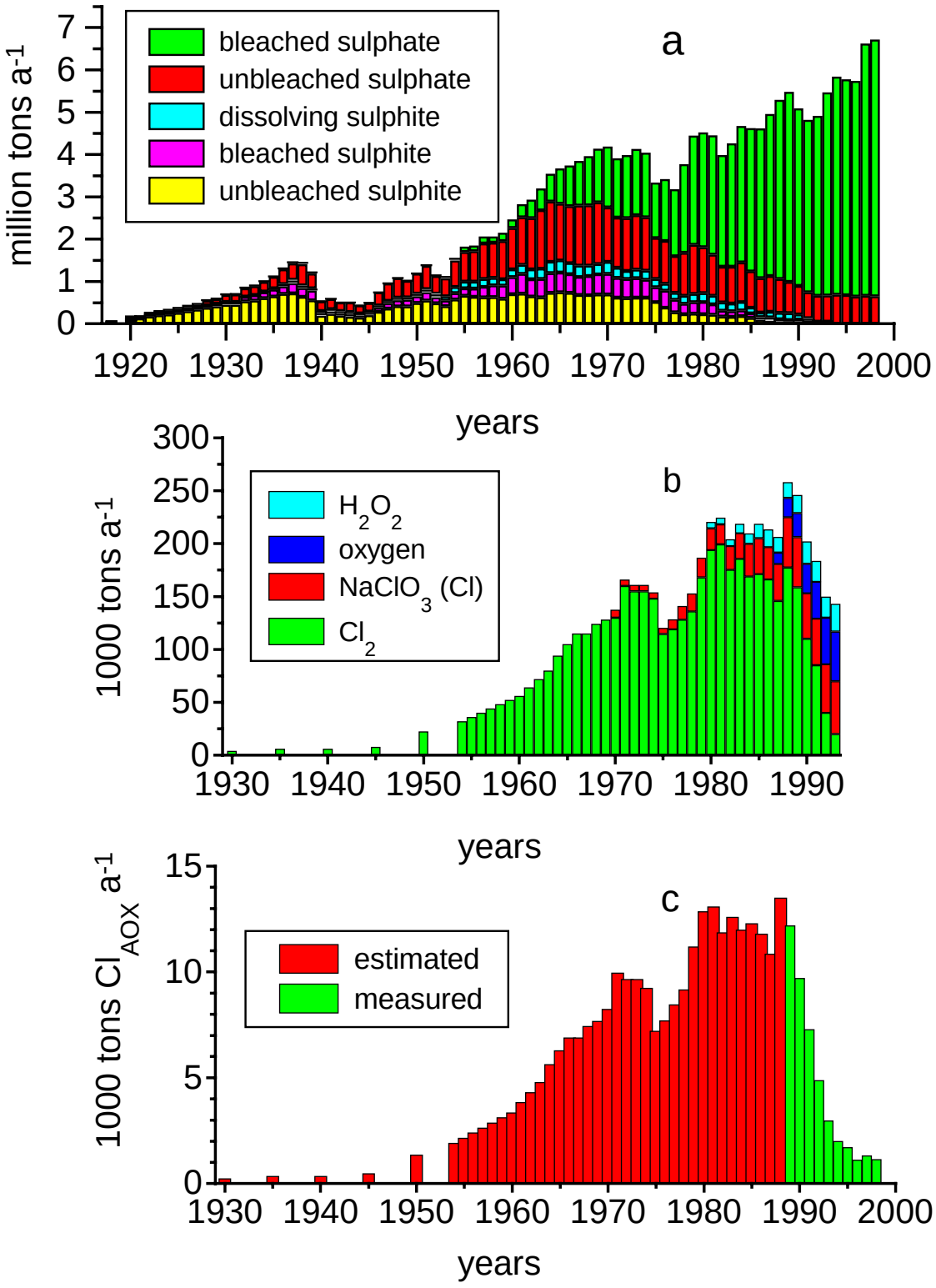

Figure 1. Inputs and outputs from pulp industry in Finland. **a:** Pulp production in Finland from 1918 to 1998. **b:** The consumption of pulp bleaching chemicals in Finland. The consumption from 1930 to 1953 was estimated from the data on total Cl_2 usage assuming that 90% of Cl_2 was used for pulp bleaching. **c:** The AOX discharges from Finnish pulp and paper mills. The discharges from 1930 to 1988 were estimated assuming that 6% of the chlorine used for bleaching was incorporated as organic halogen. From 1989 onward results are based on measurements. Data from Finnish Forest Industries Federation (Helsinki).

total amount of organohalogens in Swedish soil (0 to 2 m layer), sediment and vegetation ($7{,}100 \times 10^3$ tons).

1.2.1 Anthropogenic sources of organohalogens

A "hot spot" pollution of organic halogen may cause local damage in the recipient water ecosystem. Pulp bleaching is an important source of organic halogens in the environment in Finland. Since early in the 20th century, more than 5.5 million tons of chlorine were used for pulp bleaching. The chlorine consumption for pulp bleaching was highest in mid 1980's, 200,000 tons a^{-1} of chlorine gas (Cl_2) and 100,000 to 150,000 tons a^{-1} of $NaClO_3$ (containing 30,000-50,000 tons of chlorine) (Figure 1b) (Salkinoja-Salonen, *et al.,* 1998). Discharges of AOX from Finnish pulp mills were largest in 1988, approx. 14,000 tons Cl_{AOX} a^{-1} (Figure 1c) (Salkinoja-Salonen *et al.,* 1998). Chlorine use for pulp bleaching has resulted into a discharge of approximately 500,000 tons of Cl_{AOX} into the watercourses in Finland (Salkinoja-Salonen *et al.,* 1998). Much has been done since the 1980's to reduce the environmental load from pulp mills. In 1998, AOX discharges from Finnish pulp mills were 1,144 tons Cl_{AOX} a^{-1} (Karessuo, 1999). However, the old discharges are still present in receiving sediments (Maatela, *et al.,* 1990; Pellinen and Soimasuo, 1993; Palm and Lammi, 1995; Saski *et al.,* 1997; Kankaanpää *et al.,* 1997).

Pesticides are an important source of organic halogens in Finland. Chlorinated compounds are an important group of pesticides. In 1997, totally of 2,775 tonnes of pesticides containing 1,034 tons of active ingredients were sold in Finland (Hynninen and Blomqvist, 1998). Consumption of pesticides in Finland has decreased by 60% since 1979, when the consumption was highest (Hynninen and Blomqvist, 1998). Pesticides or their commercial impurities may have harmful properties such as poor biodegradability, bioaccumulability or toxicity.

Chemical industry has caused several local pollution problems in Finland. For instance River Kymijoki sediment in south-east Finland is heavily polluted by polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/DF) and related compounds (Koistinen *et al.,* 1995), due to a factory, which produced chlorophenolic wood preservative from 1940 to 1984. Soils in the vicinity of saw mills have become polluted by chlorophenolic wood preservative and polychlorinated dibenzo-p-dioxins and -furans (Valo *et al.,* 1984; Kitunen *et al.,* 1985; Kitunen, 1990; Laine, 1998).

Chlorine is an effective agent for eradicating pathogenic microorganisms for instance in drinking water. Organohalogen compounds are known to be formed in desinfection of humus containing drinking water by chlorine chemicals (Isaac, 1996). Therefore drinking water may contain organohalogens (Jokela *et al.,* 1992; Kostyál *et al.,* 1994). Total load of AOX in drinking water in late 1980's was estimated as 10 tons a^{-1} (Salkinoja-Salonen *et al.,* 1990). Due to toxicity and genotoxicity of the chlorinated compounds (Holmbom, 1990), better water purification of raw water prior to chlorination is now required by the health authority.

1.2.2 Natural production of organic halogen

Many authors have shown the formation of organic halogens by microorganisms (Siuda and DeBernardis, 1973; Neidleman and Geigert, 1986; Asplund and Grimvall, 1991; Asplund, 1992; Gribble, 1992, 1994; van Pée, 1996; Hjelm, 1996, Johansson; 1996; Hoekstra *et al.,* 1999). Many bacteria can synthesise halogenated metabolites (Neidleman and Geigert, 1986; van Pée, 1996). Fungi may produce organohalogens concomitantly to degrading organic matter in the terrestrial ecosystem (Neidleman and Geigert, 1986; Asplund, 1992). Marine algae were reported to produce halometabolites, mainly brominated compounds (Neidleman

and Geigert, 1986). Plant organic matter can contain 10 to 100 µg of Cl g^{-1} d.w. (Asplund, 1992). It is uncertain, whether higher plants are able to produce halometabolites. The spectra of halogenated compounds produced by bacteria, fungi and algae is large and includes aromatic and aliphatic compounds (Siuda and DeBernardis, 1973; Neidleman and Geigert, 1986; Gribble, 1992, 1994; van Pee, 1996; Hjelm, 1996). The number of naturally produced halometabolites is estimated to exceed 2000 (Gribble, 1994).

Two enzymes catalysing biohalogenation are known: heme-containing haloperoxidase and non-heme haloperoxidase (van Pée, 1996; Neidleman and Geigert, 1986), both belonging to peroxidases. Peroxidases catalyse the oxidation of a wide spectrum of substrates with hydrogen peroxide or other hydroperoxides as oxidant (Neidleman and Geigert, 1986). From Equation 1 (Neidleman and Geigert, 1986) it can be seen that a hydrogen ion (acidity) is needed for haloperoxidases to work.

Reaction mechanisms of haloperoxidases are described in detail by Neidleman and Geigert (1986).

Haloperoxidases prefer acid or neutral pH region (pH 4 to 7.5) (Neidleman and Geigert, 1986). Production of organohalogens in extracts of soil by fungal haloperoxidase was reported to occur maximally at pH 3 to 4, and the production at >pH 5 was virtually zero (Asplund, 1992).

Asplund and Grimvall (1991) observed that concentrations of organohalogens in lakes were related to the concentration of humic substances. Major part of the organic matter in humic forest lakes may originate from the drainage area (Meili, 1992). Therefore the terrestrial ecosystem in the drainage area is a likely source of halogenated organic material in the lake.

1.2.3 Atmospheric sources of organohalogens

Combustion processes, metal industry and car traffic are important sources of organohalogens into the atmosphere (Quaß *et al.,* 1998). Asplund (1992) estimated the emissions of organohalogens into the atmosphere as 20×10^3 tons in 1987 in Sweden. This was two fold as compared to Swedish industrial discharges into watercourses (Asplund, 1992). Since then anthropogenic emissions of organic halogens into the atmosphere have decreased in Europe, due to improved technology in municipal waste incineration plants and other sources (Quaß, *et al.,* 1998; Cleverly *et al.,* 1998). Atmospheric precipitation of organic halogen was estimated in Finland in 1980's as 1,000 tons of organic bound chlorine and 150 tons of organic bound bromine per year assessed by adsorption to activated carbon followed by measurement by neutron activation analysis (Manninen, 1990).

Equation 1:
$$\text{substrate} + H_2O_2 + Cl^- + H^+ \xrightarrow[\text{enzyme}]{\text{haloperoxidase}} \text{halogenated product} + 2H_2O$$

1.3 Different ways of measuring organic halogen in water and in sediment

Organic halogen concentration in water is commonly measured as activated carbon adsorbable organic halogen (AOX) (ISO, 1989). AOX is a group parameter and includes organic halogens of many types, small and large molecular size, hydrophilic and hydrophobic. Halogen compounds sorbed on the activated carbon are combusted and the

halides are measured by coulometric titration (*e.g.* Jokela, 1997) or by neutron activation analysis (Manninen, 1990).

A modification of the AOX method can be used to measure sediment organic halogen (Palm and Lammi, 1995; Kankaanpää, 1997). Prior to the AOX analysis, sediment can be digested either by sonicating (Kankaanpää, 1997) or by a mild hydrolysis (0.1 M Na_2HCO_3, 2 h) (Palm and Lammi, 1995). AOX after strong alkaline treatment (KOH 1 M, 80 °C, 1 h) (Pellinen and Soimasuo, 1993) is commonly used to assess sediment organic halogen. In this thesis I name this assay bound organic halogen (BOX). Titration after wet combustion (Salkinoja-Salonen *et al.,* 1981; Salkinoja-Salonen *et al.*, 1984; Maatela *et al*., 1990) may be used to assess sediment total organic halogen (TOX).

Extraction with organic solvent followed by combustion and microcoulometric titration is a widely used method for analysing sediment organic halogen (EOX, extractable organic halogen). The yield and the quality of organic halogen assayable in this way depends on the solvent used. Acidified THF (pH 2, HNO_3) solubilised 70 to 95% the AOX in untreated bleached kraft pulp mill waste waters (Jokela and Salkinoja-Salonen, 1992). Polar solvents, such as THF dissolved more organic halogen from pulp mill waste waters effluents than did nonpolar solvents such as heptane, hexane or cyclohexane (Jokela and Salkinoja-Salonen, 1992). THF extraction has been applied for material sedimenting in mesocosms (Saski *et al.,* 1995, 1996a,b) and for lake sediments (Saski *et al.,* 1997). Acidified THF extracted an average 0 to 80 % of the sedimenting BOX material in lake mesocosm (Saski *et al.,* 1996a). Acidified hexane extracted 1/5 of the organic halogen assessable by acidified tetrahydrofuran (THF) (Saski *et al.,* 1997). Kankaanpää and Tissari (1994a) reported that the nonpolar solvents hexane, cyclohexane and toluene were less effective than polar solvents, THF and cyclohexane-isopropanol (8:2 v/v) for extracting organic halogens from the sediments. Cyclohexane-isopropanol dissolved 2.3 to 9.4 (mean 5.6%) of the sediment AOX in the Gulf of Finland (Kankaanpää and Tissari 1994a,b). Pellinen and Soimasuo (1993) found that cyclohexane-isopropanol (1:1) extracted 30 to 45% (pulp mill affected) and 22 % (non recipient) of the BOX in the sediment.

Solubility into different solvents give information about bioaccumulative potential of sediment organic halogen (Reeve, 1996; McKague and Carlberg, 1996). Water soluble chemicals are easily and quickly distributed in aquatic environments. Hydrophilic pollutants may sorb to soils and sediments less efficiently than hydrophobic pollutants (Neilson, 1994). Hydrophobic compounds can pass the cell membrane by passive transport. Of hydrophilic compounds, only those for which cells possess an active a transport mechanism, can pass the cell membrane, for instance sugars or amino acids. The bioaccumulability of chemicals was shown to increase with the hydrophobicity of the compound (Bysshe, 1990; Meylan *et al*., 1999). Saski (1998) showed that organic halogen compounds in pulp mill effluents are hydrophilic, but they may be transformed into hydrophobic matter in recipient sediment ecosystem.

1.4 Sediment dating

Undisturbed, laminated sediments record the changes in the water column and the discharge area. If the age of each sediment layer can be determined, analysis of sediments can be used for tracking events from the past. Dated sediments can be read like a history book. Methods of sediment dating, such as ^{210}Pb method (Appelby and Oldfield, 1978) are important tools in sedimentology. The ^{210}Pb method extends over a time period of 150 years, covering most part of the industrial history of Finland.

The principle of the ^{210}Pb dating is illustrated in Figure 2. The dating is based on the radioactive isotopes of ^{238}U decay series. ^{238}U

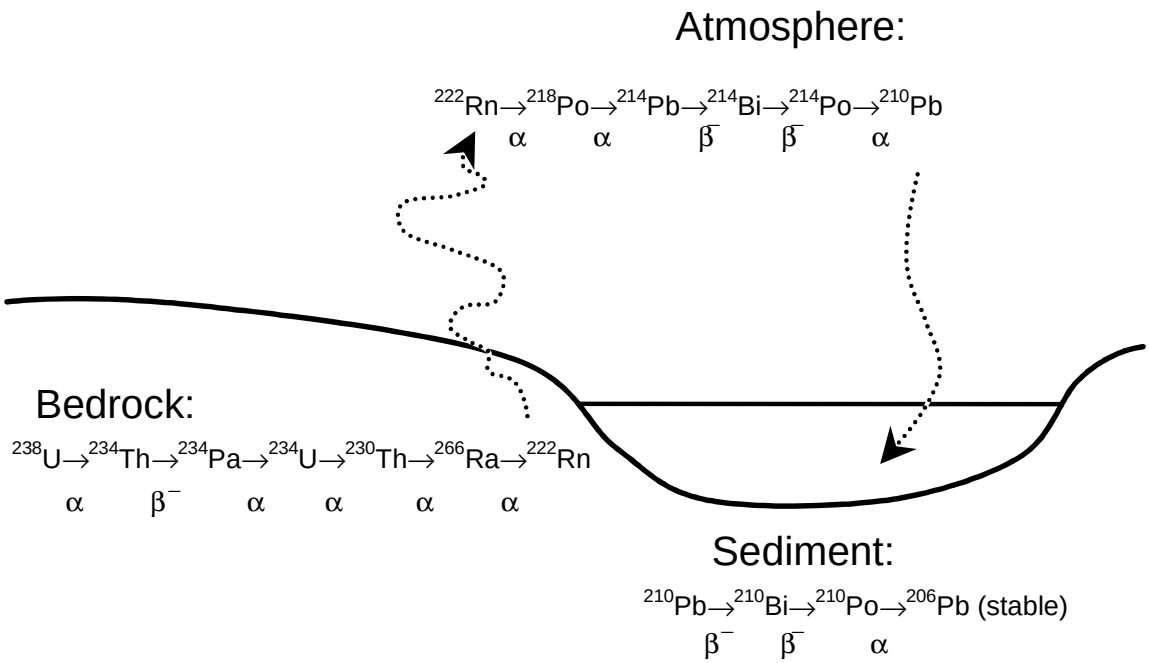

Figure 2. Principle of the ^{210}Pb sediment dating method: ^{238}U in the bedrock is decays to noble gas ^{222}Rn. ^{222}Rn evaporates into the atmosphere and decays further via multiple steps into ^{210}Pb. ^{210}Pb deposits on the sediment. Assuming the average annual deposition rate of ^{210}Pb onto the sediment to be constant, age of the sediment can be determined. In principle, the sediment layer containing 50% of the ^{210}Pb found in the top of the sediment, has an age of one half life, 22 years.

in the bedrock decays through several steps to a noble gas, ^{222}Rn. ^{222}Rn evaporates to the atmosphere, where it further decays to ^{210}Pb, which deposits. Assuming that the rate of deposition of ^{210}Pb in the sediment is constant, the ^{210}Pb contents of the sediment strata can be used for dating. In principle, the sediment layer, where the activity of ^{210}Pb is 50% of that on the sediment surface, has an age equal to one half life of ^{210}Pb, which is 22 years. In sediment strata older than 150 years, the estimate becomes unacceptably uncertain, due to a low activity of the studied nuclide. Sediment dating makes it possible to calculate historical sedimentation rates. This is needed when determining the flow and rates of accumulation of different materials into the sediment.

Chernobyl accident in Ukraine in 1986 and the nuclear weapon tests in the atmosphere in the early 1960's caused deposition of radioactive isotopes in the northern hemisphere. One of these isotopes is commonly used in sedimentology, ^{137}Cs. It is easy to measure, due to its gamma active daughter, ^{137m}Ba. The half life of ^{137}Cs is 30.2 years, which is sufficient for many sedimentological purposes. The shape of the peak of ^{137}Cs activity is an indicator of disturbance and rates of diffusion in the sediment profile. A sharp peak of ^{137}Cs is an indicator of an undisturbed sediment (Kansanen *et al.*, 1991). When two sharp peaks of ^{137}Cs, one from the atomic bomb experiments from the mid 1960's and the other from the Chernobyl accident in 1986 are found, and when the information obtained from these supports that from ^{210}Pb dating, the results of dating can be considered very reliable.

1.5 Ecotoxicological and biogeochemical assessment of the sediment

Sediments act as major sink for the organic material from the water column. Sediments contribute biogeochemical cycles and mineralisation, a major source of inorganic nutrients for the primary producers in aquatic

ecosystem. In the sediment, the mineralisation work is done by bacteria. Sediment is a sink for xenobiotics and pollutants, which may affect the microbial functioning of the sediment. Microbes in the sediment may also degrade xenobiotic or polluting compounds (Neilson, 1994; Kong, 1998; Albrecht *et al*., 1999; Bradley and Chapelle, 1999). Welfare of the microbial community in the sediment is of primary importance for the functioning of a lake ecosystem.

Microbial tests and metabolic activities may be used as tools to assess disturbance in aquatic ecosystems. One of the most commonly used assay is the light inhibition test of *Vibrio fischeri*. Also the amount of biomass (ATP, bacterial count), the growth related parameters (photosynthesis, thymidine incorporation) or mineralisation activity (methanogenesis, endogenous respiration, methane oxidation, enzymatic activities, nitrification or denitrification; Kemp *et al*., 1993) can be used to assess disturbance in an aquatic ecosystem. Methods for assessing genotoxicity have been developed (Ames *et al.,* 1975; Quillardet and Hofnung, 1985). Aquatic animals, such as *Daphnia magna* can be used to detect toxicity (Landis *et al*., 1989). Any change in these functionalities, it may be a drop or a rise, may indicate disturbance of the studied system. There is plentiful literature on different toxicity tests (*e.g.* Richardson, 1993; Liu and Dutka, 1984; Neilson, 1994; Suter and Lewis, 1989; Zakrzewski, 1991).

It should be kept in mind that in order to get a full picture the toxicity of a complicated matrix, such as pulp mill recipient sediment containing a myriad of different potentially toxic components, independent, principally different tests together with chemical analyses are needed. Developing new toxicity test systems may reveal hitherto unexpected types and degrees of toxicity, so that current assessment norms should be regarded as provisional and possibly conservative (Neilson, 1994).

1.5.1 Microbial toxicity tests

Marine luminescent bacterium *Vibrio fischeri* is a frequently used tool for measuring toxicity (Isenberg, 1993; ISO, 1998). This bacterium is capable of emitting light. Because light emission from *Vibrio fischeri* is one end point of ATP consuming reactions, the luminescence measurement reflects the energy status of the cell. Inhibition of luminescence indicates impairment of energy metabolism in the *Vibrio fischeri*. Thus the bioluminescence inhibition of *Vibrio fischeri* can be used as an indicator of toxicity. This Microtox® test has widely been used for assessing toxicity in soils (Vanhala and Ahtiainen, 1994) and sediments (Brouwer *et al.,* 1990; Heida and van der Oost, 1996; Pedersen *et al.,* 1998; Salizzato *et al.,* 1998).

Vibrio fischeri bioluminescence bioassay provides an inexpensive and rapid screening system. However, as a marine species it requires highly saline conditions for the bioassay. This may compromise its applicability to fresh water or terrestrial ecosystems. Another bioluminescence-based bioassay has been developed by inserting the lux gene (encoding the enzymes of bioluminescence in *Vibrio fischeri*) into a terrestrial bacterium *Pseudomonas fluorescens* (Boyd *et al.,* 1998). This bioassay has been developed and utilised to screen toxicity of organic xenobiotics in aqueous solution and in groundwater (Boyd *et al.,* 1998).

The results of a luminescent bacterial toxicity test are expressed as the effective concentration, *e.g.* the concentration inhibiting 50% of luminescence (EC_{50}). An informative way to show toxicity results is to use toxic equivalents (Neilson, 1994). Using toxic equivalents, the inhibition of light production by a compound or a matrix (for instance, polluted sediment) is compared to that of a reference compound. A frequently used reference compound is 3,5-dichlorophenol (ISO, 1998). The toxicity is expressed as the amount (milligrams) of the

reference compound required to give the same degree of inhibition as the studied material (ISO, 1998).

A variety of chemicals and pollutants, natural and artificial, may damage DNA of cells, thus being genotoxic, mutagenic or carcinogenic. As the sensitivity with which selectable mutants can be detected in a large population of bacteria is very high, bacteria can be used as screening agents for the potential of genotoxicity or mutagenity of chemicals. Quillardet and Hofnung (1985) presented a method for measuring genotoxicity. This method, known as the SOS-chromotest, has gained wide popularity. The test is based on the induction of SOS-gene in *E. coli* PQ37. The SOS-gene may be activated as a result of a mutation in any gene in the cell. The SOS-gene is placed upstream of β-galactosidase-inducing gene. Activation of the SOS-gene thus activates the promotor of β-galactosidase and the synthesis of ß-galactosidase enzyme. The ratio of ß-galactosidase activity to the constitutive phosphatase activity is called induction ratio I (Quillardet and Hofnung, 1985). I_c is the induction ratio for the cells exposed to the studied material, and I_0 is the induction ratio for the unexposed cells. An $I_c/I_0 > 1.5$ is considered as an indicator of genotoxicity (Quillardet and Hofnung, 1993).

The Salmonella AMES-test (Ames *et al.*, 1975) is another commonly used test to quantify mutagenity of materials. The test is based on histidine-requiring mutants of *Salmonella typhimurium*. Growth medium used for the test contains a low, growth limiting concentration of histidine, and the mutated test strain is not able to produce it. If a pre-existing mutation is reversed by a mutagen, the production of histidine will be restored, and the cells can grow.

1.5.2 ATP in the sediment

Adenosine triphosphate (ATP) is an important carrier of chemical energy in biological reactions. It can release sufficient energy during the cleavage of the tris-phosphate anhydride bond to drive many of the energy-requiring reactions in the cell. ATP is of primary importance to the functioning of the cell. ATP is present (0.1 to 0.5 ‰ of d.w.) in all living cells, and it is rapidly destroyed when a cell dies (Gregg, 1991). ATP is an ideal cell component for quantitating microbial biomass in ecosystems. ATP assay has been used to measure microbial biomass in many different areas, such as clinical research, food microbiology, brewing technology. It has also been used in biodeterioration studies and to assess airborne microorganisms (Nelson, 1991). ATP has been used to measure biomass and viability of microbes, as well as toxicity in soils (Paul and Clark, 1989; Vanhala and Ahtiainen, 1994; Frostegård *et al.*, 1996*)* and in aquatic ecosystem (Kemp *et al.*, 1993; Saski, 1997).

ATP can be quantified using firefly luciferin-luciferase system. Luciferin is a protein extracted from fireflies, for instance the European species *Lampyris noctiluca* or the North American one *Photinus pyralis* (Campbell, 1988). The assay is based upon the quantitative measurement of light produced during the reaction catalysed by the firefly luciferase enzyme. In the presence of O_2, the addition of ATP into luciferin-luciferase system produces AMP, oxyluciferin and light (Campbell, 1988) (Equation 2).

The direct effect of ATP on the firefly luciferin-luciferase is not an energy source. The energy for the reaction comes from oxidation of luciferin to oxyluciferin. ATP

$$\text{Equation 2:}\quad \text{ATP} + \text{luciferin} + O_2 \xrightarrow[\text{enzyme}]{\text{luciferase}} \text{oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{light}.$$

converts luciferin into a form capable of being catalytically oxidised by luciferase to oxyluciferin in a high quantum yield chemiluminescent reaction. The luciferase reaction is highly selective to ATP (Gregg, 1991). Firefly luciferin-luciferase can detect ATP down to 0.01 to 0.1 fmol, which is million times more sensitive than conventional spectrophotometric analysis (Campbell, 1988). ATP assays are standardised methods for the analysis of water and waste water (APHA, 1992). ATP assay kits are commercially available for analysis of living microbial biomass on various substrata and for ecotoxicologic monitoring microbial biomass in sediment. Cell membrane or cell wall components, such as phospholipid fatty acids, lipolysaccharides or muramic acid, has been used to quantitate microbial biomass in soils and sediments (*e.g.* Balkwill *et al.,* 1988; Frostegård *et al*., 1996).

1.5.3 Diatoms as indicator of water quality

Diatoms (Bacillariophyta) are algae present in the marine and freshwater ecosystems. During growth they generate a silica frustule with a significant morphological features. When cells die, the frustules sink to the lake bottom, where they can be identified to species level even centuries later. Planktonic diatoms live in the water column, and they are most sensitive of all diatoms to changes in water quality, such as pH, eutrophication or the presence of pollutants. According to literature, diatoms can be used as indirect indicator of water quality (Järnefelt, 1956; Hutchinson, 1967; Wetzel, 1983; Tikkanen, 1986; Riemann and Søndergaard, 1986). Figure 3 shows examples of diatom indicator species occurring in the sediments of Vatavalkama basin of Lake Saimaa, and Lakes Pyylampi and Mustalampi, forest lakes in south-eastern Finland.

Paleolimnological analysis of diatoms can give indirect information on the nutritional status of the water column. They can be used to track the eutrophication history of the lake (Hutchinson, 1967; Wetzel, 1983; Stoermer *et al.,* 1985). For instance, Liukkonen *et al.,* (1993) studied the eutrophication history and recovery of Lake Vesijärvi, south Finland, by the means of diatom analysis. Liukkonen *et al.* (1993) considered *Fragilaria crotonensis* and *Aulacoseira islandica* as eutrophication indicator species and *Cyclotella rossii* as an indicator of oligotrophy. Diatoms have been used to assess acidification history of lakes (Battarbee, 1984; Battarbee and Charles, 1986; Smol *et al.,* 1986; Huttunen *et al.,* 1990). For instance *Aulacoseira ambigua* is considered an alkaliphilic and *Aulacoseira lirata* an acidophilic species. Diatoms have been studied in the recipient waters of the industrial effluents (Granberg, 1972; Slepukhina *et al*., 1996; Liukkonen *et al.*, 1999). Slepukhina *et al.* (1996) found *Diatoma elongatum* and *Asterionella formosa* to profit from industrial effluents in an pulp mill burdened basin of Lake Ladoga. Liukkonen *et al*. (1999) found that the relative abundance of *Asterionella formosa* increased and that of *Cyclotella rossii* decreased in the recipient pelagic area of a pulp mill during the time of the heaviest pollution of organic halogen.

Many factors derived from the drainage area or as precipitation may influence the development of diatom flora (Stoermer *et al.*, 1985). Also changes of internal factors of the lake ecosystem, such as grazing, light conditions or water temperature influence the algal population in lakes (Hutchinson, 1967; Wetzel, 1983; Round *et al.*, 1996).

1.5.4 Biogeochemical functioning of lake sediments

Lake sediments are major sink of organic matter in the boreal aquatic ecosystem. Sediments also act as a store and source of organic matter. When organic matter from the water column reaches the sediment, it is mineralised by the sediment bacteria. The end products of the mineralisation process are CO_2 in the aerobic and CO_2 plus CH_4 in the

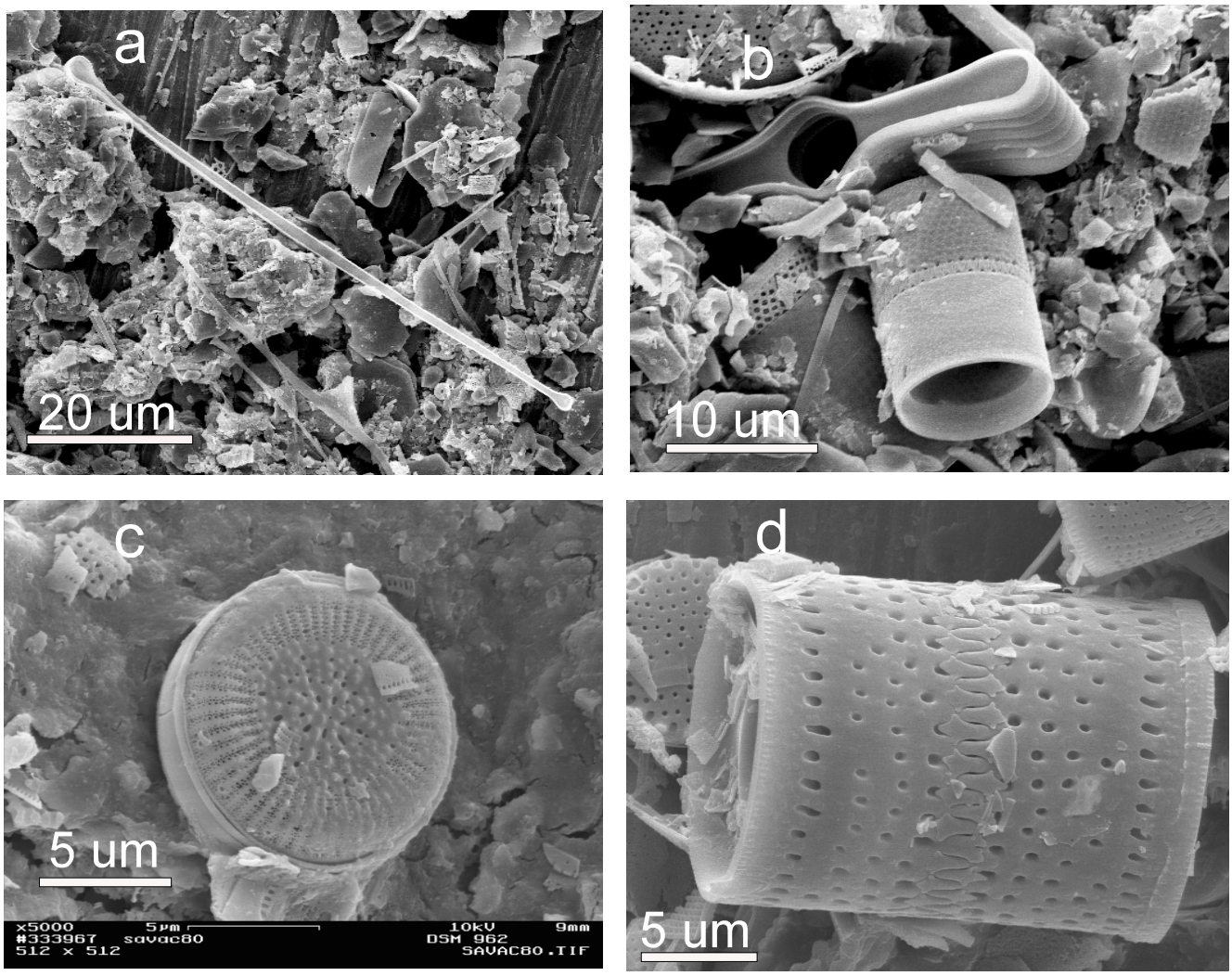

Figure 3. Portraits of four species of diatoms analysed as lake water column quality indicators: **a:** *Asterionella formosa,* a planktonic species recovered from the depth of 0 to 10 cm of Lake Mustalampi. *Asterionella formosa* is abundant in water courses under human impacts, *e.g.* eutrophication or pollution. **b:** *Aulacoseira ambigua,* an alkaliphilic planktonic species recovered from sediment at the depth of 0 to 10 cm of Lake Mustalampi. **c:** *Aulacoseira lirata* an acidophilic benthic species recovered from 0 to 10 cm sediment layers of Lake Pyylampi. **d:** *Cyclotella rossii*, an oligotrophic planktonic species recovered from the depth of 40 to 45 cm of Vatavalkama sediment. The age of this layer is about 1,000 years, based on the ^{210}Pb dating and assuming constant sedimentation rate at depths below 19 cm. (Micrographs: Mikko Liukkonen).

anaerobic ecosystem. Inorganic nutrient salts are also formed. These compounds can be utilised by bacteria or by primary producers, thus the nutrients are recycled into aquatic food chain.

1.5.4.1 Extracellular hydrolytic enzymes of microorganisms

1.5.4.1.1 General backgrounds of extracellular enzymes

The major part of the organic matter entering sediment is macromolecular and has to be extracellularly cleaved by hydrolytic enzymes into monomers or oligomers in order to be transportable into microbial cells (Meyer-Reil, 1987, 1991). Enzymes released into sediments by active growing microbes are catalysing these chemical degradation reactions. Extracellular enzymatic hydrolysis of higher-molecular weight material is considered to be the rate limiting step in the process of organic matter utilisation in sediments (Billen, 1982; Meyer-Reil, 1987).

The concept about the role of an extracellular enzymes is ambiguous. By definition, an intracellular enzyme is one that hydrolyses substrates inside the cell, inside the cytoplasmic membrane (Wetzel, 1991, Chróst, 1991). According to Chróst (1991), ectoenzyme indicates an enzyme that is exposed towards the outer space of the cytoplasmic membrane being able to react with substrates outside the cell, while the enzyme remains associated to the cell. According to Chróst (1991), extracellular enzymes may occur in a free form detached from the cell, free in water, or as attached to surfaces other than its producer cell, such as sediment particles. However, other researchers (Wetzel, 1991; Meyer-Reil, 1991; Münster and de Haan, 1998) consider as extracellular enzymes all enzymes that attack substrates external to the cells, irrespective of the enzyme occurs bound to the cell membrane or any other particle, or is free in the water. When I speak about extracellular enzymes in this thesis, it refers to any enzyme exposed to outside of the cell wall, whether associated on the outer surface of its producer, sorbed onto sediment particles, or occurring free in the sediment interstitial water.

It would be energetically inefficient for the cell to release enzymes to the extracellular space thus increasing the distance between the sites of enzymatic reactivity and the cell. The probability of a cell being able to utilise the hydrolysis products decreases precipitously as the distance between the cell and the enzyme increases (Wetzel, 1991). Surface bound enzymes may exhibit boundary layer diffusion problems. The diffusion rate of substrate to a bound enzyme is slow, and the substrate concentration is lower in the micro-environment at the cell boundary than in the free macroenvironment. Diffusional limitation can be reduced by releasing enzymes into the immediate medium surrounding the cells, although losses may result from products diffusing away (Wetzel, 1991).

Microbial activity may be present in very old sediments (Morita, 1997). Wittmann *et al.* (1999) and Kähkönen *et al.* (1999) recorded measurable activities of phosphatase and other extracellular enzymes in lake sediment layers formed hundreds of years ago. Opposite to ATP, intracellular or extracellular enzymes may retain their activity function for a long time. Enzymes may become associated with cell debris (Alef and Nannipieri, 1995). The formation of humic-enzyme complexes may inactivate enzymes (Wetzel, 1991). Often this reaction is reversible. Enzymes can be more stable in enzyme-humic acid complexes than in free enzyme, in part because of steric effects that interfere with proteolytic activity (Wetzel, 1991).

1.5.4.1.2 Examples of specific extracellular hydrolytic enzymes

Enzymes show certain specificity in catalysing cleavage of biological molecules. Their trivial names are created by adding

suffix "-ase" to the name of the substrate, the molecule on which the enzyme exerts it cleavage action. "Phosphatase" indicates the activity required to mineralise an organic phosphate to yield inorganic phosphate, an available nutrient for microbes, phytoplankton and plants. Phosphatase is a group name of several enzyme activities hydrolysing phosphorus monoesters (Alef *et al.*, 1995; Schomburg and Stephan, 1998). Different phosphatase enzymes have received different names, according to their substrates: phytase, nucleotidases, sugar phosphatases and glycerophosphatases (Alef *et al.*, 1995), all belonging to the group of phosphoric monoester hydrolases (EC 3.1.3) (Schomburg and Stephan, 1998). In addition to phosphoric monoesterases, there are phosphoric diesterases (EC 3.1.4) (Schomburg and Stephan, 1998) and phosphoric triesterases (EC 3.1.5). The term phosphatase may also include enzymes hydrolysing organic pyrophosphates, metaphosphates and inorganic polyphosphates (Alef *et al*., 1995). Methyl umbelliferyl (MUF) conjugated substrate, MUF-phosphate is a commonly used fluorogenic artificial substrate to assesses the activity of phosphomonoesterase (Alef and Nannipieri, 1995). In earlier days, chromogenic substrates, such as 2,4-dinitrophenylphosphate, were commonly used for assaying phosphatase activity. Fluorogenic substrates allow a detection 1,000 times more sensitive than chromogenic substrates.

Esterase is a group of enzyme activities, hydrolysing ester bonds (Schomburg and Stephan, 1998). Synthetic butyrate esters are used to measure various esterases (EC 3.1.1.56) with low substrate specificity. Reaction products are an organic acid and an alcohol.

Cellulose is the most abundant structural polysaccharide of plant cell walls. Cellulose is a linear polymer of D-glucose with ß-(1,4)-glucoside linkages. Cellulose needs at least three types of different enzymatic activities to be degraded: endo-ß-(1,4)-glucanase (EC 3.2.1.4), exo-ß-(1,4)-glucanase (EC 3.2.1.91) and ß-glucosidases (EC 3.2.1.21; EC 3.2.1.22) (Alef and Nannipieri, 1995). ß-glucosidase activity (ß-D-glucosidic-glucohydrolase, EC 3.2.1.21) is the rate limiting step in microbial degradation of cellulose to glucose (Alef and Nannipieri, 1995). This enzyme catalyses the hydrolysis of ß-glucosidic linkages in the cellulose chain.

In nature, α-glucosidase cleaves the α-(1,4)-bonds in starch and other storage carbohydrates of plants (Alef and Nannipieri, 1995; Olsen, 1995). The reaction products are oligosaccharides, which are further metabolised in the cell. The substrate for fluorometric measuring α-glucosidase (EC 3.2.1.133) is 4-methylumbelliferyl α-D-glucoside.

Chitin is a homopolymer of *N*-acetyl-glucosamine, with ß-(1,4)-linkages. It is a major organic component in the exoskeleton of insects, crustaceans and fungi (Cabib, 1987; Wood and Kellogg, 1988; Alef and Nannipieri, 1995). Enzymatic hydrolysis of chitin to *N*-acetyl glucosamine is mediated by two hydrolases, chitinase (EC 3.2.1.14) and chitobiase (EC 3.2.1.29). Chitinase hydrolyses chitin to dimers and trimers, which are further hydrolysed by chitobiase to the monomeric units (Cabib, 1987). Chitinase and chitobiase are common in nature and are produced by bacteria, fungi, plants and digestive glands of the animals that consume chitin containing material (Cabib, 1987; Wood and Kellogg, 1988; Alef and Nannipieri, 1995).

Chromogenic and fluorogenic substrates are commercially available for many enzyme tests. In these substrates, a chemical bond imitates the bond in natural substrates. These substrates may be used to measure extracellular, but also intracellular activities in environmental samples. Synthetic hydrophilic substrates, *i.e.* compounds with a low octanol-water partition coefficient (K_{ow}) ($\log K_{ow} < 1$), will not pass biological membrane. Such substrates can react with extracellular enzymes only. Hydrophobic substrates, *i.e.* compounds with a high K_{ow} ($\log K_{ow} >> 1$),

may penetrate biological membrane by passive diffusion. These substrates will react with extracellular and intracellular enzymes.

In some studies, phosphatase has been shown to positively correlate with the amount of microbial biomass in the aquatic ecosystem (Sinsabaugh, 1991, Overbeck, 1991). However, in many studies no correlation was found. For instance, Münster and de Haan (1998) studied enzymatic activity in humic forest lakes in southern Finland. They showed that in some of their lakes, the activity of phosphomonoesterase correlated with the microbial community respiration and with bacterial respiration, but not with microbial biomass parameters, such as bacterial numbers. Kähkönen *et al.* (1999) showed that lake sediment phosphatase activity did not correlate or correlated negatively with microbial biomass (ATP) in the sediment. Butyrate-esterase and aminopeptidase activities correlated positively with the sediment content of ATP (Kähkönen *et al.*, 1999).

To assess the quantity of enzyme activity in a given sample, the measurement must be done under saturating concentration of substrate. With poorly water soluble substrates, such as methyl umbelliferyl (MUF) derivates, measurements require a relatively high temperature (Alef and Nannipieri, 1995). Substrate must be present in excess, and the pH may be buffered to a high value (>7). The results from enzyme activity measurements therefore represent the potential rather than the actual activity.

1.5.4.2 Aerobic mineralisation

An important function of the sediment microbes is to oxidise organic matter to inorganic compounds. This reaction is called mineralisation. In aerobic mineralisation process, part of the organic carbon in the substrate is converted to biomass, and part is converted to carbon dioxide. Carbon dioxide is formed also in anaerobic heterotrophic mineralisation. In absence of molecular oxygen, nitrate, sulphate, iron and manganese ions function as electron acceptors. Many environmental stressors, such as pollutants or xenobiotics may inhibit mineralisation activity.

Aerobic mineralisation activity can be determined by monitoring for the formation of CO_2 or for the consumption of O_2 (OECD, 1992). Atmospheric carbon dioxide concentration is 0.035% (v/v) and that of oxygen 20% (v/v). Because of the low carbon dioxide concentration in the atmosphere, carbon dioxide measurement provides a sensitive tool to assess aerobic mineralisation (Paul and Clark, 1989). Measuring carbon dioxide is easy. The carbon dioxide can be trapped into a sorbent, such as a solution of NaOH or an organic base, and titrated by acid or analysed by carbon analyser (OECD, 1992). For detecting carbon dioxide in the gas phase, a gas chromatograph equipped with thermal conductivity detector can be used. Infra red detector gas analysers are sensitive to carbon dioxide and can be used for both static and flow systems (Paul and Clark, 1989). Measurement of carbon dioxide can be augmented by the incorporation of ^{14}C into the chosen substrates (radiorespirometry) (Atlas and Bartha, 1993). The ^{14}C may be in known molecules, such as glucose, amino acids, or herbicides, or in a complex materials such as microbial cells or plant residues. Because being the basic property in every ecosystem, and because of the easiness of carbon dioxide measurement, the assays of mineralisation and respiration are a popular and widely used tool in ecological and exotoxicological studies (for the standards, see Schinner *et al.*, 1993).

1.5.4.3 Methane: a global and ecological perspective

Methane is the end product from the anaerobic mineralisation of organic carbon. Methane is produced in aquatic sediments, rice fields, land fills and many other anaerobic

environments. Of the global production of methane, 1,130 Tg a^{-1}, 80% is of biogenic origin (Nedwell, 1996). About half of the global biogenic methane production becomes microbially oxidized at the site, where it is produced: wetlands, sediments, rice paddies, landfills (Nedwell, 1996). A net emission of 500 Tg a^{-1} is estimated to be released into the atmosphere (Hanson and Hanson, 1996; Nedwell, 1996). It has been estimated that 90% of the annual methane emissions into the atmosphere are oxidised through photo-chemical reactions in the troposphere, and approximately 10% is resorbed and oxidised by microbes in soils (Hanson and Hanson, 1996). The net annual increase of atmospheric methane content is about 40 Tg a-^{1} (Hanson and Hanson, 1996). The global methane budget is illustrated in Figure 4.

Atmospheric methane is of world wide concern because it is a greenhouse gas, 26 fold more effective as compared to carbon dioxide (mol/mol) (Hanson and Hanson, 1996). Reduction in methane emissions would be 20 to 60 times more effective in reducing the potential warming of the Earth's atmosphere over the next century than an equivalent molar reduction in CO_2 emissions would be (Hanson and Hanson, 1996).

1.5.4.3.1 Methane formation: a tool in environmental assessment

1.5.4.3.1.1 Methane formation: ecological significance

Methane is the microbial end product of anaerobic energy metabolism, formed in environments with no available electron acceptor other than carbon dioxide. The most important carbon dioxide reducing prokaryotes are the methanogens, a major group of *Archaea*. Many of these may utilize H_2 as the electron donor. The over all reaction is described in Equation 3.

In general, the production of methane from carbon dioxide is driven by molecular hydrogen (H_2) (Conrad, 1996; Madigan *et al.*, 1997). Methanogens can also grow and form methane from methanol, methyl amines, formic acid, carbon monoxide and acetic acid. This may happen especially in high sulphate concentration, like in marine sediments, where methanogenesis from carbon dioxide is suppressed by sulphate reducing bacteria, who compete with the methanogenic population for available acetate and H_2 (Conrad, 1996).

Sulphate reducing bacteria have a greater affinity for the electron donor H_2 than methanogenic bacteria (Conrad, 1996). Thus sulphate, sulphur or some organosulphur compounds may competitively inhibit methanogenesis (Oremland, 1988; Londry and Suflita, 1998). Nitrate and its denitrification products, nitrite, NO and N_2O are also known to inhibit methanogenesis, due to competitive inhibition as electron acceptors (Kluber and Conrad 1998).

1.5.4.3.1.2 Methane formation in polluted environment

Heavy metals may inhibit methanogenesis (Oremland, 1988; Codina *et al.*, 1998). In some cases, heavy metals may increase methanogenesis by inhibiting the metal sensitive substrate competitors, such as sulphate reducers (Oremland, 1988). Chlorinated methanes and other organic halogen compounds also inhibit methano-genesis (Oremland, 1988). Methanogenic bacteria are not as sensitive to organic halogen compounds as methanotrophic

Equation 3: $4H_2 + H^+ + HCO_3^- \longrightarrow CH_4 + 3\,H_2O$

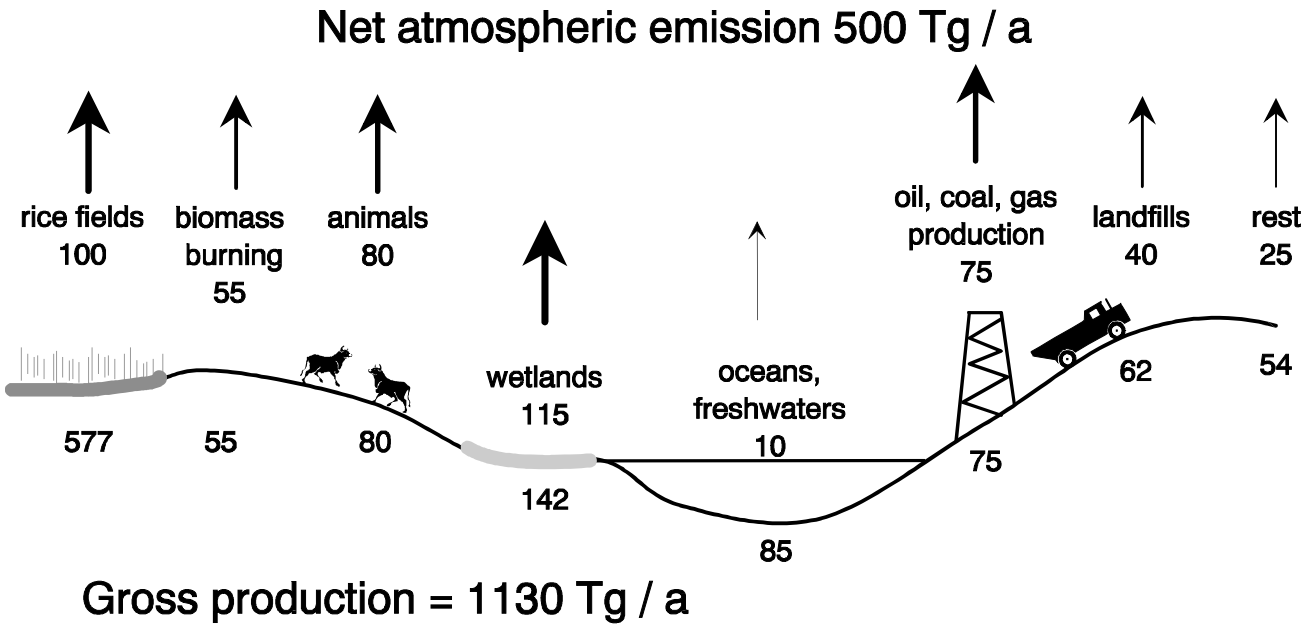

Figure 4. Schematic presentation of the global methane budget. Data from Nedwell (1996) and Hanson and Hanson (1996).

bacteria (Anderson and McCarty, 1996; Miller *et al.*, 1998; van Eekert *et al.*, 1999). Miller *et al.* (1998) recorded an inhibition of methanotrophic activity in the presence of 0.03 kPa difluoromethane. The same concentration did not decrease methanogenic activity. Ennik-Maarsen *et al.* (1998) recorded a 50% inhibition of fatty acid degradation by methanogenic granular sludge when exposed to 0.6 to 1.0 mM of monochlorophenols. Organosulphur compounds, thiophenes, thiols and aromatic sulphides inhibited methanogenesis at concentration greater than 5 mM (Londry and Suflita, 1998).

1.5.4.3.2 Methane oxidation activity: a tool in environmental assessment in aquatic ecosystem

1.5.4.3.2.1 Ecological significance

Lake and ocean ecosystems produce 85 Tg of methane a^{-1}, which contributes 8% of the global methane production (Nedwell, 1996). The major part, 90% of the methane formed in aquatic ecosystem is oxidised in anaerobic and aerobic sediments, and in the water column (Nedwell, 1996) (Figure 4). Methane in lake sediment and in the water column is oxidised exclusively by methanotrophic bacteria (Hanson and Hanson, 1996). However, these bacteria may survive and retain their aerobic methane oxidation capacity for long times even in the absence of molecular oxygen (Iversen, 1996).

All known methanotrophic bacteria are obligatorily aerobic. The enzyme responsible for methane oxidation is methane monooxygenase (MMO). It utilises two reducing equivalents to split the O=O bond of dioxygen to incorporate one oxygen atom into a methane molecule (Lipscomb, 1994; Hanson and Hanson, 1996). The product is CH_3OH, which is further oxidised in the cell into carbon dioxide, with formaldehyde as an intermediate. The other oxygen atom forms H_2O (Lipscomb, 1994; Hanson and Hanson, 1996). The reducing power required for the reduction of one oxygen atom to water is produced from the dehydrogenating methanol (Anthony and Dales, 1996), and subsequent oxidation of formaldehyde into formate and finally to carbon dioxide (Lipscomb, 1994; Hanson and Hanson, 1996).

Methane oxidation has been reported also in anaerobic sediments (Hoehler and Alperin, 1996; Iversen, 1996). No anaerobic methane oxidisers has yet been isolated in pure culture. It remains uncertain which electron acceptor is used for anaerobic methane oxidation. Sulphate has been suggested as the electron acceptor (Hoehler and Alperin, 1996; Iversen, 1996), mainly because sulphate reduction zone in sediments coincide with anaerobic methane oxidation (Iversen, 1996). Methane producers are hypothesised to reversibly convert methane to carbon dioxide and hydrogen gas (Hoehler and Alperin, 1996; Iversen, 1996). To make this process thermodynamically favourable, hydrogen must continuously be removed from the system, which could be done by the sulphate reducers. However, inhibitors of methane production failed to inhibit anaerobic methane oxidation suggesting that methane producers participating in this consortium behave differently from the methane oxidisers (Hoehler and Alperin, 1996; Iversen, 1996).

1.5.4.3.2.2 Methane oxidation potential in polluted aquatic environment

Methanotrophic bacteria are extremely sensitive to environmental disturbance, including pollutants, for example organic halogens (Anderson and McCarty, 1996). Ammonia fertilisers inhibit the oxidation of methane in soil by eradicating methanotrophic bacteria (Conrad, 1996; Hanson and Hanson, 1996). This inhibition may be due to competition between ammonia and methane for methane monooxygenase, or ammonia oxidation may deplete intracellular pools of reduced $NADH+H^+$ (Conrad, 1996; Hanson and Hanson, 1996; King, 1996). Disappearance of methanotrophs may also be due to displacement of the methane oxidising methanotrophs by nitrifying bacteria (Conrad, 1996), who have lower affinity for methane than methanotrophs (Hanson and Hanson, 1996). Nitrite produced from the ammonia by methanotrophs has been found to cause inhibition of methane oxidation by an unknown mechanism (Hanson and Hanson, 1996; King, 1996). Runoff from fertilised cropland may inhibit methanotroph activity in lake sediments. The disappearance of methane oxidation activity is reversible only slowly, if at all (Hanson and Hanson, 1996). Thus environmental pollution and human activities, like the use of fertilisers or pesticides may damage the exposed population of methanotrophic bacteria resulting into an increase of the net flow of methane into the atmosphere.

MMO is a biochemical missile, which catalyses oxidation of a broad range of hydrocarbons (Lipscomb, 1994; Hanson and Hanson, 1996). These include saturated, unsaturated, linear, branched and cyclic hydrocarbons up to approximately C_8 in size (Lipscomb, 1994). Also single- and double-ring aromatics, heterocycles, halogenated alkenes and ethers are turned over (Lipscomb, 1994). MMO in microbial cells can exist in either of two forms: particulate (membrane-bound) MMO (pMMO) or soluble MMO (sMMO). All methanotrophic bacteria contain pMMO, but production of sMMO is restricted only to type I methanotrophs (Lipscomb, 1994; Hanson and Hanson, 1996). The sMMO has a broader substrate specificity than pMMO, and sMMO not only oxidises methane, but other organic compounds, including halogenated C_1 and C_2-compounds (Hanson and Hanson, 1996). Thus methanotrophs may represent an important component of the self cleaning machinery of lake sediments. This capacity may be inhibited by heavy metals (Hanson and Hanson, 1996). Heavy metal pollution may thus impair the self remediation capacity of lake sediment for organic pollutants.

1.5.4.4 Dehalogenation: a mechanism for self remediation of the environment

1.5.4.4.1 Organic halogens and their dehalogenation in the sediment

Chlorinated compounds are commonly found in the environment. Organohalogens have accumulated into pulp mill recipient sediments (Maatela *et al.*, 1990, Palm and Lammi, 1995, Kankaanpää *et al.,* 1997, Saski *et al.*, 1997). Sediments are sinks for organohalogens from industry and pesticide use. Halogenated hydrocarbons are used as solvents in industry and as dry cleaning agent. Due to environmental hazard of halogenated hydrocarbons, attempts are being made in European Union to reduce the use of them (Klein *et al.,* 1999).

Sediments may have potential to dehalogenate the accumulated organohalogens. Dechlorination in sediments has been reported for chlorinated methanes and ethenes (Bradley and Chapelle, 1999; Wittmann, *et al.,* 1999). Degradation of chlorophenols (Kong, 1998), chlorobenzenes (van Hoof and Jafvert, 1996), PCB compounds (Williams, 1994; Wu *et al.*, 1997) and of polychlorinated dibenzo-*p*-dioxins (Albrecht *et al.,* 1999) in freshwater sediments has been reported. However, the degradability of pulp mill originating organic halogens in the receiving lake sediments appears not to have been studied prior to our work.

Tetrachloromethane, CCl_4, is a commonly used substrate in studies of reductive dehalogenation (Doong *et al.,* 1996a,b; van Eekert *et al.,* 1998). Tetrachloromethane, like many other halogenated hydrocarbons, is toxic, a suspected carcinogen and destroys stratospheric ozone layer. Oxidation products of tetrachloromethane may cause cross-linking of the unsaturated fatty acids in membrane lipids (Frank *et al.,* 1989).

1.5.4.4.2 Microbially catalysed reductive dehalogenation

Degradation of halogenated compounds under anaerobic compounds can be carried out by halorespiring bacteria (van Eekert *et al.,* 1998). Also acetogenic and methanogenic bacteria are able to transform chlorinated compounds via aspecific reactions. Both intracellular and extracellular microbial processes are important in the dechlorination of chlorinated organics. Reductive dehalogenation of chlorinated organic compounds has been reported under sulphate reducing (Gupta *et al.,* 1996a), methanogenic (Gupta *et al.,* 1996b; Vogel and McCarty, 1985; van Eekert *et al.,* 1998, 1999; Bradley *et al.,* 1999) and denitrifying (Häggblom *et al.,* 1993) conditions. Not only obligate anaerobes, but also facultative anaerobic bacteria, like *E. coli* were reported to perform reductive dechlorination of haloaliphatic compounds (Fetzner and Lingens, 1994). Degradation of tetrachloromethane and other chlorocarbons has been reported to occur in anaerobic sludges (Doong *et al.,* 1996a,b; van Eekert *et al.,* 1998, 1999) and sediments (Bradley and Chapelle, 1999).

For reductive dechlorination a redox potential below +70 mV (E^o_H) of the microenvironment is needed. For complete dechlorination of tetrachloromethane a redox potential below -200 mV is required (Doong *et al.,* 1996b).

1.5.4.4.3 Biotic vs. abiotic dehalogenation

Degradation of tetrachloromethane may be microbially catalysed (van Eekert, *et al.,* 1998; Workman *et al.,* 1997). Degradation of tetrachloromethane and other chlorinated hydrocarbons in anaerobic studies was shown to continue also after living bacterial cells were killed by autoclaving (van Eekert *et al.,* 1998; 1999). Degradation of tetra-chloromethane thus was mediated by heat stable components (van Eekert *et al.,* 1998, 1999). Workman *et al*. (1997) used *Shewanella alga* BrY strain to reduce Co(III)

in the vitamin B_{12a} (cob(III)alamin) in to (cob(II)alamin), B_{12r}, which then reduced tetrachloromethane to trichloromethane. Tetrachloromethane was reduced also by extracts from which bacterial cells were removed by 0.2 µm filtration (Workman *et al.*, 1997). Cellular components, passing the 0.2 µm filter, therefore were responsible reducing the vitamin B_{12a}, even after the cells of *Shewanella alga* were removed from the system. Metal containing cofactors, like vitamin B_{12} or F_{340}, which contain cobalt, nickel or iron, make part of enzymes that drive many metabolic processes in anaerobic bacteria. These processes involve acetyl coenzyme A pathway and methane formation (Schlegel, 1992). Thus these cofactors are likely to be present in the methanogenic sediment.

Curtis and Reinhard (1994) suggested that iron porphyrines or hydroquinones can act as mediators in reductive dehalogenation. These compounds form highly reactive, reduced intermediates that reduce the oxidised contaminants, such as tetrachloromethane. Hydroquinones are present in humic material, and humic material may act as this kind of mediator (Curtis and Reinhard, 1994). Once the electron transfer mediator is oxidised by the contaminant, the mediator is assumed to be re-reduced back to the initial state by a "bulk" electron donor, *e.g.* Fe^{+2} or HS^- (Curtis and Reinhard, 1994). Carbon tetrachloride can also be transformed in the presence of pyrite (FeS_2), metallic iron or sulphide as the bulk electron donor (Curtis and Reinhard, 1994; Kriegman-King and Reinhard, 1994).

The end products from dechlorination of tetrachloromethane or of chlorinated ethenes may be Cl^-, CH_4, CS_2, CO_2 or CO (Workman *et al.*, 1997; van Eekert *et al.*, 1998; Bradley and Chapelle, 1999). In degradation of tetrachloromethane and chlorinated ethenes, less chlorinated metabolites emerge.

Adaptation of microbial sediment community to degrade organic halogen compounds has been studied widely. Kong (1998) reported that dechlorination of 2-monochlorophenol and 3-monochlorophenol occurred without lag phase, with a half life of 1 to 1.2 days in a sediment acclimated by monochlorophenol. In unacclimated sediment, a lag period of 10 days was recorded for 2-monochlorophenol and of 56 days for 3-monochlorophenol. Half lives for the degradation of 2-monochlorophenol and 3-monochlorophenol in unacclimated sediments were 17 and 26 days, respectively. These results suggest that adaptation may be important in the degradation of organic halogens in anaerobic conditions. Also Fulthorpe *et al.* (1989) and Reineke and Knackmuss (1988) pointed out the role of adaptation on the growth and degradation potential of bacteria in sediment and water. Several hypotheses have been presented to explain the lag period: enzyme induction, mutation/genetic exchange, change of microbial population, or the structure of the chemicals (Kong, 1998). Microorganisms may adapt to degrade halogenated compounds by developing taylor made enzyme systems for degrading these compounds (*e.g.* Pries *et al.*, 1994; Reineke and Knackmuss, 1988). Pries *et al.* (1994) suggested that lack of enzymes that could catalyse the required catabolic steps is the most important reason for the persistence of organic halogens.

2. AIMS OF THIS STUDY

The aim of this work was to answer the following questions:

1. How much organic halogen is there in industrially non affected lake sediments, and is the organic halogen of biogenic origin?

2. What are the ecotoxicological consequences of pulp bleaching chlorine discharges to the sediment?

3. How does the organic halogen in pulp mill discharges affect the biogeochemical functioning of recipient lake sediment?

4. How is the reduction of discharges from a pulp mill visible in the chemical and biochemical qualities of the recipient sediment?

5. What ecotoxicological benefits are there from the reduction of discharges from a pulp mill as judged from the recipient sediment?

6. Did the biogeochemical functioning of the sediment change after the industry dramatically decreased its discharges?

7. Is pulp mill organic halogen biodegradable in a recipient sediment?

3. MATERIALS AND METHODS

Methods used in this study are presented in Table 1. For the closer description of the materials and methods, see papers I to IV.

Table 1. The summary of methods used in this work. For the closer description of the materials and methods, see papers I to IV.

Analysis	Method	Description	Reference (manufacturer)
Sediment sampling and dating			
Sampling	Freeze finger	I, II, III, IV	Huttunen and Meriläinen, 1978
	Limnos corer	I, II, III, IV	Kansanen *et al.*, 1991
Sediment dating	^{210}Pb-method	I, III	Appelby and Oldfield, 1978
	^{137}Cs-method	I, III	Kansanen *et al.*, 1991
Sediment physico chemical analysis:			
EOX	Tetrahydrofuran soluble organic halogen	I, II, III, IV	Jokela and Salkinoja-Salonen, 1992
C, N, S	LECO C-H-N-S-analyser	II, III, IV	(Leco Incorporation, St. Joseph, USA)
Dry weight	Freeze drying	I, II, III, IV	(B. Brown Christ Gamma 2-20, Melsungen, Germany)
Organic matter	Ignition loss at 650 °C	I	
Heavy metals	ICP/mass spectrometry	II, III, IV	(Fison Plasma Quard, Winsfont, UK)
Redox potential	Standard calomel electrode	IV	APHA, 1992
Water physico-chemical analysis:			
AOX	Activated carbon adsorption method	I	ISO, 1989 (Euroglas, Delft, NL)
Dissolved oxygen:	Titrimetric method	I	APHA, 1992
	Oxygen electrode	I	(YSI incorporation, Ohio,USA)
Turbidity	Nephelometric method	I	APHA, 1992
Phosphorus	Ascorbic acid method	I	APHA, 1992
Ammonium	Indofenol method	I	SFS, 1976
Nitrate	Cd-reduction method	I	APHA, 1992
Total nitrogen	Peroxodisulfate+N reduction	I	SFS, 1990, APHA, 1992
Total organic carbon, TOC	TOC-analyser	I	ISO, 1987 (Shimadzu)
Conductivity	Specific conductance	I	APHA, 1992
Colour	Comparator method	I	APHA, 1992
Alkalinity	Titration method	I	APHA, 1992
Chemical oxygen demand, COD_{Mn}	Permanganate method	I	SFS, 1981
Chemical oxygen demand, COD_{Cr}	Dichromate method	thesis	APHA, 1992
Ecological and ecotoxicological tests:			
Toxicity	Microtox® *Vibrio fisheri* bioluminescence test	IV	ISO, 1998
Genotoxicity	SOS-chromotest with *E. coli* PQ37	IV	Quillardet and Hofnung, 1985
Enzyme activities	Kinetic fluorometry	II, IV	Wittmann *et al.*, 1999
Methane oxidation potential	Head space test, GC+FID	II	Wittmann *et al.*, 1999
Endogenous methane production	Head space test, GC+FID	thesis	
Endogenous carbon dioxide production	Head space CO_2 formation test, GC+TCD	II	Schinner *et al.*, 1993
Reductive dehalogenation	Head space incubation, GC+ECD	thesis	Wittmann, *et al.*, 1999
Diatom counting	Light microscope, scanning electron microscope	IV	Liukkonen *et al.*, 1993, 1999

4. RESULTS AND DISCUSSION

4.1 Halogen and sulphur in Lake Saimaa and forest lake sediments

4.1.1 Background

The aim was to compare accumulation of organic halogens in sediments of industrially affected and non-affected Finnish lakes. The chosen industrially affected sites were two basins, Vatavalkama and Tattari, recipient areas of pulp mills for 100 years, in the oligotrophic Lake Saimaa. The chosen non-affected lakes were Lake Mustalampi and Lake Pyylampi in the forest in south eastern Finland. The forest lakes were near each other (< 1 km) in a geographically uniform area. One lake, Lake Mustalampi had received an experimental discharge of 30 m^3 of biologically purified kraft pulp mill bleaching wastewater in 1979 (Salkinoja-Salonen *et al.*, 1981, 1983). The other lake, Lake Pyylampi has no known history of any type of discharges. The lakes and their history are described in papers I and III. All sampled sediments were clearly stratified and isotopic dating (^{210}Pb, ^{137}Cs) showed that the sampling had not disturbed the stratification (Figure 6 in Paper I, Figure 2 in Paper III).

We measured the amount and the accumulation of solvent soluble part of organic halogen (EOX) at four sites in the three different lakes. Tetrahydrofuran was chosen as the extraction solvent because it dissolves a wide spectrum of different organic halogens from hydrophilic to hydrophobic compounds (Jokela and Salkinoja-Salonen, 1992; Jokela, 1997). The sediment bound organic halogen (BOX) was measured in Lakes Mustalampi and Pyylampi. The historical pH of the lake was analysed by paleolimnological analysis of diatom frustules.

The results of this work are summarised in Table 2.

4.1.2 Organic halogen in forest lake sediments

The sediment concentrations of EOX were highest at the depth of 17 to 19 cm (440 mg of Cl_{EOX} (kg C^{-1}) in Lake Mustalampi and at the depth of to 21 to 23 cm (650 mg of Cl_{EOX} (kg C)$^{-1}$) in Lake Pyylampi (Figure 5a,c). Both EOX-rich layers dated to before the year 1900 (Figure 6 in Paper I). The layers deposited most recently ($\leq$ 30 years), located at the depth of 0 to 7 cm, contained 150 to 200 mg of Cl_{EOX} (kg C)$^{-1}$ in Lake Mustalampi and 200 to 260 mg of Cl_{EOX} (kg C)$^{-1}$ in Lake Pyylampi. Lake Mustalampi had in 1979 received an experimental discharge of 30 m^3 of biologically purified kraft pulp bleaching waste water, containing in total 2 kg of Cl_{AOX} (Salkinoja-Salonen *et al.*, 1981). No trace of this AOX input was traceable in the sediment of Lake Mustalampi 15 years later (Fig 5 a,b, Figure 4 in Paper I). The detection limit was 5% of the added Cl_{AOX} (<100 g). If this amount of Cl_{AOX} had deposited in a 1 cm layer of Lake Mustalampi area (6,800 m^2; 15 mg of Cl_{AOX} m^{-2}), an excess of the sediment concentration of EOX by 50 mg Cl_{AOX} (kg C)$^{-1}$ would have been observed in the 4 to 5 cm layer as compared to the layers flanking it.

Figures 5b and d show the concentrations of bound organic halogen (BOX) in Lakes Mustalampi and Pyylampi, assessed as AOX after alkaline hydrolysis. Sediment concentrations of BOX ranged between 300 and 1,400 mg of Cl_{BOX} (kg C)$^{-1}$ in Lake Mustalampi and 600 to 2,000 mg Cl_{BOX} of (kg C)$^{-1}$ in Lake Pyylampi. Comparison of the EOX content to BOX in the sediment layers shows that acidified (pH 2) tetrahydrofuran (EOX), extracted 20% to 54% (mean 30%) of the BOX (sediment bound organic halogen). This percentage was used to compare EOX values with BOX and vice versa (EOX= 0.3$\times$ BOX).

Table 2. Summary of the results of sediments from two pulp mill recipient basins of Lake Saimaa (Vatavalkama, 3 km from the mill and Tattari, 5 km from the mill) and from two non recipient forest lakes (Lakes Mustalampi and Pyylampi). The sediments were sampled at the depths indicated in the title row, except those mentioned as superscript after each value.
n.d. = no data available.

Site	**Vatavalkama**			**Tattari**		
Depth (cm) **Age of the layer**	0-1 cm 1995-97	4-6 cm 1980-85	below 18 cm before 1900	0-1 cm 1995-97	4-6 cm 1980-85	below 18 cm before 1900
Toxicological parameters:						
EC_{50} *V. fischeri* (% v/v)	18	1.0	$16^{18\text{-}20\ cm}$	n.d.	n.d	n.d.
ATP (nmol $(g\ C)^{-1}$)	39	0.03	$0.1^{22\text{-}24\ cm}$	n.d.	n.d	n.d.
Genotoxicity induction ratio I_c/I_0	1.1	1.3	n.d.	n.d.	n.d.	n.d.
Biogeochemical parameters:						
endog. CH_4 production ($\mu mol\ CH_4\ (g\ C)^{-1}\ d^{-1}$)	3.8	0.5	$<0.1^{6\text{-}31\ cm}$	n.d	n.d	n.d.
CH_4 consumption ($\mu mol\ CH_4\ (g\ C)^{-1}\ d^{-1}$)	130	6	$<0.1^{26\text{-}31\ cm}$	n.d.	n.d.	n.d.
endog. CO_2 production ($\mu mol\ CO_2\ (g\ C)^{-1}\ d^{-1}$)	$300^{0\text{-}3\ cm}$	$140^{3\text{-}6\ cm}$	n.d.	n.d.	n.d.	n.d.
Enzyme activities:						
ß-glucosidase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	$0.17^{0\text{-}3\ cm}$	$0.11^{3\text{-}6\ cm}$	n.d.	n.d	n.d	n.d.
Phosphatase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	$18^{0\text{-}3\ cm}$	$10^{3\text{-}6\ cm}$	n.d.	n.d.	n.d.	n.d.
Butyrate-esterase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	$18^{0\text{-}3\ cm}$	$6.7^{3\text{-}6\ cm}$	n.d.	n.d.	n.d.	n.d.
Chemical parameters:						
EOX (mg Cl $(kg\ C)^{-1}$)	7,000	23,500	$130^{28\text{-}30\ cm}$	10,800	15,900	$720^{20\text{-}22\ cm}$
C (g C $(kg\ d.w.)^{-1}$)	190	208	$81^{28\text{-}30\ cm}$	170	180	$74^{20\text{-}22\ cm}$
N (g N $(kg\ C)^{-1}$)	53	44	$93^{28\text{-}30\ cm}$	59	56	$93^{20\text{-}22\ cm}$
S (g S $(kg\ C)^{-1}$)	25	45	$5.7^{26\text{-}28\ cm}$	n.d.	n.d.	n.d.
dry weight (%)	4.3	9.5	$11.7^{28\text{-}30\ cm}$	2.1	5.5	$9.8^{20\text{-}22\ cm}$

Table 2 continued.

Site	**Pyylampi**			**Mustalampi**		
Depth (cm) **Age of the layer**	0-2 cm 1993-95	4-6 cm 1970-82	below 18 cm before 1900	0-2 cm 1993-95	4-6 cm 1982-87	below 18 cm before 1900
Toxicological parameters:						
EC_{50} *V. fischeri* (% v/v)	35	4.5	$4.4^{18\text{-}20\ cm}$	n.d.	n.d.	n.d.
ATP (nmol $(g\ C)^{-1}$)	47	20	$0.03^{24\text{-}26\ cm}$	n.d.	n.d.	n.d.
Genotoxicity induction ratio I_c/I_0	1.0	1.0	n.d.	n.d.	n.d.	n.d.
Biogeochemical parameters:						
endog. CH_4 production ($\mu mol\ CH_4\ (g\ C)^{-1}\ d^{-1}$)	14	0.5	$<0.1^{37\text{-}42\ cm}$	n.d.	n.d.	n.d.
CH_4 consumption ($\mu mol\ CH_4\ (g\ C)^{-1}\ d^{-1}$)	130	27	$16^{37\text{-}42\ cm}$	n.d.	n.d.	n.d.
endog. CO_2 production ($\mu mol\ CO_2\ (g\ C)^{-1}\ d^{-1}$)	670	170	n.d.	n.d.	n.d.	n.d.
Enzyme activities:						
ß-glucosidase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	0.24	0.03	n.d.	n.d	n.d	n.d.
Phosphatase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	9.0	1.5	n.d.	n.d.	n.d.	n.d.
Butyrate-esterase $\mu mol\ (g\ C)^{-1}\ h^{-1}$	20	7.4	n.d.	n.d.	n.d.	n.d.
Chemical parameters:						
EOX (mg Cl $(kg\ C)^{-1}$)	250	$240^{4\text{-}5\ cm}$	$650^{18\text{-}20\ cm}$	190	$160^{4\text{-}5cm}$	$310^{19\text{-}21\ cm}$
C (g C $(kg\ d.w.)^{-1}$)	270	$230^{4\text{-}5\ cm}$	$250^{18\text{-}20\ cm}$	400	$390^{4\text{-}5\ cm}$	$410^{19\text{-}21\ cm}$
N (g N $(kg\ C)^{-1}$)	98	$74^{4\text{-}5\ cm}$	$80^{18\text{-}20\ cm}$	65	$54^{4\text{-}5\ cm}$	$63^{19\text{-}21\ cm}$
S (g S $(kg\ C)^{-1}$)	31	$43^{4\text{-}5\ cm}$	$7.9^{20\text{-}22cm}$	n.d.	n.d.	n.d.
dry weight (%)	1.9	$6.5^{4\text{-}5\ cm}$	$7.1^{18\text{-}20\ cm}$	$0.95^{0\text{-}1\ cm}$	$6.7^{4\text{-}5\ cm}$	$5.1^{19\text{-}21\ cm}$

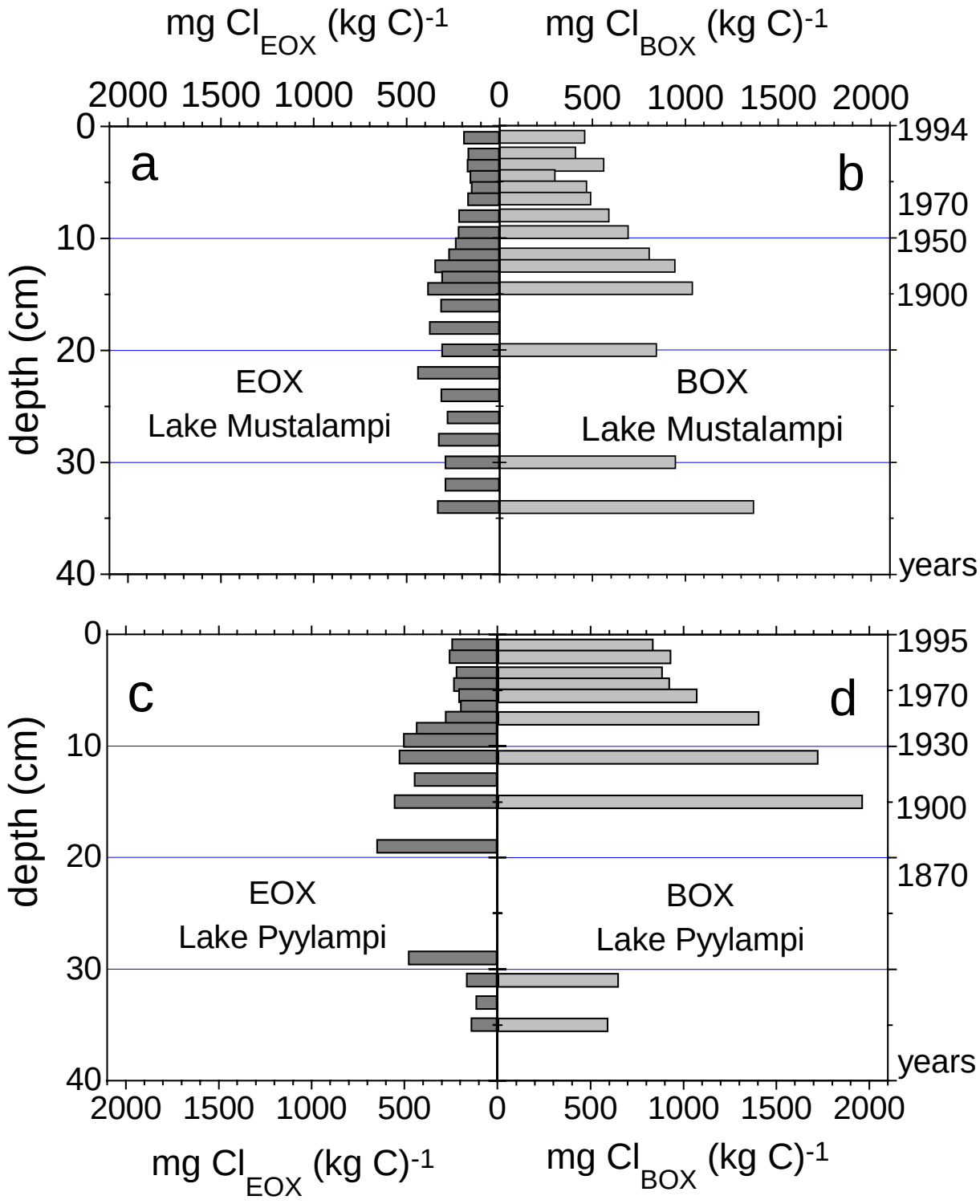

Figure 5. Sediment concentrations of different fractions of organic halogen in Lakes Mustalampi and Pyylampi. Sediment concentrations of extractable organic halogen (EOX) **(a,c)** and bound organic halogen (BOX) **(b,d)** in Lake Mustalampi **(a,b)** and in Lake Pyylampi **(c,d)**. Sediment concentration of EOX was determined as the organic halogen soluble in acidified tetrahydrofuran. The concentration of BOX was determined as the AOX released after alkaline hydrolysis of the same sediment. The years in the Figures 5 b and d refer to the ages of the sediment layers (for details in dating, see Figure 6 in Paper I).

Sediment layers older than 100 years (located deeper than 20 cm depth) of the oligotrophic Lake Saimaa contained 130 to 490 mg of Cl_{EOX} (kg C)$^{-1}$ in Vatavalkama basin and 130 to 250 mg of Cl_{EOX} (kg C)$^{-1}$ in Tattari basin (Figure 6). The sediment concentration of BOX in Lake Saimaa may be estimated using the factor 0.3 to convert BOX into EOX. The sediment concentration of BOX in the layers of Lake Saimaa formed early in the 20th century, *e.g.* before the arrival of the local pulping industry, can be estimated as 430 to 1630 mg of Cl_{BOX} (kg C)$^{-1}$ in Vatavalkama and 430 to 830 mg Cl_{BOX} (kg C)$^{-1}$ in Tattari. The sediment concentration of EOX and BOX in the layers between 2 and 20 cm (deposited from 1930's to 1980's) will be dealt in chapter 4.1.4 and that in the 0 and 1 cm layer (deposited in 1990s) in chapter 4.3.

Saski *et al.* (1997) recorded 710 mg of Cl_{EOX} (kg C)$^{-1}$, extractable into acidified THF, in a large lake Höytiäinen sediment. Lake Höytiäinen receives rural discharges, but no significant industrial discharges. If the acidified THF dissolved 30% of the BOX in that sediment, the sediment concentration of BOX may have been 2,400 mg of Cl_{BOX} (kg C)$^{-1}$ in Lake Höytiäinen. Pellinen and Soimasuo (1993) reported 990 mg of Cl_{BOX} (kg C)$^{-1}$ in the lake sediment upstream of a pulp mill, assessed as AOX after alkaline hydrolysis. Maatela *et al.* (1990) measured sediment total concentration of total organic halogen (TOX) from a small polyhumous lake receiving rural pollution. Their method was titration of chloride ions after wet combustion. They found 500 to 1,900 mg of Cl_{TOX} (kg C)$^{-1}$, assuming that organic matter contained 40% of carbon. Asplund (1992) investigated sediment concentrations of organic halogen in Swedish lakes, and found a relatively constant concentration of 800 to 3,400 mg of organic Cl per kg of carbon. We conclude from the above that organic halogen concentrations were similar in pulp mill non-recipient lake sediments, irrespective of the type of the lake, small (Lake Mustalampi, 0.000021 km^3) and large (Lake Vättern, 74 km^3), in Finland and in Sweden.

4.1.3 Source of the organic halogen in forest lake sediment

The water column of Lake Mustalampi and of Lake Pyylampi contained between 10 and 29 µg of Cl_{AOX} l^{-1} (adsorbable organic halogen, AOX) (Figure 3 in Paper I) and between 8.3 and 11.6 mg of TOC l^{-1} (total organic carbon, TOC) (Table 3 in paper I). The colour of water in Lakes Mustalampi and Pyylampi ranged between 52 to 104 mg of Pt l^{-1} (Table 3 in Paper I). We found a moderate correlation between the colour and the concentration of AOX ($r=0.63$; $n= 13$) of lake water, and between water concentration of total organic carbon (TOC) and water concentration of AOX ($r=0.62$; $n=9$) in Lakes Mustalampi and Pyylampi (Figure 7). The correlations between water colour and water concentration of AOX, and water concentration of TOC and water concentration of AOX in Finnish humic lakes were similar to those observed in Swedish lakes by Asplund and Grimvall (1991).

We noticed that the changes in the organic halogen contents of the sediment strata in Lakes Mustalampi and Pyylampi followed the changes in the paleolimnologically estimated historical pH of the water column of these lakes (Figure 8a,b). Sediment concentration of EOX increased with the acidity of the lake. The pH of the water column was deduced from diatom frustule diversity in dated sediment layers of Lakes Mustalampi and Pyylampi. Many soil fungi have been shown to possess capacity to synthesise halogenated organic compounds. This process is catalysed by the fungal haloperoxidases (Neidleman and Geigert, 1986). Haloperoxidases are active in acid environment (Asplund, 1992; Asplund *et al*., 1993; Hoekstra *et al*., 1999). Asplund and Grimvall (1991) and Asplund (1992) suggested that organic halogen in forest lake sediment may have its origin in the runoff from the forest humus with low pH (ca. 3.5). This may be the case also in Lakes Pyylampi and Mustalampi.

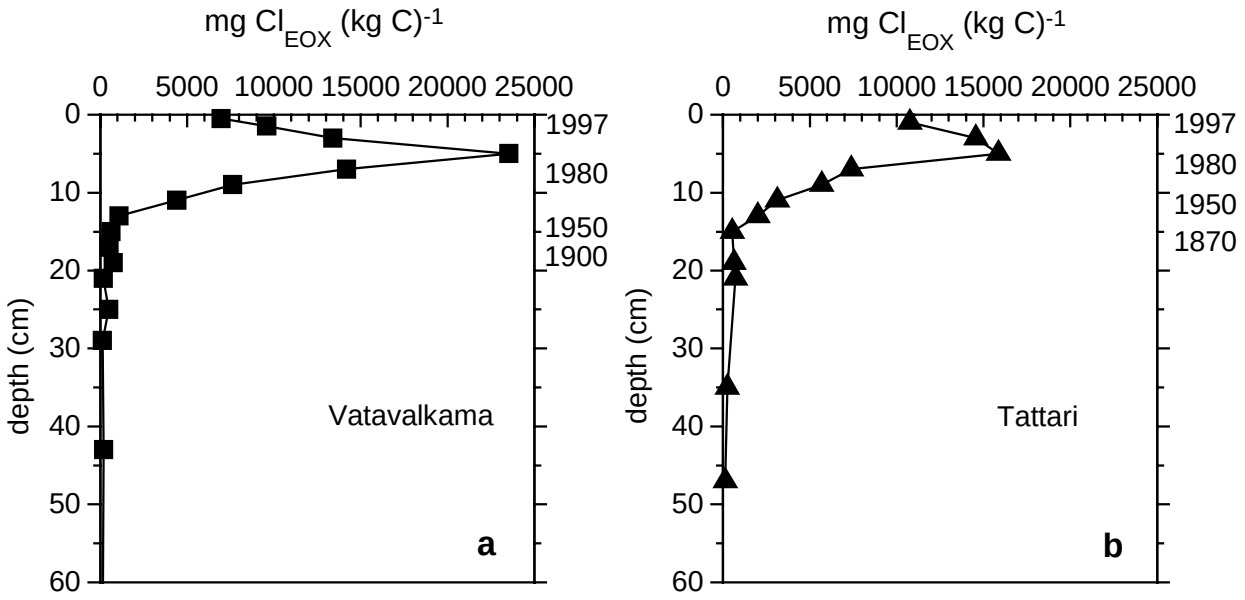

Figure 6. Sediment concentrations of EOX in two pulp mill burdened basins of the oligotrophic Lake Saimaa, SE-Finland, Vatavalkama **(a)** and Tattari **(b)**. EOX was assessed as the organohalogen soluble in acidified tetrahydrofuran. The years in the figure refer to the ages of the sediment layers (for dating details, see Figure 2 in Paper III).

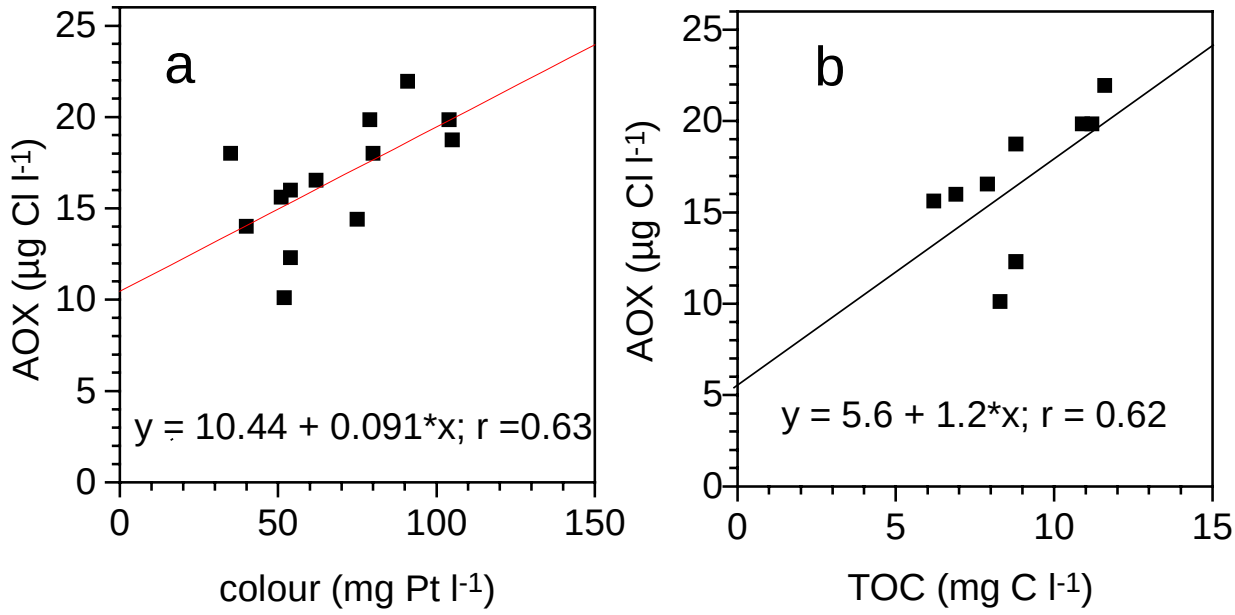

Figure 7. Correlations between the water colour and concentration of AOX **(a)** in water column, and between the water concentration of TOC and water concentration of AOX **(b)** in two forest lakes, Mustalampi and Pyylampi in SE-Finland.

Accumulation of organic halogen in forest lake sediments followed that of organic matter (Figure 8 in Paper I) suggesting that the halogenated organic matter may have originated from the runoff. Sediment accumulation of dry matter (d.m.) and of organic matter OM were in Lake Pyylampi 70 to 100 years ago (depth of 10 to 20 cm) 200 g of d.m. $m^{-2}\,a^{-1}$ and 100 g of OM $m^{-2}\,a^{-1}$, respectively, and that of organic halogen 25 mg of Cl_{EOX} m^{-2} a^{-1} (Figure 3 in Paper III; Figures 2 and 7 in paper I). In the period 30 years to 70 years ago (depth of 7 to 10 cm), these deposition rates decreased to 1/3 as compared to rates 70 to 100 years ago. The high accumulation rates of dry matter, organic matter and organic halogen for 70 to 100 years ago as compared to those for 30 to 70 years ago indicate high amount of runoff into lake Pyylampi from the catchment area. This may have been the result of a fire, which destroyed large area of forest around the lake Pyylampi at the end of 19th century (S. Siitonen, personal communication).

From 30 years ago to the year of the sampling (1995) (0 to 7 cm) the accumulation rates of dry matter, organic matter and organic halogen in Lake Pyylampi increased from 80 g of d.m. m^{-2} a^{-1}, 38 g of OM m^{-2} a^{-1} and 4 mg of Cl_{EOX} m^{-2} a^{-1} to 100 g of d.m. m^{-2} a^{-1}, 60 g of OM m^{-2} a^{-1} and 7 of mg Cl_{EOX} m^{-2} a^{-1} in 1995 (Figure 7 in Paper I, Figure 3 in Paper III). The sediment organic matter content increased from 45% at the depth of 5 to 8 cm (formed in the 1960's) towards the top 0 to 2 cm of the sediment to 55% (1995) (Figure 4 in Paper I). Similar increases in the accumulation rates of dry matter, organic matter and organic halogen were observed in the same time period also in Lake Mustalampi sediment. In Lake Mustalampi the sediment concentration of organic matter increased from 70% at the depth of 7 cm (deposited in 1970's) to 80% at the top of the sediment. The changes cited above coincided with forest harvesting around the Lakes Mustalampi in 1960's and Pyylampi in 1975-1976. Forest ground around Lake Mustalampi was burned in 1965, and soil around Lake Pyylampi was ploughed in 1976-1977. Soils around Lakes Mustalampi and Pyylampi were drained in 1960's (J.-V. Hyytiäinen, personal communication). Clear cutting, forest burning, soil ploughing and drainage are known to increase the runoff from the drainage area (Wells, *et al.,* 1979; Seuna, 1982; Ahtiainen, 1992). This may explain the increased deposition rates of dry matter, organic matter and organic halogen into the receiving Lakes Mustalampi and Pyylampi.

In the oldest layers of Lake Pyylampi sediment, deposited more than 150 years ago and situated below 30 cm depth, the ratio of Cl_{EOX}:C ranged from 1:17,000 to 1:25,000 (Figure 9). Between 7 and 30 cm depth, the Cl_{EOX}:C ratio of the Lake Pyylampi sediment ranged between 1:4,600 to 1:6,700. Our result shows no effect of the forest fire around Lake Pyylampi for 100 years ago (approx. 17 cm depth) on the Cl_{EOX}:C ratio of the sediment. Organic halogens are known to be formed in a combustion process of organic matter (Clement *et al.*, 1985; Reinhardt and Ward, 1995) and in forest fires (Palmer, 1976; Thomas and Spiro, 1996). Forest burning may increase the pH of the soil by one or two pH units (Wells *et al.,* 1979; Fritze *et al.,* 1993; Korhola *et al.,* 1996). This increase of the soil pH may depress the haloperoxidase activity and intrinsic formation capacity of organohalogens in soil, both being largest at low pH (3.5 to 4) (Asplund, 1992).

The Cl_{EOX}:C ratio was between 1:14,700 and 1:10,500 (Figure 9a) in the 0 to 7 cm depth layers Lake Pyylampi sediment, deposited during the past 30 years. In Lake Mustalampi sediment, the Cl_{EOX}:C ratio was stable at 1:13,000...1:7,000 at the depths from 34 to 8 cm (deposited before 1965) (Figure 9b). In the sediment layers formed after 1965 (0 to 8 cm depth), the Cl_{EOX}:C ratio decreased in Lake Mustalampi to 1:19,000...1:13,000. The decrease of Cl_{EOX}:C ratio in the layers of 0 to 8 cm depth in Lakes Pyylampi and Mustalampi as compared to the older sediment layers indicates that the organic matter deposited recently contained less

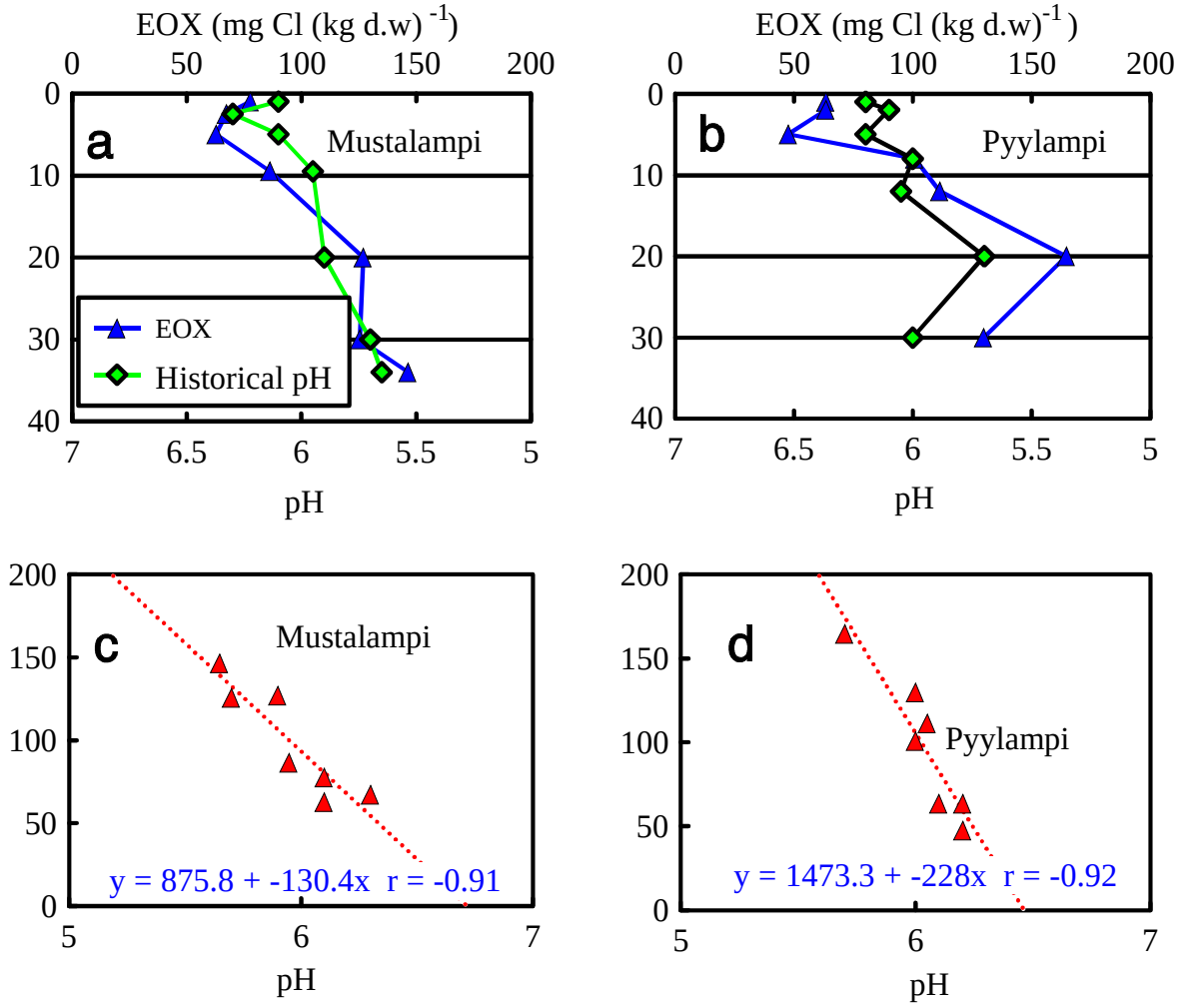

Figure 8. Paleolimnological assessment of water column pH in Lakes Mustalampi and Pyylampi. Historical pH of the water column and the sediment concentration of EOX in Lake Mustalampi **(a)** and Lake Pyylampi **(b)**. Sediment concentrations of EOX were assessed as organic halogen soluble in acidified tetrahydrofuran. The historical pH was deduced from diatom frustule diversity in dated sediment layers. Correlation between historical pH of water column and sediment concentrations of EOX in Lake Mustalampi **(c)** and Lake Pyylampi **(d)**. (Figure 8 a,b from Liukkonen *et al.* 1997, with the permission of the author).

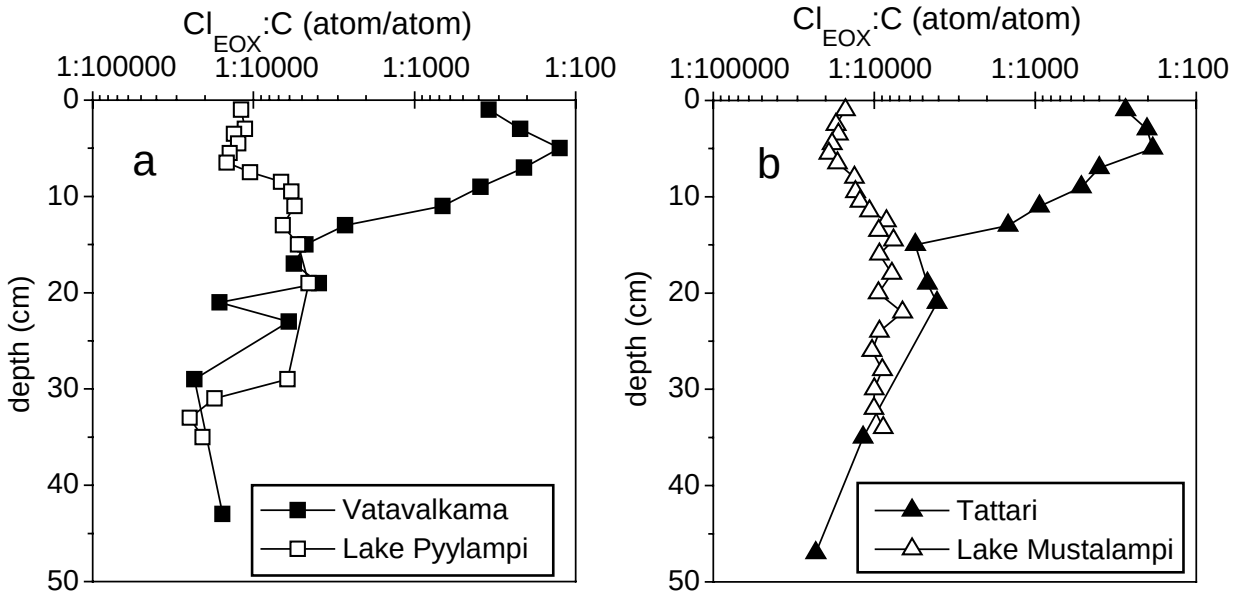

Figure 9a,b. Ratio of Cl_{EOX} to C (atom:atom) in sediments of Vatavalkama and Tattari basins and from forest lakes, Lakes Pyylampi and Mustalampi. The Vatavalkama and Tattari basins the oligotrophic Lake Saimaa were burdened by bleached pulping discharges from a large mill since 1954.

halogen than earlier. Our explanation is that the humic organic matter, which came from the drainage area after clear cutting and forest soil burning and ploughing, was less mature and possibly less attacked by haloperoxidases in the acid forest soil. Forest clear cutting may increase the forest soil pH (Zobel, 1993; Pietikäinen and Fritze, 1995; Smolander *et al.,* 1998), which is unfavourable to haloperoxidase activity in soil, and thus may reduce intrinsic capacity for organic halogen formation. This may be an additional explanation for the low Cl_{EOX}:C-ratio in the youngest sediment layers of Lakes Mustalampi and Pyylampi. A further explanation for the low concentration of organic halogen in the youngest sediment layers as compared to the older ones may be the enrichment of halogenated matter along with the biodegradation of the organic matter in the sediment.

Atmospheric deposition may be a source of organic halogen in the sediment. Wet precipitation of AOX in south eastern Finland ranged from 4.1 to 6.7 mg of Cl_{AOX} m^{-2} a^{-1} in 1980's and 1990's (Manninen, 1990; Jokela *et al.,* 1992; Piutunen and Silakoski, 1995). If all of this AOX would enter the lakes Mustalampi and Pyylampi as runoff and bind to the sediment, it would explain the quantities of organic halogen accumulated in the sediments, which were in 1989 - 1990 7 to 10 mg of Cl_{EOX} m^{-2} a^{-1} (Figure 7 in paper I), or 21 to 24 mg of Cl_{BOX} m^{-2} a^{-1}. The actual proportion of the atmospheric deposition of organic halogen in the forest lake sediments is difficult to estimate, because there are no historical data on the deposition of organic halogen.

In conclusion, the sediment accumulation and its organic halogen content reflect closely the quality of the water column, the quality and quantity of runoff from the drainage area and the changes in the drainage area. We consider soil organic matter as the main source of sediment organic halogen in Lakes Mustalampi and Pyylampi.

4.1.4 Sediment organic halogen in two pulp mill recipient basins of Lake Saimaa: Vatavalkama and Tattari

The kraft pulp mill in the drainage area of Vatavalkama and Tattari basins of Lake Saimaa started operation in 1935, and bleaching with elemental chlorine started in 1954. Sediment layers formed since 1950's

(above 15 cm depth) contained large amounts of EOX (Figure 6). The accumulation rates of organic halogen in these layers followed the rates of AOX discharges from the pulp mill (Figure 5 in paper III). Peak values of EOX, 23,500 mg of Cl_{EOX} (kg C)$^{-1}$ in Vatavalkama (3 km from the mill) and 15,900 mg of Cl_{EOX} (kg C)$^{-1}$ in Tattari (5 km from the mill) were found at the sediment depth of 4 to 6 cm, dating from the mid 1980's. The peak concentrations of BOX can be estimated as 78,000 mg of Cl_{BOX} (kg C)$^{-1}$ in Vatavalkama and 53,000 mg of Cl_{BOX} (kg C)$^{-1}$ in Tattari. The concentrations of organic halogen in the most polluted layers of the pulp mill recipient sediment were 90 to 190 times higher than those in the contemporary layers of the forest lakes Pyylampi and Mustalampi sediments (Figure 5) or in historic sediment layers (>100 years old, below 20 cm depth in Figure 6) of the Lake Saimaa. Our results thus show that organic halogens from the pulp mill accumulated into the sediments of the recipient lake Saimaa.

Maatela *et al.* (1990) recorded an increase in sediment content of organic halogen towards the more recent layers in Lake Kuhnamo, a recipient area of bleaching pulp mills in Äänekoski area in central Finland. The highest recorded value, 64,000 mg of Cl_{TOX} (kg C)$^{-1}$, was 1 km downstream from a bleaching pulp mill in the sediment layers deposited in 1980's. This was calculated by us from the data of Maatela *et al.* (1990) assuming that organic matter in the sediment contained 40% of carbon. In 1995, Saski *et al.* (1997) measured at the same site in Lake Kuhnamo a sediment concentration of 12,000 mg of Cl_{EOX} (kg C)$^{-1}$ using acidified tetrahydrofuran as solvent. Using the conversion factor EOX = 0.3 × BOX, Saski's (1997) result translates to 40,000 mg of Cl_{BOX} (kg C)$^{-1}$. Kankaanpää *et al.* (1997) observed a sediment concentration of EOX of $\leq$ 1340 mg Cl_{EOX} (kg C)$^{-1}$ in the recipient areas of pulp mills in southern Lake Saimaa and in Gulf of Finland, using cyclohexane-isopropanol (4:1 v/v) as a solvent. This procedure dissolved 2.3 to 9.4 (mean 5.6 %) of the AOX in the sediment (Kankaanpää and Tissari 1994b). Thus the EOX data by Kankaanpää *et al.* (1997) would translate into sediment content of AOX of 15,000 to 58,000 mg of Cl_{AOX} (kg C)$^{-1}$.

In conclusion, our data from south-eastern Lake Saimaa and those reported by other authors for other recipient areas show that sediment organic halogen in the recipient waters in a distance between 1 to 7 km from the mills, are relatively similar.

In the most polluted 4 to 6 cm layer of Lake Saimaa Vatavalkama sediment, the ratio of Cl_{EOX}:C was 1:120 (Figure 9a). This translates to Cl_{BOX}:C, (atom/atom) ratio of 1:36. The atom/atom ratio of Cl_{AOX}:C in organic matter in untreated and biologically purified waste water from Finnish bleached kraft pulp mills was reported as 1:(20...80) and 1:(15...60), respectively (Jokela *et al.*, 1993; Jokela, 1997). Summarising our results and those of other authors (Maatela *et al.*, 1990; Kankaanpää and Tissari, 1994b; Kankaanpää *et al.*, 1997; Saski *et al.*, 1997) show that pulp mill recipient sediment organic matter can be chlorinated to the same high degree as that found in the organic matter of waste water from a bleached kraft pulp mill.

4.1.5 Sulphur in Lake Saimaa and forest lake sediments

Kraft pulping process involves a digestion of wood chips in sulphurous soda lime at ca. +180 °C. A considerable portion of wood components converts into organic sulphur compounds during the digestion. We therefore analysed the sediment contents of sulphur in a recipient (Vatavalkama) and non- recipient sediment (Lake Pyylampi). Sulphur was analysed from freeze-dried sediments by combustion of the whole sediment.

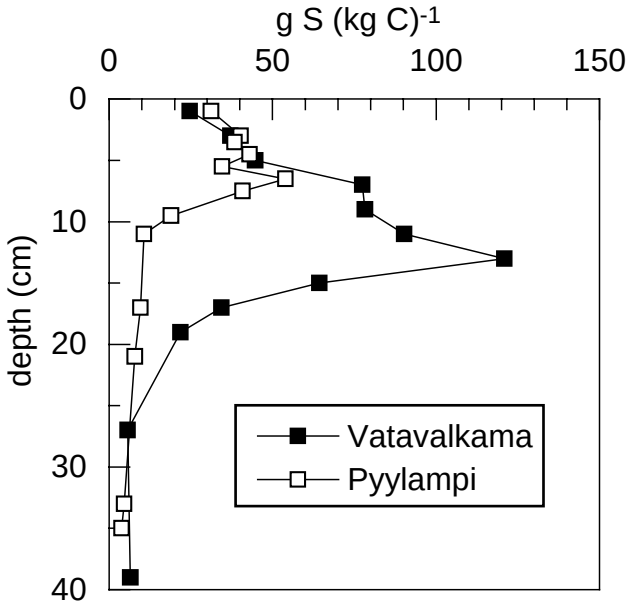

Figure 10. Sediment concentrations of total sulphur in Vatavalkama (Lake Saimaa) and Lake Pyylampi.

Figure 10 displays the concentrations of total sulphur in the sediments of Vatavalkama basin and Lake Pyylampi. The concentrations were low, below 10 g S (kg C)$^{-1}$, in the layers below 20 cm depth (> 100 years old). The low concentration of sulphur indicates that organic sulphur may have become mineralised in low redox environments (Pyylampi <-100 mV at 10 cm depth, Vatavalkama -390 mV at 20 cm depth, Figure 3a in Paper IV) and become reduced to H_2S. It may have evaporated during freeze drying of the sediment, prior to sulphur measurement.

Our results show that sulphur accumulated in pulp mill recipient sediment to concentration 2 to 5 fold (80 to 120 g S (kg C)$^{-1}$) as compared to non-recipient sediment (30 to 50 g S (kg C)$^{-1}$. Saski *et al.* (1997) showed that there was about 3 fold accumulation of tetrahydrofuran extractable organic sulphur (EOS) in the Lake Kuhnamo sediment downlake of the pulp mill as compared to sediments uplake of the mill. The accumulation tendency of kraft pulping sulphur compounds thus was very much lower than that of organic halogen, of which the concentration in bleached kraft pulp mill recipient sediment was up to 190 fold higher than in a non-recipient sediment.

Sediment concentrations of sulphur at the top 0 to 2 cm were similar in Vatavalkama and Lake Pyylampi, 20 to 30 g S (kg C)$^{-1}$, and similar to that in living cells (Madigan *et al*., 1997). This suggest that sulphur in the top 0 to 2 cm layers of Vatavalkama and Lake Pyylampi was of biomass origin.

The depth profiles of sediment sulphur of the Vatavalkama and Lake Pyylampi sediments were of similar shape (Figure 10). This may indicate that even the amount of sulphur was higher in the Vatavalkama as compared to Lake Pyylampi sediment, the redox dependent sequences of sulphur transformations were similar in both sediments.

4.2 Ecotoxicological and biogeochemical analysis of the sediment from Lake Saimaa and the forest lake

4.2.1 General background

We used microbial toxicity indicators to study the ecotoxicological quality of lake sediment generated under the influence of pulp mill discharges. Toxicity towards bacteria was measured using the inhibition of luminescence of *Vibrio fischeri* (ISO, 1998). Genotoxicity of the sediment was assessed by SOS-chromotest using *E. coli* PQ37 as a test strain (Quillardet and Hofnung, 1985). These tests were selected because they use bacteria

as indicator organisms, and because *Vibrio fischeri* and *E. coli* are widely used in toxicological assessments. In addition, we quantitated direct damage to sediment biomass by measuring the ATP content of the sediment. ATP was selected because it is a direct indicator of living cells, widely used, and large amount of data is available to compare results.

We selected six indicator activities to assess the biogeochemical functioning of Vatavalkama and Lake Pyylampi sediments. The selected activities were: three extracellular enzyme activities (ß-glucosidase, phosphatase and butyrate-esterase), endogenous aerobic mineralisation (carbon dioxide production), endogenous anaerobic mineralisation (methane production) and methane oxidation.

4.2.2 Microbial toxicity tests and ATP content of lake sediments

The most polluted (4 to 6 cm depth) layer of Vatavalkama sediment gave an EC_{50} value of 1% (v/v) of the sediment slurry for light production by *Vibrio fischeri* (Figure 2b in paper IV). Lake Pyylampi sediment layer from the same depth had an EC_{50} of 4.5% (v/v). The high toxicity of Vatavalkama sediment as compared to Lake Pyylampi sediment may be due to the high concentration of organic halogen in (Figure 6a). Less than 10% inhibition of the light production was observed when *Vibrio fischeri* cells were exposed to 25% (v/v) interstitial water (permeate 0.45 µm) prepared from the toxic 4 to 6 cm depth layer of the Vatavalkama sediment. The absence of toxicity in the permeate as compared to the total sediment indicates that the toxic components sorbed to sediment particles.

Sediment genotoxicity was assessed from the ratios of induced ß-galactosidase activity in relation to phosphatase (I) of *E. coli* PQ37. The results indicated close to significant genotoxicity in the most polluted 4 to 6 cm layer of the Vatavalkama sediment, with ß-galactosidase induction ratio I_c/I_0 = 1.3 (Figure 2c in paper IV).

The ATP content of the most polluted 4 to 6 cm layer in Vatavalkama sediment was 0.03 nmol of ATP $(g\ C)^{-1}$, *i.e.* 1/1,000 of that in the same depth of the Lake Pyylampi sediment (20 nmol of ATP $(g\ C)^{-1}$ (Figure 2a in paper IV). The low level of ATP shows that microbial life had been seriously damaged in the Vatavalkama sediment layers of 4 to 6 cm depth. The content of organic halogen was highest in this layer, 90 to 190 fold higher than in the contemporary sediment layers of Lake Pyylampi or in Vatavalkama deep layers (below 20 cm depth, > 100 years old).

Our results show that the three toxicity methods gave useful information for evaluating the ecotoxicity of pulp mill discharges on the recipient lake sediment. It is interesting that the ATP content of the sediment was more severely affected in Vatavalkama sediment layers at the depth of 4 to 6 cm, than was expectable from the outcome of the exogenous toxicity indicators *Vibrio fischeri* or *E. coli* PQ37 (Figure 2 in Paper IV). Since 97% of organic halogen of the 4 to 6 cm layer of Vatavalkama was associated with particles > 5 µm (paper IV) the pollutants may have poisoned bacteria inhabiting the sediment, while exogenously introduced test bacteria *Vibrio fischeri* or *E. coli* were in contact with sediment interstitial water only. The organic halogen of pulp mill polluted sediment has been shown to be lipophilic (log K_{ow} >4) (Saski *et al.*, 1997, Saski 1998), explaining efficient sorption to sediment (Lyman, 1990). The hydrophobic organic halogen in the sediments may thus have been available for the microbes habitating the sediment particles but less so to the exogenously introduced bacteria, *Vibrio fischeri* and *E. coli.*
We conclude that strong sorption of the toxicants to sediment particles protected the exogenous indicator bacteria towards the toxicity and explains the underestimation of sediment toxicity by *Vibrio fischeri* and *E.*

coli. This finding corresponds the observations by Vanhala and Ahtiainen, (1994), who showed that in soil ATP was a more sensitive indicator assessing toxicity of heavy metals than *Vibrio fischeri* bioluminescence test. Heida and van der Oost, (1996) and Pedersen *et al.* (1998) observed that sediment content of heavy metals, PAHs and PCBs did not always correlate with the toxicity measured with the *Vibrio fischeri* and recommended to use toxicity test in combination with chemical analyses.

4.2.3 Diatoms as indicator of pulp mill discharges

The sediment strata of Vatavalkama were analysed microscopically for the diatom frustules. It was found that *Asterionella formosa* increased from a frequency of <0.5% to 24-26% and that of *Aulacoseira subarctica* (in the paper IV identified as *Aulacoseira alpigena)* decreased from 18-19% to 2% in the layers of highest sediment concentration of EOX (23,500 mg Cl_{EOX} $(kg\ C)^{-1}$) at the depth of 4 to 6 cm as compared to the layers below 25 cm depth (Figure 4 in Paper IV). The prevalence of *Asterionella formosa* decreased and that of *Aulacoseira subarctica* recovered above the 4 to 6 cm layer, *i.e.* after the discontinuation of elemental chlorine in the bleaching process in the pulp mill (years 1987 to 1991) and the introduction of full scale biological purification plant of the mill's total effluents in 1993. No other changes in the waste water quality (suspended solids, COD_{Cr}, BOD_7, tot P, tot N) coincided with the changes in the abundance of these diatom species (Table 1 in Paper IV). The successions of a known oligotrophy indicator *Cyclotella rossii* (in the paper IV identified as *Cyclotella kützingiana*) and an eutrophy indicator *Aulacoseira islandica* also showed temporal changes, but these changes coincided rather with the changes of sediment concentration of nitrogen and carbon than that of organic halogen (Figure 3b and 4 in paper IV).

Diatoms are sensors for the water quality at the time of deposition. Also other authors have studied the response of diatoms to industrial discharges. For instance, Eloranta (1972) suggested a connection between sulphite pulp mill discharges and the diatoms diversity in Lake Kuorevesi, Central Finland. Slepukhina *et al.* (1996) studied the diatom succession in Lake Ladoga, in the recipient area of a non bleaching pulp mill Russia. Müller (1997) suggested a connection between organohalogens, heavy metals and the diatoms in Lake Constance, Germany.

We conclude that *Asterionella formosa* and *Aulacoseira subarctica* may have value as indicators of industrial effluents or organic halogen discharges.

4.2.4 Biogeochemical functioning of pulp mill recipient and non-recipient sediments

4.2.4.1 Extracellular hydrolytic enzymes in the sediment

We measured the activities of ß-glucosidase, phosphatase and butyrate-esterase in different layers of Vatavalkama and Lake Pyylampi sediments. Highest activities were found near the sediment surface (0 to 3 cm depth) (Figure 3 in Paper II). The activity of ß-glucosidase was 30% lower at the top 0 to 3 cm layer of Vatavalkama (0.17 µmol of substrate $(g\ C)^{-1}\ h^{-1}$) than in 0 to 2 cm layer of Lake Pyylampi (0.24 µmol $(g\ C)^{-1}\ h^{-1}$) sediment (Figure 3 in Paper II). In deeper layers, below 3 cm depth, the activity of ß-glucosidase was higher in Vatavalkama (0.09 to 0.11 µmol $(g\ C)^{-1}\ h^{-1}$) than in the Lake Pyylampi (0.02 to 0.06 µmol $(g\ C)^{-1}\ h^{-1}$) sediment, possibly because there may have been more substrate (cellulose) in Vatavalkama than in Lake Pyylampi sediment. The pulp mill may have discharged cellulosic fiber containing waste water to Vatavalkama before the purification plant was built in 1993.

Activities of phosphatase (*i.e.* phospho-monoesterase) in the top 0 to 3 cm layer of

Vatavalkama and 0 to 2 cm layer of Lake Pyylampi sediments were 18 and 9 µmol $(g\ C)^{-1}\ h^{-1}$, respectively. Between 3 to 12 cm depth, the activity of phosphatase ranged between 10 and 15 µmol $(g\ C)^{-1}\ h^{-1}$ in Vatavalkama and between 1 and 2 µmol $(g\ C)^{-1}\ h^{-1}$ in Lake Pyylampi sediment. The activity of phosphatase was higher in Vatavalkama than in Pyylampi sediment, maybe in response to phosphorus limitation in Vatavalkama sediment (Chróst, 1991). Liukkonen (1999) showed, that Vatavalkama sediment content of phosphorus indeed was low (< 2 g of P $(kg\ d.w.)^{-1}$) at 0 to 18 cm depth. There was a gap in phosphatase activity at the depth of 3 to 6 cm of Vatavalkama sediment with a minimum of 10 µmol $(g\ C)^{-1}\ h^{-1}$. This may indicate toxicity because this layer also contained the highest concentration of organohalogens in Vatavalkama sediment.

Activity of butyrate-esterase in the top layer (0 to 3 cm) of Vatavalkama sediment (18 µmol $(g\ C)^{-1}\ h^{-1}$) was almost similar to that in Lake Pyylampi (0 to 2 cm) (20 µmol $(g\ C)^{-1}\ h^{-1}$ sediment. The activities expectedly declined towards depth in both lakes, but there was transient depression of butyrate-esterase in the most polluted 3 to 6 cm depth layer of Vatavalkama sediment (Figure 3 in Paper II). It was similar to the depression observed for phosphatase activity. Similar or more severe depressions were also observed for chitinase, sulphatase and aminopeptidase activities in these same sediments, as shown elsewhere (Wittmann *et al.*, 1999). We support the view of Wittmann *et al.* (1999) that extracellular enzymes may be useful in documenting disturbance of sediment microbial ecosystem by industrial effluents. These depressions in enzyme activities were mild and not 100 to 1,000 fold as were the accumulations of organic halogen and the damages to ATP. Thus the metabolic activity was not totally blocked even in the most polluted layers of pulp mill recipient sediment.

Phosphatase (phosphomonoesterase) activity has been shown to correlate with the living biomass in aquatic ecosystem (Overbeck, 1991; Sinsabaugh, 1991). In our study, the sediment concentration of ATP (Figure 2a in Paper IV) did not follow the phosphatase activity (Figure 3 in Paper II) of the sediment. Münster and de Haan (1998) showed that activity of phosphomonoesterase did not correlate with the bacterial numbers (acriflavine direct counts-AFDC) in humic lakes in southern Finland.

4.2.4.2 Sediment carbon cycling

Rates of carbon dioxide formation, anaerobic mineralisation (methane production rate) and of methane oxidation were selected as tools for describing rates of organic carbon transformation. These reactions represent the final steps in mineralisation of organic carbon. The rates of mineralisation and methane oxidation were analysed in the laboratory at +7 °C, close to *in situ* temperature.

4.2.4.2.1 Mineralisation of endogenous organic matter

Aerobic mineralisation of Vatavalkama and of Pyylampi sediments was assessed by measuring the endogenous carbon dioxide (CO_2) production. Rate of carbon dioxide production in the top layer of Vatavalkama sediment (0 to 3 cm) was 300 µmol of $CO_2\ (g\ C)^{-1}\ d^{-1}$ and that in Lake Pyylampi (0 to 2 cm) 670 µmol of $CO_2\ (g\ C)^{-1}\ d^{-1}$ (Figure 4 in paper II). The lower carbon dioxide production in Vatavalkama may reflect toxicity, or poor biodegradability of the sediment contained organic material. Carbon dioxide production rates were similar (70 to 180 µmol of $CO_2\ (g\ C)^{-1}d^{-1}$) in 2 to 12 cm layers of Vatavalkama and Lake Pyylampi sediments. Despite the high amount of organic halogen in Vatavalkama (23,500 mg of $Cl_{EOX}\ (kg\ C)^{-1}$), high toxicity towards *Vibrio fischeri* (EC_{50} = 1% (v/v)) and low

amount of ATP (0.03 nmol of ATP (g C)$^{-1}$), the aerobic mineralisation of endogenous organic matter was only moderately inhibited in Vatavalkama sediment as compared to Lake Pyylampi sediment.

Anaerobic mineralisation of sediment carbon (methane production) occurred at the rate of 4 µmol of CH_4 (g C)$^{-1}$ d^{-1} in the top 1 cm of Vatavalkama and 14 µmol of CH_4 (g C)$^{-1}$ d^{-1} in Lake Pyylampi (Figure 11a). Different methane production rates in the two lakes may indicate toxicity or different amounts of fermentable organic matter in the two sediments.

Endogenous methane production in Vatavalkama and in Lake Pyylampi sediments decreased towards deeper layers. The endogenous methane production rates of Vatavalkama and Pyylampi sediments were similarly low between 5 and 40 cm depth, <1 µmol of CH_4 (g C)$^{-1}$ d^{-1}. The low endogenous methane production may indicate decreasing amount of fermentable organic matter towards sediment depth (below 5 cm depth).

Especially in fresh water environment, H_2 is considered as a more important electron donor in methanogenesis than methyl groups or acetate (Conrad, 1996; Madigan *et al.*, 1997). We measured methane production in N_2 atmosphere, with no exogenous H_2 added. The measured values may therefore express methane production relying on endogenously formed H_2. Our results may be biased (*i.e.* too low) if the endogenous H_2 was lost from the sediment strata when prepared for the laboratory measurement, for instance during the evacuation and refilling the vials with N_2.

4.2.4.2.2 Methane oxidation potential in sediment strata

Methane oxidation potential was measured in 20 ml head space bottles containing 2 g of the sediment slurry and methane in the atmosphere (1,500 ppm (v/v)). Incubation was carried out at +7 °C, close to *in situ* temperature. Consumption of methane was followed by gas chromatograph equipped with a flame ionisation detector for 10 days.

Methane oxidation potential was observed at all measured depths, 0 to 40 cm depth of Lake Pyylampi sediment, but only in the top 0 to 7 cm sediment layers of Vatavalkama sediment (Figure 11b). Methane oxidation potential was low, <0.1 µmol CH_4 (g C)$^{-1}$ d^{-1}, at the depth of 15 cm and 40 cm in Vatavalkama sediment (Figure 11b). In the Lake Pyylampi sediment the same depth was more active: 15 to 27 µmol CH_4 (g C)$^{-1}$ d^{-1}. Methane oxidation started after a lag phase shorter than one day. Iversen (1996) observed a rapid consumption of methane without a lag phase, when anaerobic sediments were exposed to oxygen and methane. This and our results suggest, that aerobic methane oxidisers survived and retained their aerobic methane oxidation potential in anaerobic sediments maybe for centuries. Vatavalkama sediment appeared less favourable niche for the obligatory aerobic methanotrophs than Lake Pyylampi sediment, possibly due to low redox potential in the Vatavalkama sediment (<-300 mV s.c.e. in Vatavalkama).

The lower methane oxidation potential at the 2 to 15 cm depth in Vatavalkama sediment (4 to 7 µmol CH_4 (g C)$^{-1}$ d^{-1}) as compared to that at the same depth in Lake Pyylampi sediment (23 to 36 µmol CH_4 (g C)$^{-1}$ d^{-1}) may reflect toxicity in the most pulp mill burdened layers of Vatavalkama sediment. In aerobic conditions, methane is believed to be oxidised exclusively by methanotrophic bacteria. Methanotrophic bacteria were reported to be sensitive to organic halogens (Andersson and McCarty, 1996). Methane oxidation potentials were similar in the top 0 to 1 cm depth layers of Vatavalkama and in Lake Pyylampi sediments, 130 µmol CH_4 (g C)$^{-1}$ d^{-1}. This shows that if the low activity at 2 to 10 cm was caused by toxicity, the sediment methanotrophic population was recovered. This is an important finding, since sediments are a vital link in the global recycling of

methane (Nedwell, 1996). Methane is an important greenhouse gas (Kelly, 1996).

4.2.4.2.3 Are Vatavalkama and Lake Pyylampi sediments net sources of methane?

The total endogenous production of methane in the sediment strata (0 to 200 cm) was 3 mmol of CH_4 m^{-2} d^{-1} in Vatavalkama and 6 mmol of CH_4 m^{-2} d^{-1} in Lake Pyylampi. The methane oxidation potentials of the topmost 1 cm sediment layer of Vatavalkama was 8 mmol CH_4 m^{-2} d^{-1} and that of Lake Pyylampi 10 mmol CH_4 m^{-2} d^{-1}. If oxygen is not a limiting factor, the methane oxidation potential in a <6 mm layer of the sediments will be enough to oxidise all methane formed in Vatavalkama and Lake Pyylampi sediments (0 to 200 cm depth). Additional methane oxidation may occur in the water column of a lake (Nedwell, 1996). Our results indicate, that Pyylampi and Vatavalkama sediments were unlikely net sources of methane into the atmosphere.

The known methanotrophic bacteria are strictly aerobic (Hanson and Hanson, 1996). Thus oxygen depletion may depress methane oxidation activity in the sediment and in the water column. The amount of oxygen required for the oxidation of the endogenously generated methane of Vatavalkama was calculated as 190 mg O_2 d^{-1} and of Lake Pyylampi as 360 mg of O_2 d^{-1}. From Figure 12a,b it can be seen, that the concentration of dissolved oxygen at the bottom-near layers (16 m depth) of Lake Vatavalkama ranged between 8 and 12 mg O_2 l^{-1} (80 to 100% saturation). In Lake Pyylampi, during the stagnation periods in winter and in summer, the concentration of dissolved oxygen in the bottom near layer (5 to 6 m dept) was less than 1 mg O_2 l^{-1} (< 10% saturation). Thus oxygen may become a limiting factor for methane oxidation in Lake Pyylampi sooner than in Vatavalkama.

Results shown here are indirect and not necessarily true reflection of the functioning of the lake bottom in Vatavalkama and Lake Pyylampi. Laboratory conditions (*e.g.* the availability of oxygen and substrate) are different from those *in situ* in the sediment. *In situ* studies are therefore needed to extrapolate the methane budget to lake sediments and to compare our results with those of other authors (*e.g.* Conrad, 1996; Hanson and Hanson, 1996; Murrell and Kelly, 1996; Lidstrom and Tabita, 1996).

4.2.5 Biodegradability of organic halogen in the sediment

In order to get an idea of the dehalogenation potential of lake sediments, we measured the dechlorination potential of pulp mill recipient and non recipient sediments using tetrachloromethane (CCl_4) as a model substrate. When the 4 to 6 cm depth layer of the Vatavalkama sediment was challenged with tetrachloromethane, over 90% of the of tetrachloromethane challenge (5 ppm, w/w) became removed in 120 hours under anaerobic, but only <1% under aerobic conditions (Figure 13a). About 5% of the consumed tetrachloromethane was recovered as trichloromethane indicating that reductive dechlorination had occurred (Figure 13b). Lake Pyylampi sediment consumed less than 1% of the 5 ppm (w/w) tetrachloromethane in 120 hours and no (<0.5%) trichloromethane formation was observed. Autoclaved sediments catalysed no tetrachloromethane consumption, nor trichloromethane formation, indicating that the dechlorination was microbially catalysed.

The catalytic potential of Vatavalkama thus resembled that observed for anaerobic sludge described for example by Doong *et al.* (1996 a,b) and van Eekert *et al.* (1998, 1999), or that observed in anaerobic sediments by Bradley and Chapelle (1999). Concentration of tetrachloromethane in granular sludges decreased by >95% during 4 to 16 days digestion (van Eekert *et al.*, 1998). Lower chlorinated methanes temporarily accumulated (van Eekert *et al.*, 1998).

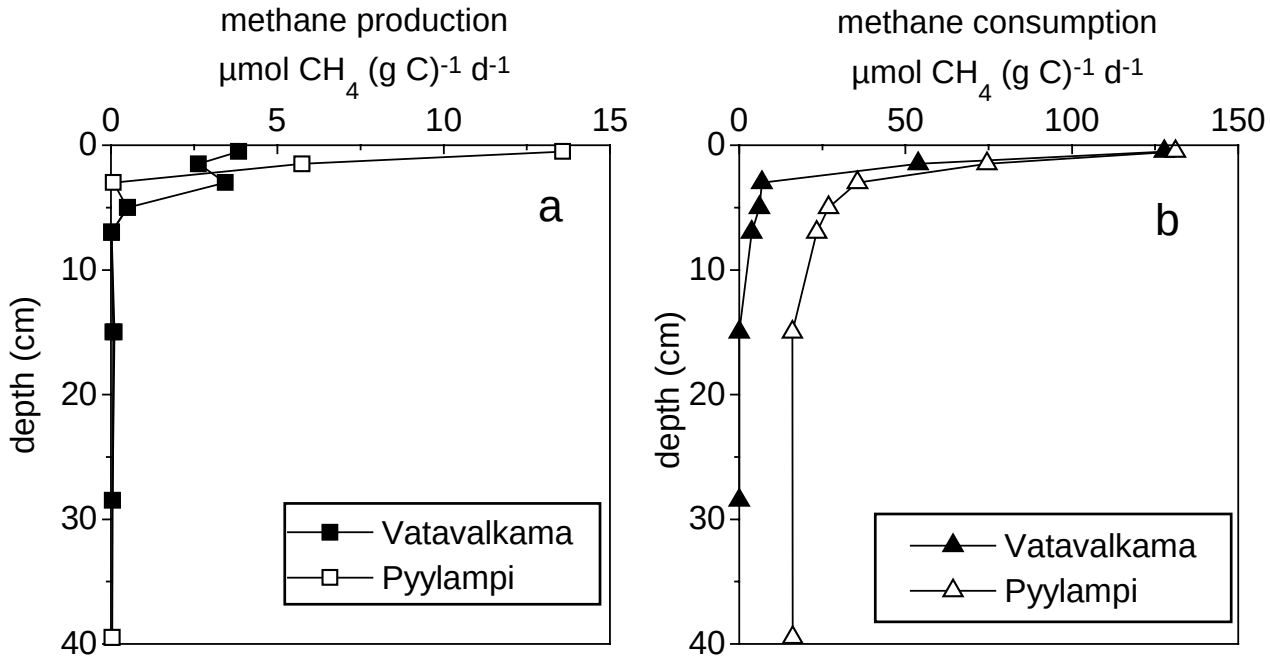

Figure 11. Vertical profiles of endogenous production of methane **(a)** and consumption of methane **(b)** in pulp mill recipient (Lake Saimaa, Vatavalkama basin) and forest lake (Lake Pyylampi) sediments. Data in Figure 11b from Wittmann *et al.,* (1999).

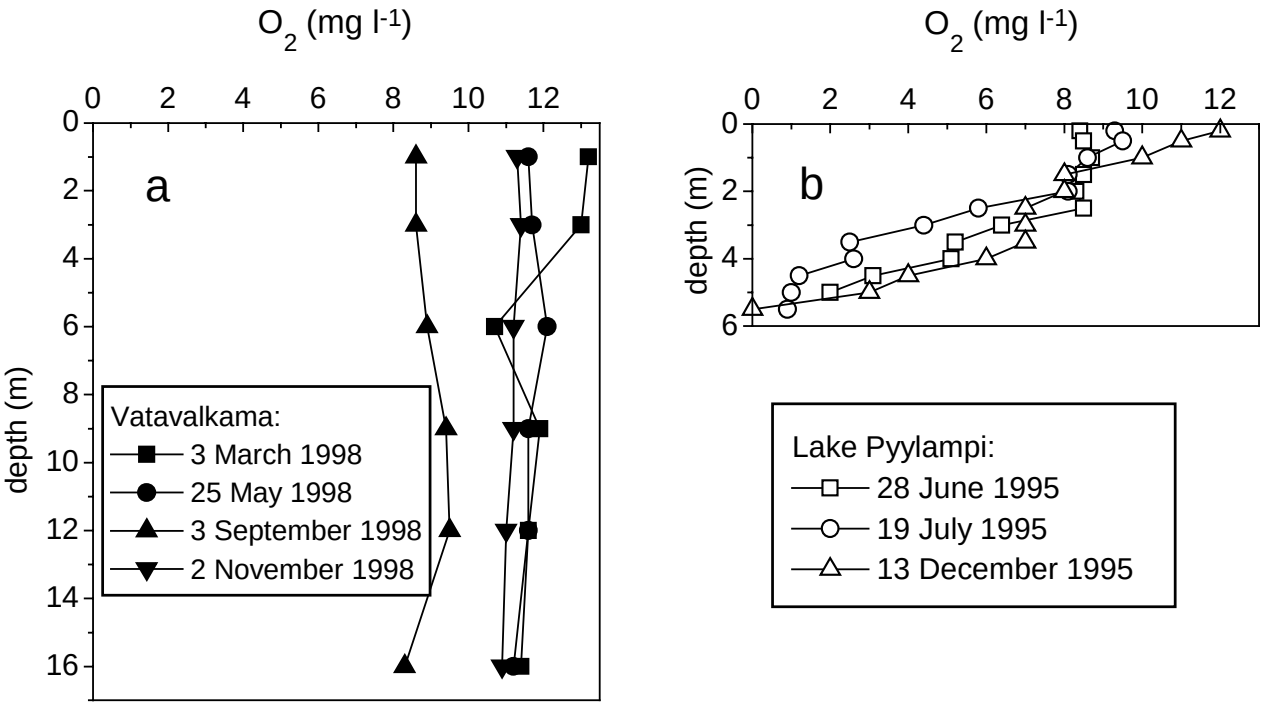

Figure 12. Vertical profiles of concentrations of dissolved oxygen in the water columns of a pulp mill recipient basin of Lake Saimaa (Vatavalkama) **(a)** and in a forest Lake Pyylampi **(b)**.

Bradley and Chapelle (1999) showed that the carbon from 1,2-trichloroethene and from vinyl chloride was ultimately incorporated into CH_4 by a methanogenic sediment, indicating that complete dechlorination may occur. The published literature shows that reductive dechlorination of chlorinated short chain hydrocarbons commonly occurs in anaerobic conditions.

It is an interesting question, why dechlorination occurred in Vatavalkama, but not in Lake Pyylampi sediment. The redox potentials in 4 to 6 cm layers of Vatavalkama (-200 mV s.c.e) and of Lake Pyylampi (-91 mV s.c.e.) sediments (Figure 3a in paper IV) should have been sufficiently low for reductive dechlorination to occur (Doong *et al.*, 1996b; Fetzner and Lingens, 1994). One reason for the difference in dechlorination activities of the two sediments may be the difference in availability of electron donors in the sediment. Organic matter in the sediment has an important role as a mediator (Curtis and Reinhard, 1994) or as a donor of electrons (Doong *et al.*, 1996a) for the reductive dehalogenation process. The dechlorination without a lag phase of tetrachloromethane in Vatavalkama sediment, but not in Pyylampi sediment may relate to the fact that Vatavalkama microbial community was adapted to a high concentration of organic halogen. Kong (1998) offered a similar explanation for the rapid dechlorination of 2-monochlorophenol and 3-monochlorophenol in sediments acclimated to monochlorophenol in contrast to an unacclimated sediment.

Our results showed that there was potential for natural attenuation of small halogenated molecules in the lake sediment receiving pulp mill waste water. Furthermore, calculations where pulp mill discharge data were compared with sediment accumulation data of organic halogens, showed that the half life of the organic halogens in the recipient sediment was 60 to 80 years (Figure 6 in Paper III).

4.3 Recovery of the chemical and biological quality of the recipient lake sediment after reduction of the mill discharges

4.3.1 Changes in the accumulation of the organic halogen after the reduction of the mill discharges

The pulp mill discharging into Vatavalkama and Tattari area of Lake Saimaa made large environmental investments since the late 1980's to reduce its environmental burden. Oxygen delignification was introduced and elemental chlorine in bleaching was stepwise substituted with chlorine dioxide and oxygen chemicals between 1987 and 1991. This together with the start of operation of a full scale biological purification plant in 1993 decreased the discharge of BOD_7 from 25,200 tons of O_2 a^{-1} (in 1986) to 2,100 tons of O_2 a^{-1} (in 1995), and that of COD_{Cr} from 55,000 tons of O_2 a^{-1} (in 1986) to 23,000 tons of O_2 a^{-1} (in 1995) (Figure 14a). The discharge of AOX from the mill decreased from approximately 2,200 tons of Cl_{AOX} a^{-1} in 1986 to 300 tons of Cl_{AOX} a^{-1} in 1995 and 150 tons of Cl_{AOX} a^{-1} in 1997 (Figure 5 in Paper III). Consequently, sediment accumulation rate of organic halogen in Vatavalkama, 3 km from the mill, decreased from 1350 mg Cl_{EOX} m^{-2} a^{-1} in mid 1980's to 390 mg of Cl_{EOX} m^{-2} a^{-1} in 1995 Figure 3 in Paper III). The sediment concentration of EOX in the top 1 cm layer of Vatavalkama was 7,000 mg of Cl_{EOX} (kg C)$^{-1}$, which is 30% of the maximum concentration in the layers formed mid 1980's (Figure 6).

It is interesting that discharge of AOX from 1986 to 1995 decreased by over 90%, while the sediment accumulation of EOX decreased simultaneously by 70%. The decrease in the sediment accumulation of EOX (70%) was similar to that of COD_{Cr} discharge (58%) and less than that of BOD_7 discharge (92%) in the same period (Figure 13a). In the study of Jokela *et al.* (1993), the biological purification plant removed 50% of AOX from the waste water from the mill. Biological purification normally removes 50% of the

COD_{Cr} of the waste water (Tchobanoglous and Burton, 1991). The ratio of BOD_7:COD_{Cr} (part of biodegradable oxygen demand in the total oxygen demand) in the waste waters discharged to Vatavalkama decreased from 0.46 in 1986 to 0.09 in 1995 (Figure 14b). Our results tell that the organic matter and AOX discharged in 1995 was more recalcitrant to biological degradation in lake water column than that discharged in 1986.

Jokela *et al.* (1993) showed that the ratio of EOX to AOX in the waste water decreased during anaerobic-aerobic treatment as compared to the untreated effluent. Jokela *et al.* (1993) concluded that the THF insoluble, but activated carbon adsorbable (AOX) fraction of the pulp mill organic halogen was the most recalcitrant to the degradation. It is likely, that AOX in biological treated waste water represents the water soluble organic halogens with molecular masses of 1,000±700 g mol^{-1}, with poor bioavailability. The discharged AOX had a remarkable tendency to accumulate into the sediment.

Palm and Lammi (1995) observed that sediment accumulation of AOX in Gulf of Bothnia in Pietarsaari area decreased from 2,700 mg of Cl_{AOX} m^{-2} a^{-1} in 1985 to about 2,000 mg of Cl_{AOX} m^{-2} a^{-1} in 1991, after cessation of chlorine gas bleaching and introducing a biological waste water treatment plant in the discharging mill. In their reference area, less than 100 mg of Cl_{AOX} accumulated m^{-2} a^{-1}. The ratio of EOX to AOX was higher (0.44 in 1982) in the pulp mill recipient sediment layers formed before the introduction of the purification plant as compared to the layers dating to the time after the treatment plant (0.08 in 1991). No similar change was observed in the non recipient area of the Gulf of Bothnia (Palm and Lammi, 1995). Assuming that EOX represented the bioavailable fraction and AOX mainly the recalcitrant fraction of the organic halogen in the sediment, the result of Palm and Lammi (1995) could be interpreted to mean that organic halogen discharged in 1990's was more recalcitrant than that discharged in the preceding decade. As a conclusion of our data and the data from the authors cited above, we state that the AOX discharged by a modern pulp mill equipped with a biological purification plant is different from that discharged in 1980's. The modern AOX discharges are more accumulable into sediment and less biodegradable than the discharges in 1980's.

4.3.2 Ecological recovery of the pulp mill recipient sediment ecosystem

In order to assess the ecological benefits from the environmental investments carried out in the mill discharging into Vatavalkama basin of Lake Saimaa, we analysed recipient lake sediment layers dated to have formed before (4 to 15 cm depth) or after (0 to 1 cm depth) the environmental investments. Biological and chemical parameters were measured.

The EC_{50} values for the inhibition of luminescence of *Vibrio fischeri* in the top 1 cm layer sediment were similar in Vatavalkama and Lake Pyylampi, 18% (v/v) and 35% (v/v), respectively (Figure 2b in Paper IV). This indicated no toxicity of industrial origin in the 0 to 1 cm layer of Vatavalkama sediment. The sediment concentration of ATP at the top 0 to 1 cm layer of the Vatavalkama sediment (39 nmol of ATP $(g\ C)^{-1}$) was 1,000 fold higher than in the most polluted 4 to 6 cm depth layer (0.03 nmol of ATP $(g\ C)^{-1}$) of the same sediment (Figure 2a in paper IV). The concentration of ATP in the top 0 to 1 cm layer of the Vatavalkama sediment was the same as in Lake Pyylampi sediment (47 nmol of ATP $(g\ C)^{-1}$). No genotoxicity was found by SOS chromotest by *E. coli* PQ37 in the top 0 to 1 cm layer of Vatavalkama sediment (I_C/I_0=1.1, Figure 2c in paper IV). Activity of butyrate-esterase showed the strongest decrease of the three studied activities (ß-glucosidase, phosphatase, butyrate esterase) at the most polluted 3 to 6 cm layer of the Vatavalkama sediment. This activity was almost similar in the top 0 to 3 cm layers of Vatavalkama

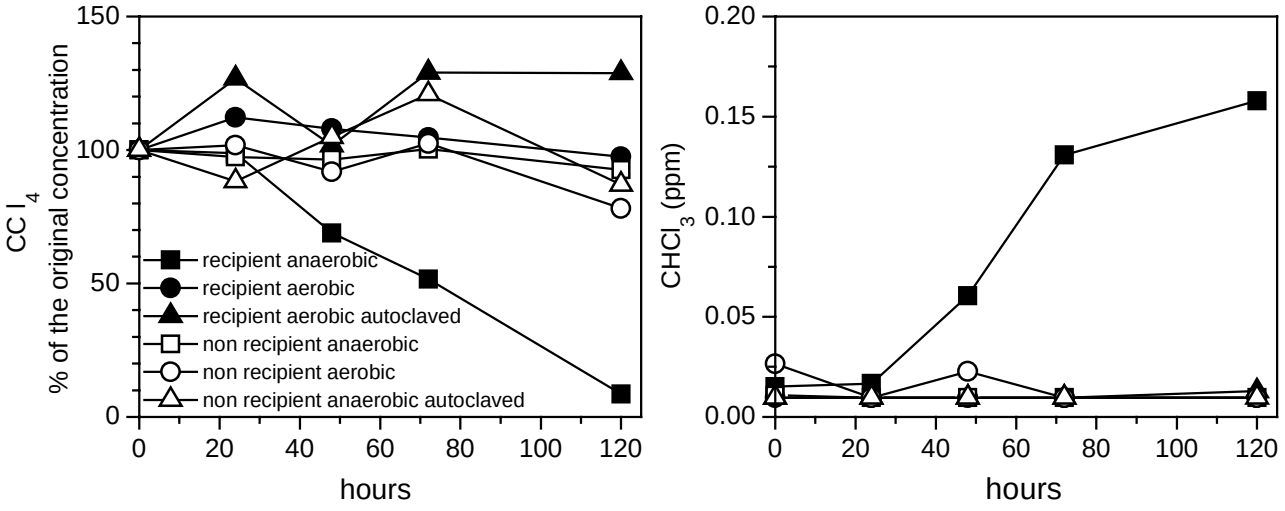

Figure 13. Potential dechlorination of tetrachloromethane by sediments from pulp mill recipient (Vatavalkama, Lake Saimaa) and a forest lake (Lake Pyylampi) at the depth of 4 to 6 cm. Consumption of tetrachloromethane **(a)** and accumulation of trichloromethane **(b)**. Initial sediment concentration of tetrachloromethane was 5 ppm (w/w) (Data from Wittmann *et al.*, 1999).

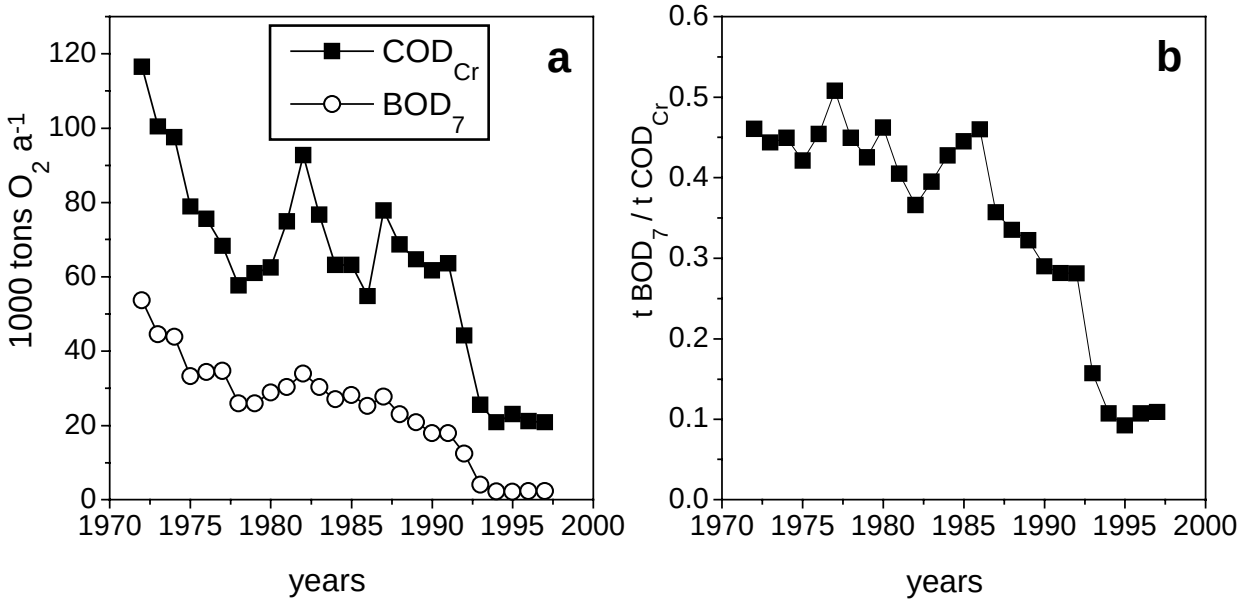

Figure 14. Burdening Vatavalkama and Tattari area of Lake Saimaa by organic discharges from Kaukopää pulp and paper mill. **a:** Discharge of BOD_7 and of COD_{Cr} from the Kaukopää pulp mill from 1972 to 1997. **b:** Ratio of BOD_7 to COD_{Cr} in the discharges from the Kaukopää pulp mill into the Lake Saimaa. Data from Water Protection Society of Lake Saimaa (Laine, P.,1999).

(18 µmol CH_4 $(g\ C)^{-1}$ h^{-1}) to that in Lake Pyylampi sediments (20 µmol $(g\ C)^{-1}$ h^{-1}) (Figure 3 in Paper III). As can be seen in the Figure 11, the methane oxidation potentials were similar (130 µmol CH_4 $(g\ C)^{-1}$ d^{-1}) in the top 1 cm of Vatavalkama and Lake Pyylampi sediments, suggesting a recovery of the methanotrophic bacterial population in the sediment. It is interesting that a full restoration of the sediment ecosystem had taken place in the 0 to 1 cm depth layer, although concentration of organic halogen in this layer was 30 times higher in Vatavalkama (7,000 mg Cl_{EOX} $(kg\ C)^{-1}$) than in corresponding layers Lake Pyylampi (250 mg of Cl_{EOX} $(kg\ C)^{-1}$) sediment (Figures 5 and 6).

As a conclusion, despite persistently high contents of EOX at the top of the Vatavalkama sediment halogen (30 fold as compared to Lakes Pyylampi 0 to 1 cm depth), our data showed full recovery in the sediment microbial activity after the discharging pulp mill had abandoned elemental chlorine in bleaching and introduced a full scale biological purification of its waster waters.

Many studies deal with the toxicity of untreated and secondary treated elemental chlorine free (ECF) and total chlorine free (TCF) kraft pulp mill effluents. Archibald *et al.* (1998) concluded that biologically purified (secondary treatment) pulp mill effluents containing recalcitrant halogenated organic matter was apparently virtually non toxic in the environment. Ahtiainen *et al.* (1996) showed that secondary treated effluents of a bleached kraft pulp mill did not inhibit light production of *Vibrio fischeri*. Saski *et al.* (1996b) observed that geochemical cycling, mineralisation of nutrients, photosynthesis and nitrification were not inhibited in the 2 m^3 outdoor mesocosm holding biologically purified bleached pulp mill waste water in concentrations up to 11% (v/v) when compared to a mesocosm with lake water only. Engwall *et al.* (1997) reported a time-dependent decrease in dioxinlike potency in the settling particulate matter collected near a bleaching pulp mill in Sweden, coinciding with the cessation of chlorine gas bleaching in the discharging pulp mill. Our data were the first to report on the microbial quality of the recipient sediment after the environmental improvements in the discharging mill.

5. SUMMARY AND CONCLUSIONS

5.1 Organic halogen in lake sediments: alien to life?

Two forest Lakes Mustalampi and Pyylampi carried 400 to 650 mg of Cl_{EOX} (kg C)$^{-1}$ in their sediments dating to before year 1900 (below 15 cm depth). The younger layers, deposited most recently ($\leq$ 30 years, 0 to 7 cm depth), contained less organic halogen, 150 to 260 mg of Cl_{EOX} (kg C)$^{-1}$. The older than 100 years (below 20 cm depth) layers of the oligotrophic Lake Saimaa sediment contained 130 to 490 mg Cl_{EOX} (kg C)$^{-1}$ (Vatavalkama and Tattari basins).

The Cl_{EOX}:C ratio (atom/atom) in the Lake Pyylampi sediment decreased from 1:6,700-1:4,600 at the depth of 8 to 30 cm to 1:14,700-1:10,500 at the depth of 0 to 7 cm. This change coincided with forest clear cutting and soil ploughing in the drainage area of Lake Pyylampi in 1960's to 1970's. Similar shift in the Cl_{EOX}:C ratio was observed also in Lake Mustalampi sediment.

Sediment accumulation of organic halogen in the forest lakes was analysed. It was found to correlate positively with sediment accumulation of organic matter for the past 30 years, suggesting that major part of the organic halogen in the sediment of these lakes originated from the organic runoff of the catchment area.

Historical pH of the water column was deduced from the diatom frustules. The sediment concentrations of EOX in the Lakes Mustalampi and Pyylampi correlated positively with water column acidity. This suggests that biological processes favoured by acidity, such as fungal haloperoxidase in a forest soil, may have been a source of organohalogens in these lakes. The decrease in Cl_{EOX}:C ratio in the sediment layers deposited after 1970's (0 to 7 cm depth) may indicate that the halogen content of the organic matter running into the lake from the drainage area was lower than earlier. Forest management, may have mobilised humus that was not fully attacked by haloperoxidases. The above mentioned decrease in Cl_{EOX}:C ratio may be due to the increase of forest soil pH typically occurring after clear cutting and forest floor burning, and a consequent decrease in haloperoxidase catalysed organic halogen formation on the forest floor.

We conclude that organic halogen in forest lake sediment most likely originated from the runoff from the soil ecosystem of the drainage area. Various factors (soil pH, forest cutting, soil treatment, draining, forest burning) in the drainage area influenced the quantity and quality of humus runoff and thus affected the organic halogen content of the accumulated sediment.

5.2 How pulp mill discharges affected the chemical quality of the recipient sediment?

A large pulp mill started chlorine bleaching of kraft pulp ashore of south-eastern of Lake Saimaa in 1954. Organic halogen was found in the sediments in highest densities at 4 to 6 cm depth, layers deposited in mid 1980's: 23,500 mg of Cl_{EOX} (kg C)$^{-1}$ at Vatavalkama, 3 km from the mill and 15,900 mg of Cl_{EOX} (kg C)$^{-1}$ at Tattari, 5 km from the mill. The Cl_{EOX}:C-ratios (atom/atom) in these layers were 1:120 and 1:190, respectively. Using a conversion factor (EOX=0.3×BOX), the ratio of bound organic halogen to carbon (Cl_{BOX}:C) in the sediment was estimated as 1:36 and 1:60 in Vatavalkama and Tattari, respectively. This is similar to that in the untreated bleached kraft pulp mill waste water.

We conclude that chlorinated organic matter accumulated in the pulp mill recipient sediment. This matter was chlorinated to the same degree as that in the discharged pulp mill waste water.

Sediment concentration of total sulphur was low, < 10 g S (kg C)$^{-1}$ in the deep layers (below 20 cm depth) of Vatavalkama and Lake Pyylampi sediments. Organic sulphur in these layers with low redox potential may have mineralised to H_2S and evaporated during the freeze-drying of the samples, prior to analysis. Vatavalkama sediment accumulated sulphur to concentrations of 3 to 5 fold (80 to 120 g S (kg C)$^{-1}$) as compared to those accumulated by Lake Pyylampi sediment (30 to 50 g S (kg C)$^{-1}$. Sediment concentrations of sulphur in the top 0 to 2 cm layers of Vatavalkama and of Pyylampi sediments were 20 to 30 g S (kg C)$^{-1}$, similar to that in living cells suggesting that sulphur was of biomass origin.

We conclude that sulphur mildly accumulated in the recipient sediment of a kraft pulp mill. In the sediment layers deposited most recently, at Vatavalkama as well as in Lake Pyylampi, sulphur was very likely of biological origin.

5.3 What do the ecotoxicological indicators tell about the polluted sediment?

The layer with the highest organic halogen pollution in Vatavalkama sediment (4 to 6 cm depth) was toxic to *Vibrio fischeri* (EC_{50} = 1%; v/v). It also was almost significantly genotoxic when assessed by SOS-chromotest by *E. coli* PQ37 (I_c /I_0 = 1.3). ATP had virtually disappeared from this layer: there was only 0.03 nmol of ATP (g C)$^{-1}$, which was 1/1,000 of that in the top 0 to 1 cm layer and 1/10 of that below (8 to 10 cm depth). ATP content was the most sensitive of the three microbiological indicators of sediment ecosystem disturbance. Exogenously introduced indicator bacteria, *Vibrio fischeri* and *E. coli* PQ37, experienced less toxicity than did the endogenous microflora, reflecting the fact that more than 90% of the microbial activity was attached to sediment particles (>5 µm), and 97% of the organic halogen was sorbed to the same size fraction of particles. The test bacteria, *Vibrio fischeri* and *E. coli* PQ37 cells were mainly in contact with the sediment interstitial water.

We conclude that ATP and both of the microbial toxicity tests (*Vibrio fischeri* and SOS-chromotest with *E. coli*) were useful in assessing sediment toxicity. *Vibrio fischeri* and *E. coli* may underestimate sediment toxicity.

5.4 Are diatoms indicators of pulp mill effluent ecotoxicity?

Asterionella formosa increased from an infrequent member (<0.5%) (below 30 cm depth) to a dominant (24 to 26%) species in the diatom community of Vatavalkama, in the period when the layers of highest sediment concentration of EOX were formed (4 to 6 cm depth). In the same layers, *Aulacoseira subarctica* decreased from 18 - 19% to 2%. The changes in diatom population coincided with the changes in discharge of AOX and sediment concentration of EOX, but not with any of the other measured changes in waste water quality of the discharging pulp mill (tot-N, tot- P, BOD_7, COD_{Cr}, suspended solids) or with the sediment concentration of carbon, nitrogen of phosphorus.

We conclude that *Asterionella formosa* may be a positive and *Aulacoseira subarctica* a negative indicator of industrial effluents or organic halogen discharges.

5.5 Biogeochemical functioning of the sediment: was the metabolic machinery affected?

The activity of ß-glucosidase (an indicator enzyme of cellulose degradation) was in the surface layer of Lake Pyylampi sediment (0 to 2 cm) 0.17 µmol of substrate (g C)$^{-1}$ h^{-1} and in Vatavalkama, Lake Saimaa sediment (0 to 3 cm) 0.24 µmol (g C)$^{-1}$ h^{-1}. Between the 2 and 12 cm depth, activity of ß-glucosidase

was higher (0.09 to 0.11 µmol (g C)$^{-1}$ h^{-1}) in Vatavalkama than in Lake Pyylampi (0.02 to 0.06 µmol (g C)$^{-1}$ h^{-1}) sediment, possibly due to higher amount of substrate (cellulose) in Vatavalkama. Phosphatase activity (an indicator enzyme of biogeochemical cycling of phosphorus) was higher at 0 to 12 cm layers of Vatavalkama (10 to 18 µmol (g C^{-1}) h^{-1}) than and Lake Pyylampi (1 to 9 µmol (g C)$^{-1}$ h^{-1}). High phosphatase activity in Vatavalkama may have been induced by phosphorus deficiency in Vatavalkama sediment (< 2 g P (kg d.w.)$^{-1}$). Sediment activitity of butyrate-esterase (a non-specific indicator enzyme of utilisation of esters other than fats) was 18 µmol (g C)$^{-1}$ h^{-1} in Vatavalkama surface layer (0 to 3 cm), and 20 µmol (g C)$^{-1}$ h^{-1} in Lake Pyylampi (0 to 2 cm). Between 3 and 12 cm depths of Vatavalkama and Lake Pyylampi sediments, the activity of butyrate-esterase ranged between 3 and 13 µmol (g C)$^{-1}$ h^{-1}. A transient depression of the activities of phosphatase and of butyrate-esterase was observed in the most polluted 4 to 6 cm layer of Vatavalkama sediment, possibly indicating toxic effect of high organic halogen pollution in this layer.

We conclude that the enzymatic indicators of biogeochemical cycling may be a tool in assessing ecological functioning of the sediment but there was no congruency in the responses of the three enzymes.

5.6 Is carbon cycling affected by organochlorine pollution?

Rates of aerobic mineralisation of endogenous organic carbon (CO_2 formation) were 300 and 670 µmol CO_2 (g C)$^{-1}$ d^{-1} in the top layer of Vatavalkama (0 to 3 cm) and of Lake Pyylampi (0 to 2 cm**)** sediments, respectively. The activities at the depth of 3 to 12 cm in both lakes were similar (70 to 180 µmol CO_2 (g C)$^{-1}$ d^{-1}).

Our results show that despite the high concentration of organic halogen, low amount of ATP, toxic towards *Vibrio fischeri* and slightly genotoxic towards *E. coli*, the aerobic mineralisation of endogenous organic carbon in Vatavalkama sediment was only moderately inhibited as compared to Lake Pyylampi sediment.

Anaerobic mineralisation of endogenous organic matter was measured as methane formation. Less methane was generated in the top 1 cm depth of Vatavalkama sediment (4 µmol CH_4 (g C)$^{-1}$ d^{-1}) than in the top 1 cm of Lake Pyylampi (14 µmol CH_4 (g C)$^{-1}$ d^{-1}) sediment. Between the depth of 7 and 30 cm, the endogenous methane formation was <1 µmol CH_4 (g C)$^{-1}$ d^{-1} both in Vatavalkama and Lake Pyylampi sediments. Low endogenous methane formation may be due to exhaustion of fermentable organic material or an analysis artefact, caused by the loss of H_2 during the sample preparation.

Sediment methane oxidation potential in the layers between 3 and 40 cm was lower in Vatavalkama (< 3 µmol CH_4 (g C)$^{-1}$ d^{-1}) than in Lake Pyylampi (15 to 27 µmol of CH_4 (g C) ^{1} d^{-1}) indicating that Vatavalkama was an unfavourable biotop for methanotrophs. This may be due to low redox potential (<-300 mV; s.c.e.), or to toxicity of Vatavalkama sediment. Methane oxidation potentials were similar (130 µmol of CH_4 (g C)$^{-1}$ d^{-1}) in the top 1 cm sediment of Vatavalkama and of Lake Pyylampi.

Our results indicate that despite the high amount of organic halogen in the top 1 cm depth layer of Vatavalkama sediment as compared to Lake Pyylampi sediment, methanotrophic potential showed full recovery of activity after discharges from the mill were reduced.

Methane oxidation potential of the top 1 cm (8 mmol CH_4 m^{-2} d^{-1} in Vatavalkama and 10 mmol of CH_4 m^{-2} d^{-1}) was large enough to oxidise all endogenously formed methane in the 0 to 200 cm layers underneath (3 mmol of CH_4 m^{-2} d^{-1} in Vatavalkama and 6 mmol of CH_4 m^{-2} d^{-1} in Lake Pyylampi).

Our results show that Vatavalkama and Lake Pyylampi sediments were unlikely a net source of methane into the atmosphere.

5.7 Is pulp mill organic halogen biodegradable in the recipient sediment?

When the 4 to 6 cm layer of Vatavalkama sediment was challenged with tetrachloromethane (5 ppm, w/w), 90% of the added tetrachloromethane became metabolised in 120 hours under anaerobic, but only <1% under aerobic conditions. About 5 % of the consumed tetrachloromethane transiently accumulated as trichloromethane indicating that the metabolic process was reductive dechlorination. Lake Pyylampi sediment consumed less than 1% (w/w) tetrachloromethane in 120 hours and no (<0.5% of the challenge) trichloromethane formation was observed. Autoclaved sediments catalysed no (<0.5%) tetrachloro-methane consumption nor trichloromethane formation, indicating that the dechlorination was microbially catalysed.

Our results show that the sediment receiving pulp mill waste water possessed potential for intrinsic remediation of small halogenated molecules. We calculated a half life of the pulp mill organic halogens accumulated into the sediment to be 60 to 80 years.

5.8 How did the decrease in the discharges of mill influence the sediment?

Changes in the accumulation and concentration of the organic halogen after the environmental investments in the mill.

Abandoning elemental chlorine in bleaching in 1991 and introducing new, biological waste water purification plant in 1993 decreased the mill AOX discharges from 2,200 tons of Cl_{AOX} a^{-1} (in 1986) to 300 tons of Cl_{AOX} a^{-1} (in 1995) and to 150 tons Cl_{AOX} a^{-1} (in 1997). Sediment accumulation of EOX in decreased from 1350 mg Cl_{EOX} m^{-2} a^{-1} in the mid 1980's to 390 mg Cl_{EOX} m^{-2} a^{-1} in 1995. Sediment concentration of organic halogen at the top 1 cm of Vatavalkama sediment was 7,000 mg of Cl_{EOX} $(kg\ C)^{-1}$. This was 30% of that in the most polluted 4 to 6 cm layer, formed in mid 1980's. The reason for the relatively low decrease (70%) in the sediment accumulation and concentration of EOX as compared to a larger decrease (90%) in the AOX discharge may be that biological purification plant took away the biodegradable fraction of the pulp mill organic halogen. Therefore the organic halogen discharged into the Lake Saimaa in mid 1990's was more recalcitrant than that discharged in the mid 1980's.

Ecological recovery of the ecology of the pulp mill recipient sediment

The EC_{50} value for light production of *Vibrio fischeri* was 18% in the top 1 cm layers of Vatavalkama and 35% (v/v) in the Lake Pyylampi, indicating that there was practically no toxicity of industrial origin in the surface of Vatavalkama sediment. Sediment concentration of ATP at the top 1 cm layer of Vatavalkama (39 nmol of ATP $(g\ C)^{-1}$) was close to that in Lake Pyylampi (47 nmol of ATP $(g\ C)^{-1}$). The top 1 cm of Vatavalkama sediment showed no significant genotoxicity by SOS-chromotest by *E. coli* PQ37 (I_c /I_0 = 1.1). Methane oxidation potentials were similar (130 µmol CH_4 $(g\ C)^{-1}$ d^{-1}) in the top 1 cm layer of Vatavalkama and Lake Pyylampi sediments.

Our results show that despite the relatively high (30 fold) concentration of organic halogen at the top 0 to 1 cm layer of Vatavalkama sediment as compared to the same layer in Lake Pyylampi sediment, a full recovery of microbial ecosystem had occurred in the Vatavalkama sediment.

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