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Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River

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Abstract. In sedimentation areas of polluted rivers, microbial dechlorination of chlorinated aromatics may be of great environmental significance. This reaction may take place in the deeper, anaerobic sediment layers and involves replacement of a chlorine in the pollutant molecule by hydrogen. In this study, the microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River is evaluated by using Rhine water pollution data, concentrations in historical sediment samples and in recent sediment cores, and the results of anaerobic laboratory incubations with Lake Ketelmeer sediment. The various data support the conclusion that microbial dechlorination of hexachlorobenzene has occurred in the anaerobic sediment. Up to 80% of the hexachlorobenzene deposited in the early 1970s has been dechlorinated. The maximum half-life of hexachlorobenzene in the sediment is found to be 7 years.

Two limitations of microbially mediated dechlorination in the natural environment have become clear. In the first place, a residual concentration of about 40 μg/kg remains unaltered in the sediment or transformation rates of this fraction are at least extremely low. Secondly, the lower chlorinated benzenes that are produced from hexachlorobenzene appear to accumulate in the anaerobic sediment.

Introduction

The Rhine River and its tributaries drain a large, industrialized area in Europe (Fig. 1). The Rhine River is heavily polluted with metals (Van der Weijden & Middelburg 1989) and organic micropollutants including chlorinated benzenes (CBs) (Meijers & Van der Leer 1976). Hexachlorobenzene (HCB) was formerly in widespread use as a fungicide in agriculture and many CBs are still applied in industry and domestic products. Among the various direct and indirect sources of CBs, processes in an aluminum foundry have been identified as sources of undeliberate HCB pollution in the Rhine River (Vogelgesang et al. 1986). CBs have been found in sewage sludges (Vogelgesang et al. 1986), sediments (Kypke-Hutter et al. 1986), river water (Kühn & Brauch 1988), and fish (Kypke-Hutter et al. 1986) from the Rhine River area. Due to their hydrophobic character, CBs accumulate in sediments and biota and seem to be rather persistent in the aquatic environment, although abiotic and biotic transformations of CBs have been shown in laboratory tests. Transformations of CBs may occur in the aerobic water column as well as in the sediment.

Photochemical transformations of CBs have been demonstrated (Choudhry & Hutzinger 1984; Hirsch & Hutzinger 1989); nevertheless the overall contribution of photolysis to the transformation of CBs in the water column will probably not be very significant as a result of the limited light transmission in Rhine River water.

Under aerobic conditions, biomineralization has been shown for monochlorobenzene (Pfaender & Bartholomew 1982; Reineke & Knackmuss 1984), all dichlorobenzenes (Van der Meer et al. 1987; Schraa et al. 1986; Spain & Nishino 1987; De Bont et al. 1986; Haigler et al. 1988), 1,2,3-trichlorobenzene (Marinucci & Bartha 1979), 1,2,4-trichlorobenzene (Van der Meer et al. 1987; Pfaender & Bartholomew 1982) and 1,2,4,5-tetrachlorobenzene (Sander et al. 1991). Most of these studies were performed with organisms originating from aquatic environments, in either undefined mixed populations or pure cultures obtained by selective enrichment. The 1,3,5-trichlorobenzene and other highly chlorinated benzenes, except 1,2,4,5-tetrachlorobenzene, seem to resist aerobic biomineralization.

Since the majority of hexa-, penta- and tetrachlorobenzenes probably remains unaltered in the aerobic river water, their concentrations in water are mainly influenced by volatilization, downstream dilution and particle associated sedimentation. In the Rhine River delta, high deposition of the CBs associated with the settling particles will occur as a result of decreasing streaming velocities. Consequently, increased concentrations of the higher chlorinated benzenes can be expected in sediments of sedimentation areas. In addition, the lower chlorinated benzenes may also be found in sedimentation areas if aerobic biodegradation processes and volatilization fail to eliminate these compounds from the water column.

Under anaerobic conditions, microbial transformations have been shown for all CBs in laboratory tests, except for mono- and 1,2,3,4-tetrachlorobenzene (Tiedje et al. 1987; Fathepure et al. 1988; Bosma et al. 1988). The anaerobic microbial transformation is a reductive dechlorination reaction. In this reaction a chlorine is replaced by a hydrogen, producing lower chlorinated benzenes. Hexachlorobenzene is mainly dechlorinated to 1,3,5-trichlorobenzene in sewage sludge (Tiedje et al.

1987; Fathepure et al. 1988). In river sediments, tri- and dichlorobenzenes are dechlorinated to monochlorobenzene (Bosma et al. 1988).

Although the origins of the anaerobic microbial consortia suggest that the results of these laboratory studies can easily be applied to the natural aquatic environment, there are some aspects that complicate the translation to field conditions: (1) long adaptation times, up to several months, are observed in laboratory tests; (2) the use of selected populations; (3) optimized incubation conditions: high temperatures, high nutrient concentrations; (4) high concentrations of the artificially added CBs. These aspects may stimulate the development of microbial populations that do not resemble the composition and capabilities of the naturally occurring populations.

Long term persistence of CBs for more than several decades has been shown in aquifers (Barber 1988) and in lake sediments (Oliver 1987). Based on CB concentration profiles in sediment cores from the Great Lakes, reported by Oliver & Nicol (1982), reductive dechlorination of CBs has been suggested by Bailey (1983). However, Oliver & Nicol (1983) did not support the suggestions of Bailey. Historic information on the concentrations of the various CBs at the time of deposition is essential to solve this disagreement. In fact, the microbial dechlorination of CBs in the natural environment, has remained uncertain.

The objective of the present study is to evaluate the possibility of reductive dechlorination of CBs in a sedimentation area of the Rhine River. The present data provide new information on the fate of CBs in sediments, taking into account the contamination levels in the Rhine River in the past, CB concentrations in the sediment at the time of deposition, the actual concentrations in sediment cores and the dechlorination capabilities of native microbial populations in the sediment, tested in the laboratory under conditions that are as close to the field situation as possible.

Materials and methods

Study area

Lake Ketelmeer is a shallow fresh-water lake (surface area 38 km^2), located in the central part of The Netherlands. It receives water originating from the Rhine River through a northern Rhine branch, called the IJssel River. The lake has an open connection with Lake IJsselmeer (Fig. 1). The lakes formed part of a coastal sea (the Zuiderzee) until the closure of a barrierdam in 1932, creating the Lake IJsselmeer. Land reclamation

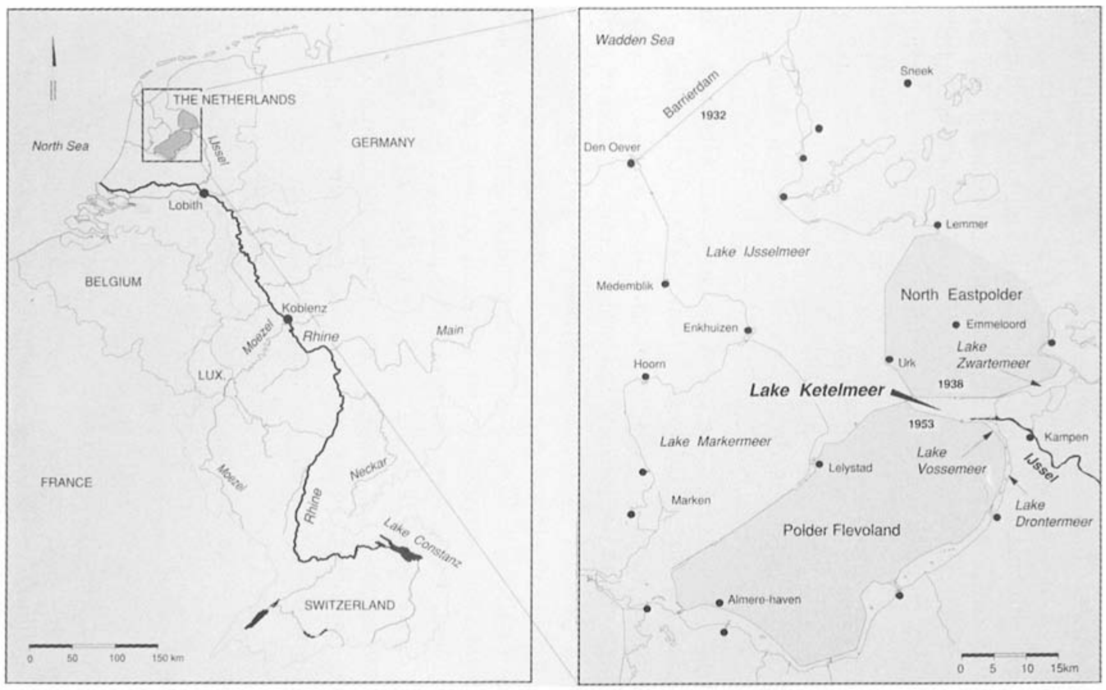

Fig. 1. The Rhine River drainage basin and the northern branch, the IJssel River, discharging into Lake Ketelmeer. The years of completion of the different dikes are indicated.

created two polders within Lake IJsselmeer, with Lake Ketelmeer as the outlet of the IJssel River, situated in between these polders. The northern dike of Lake Ketelmeer was completed in 1938 and the southern dike in 1953. The most recent geological layer in this area, the IJsselmeer (IJm) deposit, is defined as the silty sediment deposited since 1932. The underlying Zuiderzee (Zu) deposit, a salt-water clay deposit, can be identified by the presence of shell fragments (*Mya arenaria*). The average thickness of the IJm-deposit is 0.5 m. The sediment cores were taken at locations known for their thick IJm-deposits. The majority of Lake Ketelmeer sediment is anaerobic, only the top layer (1—3 cm) at the interface with the surface water is aerobic.

Sample collection and treatment

Rhine River water samples have been collected since the early 1970s at the gauging station Lobith, which is situated on the Dutch side of the German-Dutch border. Water samples are collected twice a month.

In 1988, three undisturbed sediment cores (0.15 m in diameter and with an average length of c.1 m) were taken with an open auger. The cores, which mainly contained IJm-deposit, were sectioned into 0.10 m layers. Samples were put in glass jars with screw caps, refrigerated at 4 °C and transported to the laboratory. Before subsamples were taken for the different chemical analyses, samples were freeze dried and homogenized.

The Institute for Soil Fertility Research (Haren, The Netherlands), collected sediment top layer samples (5 cm) from 1972. The 10 historical samples used in this study originate from the same areas as the three sediment cores taken in 1988. After collection in 1972, the top layer samples were dried overnight at 40 °C; since, they have been stored in jars with screw caps at room temperature in the dark.

Sediment dating

The age of the different layers in the sediment cores was estimated by several methods. The well known geological history of this area offers some valuable recognition points in cores. The interface between Zu-deposit and IJm-deposit is visually recognizable (shells) and indicates the early 1930s. The polders were under construction until the 1950s. Sedimentation occurred over a much larger area before the completion of the surrounding dikes created Lake Ketelmeer. Consequently, only a thin layer represents the 1930—1950 period. Due to the thickness of the sampled layers, no subdivision in this period could be made and therefore the 1930—1950 period has been omitted in this study. The ^{137}Cs and

^{134}Cs gamma-activities are used as historical markers. A detailed description of the applied analytical method is presented by Beurskens et al. (1993). Estimations of the year of deposition was only possible for the layers of the IJm-deposit since 1950. The underlying Zu-deposit could not be dated. However, the top of the Zu-deposit has to correspond with the year 1932.

Lead as a non-volatile element in the water column and rather immobile element in anoxic sediments will serve as a tracer in this study. The long-term changes in the concentrations pattern of lead in Rhine water samples is expected to be found in the sediment cores from Lake Ketelmeer. Comparison of the lead concentrations in the top layer samples from 1972 with the levels in the sediment cores offers an additional recognition point.

Chemical analyses

The contaminant concentrations in water samples from Lobith are based on data from the long term water quality research, conducted at our institute (Heymen 1990). Since the early 1970s, polutants have been monitored in the Rhine River. As a result of the developments in the analytical methods, several analytical techniques have been applied during the past two decades. Particularly with respect to the organic micropollutants, clear improvements have been obtained. Consequently, the reported concentrations of organic micropollutants in Rhine water are probably less accurate for the 1970s than for the 1980s. The most recently applied technique for CB quantification is described below.

Water samples were extracted three times with petroleum ether. The combined petroleum ether extracts were dried with sodium sulphate and concentrated with a Kuderna-Danish condensor to a volume of 5 ml. The extract volume was further reduced to 1 ml with a gentle stream of clean nitrogen. Extract cleanup consisted of passing the extract through a column of 2 g 11% deactivated alumina. Evaporation with nitrogen concomitant with a solvent change to iso-octane was used to reduce the volume. Sample extracts were analyzed with a HP 5890A gas chromatograph, equipped with dual ^{63}Ni electron capture detector using automated splitless injection (HP 7673A). The separations were carried out on dual capillary columns (CPsil8 and CPsil19, length 50 m, 0.22 mm i.d., film-thickness 0.12 μm). Operating conditions were as follows: temperature program of 60 °C for 2 min, 10 °C/min to 140 °C, 5 min at 140 °C, 5 °C/min to 250 °C, 30 min at 250 °C, 5 °C/min to 280 °C, 15 min at 280 °C; injector temperature of 250 °C; detector temperature of 300 °C. Helium was used as a carrier gas and nitrogen as a detector-quench gas.

CBs and polychlorinated biphenyls (PCBs) in the sediment samples have been analyzed as follows. The dried sediment samples were extracted twice with acetone for 15 min. The acetone extracts were shaken with a sodium sulphite solution to remove elemental sulphur. The extracts were mixed with petroleum ether and washed with water to remove the acetone. After separation, the aqueous phase was extracted with a second portion of petroleum ether. The petroleum ether extracts were combined and further treatment and gas chromatographic analysis were identical to the procedures for the extracts obtained from water samples, as described above. Only an additional extract cleanup through a column of 2 g 6% deactivated silica was applied.

Lead concentrations in Rhine water samples were determined after acidification of the samples to pH 2 with nitric acid. The acidified samples were analyzed by graphite furnace atomic absorption spectrometry (Perkin Elmer 5000).

Lead concentrations in the sediment core samples were determined after treatment with hydrochloric acid. A graphite furnace AAS (Perkin Elmer 5000) was used for the lead analyses. Lead concentrations in the top layer samples from 1972 have been determined at the Institute for Soil Fertility Research. The applied analytical method (Japenga et al. 1990) is similar to the method described above.

All concentations of radionuclides and pollutants in sediment samples are reported on a per dry weight of sediment basis. For purposes of numerical calculation and graphical display, all concentrations below detection levels were assumed to be one-half the detection level.

Anaerobic incubations

The dechlorination capabilities of native microbial populations in Lake Ketelmeer sediment have been tested in laboratory incubations for chlorinated compounds that already existed in the polluted sediment and for CBs and PCBs that were added to the same sediment in the laboratory. The following procedure was used, 60 ml of Lake Ketelmeer sediment slurries (30% d.w.) was added to 100 ml serum bottles, sealed with viton stoppers (Eriks b.v., Alkmaar, The Netherlands). Incubation was in the dark at 20 °C. Headspace samples were taken twice a week for methane analysis. Methane was determined with a gas chromatograph (HP 5890), equipped with a Carbosieve SII column and flame ionization detector. Helium was used as a carrier gas, gas flow was 30 ml/min. After 4 weeks of incubation, increased methane concentrations indicated that anaerobic conditions were established. At that moment, 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), hexachlorobenzene (HCB), 2,4,4′-trichlorobiphenyl (PCB 28), 2,2′,5,5′-tetrachlorobiphenyl (PCB 52) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) in 100 μl of methanol were added separately to series of serum bottles. In another series of serum bottles no chlorinated compounds were added. In these bottles, the degradability of chlorinated compounds already present in the sediment, was studied. Pure methanol (100 μl) was added to these bottles, in order to obtain identical treatment for both series. Sterile controls were obtained by adding formalin to the slurries, the final concentration being 4% (v/v). Duplicates were sacrificed at each time point and 6 g (w.w.) slurry samples were extracted with acetone by the method described above in order to obtain the CB and PCB concentrations.

Dechlorination products of HCB were identified in another series of incubations with a similar experimental set-up. In order to obtain detectable changes in possible dechlorination products, the concentration level of the laboratory added HCB has been increased to 1340 nmol/kg in these experiments.

Results

Pollutants in the Rhine River

A significant decrease in concentrations of HCB and lead in Rhine water near Lobith is observed during the last two decades (Fig. 2). The concentrations of the lower chlorinated benzenes have only been monitored after 1982. The sum of the concentrations of TCBs has decreased as well.

There are no major point sources of CBs, PCBs and lead at Lake Ketelmeer, nor along the Rhine and IJssel between Lobith and Lake Ketelmeer. In Lake Ketelmeer, sedimentation of suspended solids together with the associated pollutants occurs as a result of decreasing streaming velocities. For hydrophobic, persistent and relatively non-volatile pollutants, the changes in concentrations observed in Rhine water during the last two decades are expected to be reflected by the pollutant concentration profiles in sediment cores from Lake Ketelmeer.

Pollutants in sediment cores and stored top layer samples

The thickness of the IJsselmeer-deposit is not identical at the three sampling locations, therefore concentration profiles of pollutants are constructed by combining the data of the various cores expressed against the period of deposition, instead of depth. The cesium activities in the three

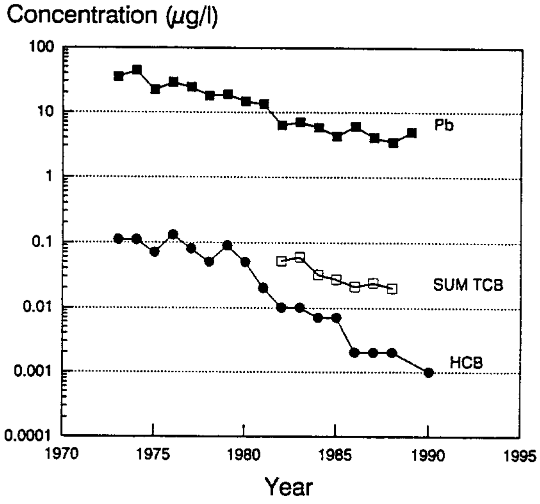

Fig. 2. The concentrations of lead (Pb), all three trichlorobenzenes (SUM TCB) and hexachlorobenzene (HCB) in Rhine River water, sampled at the German-Dutch border (Lobith).

cores are shown in Fig. 3 and clearly show the ^{137}Cs maximum in the samples from near the surface, which always correlated with elevated activities of ^{134}Cs (half-life $t_{1/2}$ = 2.06 y), indicating the fallout from the nuclear power plant accident in Chernobyl in April 1986. The second ^{137}Cs maximum, found in the deeper layers, was related to the fallout from nuclear weapons testing, which had its maximum in the early 1960s. These results are in agreement with earlier sediment dating studies in this area (Beurskens et al. 1993; Comans et al. 1989).

Concentration profiles of lead, PCB 52 and HCB are shown in Fig. 4. The PCB congener has been included as a compound that behaves rather similar as HCB in Lake Ketelmeer with regard to volatilization to the atmosphere (Henry's law constant for HCB and PCB 52 are $4 \cdot 10^{-4}$ and $1.6 \cdot 10^{-4}$ atm.m^3.mol^{-1}, respectively, Ten Hulscher et al. 1992), and with regard to sorption to suspended matter and subsequent sedimentation (Octanol/water partition constants [Log K_{ow}] for HCB and PCB 52 are 5.73 and 6.31, respectively, De Bruijn et al. 1989).

Decreasing concentrations of lead in Lake Ketelmeer sediment are observed since the 1950s (Fig. 4). The pattern is in agreement with the decreasing concentrations in Rhine water (Fig. 2). The stored top layer samples from 1972 represent the contamination levels around 1970 and are plotted in the same graphs together with the concentration profiles from the cores. The concentrations in the stored samples from 1972 confirm the age estimation, they fit rather well in the constructed lead

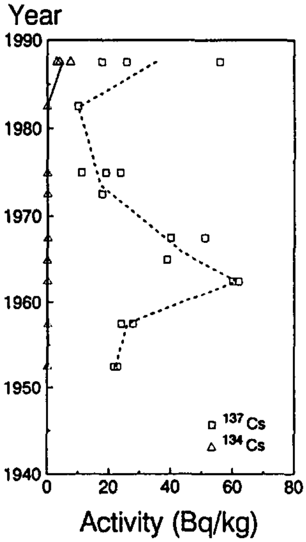

Fig. 3. Cesium activities in sediment layers from three cores vs. the estimated year of deposition. Average activities are indicated by visually fitted curves.

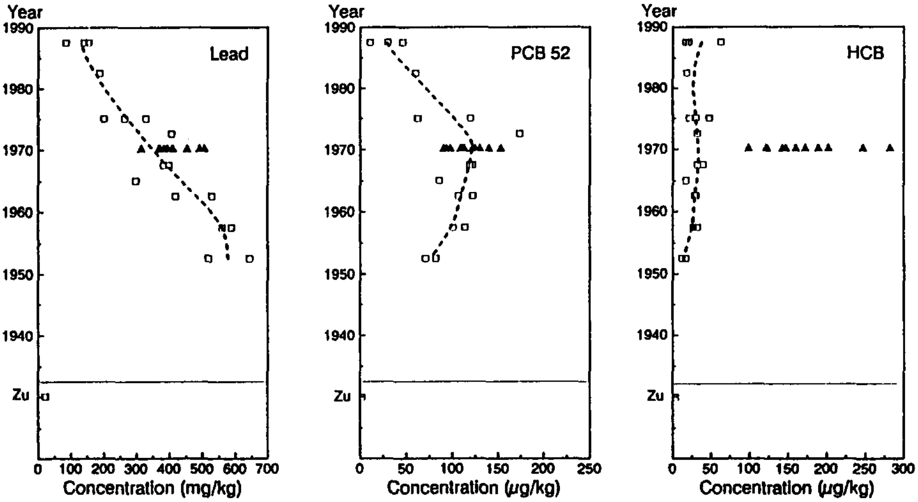

Fig. 4. The concentrations of lead, 2,2′,5,5′-tetrachlorobiphenyl (PCB 52) and hexachlorobenzene (HCB) in sediment core layers taken in 1988 (□) vs. the estimated year of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples taken in 1972 (▲) indicate the original pollution level.

profile. Highest concentrations of PCB 52 were found in layers deposited in the 1960—1975 period. The concentrations of PCB 52 in the samples from 1972 clearly fit in the average concentation level found in the sediment core layers around 1970. The concentration of HCB in the sediment cores is rather constant and relatively low. Surprisingly, there is no maximum in the deeper layers and the highest concentration is even found in a sample from near the surface (63 μg/kg). On the other hand, HCB concentrations in the stored samples from 1972 are two to seven times as high (range: 99—282 μg/kg) as the levels found in core layers that have been deposited around 1970 (± 40 μg/kg). Even higher concentrations of HCB are found by recent analysis of stored flood plain samples of the Rhine River, taken in 1970 and 1972 (Japenga et al. 1990). Somehow HCB has disappeared in the anaerobic lake sediment. The concentrations of PCB 52 and HCB in the samples from the Zu-deposit, directly beneath the IJm-deposit, were below the detection limit (1 μg/kg). The lead concentration in the Zu-deposit (20 mg/kg) equals the natural background concentration of unpolluted Rhine sediment (Salomons 1989).

The concentrations of pentachlorobenzene (QCB) and tetrachlorobenzenes (TeCBs) in the cores were rather low and showed only minimal losses as compared to the concentrations in the samples from 1972 (cf. Table 1).

The concentration profiles of the trichlorobenzenes (TCBs) are shown in Fig. 5. The concentrations of 1,2,3-TCB were rather low in the sediment cores and reached a maximum of 23 μg/kg in the 1960s. The concentrations in the top layer samples from 1972 were somewhat higher. For 1,2,4-TCB, high concentrations are found in the sediment cores around 1955—1965. Concentrations of 1,2,4-TCB in the top layer samples from 1972 fit rather well in the concentration profile of the cores. The 1,3,5-isomer shows highest concentrations around 1970 in the sediment cores. The concentrations in the samples from 1972 are much lower. This clear difference indicates that somehow 1,3,5-TCB has been produced in the anaerobic sediment. The concentrations of TCBs in the Zu-layers indicate some downward transport of 1,2,3-TCB (Fig. 5).

The concentrations of all dichlorobenzenes (DCBs) were rather high in the sediment cores and reached concentrations up to 500 μg/kg (Fig. 6). The concentration profile of 1,2-DCB in the sediment core has no clear maximum. Concentrations in the top layer samples from 1972 are somewhat lower than the concentrations in the cores. The concentration of the 1,3-isomer in the cores has a maximum around 1970, and is about seven times as high as the concentration in the stored top layer samples from

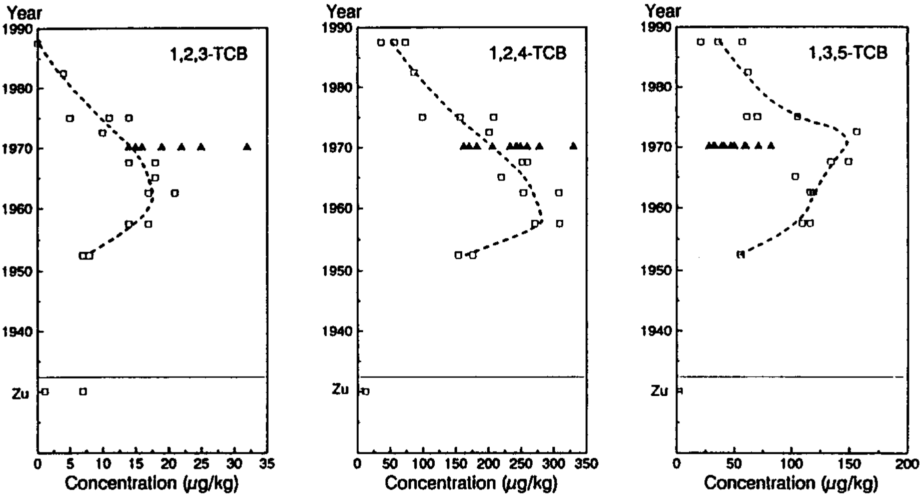

Fig. 5. The concentrations of the trichlorobenzenes (TCB) in sediment core layers taken in 1988 (□) vs. the esimated year of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples taken in 1972 (▲) indicate the original pollution level.

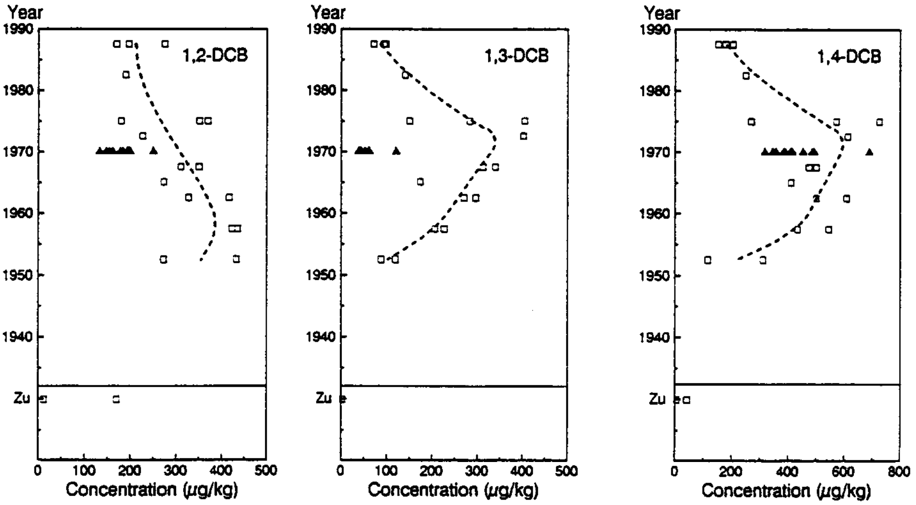

Fig. 6. The concentrations of the dichlorobenzenes (DCB) in sediment core layers taken in 1988 (□) vs. the estimated year of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples taken in 1972 (▲) indicate the original pollution level.

Table 1. Comparison between chlorinated benzene concentrations in top layer samples that have been taken in 1972 and recently sampled core layers that have been deposited around 1970 (s.d. = standard deviation). The differences are interpreted as disappearance (−) and production (+) of chlorinated benzenes in the anaerobic lake sediment.

Compound	Concentrations in nmol/kg				
	Top layer from 1972 $n = 10$		Sediment core layers from ± 1970 $n = 3$		Difference*
	mean	s.d.	mean	s.d.	
HCB	606	205	121	16	−485
QCB	107	23	70	6	−37
1,2,3,4-TeCB	94	17	93	20	ns
1,2,3,5-TeCB	40	8	25	3	−15
1,2,4,5-TeCB	189	39	236	21	ns
1,2,3-TCB	98	21	77	22	ns
1,2,4-TCB	1226	220	1308	174	ns
1,3,5-TCB	259	90	806	63	+547
1,2-DCB	1184	159	2016	431	+832
1,3-DCB	350	64	2388	315	+2038
1,4-DCB	2759	400	3608	495	ns

*: only significant differences at the 95% confidence level are denoted (t-test), ns = not significant.

1972. The concentrations of the 1,4-isomer were very high in the core layers that have been deposited between 1955 and 1975. The concentration in the top layer samples from 1972 were somewhat below the concentrations found in the sediment cores around 1970. The concentrations of the DCBs in the Zu-layers indicate that downard transport has occurred, in particular of 1,2-DCB.

As indicated by the results of Fathepure et al. (1988), the microbial dechlorination of CBs results in a stochiometric accumulation of dechlorination products. A mass balance has been calculated (Table 1) for the top layer samples from 1972, that reflect the original pollution level and the core layers dated around 1970. These core layers show the concentrations after almost 20 years of anaerobic environmental incubation. Only the significant differences at a 95% confidence level are considered. The loss in the total amount of HCB, QCB and 1,2,3,5-TeCB is far less than the increase in the amount of 1,3,5-TCB, 1,2-DCB and 1,3-DCB amount.

Anaerobic incubations with Lake Ketelmeer sediment

The studied CBs and PCBs in the field contaminated sediment did not show any clear disappearance in the anaerobic incubations by comparison with the sterile controls (Table 2). HCB was the only laboratory spiked compound that showed a clear decrease by comparison with the sterile control. The disappearance of HCB occurred mainly in the first part of the experiment (half-life of HCB was 11 weeks). A rather constant residual concentration, more or less equal to the field contamination level that already existed in the sediment, more or less equal to the field contamination level that already existed in the sediment, remained unaltered up to 78 weeks.

In an additional experiment, a high HCB addition enabled the detection of changes in the possible dechlorination products. In the sterile controls

Table 2. Anaerobic incubations of field contaminated sediment and laboratory spiked sediment ($n = 2$).

Field contaminated sediment

Compound	Initial concentration (in μg/kg d.w.)	Recovery after 78 weeks (as % of initial conc.)	
		Non sterile	Sterile
1,2,4-TCB	127	98	113
1,2,4,5-TeCB	21	95	86
HCB	41	80	124
PCB 28	140	76	76
PCB 52	100	94	94
PCB 153	104	94	121

Laboratory spiked sediment

Compound	Initial concentration (in μg/kg d.w.)	Recovery after 78 weeks (as % of initial conc.)	
		Non sterile	Sterile
1,2,4-TCB	153	84	81
1,2,4,5-TeCB	63	75	154
HCB	161	37	110
PCB 28	230	83	87
PCB 52	178	99	110
PCB 153	201	106	96

no decrease of the HCB concentration was observed. In Fig. 7 the concentrations of the added HCB and produced metabolites are presented. Only compounds that showed a clear difference from the sterile controls during the 18 week incubation period are shown. An almost linear decrease of HCB resulted in a transient increase of QCB and 1,3,5-TCB concentrations and finally an increase of 1,3-DCB. The HCB dechlorination pathway can not be identified fully from this single experiment, but it became clear that 1,3,5-TCB is a major intermediate. The 1,3-DCB may be a major endproduct of the HCB dechlorination, although it remains uncertain whether a prolonged incubation would lead to a decrease of the 1,3-DCB too.

Discussion and conclusions

Testing hypotheses for the disappearance of HCB in lake sediment

For hydrophobic, persistent and non-volatile pollutants it is expected that sediment cores from Lake Ketelmeer show concentration profiles that

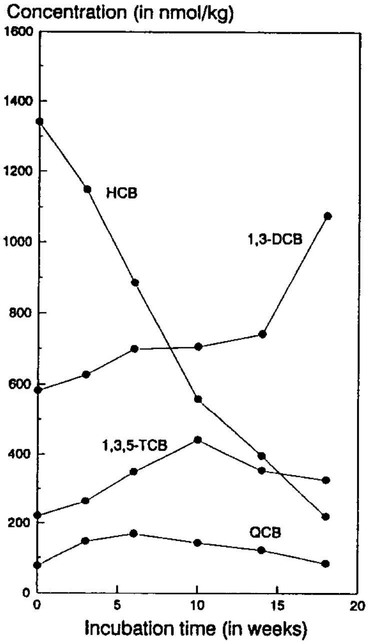

Fig. 7. Pattern of hexachlorobenzene dechlorination and the formation of dechlorination products during the anaerobic incubation of Lake Ketelmeer sediment after a hexachlorobenzene addition in the laboratory.

reflect the changes in pollutant concentrations of Rhine water during the last decades. This proved to be true for lead. For HCB the concentration profile did not reflect the decrease in concentration which is observed in Rhine water during the last two decades. Below, three hypotheses explaining the absence of a decrease in the HCB concentration in sediment cores since c.1975 will be verified. The first hypothesis is that partition processes (sorption and/or volatilization) prevent accumulation of HCB in the sediment. Secondly, the absence of a decrease is explained by the possibility of downward transport of HCB with infiltrating water. Thirdly, it is hypothesized that HCB is not persistent in the anaerobic sediment.

The first hypothesis is verified with the aid of PCB 52 concentrations in the cores. HCB and PCBs had similar discharge patterns in the Rhine River with highest levels around 1970 as demonstrated by Japenga et al. (1990) in a study using freshly deposited sediments from floodplains, sampled in 1958, 1970, 1972 and 1981. HCB and PCB 52 will behave rather similar in the water column of Lake Ketelmeer with respect to volatilization and sorption, as indicated by their water/air and water/octanol partitioning constants. For PCB 52, maximum concentrations in the sediment cores were observed in layers dated around 1970. The top layer samples from 1972 had similar PCB 52 concentrations as found in the cores around 1970 indicating that no disappearance has occurred since. Long term persistence of PCB 52 in anaerobic sediments was also found by Brown & Wagner (1990). The recalcitrance of PCB 52 in Lake Ketelmeer sediment was confirmed in the anaerobic incubations (Table 2). Consequently, the lack of a maximum in the HCB concentration in the sediment cores can not be explained by a low sorption to settling particles or a high volatilization to the atmosphere. In addition, analyses of top layer samples from 1972 showed that high concentrations of HCB have been deposited.

Transport of HCB to deeper sediment layers by infiltrating water is a second hypothesis for the observed relatively low and constant level of HCB in sediment cores and the high HCB concentrations in the top layer samples from 1972. Therefore the HCB levels in the Zu-layers directly beneath the IJm-deposit were also determined (Fig. 4). It is expected that the Zu-deposit was not polluted with chlorinated compounds at time of deposition. The Zu-layers have been deposited at least before 1932. The widespread use of chlorinated biphenyls and benzenes started somewhere in the 1940s. The concentrations of HCB in the Zu-samples were below the detection limit (1 μg/kg) and indicate that no downward transport has occurred.

The third hypothesis, HCB does not persist in the anaerobic lake sediment, is based on the findings of Fathepure et al. (1988). They showed a rapid microbial dechlorination of HCB into lower chlorinated benzenes in anaerobic sewage sludge. Sediment cores from Lake Ketelmeer show a clear loss of HCB as compared to the concentrations in the top layer samples from 1972. An increase of possibly related dechlorination products like 1,3,5-TCB is observed in the sediment cores. However, a mass balance for the CBs in sediment cores and top layer samples (Table 1) indicates that not all increases of the lower chlorinated compounds in these layers can be attributed to the dechlorination of HCB. Two explanations are possible. 1) The less hydrophobic DCBs are transported downward, as indicated by the elevated levels in the Zu-deposit. This transport may result in a downward shift of the maximum in the concentration profiles. Consequently, the amount of DCBs produced within these layers will be overestimated. 2) Possible losses of CBs during sample treatment and storage of the 1972 samples. This may result in an underestimation of the amount of HCB that has disappeared and an overestimation of the amount of lower chlorinated benzenes produced.

The anaerobic incubations with Lake Ketelmeer sediment clearly support the third hypothesis. It became clear that the native microbial population is able to dechlorinate HCB and produces lower chlorinated benzenes. Unfortunately, we were not able to demonstrate a quantitative transformation of HCB into less chlorinated benzenes in the laboratory study. This was probably caused by the rather high variation in the DCB quantification and the inability to quantify monochlorobenzene at concentrations $\leqslant 26{,}700$ nmol/kg.

Based on the various facts it is concluded that *in situ* microbial dechlorination of HCB in the anaerobic Lake Ketelmeer sediment has occurred. The rate of HCB dechlorination in Lake Ketelmeer (a mean half-life time) is difficult to determine. Assuming first order kinetics, a maximum half-life of 7 years for HCB has been calculated for the data around 1970. Half-life times for some PCB congeners have been estimated at 8 years in anaerobic estuarine sediment (Brown & Wagner 1990).

Implications of dechlorination in the environment

The disappearance of HCB in Lake Ketelmeer sediment showed clearly two limitations of the microbially mediated dechlorination in the natural environment. First, dechlorination seems not to result in a complete elimination of HCB. Although after 20 years the majority of the original input has been dechlorinated (80% of the HCB deposited in the early 1970s), a residual concentration of about 40 μg/kg was observed in all sediment cores. Transformation of the residual fraction is probably very slow or even absent. No decrease of this fraction was observed in the laboratory incubations as well (Table 2). Nevertheless, HCB added to the same sediment in the laboratory was readily dechlorinated. This indicates that the persistence of the residual concentration is probably the result of a limited microbial availability. Secondly, the dechlorination of HCB in the environment results in the formation of lower chlorinated benzenes that seem to accumulate in the anaerobic sediment, as observed in the sediment cores. Moreover, the laboratory incubations showed the persistence of 1,2,4-TCB and 1,2,4,5-TeCB (Table 2) even if they are added to the sediment in the laboratory. These results indicate a high specificity of the anaerobes in Lake Ketelmeer sediment. The produced less chlorinated benzenes are less toxic than the parent compounds (van Leeuwen et al. 1990) and are less hydrophobic and therefore more mobile than HCB. Theoretically, the lower chlorinated benzenes might enter the aerobic lake water due to their increased mobility and be mineralized by aerobic microorganisms. In Lake Ketelmeer, where convective transport through downward seepage dominates diffusive transport in the pore water, upward transport of the lower chlorinated benzenes to the aerobic upper sediment and lake water, is unlikely. Downward transport is more realistic and results in an entrance into the anaerobic ground water, probably leading to a long term persistence. Transport to the Zu-deposit has only been observed for 1,2-DCB, 1,4-DCB and 1,2,3-TCB. Although these compounds appear not to be related to the dechlorination of HCB, at least they show the potential mobility of dechlorination products.

The three PCBs tested in laboratory incubations showed no significant decrease in the 78 weeks incubation period. The persistence of PCB 52 in Lake Ketelmeer sediment was confirmed by the PCB 52 concentrations in the top layer samples from 1972, that clearly fitted in the concentration profile of the sediment cores. It is concluded that PCB 52 is highly persistent in the anaerobic Lake Ketelmeer sediment. The persistence of the three tested PCBs (Table 2) might be the result of microbial specificity or PCB concentrations being too low to induce dechlorination, even for the laboratory spiked compounds. Quensen et al. (1988) showed that dechlorination of Aroclor 1242 strongly depended on the applied dosage. Microbially mediated reductive dechlorination of PCBs in anaerobic Hudson sediment has been indicated based on PCB congener distributions in anaerobic sediments relative to the distributions in the original mixtures (Brown et al. 1987). This was confirmed in laboratory studies using microorganisms eluted from the anaerobic sediments in which reductive dechlorination of PCBs was thought to occur (Nies & Vogel 1990; Quensen et al. 1988). Although the three tested PCBs seem to persist in Lake Ketelmeer sediment, it can not be excluded that other, more reactive, PCB congeners are transformed in Lake Ketelmeer sediment.

A complete *in situ* biological decontamination of HCB in the sediment is not feasible due to the persistence of residues and the accumulation of reaction products under anaerobic conditions. As recently demonstrated by Fathepure & Vogel (1991) a complete biomineralization of HCB can be obtained in a two stage anaerobic-aerobic biofilm reactor. However, if dechlorination takes place in the natural environment with hydrological conditions as in Lake Ketelmeer, anaerobic conditions are prolonged and the relatively mobile dechlorination products persist and may be transported. In this case, environmental dechlorination becomes an additional source of persistent and mobile compounds that are of particular concern for the ground water quality.

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