ENVIRONMENTAL FATE and EFFECTS of PULP and PAPER MILL EFFLUENTS

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BIODEGRADABILITY OF DIFFERENT SIZE CLASSES OF BLEACHED KRAFT PULP MILL EFFLUENT ORGANIC HALOGENS DURING WASTEWATER TREATMENT AND IN LAKE ENVIRONMENTS

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Less than 5% of the organic halogen emissions from bleaching of pulp are molecules of known structures. The major part consists of mainly hydrophilic molecules of unknown structure, chlorohumus. This paper focuses on the impact of size of the halogenated molecules on the environmental fate. The tetrahydrofuran-soluble fraction of the wastewaters was studied because >90% of the wastewater contained organic halogen (AOX) dissolves in this solvent. We show that halogen emissions of modern kraft pulp mills range from 100 to 1000 g mol\(^{-1}\) in size. The molecular size had little limitation on biodegradation during secondary wastewater treatment. Thirty to 70% of the AOX was removed during wastewater treatment (full scale). Molecules of <500 g mol\(^{-1}\) were slightly more degraded than those >500. Fifty to 80% of the remaining halogens were biodegraded in the lake ecosystem, studied in 2 m\(^3\) outdoor mesocosms over four seasons. The molecular size distribution of the tetrahydrofuran-soluble fraction of wastewater showed little change in clear lake water mesocosms, although over 50% of the AOX was removed. Sedimentation explained only a minor fraction of AOX removal from the water column. The tetrahydrofuran-soluble halogenated material which accumulated in the sediments showed a molecular weight distribution completely different from that in the water column or in the wastewater, indicating extensive metabolism.

INTRODUCTION

The size and the polarity of xenobiotic molecules are assumed to be the decisive factors for predicting the biomobility and the effects on biota in the environment. This assumption explains why most published studies deal with the small molecular (<500 g mol\(^{-1}\)) fraction, although this fraction comprises less than 5% of the total bleached kraft pulp mill discharged organic halogens. The main part of the pulp mill halogen discharge consists of extremely heterogenous, mainly hydrophilic, molecules of unknown structure and assumed of high molecular weight, 10\(^3\) to 10\(^4\) g mol\(^{-1}\), often called chloroilignin. Chloroilignins are recognized as slowly biodegradable and considered humus-like in not being bioavailable, which would explain their recalcitrance.

Most pulp mills in Finland discharge into shallow inland waters or equally shallow Baltic bay estuaries. For this reason the mills have been required to build extensive secondary wastewater treatment. Since 1995 all mills have external secondary wastewater treatment. The data accumulating on the performance of biological secondary treatment in Finland and elsewhere show that treatment removes 30 to 60% of the mill effluent organic halogen (measured as AOX, active carbon adsorbable organic halogen), depending on the mill and the method of treatment (Jokela et al. 1993, also a review by Graves and Joyce 1994). Only a small part of the removal relies on conversion to small volatile molecules escaping AOX analyses (<1%) or adsorption to sludge and carry-away with excess sludge (<5%), indicating that the main part has truly become mineralized (Gergov et al. 1988; Jokela et al. 1993; Saski et al. 1995a). After being discharged into recipient waters, the organic halogen concentration (AOX) of the water phase decays faster than can be accounted for by dilution alone (Jokela et al. 1992).
The present paper deals with the environmental fate, with an emphasis on biodegradability, of bleached kraft pulp mill discharged organic halogens. The molecular size distribution of the halogenated compounds was studied during the biological purification of the wastewater and in the recipient lake (large enclosures placed in lakes) water column and sediment over four seasons.

EXPERIMENTAL

Molecular Weight Distribution Analysis

HPSEC. For molecular weight determination, nonaqueous high performance size exclusion chromatography (HPSEC) was used as described earlier (Jokela and Salkinoja-Salonen 1992). A comparative study and validation of the method used was performed with aqueous HPSEC, vapor pressure osmometry and ultrafiltration (Jokela and Salkinoja-Salonen 1992). Both synthetic lignin model compounds and commercial narrow molecular weight distribution polystyrenes (Pressure Chemicals, Pittsburgh, PA, U.S.A.), with average molecular weight of 498,000, 50,000, 17,500, 4000, 2200 and 800, were used. The lignin model compounds used for calibration contained 2, 3, 4 and 6 aromatic nuclei with 3C side chains, all of which were synthesized under the supervision of G. Brunow at the Dept. of Chemistry, University of Helsinki. The synthesis and structures of the 2- (320 g mol⁻¹) and 4-ring molecules (638 and 668 g mol⁻¹) were described by Jokela et al. (1985, 1987) and 6-ring (1080 g mol⁻¹) compound structures by Jokela (1993). The structures of the synthesized lignin models are shown Fig. 1. A total of 75 different chloride compounds, obtained from commercial sources, were also used for calibration (for a list of the compounds used, see Jokela and Salkinoja-Salonen 1992). The ratio of C:Cl of the compounds varied from 0.5:1 (tetrachloroethene) to 20:1 (monochlorodihydroxybetic acid) and molar weights ranged from 60 (acetic acid) to 369 (dichlorodihydroxybetic acid).

Sample Preparation

Water samples were freeze dried and residues dissolved in tetrahydrofuran containing ≤2% (v/v) of concentrated nitric acid in a bath sonicator. The solution was filtered through a 0.45 μm nylon filter and stored at -20°C under nitrogen gas until analyzed.

³⁵Cl-Chlorolignin

³⁵Cl-labeled bleaching effluent was a gift of Clas Wesen (University of Lund). Softwood kraft pulp (kappa 35, consistency 3%) was bleached with Cl₂ (70 kg tonne⁻¹), C-stage at room temperature, E-stage at 70°C and pH 12. The ³⁵Cl-labeled effluent was stored at -20°C in a polyethene bottle. Inorganic ³⁵Cl was removed using Sep-Pak C18 cartridges (Waters Associates, Milford, MA). The cartridges were conditioned with 10 mL of methanol, 10 mL of tetrahydrofuran and 20 mL of deionized water. Ten mL of the ³⁵Cl-labeled bleaching effluent was impregnated into the cartridge, then washed with 10 mL aqueous HCl (pH 1.2). The organic components were eluted from the cartridge with 5 mL of methanol. One mL of methanol was evaporated to dryness in a flow of N₂ and the residue dissolved in 100 μL of tetrahydrofuran.

Assay of Halogen

Halogen was assayed using microcoulometric assay, neutron activation analysis or scintillation counting (³⁵Cl). AOX (adsorbable organic halogen) was assayed microcoulometrically after adsorption onto active carbon (background <0.2 μg of Cl per 50 mg of C, from Euroglas, Delft, NL) according to the ISO 9562 protocol. HPSEC fractions were assayed after evaporating the solvent under nitrogen gas prior to inserting the sample into the oven of the microcoulometer. Some samples were parallel assayed by the neutron activation technique at the Technical Research Centre (VTT, Oulunen, Finland by P. Manninen). The carbon used for adsorption was ENC activated granular carbon (gran size 0.15-0.35 mm, S-bet 1020 m² g⁻¹). The samples were irradiated in a Triga Mark II reactor, usually for 300 s, in a neutron flux of 4
\(10^{12} \text{ n cm}^{-2} \text{ s}^{-1}\). Gamma rays were measured using a GeLi crystal detector (Ortec) and a multichannel analyzer (Canberra MCA 40) and a Rockwell AIM computer.

![Chemical structures](image)

**Figure 1. Synthetic lignin model compounds used for molecular weight calibration (HPSEC) along with the narrow molecular weight distribution polystyrenes and various halogenated compounds. Calibration was performed to fit both the lignin models and polystyrenes. Halogen compounds fitted reasonably well into the calibration curve that obtained when the C:F ratio was 5:1 or higher.**

**Elemental Analyses**

Organic carbon was measured (<0.18 mm particles) with a TOC-5000 total carbon analyzer (Shimadzu, Japan) according to ISO standard 8245. Calibration was done with sodium hydrogen phthalate (total carbon) and sodium hydrogen carbonate plus sodium carbonate (inorganic carbon) manufactured by Kanto Chemical Co. (3-chome Chuo-ku, Tokyo).

**Mesocosm Set-up**

Enclosures of 2 m³, made of polyethylene (translucent for daylight, black for darkened controls), were floating in the lake, aided by wood-styrofoam flotation frames. Wastewaters from two different mills, A and C, both bleaching hardwood and softwood, approx. 1:1, with elemental chlorine free (ECF) bleaching (Mill A: D-EO-D-E-D) or with oxygen delignification (Mill C: O-CD-EO-D-E-D in 1991 and O-D-EO-D-E-D in 1992), were mixed with lake water, 2%...13% (v/v, as indicated). Mill A wastewater contained 3.6 (1991) to 2.0 mg (1992) of total nitrogen, 0.9 to 0.3 mg of total phosphorus, 380 to 300 mg of total organic carbon (TOC) and 26 to 17 mg of Cl as organic halogen (AOX) per liter. Mill C wastewater contained 5.0 (1991) to 4.5 mg (1992) of total nitrogen, 1.0 to 0.9 mg of total phosphorus, 280 to 190 mg...
of TOC and 21 to 7 mg of Cl as AOX, respectively, per liter. Mill C discontinued the use of elementary chlorine after 1991 sampling and Mill A bleached ECF both years. Further details of the wastewaters are given by Sassi et al. (1996).

One lake was clear (color 60 mg Pt L^{-1}) and oligotrophic, and the other highly humic (color 380 mg Pt L^{-1}). The lake water concentrations of nitrogen, phosphorus and organic carbon were 1/5 to 1/10 (N), 1/30 to 1/100 (P) and 1/60 to 1/10 (C) of that contained by the wastewaters. More details are described elsewhere (Sassi et al. 1996).

RESULTS AND DISCUSSION

Molecular Size of Chlorinated Organic Molecules in Bleached Kraft Pulp Mill Effluents

The first thing that happens to wastewater leaving the pulp mill is dilution. At a wastewater treatment plant the bleaching wastewaters are diluted by other waste streams and sometimes cooling waters. In the receiving water, further dilution by a factor of 20 to 1000 occurs, depending on the recipient size and flow. It was found that while 70 to 80% of both the organic halogen (AOX) and carbon of undiluted wastewater (from which insolubles had been removed by G/FA filtration) was retained by the 1000 g mol^{-1} ultrafilter, the retained portion of the same wastewater decreased upon dilution in distilled water. Less than 50% was retained after 10-fold dilution, and after diluting 100-fold or more, the major part of the wastewater contained TOC and AOX permeated the 1000 g mol^{-1} filter (Jokela and Salkinoja-Salonen 1992). Since dilution with pure water may not be expected to cause chemical cleavage of covalent bonds, the phenomenon must be of a physical nature, i.e., dissolution of colloidal and/or micellar aggregates. One may therefore expect the wastewater contained organics behave like molecules of <1000 g mol^{-1} after entering the recipient water ecosystem.

Vapor pressure osmometry and different variants of HPSEC were used to analyze the molecular weight distribution organic compounds in wastewaters of six Finnish kraft pulp mills and one Swedish mill, with different pulping and bleaching protocols. The results showed that when measures were taken to counteract hydrophobic and ionic interactions between the solute molecules during analysis, the bleached kraft pulp mill organohalogens behaved as rather low molecular weight material, M_w (average number) varied from 200 to 300 g mol^{-1} and M_w (average weight) from 300 to 600 g mol^{-1} (Jokela and Salkinoja-Salonen 1992; Pellinen and Salkinoja-Salonen 1985a,b). In most wastewaters, 85 to 95% of the halogen was bound to molecules in the size range from 200 to 1000 g mol^{-1}.

The microcoulometric analyzer used as the chlorine detector of the HPLC chromatographic fractions in the above studies is halogen specific rather than chlorine specific. It also may give (although to our experience, rarely) false positive responses if the studied sample contains nitrocompounds. We used neutron activation analysis (chlorine specific) to calibrate for the mass balance (solvent extracts of organohalogens), but the sensitivity of this method is too low to allow for measuring the chlorine contents of chromatographic fractions. In this paper we describe confirmatory results obtained using labelled chlorine (36Cl) as the chlorine indicator. This is a direct chlorine-specific method. Kraft pulp was bleached with 36Cl_2, inorganic chloride removed and the molecular weight distribution of the radiochlorinated organic fraction was analyzed by the same HPLC column and eluent system as before. Figure 2 shows the result: also in this case the major part of the molecules were of sizes ranging from 200 to 2000 g mol^{-1}. We therefore conclude that the molecular size of solvent soluble chlorolignin is in this range.

Ultrafiltration technology has been applied to remove the major part of chlorolignins from wastewater at pilot scale for the purpose of purification (reviewed by Frostell et al. 1994). This operates in practice, but the large apparent molecular size in concentrated aqueous solution probably depends on intermolecular associations, may be similar in nature to the micelles formed by hydrophilic molecules with hydrophobic domains, e.g., (bio)surfactants. The surfactant nature of chlorolignins has in fact been indicated by studies where purified chlorolignin fractions ("HMM") were shown to inhibit sea urchin sperm acrosome reaction, an event prerequisite for successful fertilization (Higashi et al. 1992).
Figure 2. The distribution of $^{34}$Cl among the different sizes of molecules in the spent liquor bleached with $^{34}$Cl. The high performance size exclusion chromatogram shows the distribution of tetrahydrofuran-soluble halogens from softwood bleaching. The size calibration was done with synthetic lignin model compounds (Fig. 1) and narrow molecular weight range polystyrenes.

Impact of Molecular Weight on the AOX Degradability During Secondary Wastewater Treatment

The secondary wastewater treatment plants of bleached kraft pulp mills were operated to remove 90 to 97% of the BOD, but the concomitant removal of organic halogen (AOX) and carbon (TOC) was only 30 to 60% efficient (Gergov et al. 1988; Laine et al. 1991; Jokela et al. 1993). This was also true for the mills bleaching ECF (Mill A) and using oxygen delignification (Mill C) studied here (Jokela et al. 1993). This means that only a part of the AOX compounds contribute to BOD.

To answer the question whether molecular weight is of importance for biodegradation during wastewater treatment, we studied the molecular weight distributions of the organic bound halogens in the influent and the effluent of the secondary wastewater treatment units. Figure 3 shows examples of results from two mills, one of which had an oxygen delignification system and anaerobic-aerobic lagoon system for wastewater (Mill C), and the other conventional ECF bleaching with activated sludge treatment for wastewater (Mill A). Figure 3 shows that the removal of organic halogens (lower panel) was more efficient than removal of color (top panel). Figure 3 also shows that anaerobic-aerobic lagooning (AL) removed all size classes of the organic halogens and that the activated sludge plant (AS) removed efficiently up to 500 g mol$^{-1}$ and less efficiently beyond this size.

The patterns obtained for the halogen removal according to molecular size distribution in the treatment plants, sampled at different seasons, during pulping of hardwood or softwood, and minor changes of bleaching sequence, were surprisingly similar to the examples shown in Figs. 2 and 3 and to those observed for other types of mills and biological treatments (Jokela et al. 1993). It is interesting to observe that anaerobic biotreatment was not inferior to aerobic in dehalogenating the chlorolignins (larger molecules). Our early work showed that anaerobic pretreatment at pilot and mill scale purification dehalogenated organohalogen in bleached kraft pulp mill wastewater (Salkinoja-Salonen et al. 1981 1984). More recently, Yu and Welander (1994) showed that a laboratory scale anaerobic fixed-film process removed 50 to 60% of pulp mill wastewater organohalogens (measured as AOX) with a hydraulic
retention time of 6 to 15 h. Since the major part of the wastewater organohalogens is composed of “chlorolignins”, their finding indicates that much of the chlorolignins became anaerobically dehalogenated.

![Figure 3](image.png)

Figure 3. Changes of color (wide window absorbance, 225 to 445 nm, upper panel) and halogen content (lower panel) in different sizes of molecules in the wastewater of Mills A and C during wastewater purification. AL (Mill A) = anaerobic-aerobic lagooning; AS (Mill C) = activated sludge. Solid line = before treatment, broken lines = after treatment. Both mills (A and C) bleached ECF at the time of the observation, either after oxygen delignification (Mill C) or hemicellulose prebleaching (Mill A). Mill A had an activated sludge plant (HRT 12 h + 12 h), and Mill C an anaerobic (3d)-aerobic (7d) lagoon system. Both mills bleached alternatingly softwood and hardwood.

When the efficiencies of the different stages of wastewater treatment in halogen removal were compared over the molecular size distribution range, it was found that efficiency of the anaerobic lagoon remained constant or declined slightly with the molecular size from 100 to 2000 g mol⁻¹, while the tendency during aerobic treatment was the opposite. The efficiency of the activated sludge plant to remove halogens declined somewhat with an increasing molecular weight (Fig. 3, see also Laine et al. 1991; Jokela et al. 1993). It is interesting that approx. 50% of the total dehalogenation was already achieved during the equalization, prior to contact with biomass. This may reflect the hydrolysis of labile organohalogens (C-stage) upon pH neutralization observed in other studies (Smeds et al. 1994).

The overall carbon to chlorine ratio of the wastewater organic fraction increased from about 35:1 to 45:1 during anaerobic lagoonning, but dropped back to the original during subsequent aerobic lagoonning. Activated sludge treatment brought no significant change in the C to Cl ratio. Ninety percent of the pulp mill wastewater AOX was soluble in tetrahydrofuran before secondary treatment, but only about 60% of the AOX that resisted biotreatment was soluble to tetrahydrofuran or any other organic solvent we tried. In fact, it seemed that the concentration of tetrahydrofuran-insoluble AOX remained constant in the
wastewater during biological purification, i.e., either was not biodegraded or was both formed and degraded. The halogen content at this level (Cl: C <1:25) was less important for biodegradability than was the solubility in a hydrophilic solvent (tetrahydrofuran) (Lainé et al. 1991; Jokela et al. 1993). The chemical nature of the tetrahydrofuran-insoluble, nonbiodegradable part of the wastewater containing AOX is not known.

Biodegradability studies using chlorinated model compounds larger than two aromatic rings are rarely published in the literature and to our knowledge have not been used to test biological wastewater purification. Such compounds are not commercially available. Sandemann et al. (1990, 1993) linked chloroaniline covalently to glucosides and lignin model compounds, thereby creating molecules of 400 to 650 g mol⁻¹ and carbon to chlorine ratios similar to those in pulp mill chlorolignins. They found that the organohalogen parent compound was released in rat digestive tract and became bioavailable. They also found that linking of chloroaniline covalently to a synthetic lignin model improved its mineralization by a ligninolytic fungus, Phanerochaete chrysosporium. It may be that in some environments and for some microbes, a larger molecular size of the xenobiotic molecule may improve rather than hamper biodegradation.

Fate of Pulp Mill Organic Halogens in Finnish Fresh Water Recipient

Organic halogens become slowly removed from the water column after the wastewater is released into the lake ecosystem. We studied die-away of AOX in a Vuoksi river basin waterway, hosting 6 bleached kraft pulp mills over a distance of 350 km. The wastewater concentrations of AOX of the mills concerned ranged from 20,000 to 80,000 μg L⁻¹, enabling the following of the wastewater plume over a large dilution. The natural background of AOX in these waters ranges from 10 (clear, oligotrophic) to 30 μg Cl L⁻¹ (humic waters). Parallel halogen assays of the elevated AOX contents showed that the higher AOX figure found microcoulometrically in humic waters could not always be observed by neutron activation analysis and may therefore represent some compound other than an organohalogen. No such discrepancy was observed with any of the pulp mill wastewater containing samples.

In a study in 1989 we found that when the dilution was accounted for, the AOX was halved over a distance (D₁₀) of 47 km (Jokela et al. 1992). We repeated the study in 1990 and found a D₁₀ of 41 km (Fig. 4). In order to get more information on this environmental degradation, mass balance and the changes of molecular weights involved, we used mesocosm enclosures holding a mixture of pulp mill wastewater and lake water (Saski et al. 1991). We observed the biodegradation of bleached kraft pulp mill wastewater organic fraction in the water column and the de novo formation of sediment in 22 separate mesocosm enclosures, incubated for four seasons in two different lakes, one clear, oligotrophic, and low in color and organic matter (60 mg Pt L⁻¹), and the other rich in humic matter and dissolved carbon (color 380 mg Pt L⁻¹). The mesocosms were not seeded with external sediment or any additive other than wastewater and lake water. Some of the mesocosm enclosures were kept out of daylight (dark) to be able to assess the impact of photochemistry.

Fate of Different Sizes of Organohalogens Studied in Fresh Water Recipient Mesocosms

The mesocosmic enclosures reflected a real lake environment in many respects: the extended incubation periods allowed for observing true seasonal variation including natural cycles of changes in light intensity, temperature, oxygen, and pH and allowing for growth of periphyton on the enclosure walls (mimicking natural biofilm surfaces on stones and shorelines) and sediment production.
Figure 4. The calculated die-away of AOX discharged from 6 kraft pulp mills into the river basin Vuoksi in 1990. AOX data were collected for 12 months at the sampling points indicated in the figure. The discharge of AOX of each mill was known (as monthly averages). Correction was made for the background AOX contents of the recipient.

A clear stratification into winter and summer epi- and hypolimnion developed in the mesocosms where humic lake water was used as the diluent. The mesocosms with clear lake water diluent did not stratify; neither did the the lake where the water was taken from. Figure 5 shows the vertical gradients of temperature (5A) and oxygen (5B) in the mesocosms during one experiment over 500 d. Also, pH gradients were established in the mesocosms, vertical and temporal. The pH (not included in Fig. 5) varied from 6 (bottom of humic enclosures) up to 9...10.5 (springtime top layer of the epilimnion). The high epilimnion pH may promote alkaline dehalogenation of chlorolignins, as has been observed in wastewater treatment studies (Strehler and Welander 1994). The mesocosm enclosures mimicked a real receiving water except for water flow and plankton composition. The plankton were different in each mesocosm, so that neither phytoplankton nor zooplankton was quantitatively similar in species composition in any of the mesocosms, not even in the duplicate parallels (Salkinoja-Salonen et al. 1993).

Figure 6 shows examples of the time course of AOX concentration in some mesocosm enclosures started in June 1991. It shows that the organic halogen concentration (AOX) declined in all mesocosms, relatively rapidly in the summer, stagnating for winter and resuming the decline in the second summer. By the end of the second summer the total decline (corrected for storm water dilution on the basis of inorganic chloride concentration and conductivity) ranged from 50 to 80%. The half-life of AOX in the mesocosms thus was less than 500 d, as were those mesocosms that were kept dark. A detailed analysis of the mineralization kinetics of organic halogen and carbon in these mesocosms, and a similar set of mesocosms started in Nov. 1992, is presented elsewhere (Saski et al. 1995).

Figure 7 shows the accumulation of the organic halogens of pulp mill wastewater origin in the mesocosms into the sediments formed de novo during one year. Mass balance calculation of the sediment contribution to the organic halogen budget showed that only a minor fraction, 0.2 to 10%, of the AOX removed from the water column during the year accumulated into the sediment. Therefore, the disappearance of AOX from the water column (Fig. 6) was likely to be due to true biodegradation. These calculations are described in detail elsewhere (Saski et al. 1995, 1996).
Figure 5. The vertical and temporal gradients of temperature (A) and dissolved oxygen (B) in the mesocosms in a clear water and humic water lake. The mesocosms shown in the figure were filled on Nov 5th. The data collected for mesocosms containing 2 to 13% of wastewater of Mill A or Mill C in lake water were close to those shown above. Similar results were obtained for mesocosms filled in June (data not shown).

Several interesting observations can be made from Fig. 7. Mill C wastewater induced more halogen accumulation into the mesocosms than did Mill A wastewater, although Mill C wastewater contained less AOX. It may be correlated to the larger amount of sediment formed in Mill C mesocosms, probably because Mill C wastewater contained more nutrients (N and P). When the wastewater was introduced into the mesocosm enclosures in November ("winter to winter") and incubated over one year, more sediment bound organic halogen accumulated than happened when the same operation was started in June ("summer to summer") and also incubated over one year. Moreover, the tetrahydrofuran-soluble fraction of the "winter to winter" sediment was larger than that of "summer to summer" sediment. Therefore, the long-term behavior of the organic halogen discharge from the mill seems to be affected by the season of discharge (for data and further discussion, see Sasaki et al. 1996).

We made tetrahydrofuran extracts of the water column and the sediment in order to study the changes in molecular weight distribution during the one-year incubation in the mesocosms. We used tetrahydrofuran extraction rather than cyclohexane or other less polar solvents to analyze sediments, because tetrahydrofuran dissolves 5 to ca. 100% (mean 34%, n = 29, see Fig. 7) instead of the 2 to 5% usually found to dissolve in nonpolar solvents used in other studies (Martinsen et al. 1988, 1994).

Figures 8A and B show examples of molecular size distributions of tetrahydrofuran-extractable organic halogens in the wastewaters of Mills A and C after incubation for one year in clear water lake. They show that the integrated peak area indicating the tetrahydrofuran-soluble halogens diminished about 50% (Mill A) or 20 to 30% (Mill C) in 393 d in the water column, but the molecular weight distributions remained essentially similar. The UV profile also showed no major change.

Some but usually not the major part of the AOX disappeared from the mesocosm water column during the year (Fig. 6) and accumulated in the sediment (Fig. 7). The molecular size distributions of tetrahydrofuran-soluble halogens extracted from the sediment formed de novo are shown in the lower panels of Fig. 8. To facilitate comparisons, the molecular size distribution of compounds of the wastewater origin is drawn in the same panels.
Figure 6. The time course of the AOX contents of the water column of some lake water containing enclosures with 0 to 11% (v/v) wastewater from Mill A or Mill C. The incubation was started June 1991. Some enclosures were built from black polythene and covered with the same material to keep out daylight. Open symbols are daylight-exposed mesocosms; black symbols are mesocosms kept in the dark.

A striking difference is seen between the molecular sizes of halogenated compounds in the water column and those in the sediment of the same mesocosm: the average sizes of the halogenated molecules in the sediment were higher by almost an order of magnitude, with maxima appearing around and over 1000 g mol⁻¹ (Fig. 8A, B). Some tetrahydrofuran-soluble halogenated material also sedimented in the 0% wastewater containing mesocosms, but the amount was negligible compared to those where wastewater was added (Fig. 7). Therefore the large molecular size halogen profiles (lower panels of Figs. 8A, B) of the sediment must have originated from the wastewater introduced 290 d before.

To elucidate the role of daylight on the molecular weight changes of organohalogens in the mesocosms, parallel mesocosms were studied, one open to daylight (Fig. 9, lower panel), and the other kept dark (Fig. 9, top panel). Figure 9 shows that large-sized halogenated molecules had accumulated in the sediment in 290 d, both in the light and in the dark.

The accumulation of large-sized halogenated molecules in the sediment was observed in all the 7 different mesocosms, both humic and clear lakes (Saski et al. 1996). At this moment we can only speculate on the mechanisms behind this change. The change was much larger than the slight shift towards higher molecular weight during activated sludge treatment of bleached kraft mill wastewater, while no shift was observed during aerobic-anoxic treatment of the wastewater (Fig. 3, also Jokela et al. 1993).
It is possible that the chlorolignins may have become degraded into smaller molecules, then entered the intermediary metabolism of the mesocosm biota (Neilson 1994), and become incorporated into cellular macromolecules such as lipids and other tetrahydrofuran solubles, but also insolubles (Fig. 7).

Figure 7. De novo produced sediment and sediment bound halogen in mesocosm enclosures filled with lake water and biologically treated bleached Kraft mill wastewater. The mesocosms were filled with lake water (clear or humic) to which wastewaters of Mill A or Mill C were added to the percentage indicated in the figure. Fifteen enclosures were filled in June 1991, and another fifteen in November 1992. Some were covered to keep out daylight. All mesocosms were incubated floating in the lake for one year before the sediment was collected and analyzed. The black bars (lower panel) indicate sediment total dry weight, grey bars indicate the sediment-bound halogen (BX) and the white bars indicate the sediment contained tetrahydrofuran-soluble (EOX) halogen (upper panel).

We are not aware of other studies of the molecular weight of sediment-bound organohalogen, but in our laboratory a similar phenomenon, increase of molecular weight, was observed in microcosms of soil polluted with chlorobenzenes from wood preservation. Organic halogens occurred at old polluted sites (>10 yrs) as large molecules (similar in size to halogens in bleached pulp mill wastewater), especially if the soil was humic (high in organic matter). Earthworms exposed to such soils resorbed the smaller and the larger halogenated matter and converted it into even larger molecules, ranging from 10^6 to 10^9 g mol^-1 (Laine et al. 1995). These data, and those presented in this paper, support the hypothesis that the biota may indeed extensively incorporate halogenated organic compounds into cellular materials from which they may further migrate into other forms of life, as molecules very different from the original structure.
Figure 8. Changes in molecular weight distributions of the UV-absorbing materials and the organic halogens of wastewater (BKME) from two mills during weathering in daylight-exposed lake water mesocosms over four seasons. The vertical axis refers to tetrahydrofuran-soluble halogens calculated per m³ of the input wastewater. The mesocosm water columns were sampled on day 0 and on day 393 (top panels). The mesocosm sediments formed de novo in either the absence (lake water mesocosm) or in the presence of wastewater (holding ca. 10% BKME) were collected on day 290 (lower panels). The mesocosms with Mill A wastewater (Fig. 8A); and Mill C wastewater (Fig. 8B) were simultaneously incubated in the same lake.
Figure 9. Daylight dependence of the weathering effect on molecular weight of bleached kraft mill wastewater halogens. Mill C wastewater was incubated in lake water holding mesocosms over four seasons exposed to (lower panel) or covered from (top panel) the daylight. The vertical axis refers to tetrahydrofuran-soluble halogens calculated per m² of the input wastewater. The mesocosm water columns were sampled on day 0 and on day 393. The mesocosm sediments formed de novo in the absence (lake water mesocosm) or in the presence of wastewater (holding ca. 10% BKME) were collected on day 290. All mesocosms were simultaneously incubated in the same lake. Size exclusion chromatograms of both UV-absorbing and the halogen-containing materials are shown.
CONCLUSIONS

In the present paper the molecular size distribution and solvent extractability (polarity) of chlororolignins were determined at various stages of exposure to treatment and water ecosystems, with the aim of increasing the understanding of the factors determining the ecological fate. We studied the fate of kraft mill-generated AOX in the mill secondary treatment and in outdoor mesocosms where the mill water was mixed with lake water and incubated under realistic conditions.

Molecular size does not limit bioremovability during biological wastewater treatment. The chlororolignins very likely behave in the water ecosystem as relatively small molecules from 200 to 1000 g mol⁻¹.

After discharge to recipient water, the kraft mill organic halogens, were removed from the water column: die-away distances of 40 to 50 km, averaged over one year in the Vuoksi river basin in southeast of Finland.

In the wastewater containing mesocosms (2 m³, placed in situ outdoor), sediments with a high content of bound halogens were accumulated. The sediment accumulated <10 % of the wastewater organohalogenes in one year, while 50 to 80% of the wastewater organic halogenes were removed from the water column. Accordingly, the sediment was not the main organohalogen sink; most likely the organic halogenes mainly became mineralized.

The molecular weight distribution of kraft mill organic halogenes remaining in the water column underwent no major change during weathering in mesocosms, in clear or humic water, over four seasons. Organohalogenes accumulated in the sediment showed a higher molecular size than those present in the water column. The molecular weight was significantly higher than in the wastewater from which the organic halogenes originated, indicating extensive metabolism by the biota in the mesocosm.

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