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Toxic Effects of Pollutants on Methane Production in Sediments of the River Rhine

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High numbers of bacteria are found in Dutch river sediments, ranging from 10^9 to 10^{12} bacteria per gram dry sediment (Van Beelen and Fleuren-Kemilä 1989). These sediments have a large capacity for the biodegradation of organic compounds and play an important role in the cycling of nutrients and elements. They are of vital importance in the functioning of river ecosystems. The methanogenic bacteria, a division of the archaebacteria (Woese 1981) and a physiologically coherent group of strict anaerobes (Balch *et al.* 1979), are responsible for the production of methane. Methanogenesis is the last stage of the anaerobic decomposition of organic matter. The role of the methanogens in the decomposition process is important; without their activity the degradation of organic matter cannot be carried out completely and acetic acid (and other organic acids) would accumulate. In anaerobic freshwater sediments acetate is the substrate used in about 70% of the methane production (Lovley and Klug 1986). An inhibitory effect on any of the processes carried out by the microbial consortium degrading organic matter, can lead to a decrease of methane production. The 5 toxicants used in this study are priority pollutants in the Rhine Action Programme, which are produced in high quantities and are relatively toxic and persistant. Benzene is an organic toxicant without a specific mode of action and thus exhibiting minimum toxicity (Slooff *et al.* 1988). Chloroform is not very toxic under aerobic conditions. Van der Heijden *et al.* (1986) quote a value for initial reduction of cell multiplication of a *Pseudomonas putida* strain under aerobic aqueous conditions at 125 mg/L chloroform. Under anaerobic conditions however, toxic free radicals can be formed during the reductive dechlorination of chloroform (Klecka and Gonsior 1984). Therefore anaerobic bacteria might be more sensitive to chloroform. The mode of action of 1,2-dichloroethane might be similar to chloroform. Pentachlorophenol is an uncoupler of the proton motive force (Schultz 1987). Zinc is known to be relatively toxic to microbial processes (Doelman and Haanstra 1989). Monitoring the effect of these very different pollutants on the methane production gives information about the effect of these compunds on the microflora decomposing organic matter in a particular methanogenic ecosystem.

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MATERIALS AND METHODS

All sediment samples were taken at the same location (Gorinchem, the Netherlands, in a harbor in the estuary of the river Rhine: Eastern Longitude 4°57'50", Northern Latitude 51°49'46"), with a core sampler. Depth of sampling was at 0.5 to 1 m below the sediment-water interface. The sediment was primarily composed of methanogenic mud; its properties are presented in Table 1. In the laboratory, the cores were brought into an anaerobic glove box (Braun MB 30 G) which contained a nitrogen atmosphere with less than 1 ppm (v/v) oxygen. In the glove box a 1:1 (w/w) wet sediment/water suspension was made from the sediment samples of which the upper and lower layer were discarded. 20 mL of this suspension was pipetted into sterile incubation bottles (60 mL). All equipment used was either autoclaved or aseptic. Toxicant solutions were prepared aerobically but added in the glove box, immediately after filling the bottles with suspension. The resulting toxicant concentrations were based on sediment dry weight and calculated after the density and dry weight of the sediment had been measured.

Table 1. Properties of Gorinchem sediments.

sediment code	G-1	G-2	G-3
sampling date	08-21-90	09-27-90	11-15-90
log AODC/g dry sedim.[a]	10.1 (0.04)[b]	9.8 (0.06)	9.9 (0.16)
wet density (kg/L)	1.45	1.43	1.33
%dry weight	44.0	44.8	40.1
M-50 (μm)[c]	125	125	125
%clay	27.5	24.9	23.2
pH (H_2O)	7.6	7.9	7.8
pH (KCl)	7.4	7.4	7.3
%org. carbon	3.1	3.2	10.4
%$CaCO_3$	9.8	12.2	12.0
mineral NO_3^--N (mg/kg)	0.8	1.5	n.d.[d]
mineral NH_4^+-N (mg/kg)	388	460	n.d.
total N (mg/kg)	2300	3010	n.d.

a) Log AODC is the number of bacteria determined by fluorescence microscopy with the Acridine Orange method (Ghiorse and Balkwill 1983). b) Standard deviations are given between parentheses. c) M-50 is the median of the particle size distribution. d) n.d. = not determined.

Pentachlorophenol (PCP, Fluka, Switzerland, purity ≥ 99%) and zinc ($ZnCl_2$, Merck, Germany; purity ≥ 98%) were added as portions from a 100 g/L or a 1 g/L stock solution. PCP (100 g/L) was dissolved in 0.5 N NaOH and zinc chloride (100 g/L) was dissolved in 0.1 N HCl. Benzene (Merck, Germany; purity ≥ 99.7%) and 1,2-dichloroethane (DCE, Instra Analyzed, J.T. Baker Chemical co., Deventer, the Netherlands) were added pure at the three highest concentrations: 1010, 3030 and 10090 mg/kg dry sediment. For the other concentrations a dilution range was made in methanol (Merck, Germany; purity ≥ 99.8%). A 20 μL aliquot from a dilution was added to the incubation bottles. Chloroform (Merck, Germany; purity ≥ 99.0%) was added as a dilution in methanol at all concentrations. The bottles were incubated at 20°C in a rotary shaker

at 225 rpm. The incubation time was not standard, the experiments with benzene, 1,2-dichloroethane and chloroform were incubated for 11 days and the experiments with zinc and pentachlorophenol for 7 days. All experiments were performed in duplicate and two blank bottles without any addition were incorporated as well as two blank bottles with addition of the highest concentration of solvent.

Methane was measured using a Carlo Erba gas chromatograph (Milan, Italy; type 4130) equipped with a flame ionization detector and a capillary PLOT (fused silica) column. Ethane (purity ≥ 99.3%) was added at the end of the incubations as an internal standard. The amount of methane produced was calculated using a calibration range based on the areas of the gas chromatographic peaks. The EC10 and EC50 concentrations can be estimated directly from the data using a logistic dose-response curve which can be mathematically described as follows (Van Beelen *et al.* 1991a):

$$A(c) = \text{blank}/[1 + \exp\{\text{slope}*(\log[c]-\log[EC50])\}] \quad (1)$$

c = concentration of the toxicant in mg/kg.
A(c) = the amount of methane (in μL methane/mL slurry) produced during the incubation time at a toxicant concentration c.
blank= the amount of methane produced without addition of a toxicant.
EC50 = that concentration of the toxicant which causes a 50% reduction of the amount of methane produced at a certain incubation time.
slope= a constant which determines the steepness of the dose response curve.

The EC10 is that toxicant concentration which causes a 10% reduction of the methane production. When the EC10 and the EC50 are known, the slope can be determined by inserting c = EC10 and A(c)/blank = 90/100 in equation (1):

$$\text{slope} = \ln(9)/\log(EC50/EC10) \quad (2)$$

A nonlinear least squares method was used to determine the best fitting curve through the data. All experiments were carried out in duplicate (two bottles per concentration) and it is therefore possible to consider one complete data set as consisting of two separate experiments. Two independent logistic curves can be calculated by splitting the data of one experiment in two and by fitting both data sets separately. The standard deviations were calculated using the unbiased (n-1) method.

RESULTS AND DISCUSSION

Benzene did not inhibit methanogenesis even at added concentrations as high as 10,000 mg/kg (Table 2). The concentration of benzene in the river Rhine is below 0.1 μg/L (Slooff *et al.* 1988). The sorbed equilibrium benzene concentration can be calculated from the log Kow (2.13), the percentage organic carbon and the benzene in the waterphase using the Karickhoff equation (Karickhoff 1981):

$$Cs = Cw * \%C * Kow * 0.411 / 100 \quad (3)$$

Cs = sediment concentration in μg/kg
Cw = water concentration in μg/l
%C = percentage organic carbon (Table 1)

Table 2 shows that the calculated equilibrium sediment concentration is much lower than the EC10 concentration. Benzene is thus expected not to inhibit methanogenesis at the actual field concentrations.

The effect of chloroform (Figure 1) is far more distinct. At concentrations of 10 mg/kg and higher, chloroform completely inhibits methanogenesis in the Gorinchem sediment. However, the average chloroform concentration in the Rhine was 0.5 μg/L on average in 1989 (RIWA 1992) and the log Kow is 1.97. Table 2 shows that the calculated field concentration is much lower than the EC10. This means that effects of chloroform on methanogenesis are not expected at the present concentrations.

Table 2. The effect of added toxicants on the methane production of sediment microcosms.

Toxicant	Sed. used	Inc. time(d)	EC10 mg/kg	S.D.[a]	EC50 mg/kg	S.D.	FC[b] μg/kg
Benzene	G-2	11	>10000	-	>10000	-	0.2
Chloroform	G-2	11	5.5	0.8	6.9	1.0	0.6
1,2-Dichloroethane	G-2	11	860	290	1300	480	0.04
Pentachlorophenol	G-3	7	140	20	270	20	34
Zinc	G-1	7	48	1	110	1	800000
	G-3	7	1780	150	4490	650	800000

The reported EC10 and EC50 values are given in mg/kg dry sediment. a) S.D. = standard deviation, calculated from two possible logistic curves at a given data set. b) FC = Field Concentration, calculated from the reported concentrations in river water and the sorption properties of the sediment using equation (3). For PCP and zinc measured concentrations were available.

The methanogenic community is not very sensitive to 1,2-dichloroethane; inhibitory effects were just observed at concentrations of 1000 mg/kg or higher. A lower transformation rate of 1,2-dichloroethane (Bouwer and McCarty 1983) compared with chloroform may explain the lower toxicity of 1,2-dichloroethane, since less toxic intermediates will be formed. Since the octanol-water partition coefficient is low (log Kow = 1.45) and the concentration is in the order of 0.1 μg/L (RIWA 1992), effects on methanogenesis are not to be expected (Table 2).

The methane production is inhibited for 10% at a concentration of 140 mg added PCP per kg sediment (Table 2). This concentration is much higher than the concentration of PCP found in sediment of the river Rhine, which was maximally 34 μg/kg (Wegman and Van den Broek 1983).

The EC10 value for zinc in a second experiment was 37 times higher than the EC10 value of the first experiment (Table 2). The G-3

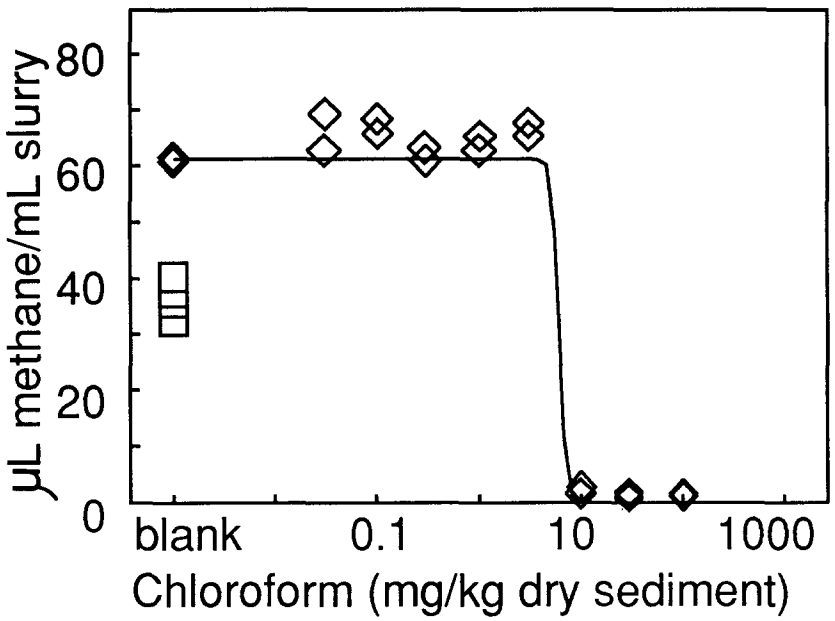

Figure 1. The effect of chloroform on the methane production in Gorinchem sediment. EC10 = 5.5 mg/kg and EC50 = 6.9 mg/kg. □ no solvent added; ◇ methanol added as a solvent. The stimulation of the methane production is caused by methanol which can be used as a substrate by certain species of methanogenic bacteria (Balch *et al.* 1979).

sediment sample had a lower methane production and a three times higher organic carbon content than sediment G-1 (Table 1). Sorption of the added zinc to organic carbon will thus be higher in the G-3 sample, but this is not sufficient to explain the difference in sensitivity. The amount of zinc that is sorbed is also dependent on the concentration of carbonates or sulfides in the sediment because zinc can form insoluble complexes with these ions. Di Toro *et al.* (1990) have shown that the toxicity of cadmium for sediment amphipods is determined by the concentration of acid volatile sulfide (AVS) in the sediment. Binding of zinc as well as other metals to AVS, is also expected to be important. The background concentration of zinc in the Gorinchem sediment is 800 mg/kg dry sediment. The major amount of zinc is present in a sorbed form, but toxic effects on methanogenesis, as well as other mineralization processes (van Beelen *et al.* 1991b), can be expected when even a small part of this zinc is or becomes bioavailable.

From the results of the experiments described here, we conclude that the present concentrations of benzene, chloroform, 1,2-dichloroethane and pentachlorophenol in sediments of the river Rhine will not inhibit methanogenesis. Present zinc concentrations in sediments are much higher than the concentrations at which effects on methanogenesis can be expected. Inhibition of the

activities carried out by members of a methanogenic consortium might lead to a deregulation of the carbon flow and the cycling of nutrients in the river ecosystem.

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Erratum

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