

SHORT COMMUNICATION

Methylation of mercury compounds in soil, sediment and sewage-sludge samples

Summary

Sediments of the rivers Rhine and Yssel and sewage sludge samples showed high mercury contents (5-30 ppm). Usually less than 1% - in one sludge sample 5% - of the mercury was in the form of methylmercury. Incubation of these samples, under varied aerobic and anaerobic conditions, with or without HgCl_2 or phenylmercuryacetate added, gave no or only very slight formation of methylmercury.

Most agricultural soils have a mercury content below 0.1 ppm and methylation will be no problem.

Introduction

Alarming reports from Sweden¹ and Japan on microbially mediated methylation of mercury in underwater sediments led us to investigate mercury methylation in soil, sediment and sewage-sludge samples.

Methods and materials

To determine total mercury content the samples were oxidized with persulfate and concentrated acids according to Hoover². Mercury in the extracts was measured by flameless atomic absorption spectrophotometry, following Hatch and Ott³. Methylmercury was extracted into benzene and cleaned up, using the cysteine acetate method of Westöb⁴, with addition of HgCl_2 to the samples. Methylmercury in the extracts was quantified by gas-chromatography. 'Easily extractable mercury' was found using 2 M NaCl or 1% Na_2EDTA solutions as extractants in amounts of 10 to 100 times the sample weight.

Samples were incubated under various conditions, with or without the addition of mercury compounds and/or nutrients. By aerating samples placed in columns and washing the eluting air through solutions of 1% Na_2CO_3 anh. + 0.5% $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and 1% HgCl_2 in 0.1 N H_2SO_4 consecutively, volatilized methylmercury was trapped. From the wash solutions methylmercury was extracted into benzene for quantification by GLC.

Rhine sediment samples were taken from anaerobic sediments that were daily flooded with Rhine water in the tidal region of this river; the Yssel sediment was an anaerobic underwater sediment; the Rhine foreland is flooded

during the winter months. Sewage-sludge samples were taken from maturing heaps of digested sewage sludge kept in the open air; some vegetation was already growing on these heaps. The polder soil samples were from the 'Dordtsche Biesbosch', a polder reclaimed from the Rhine delta 50 years ago.

Results and discussion

Total and methylmercury contents of various samples used in our experiments are shown in Table 1. From this table it can be seen that methylmercury almost always makes up less than 1% of total mercury. The Rhine polder soil has a high mercury content compared to normal agricultural soils, that have less than 0.1 ppm Hg, but much lower than the Rhine foreland and Rhine sediments (*cf* Poelstra ⁵). 'Easily extractable mercury' in these samples was at most 0.1 ppm; the recovery of HgCl_2 added to the samples was very low in the NaCl or Na_2EDTA extracts, even after oxidation of the extracts with KMnO_4 . Mercury in these samples was therefore not present in an easily extractable form.

TABLE 1

Mercury contents, in mg Hg/kg dry sample, of some soil, sediment and sewage-sludge samples, used in the experiments

Sample	Code	Total mercury	Methylmercury
Rhine sediment	R1	12-17	0.01-0.11
Rhine sediment	R2	5	0.01
Yssel sediment	Y1	12	0.07
Rhine foreland	Rf	7	0.02
Rhine polder	Rp	0.4-1.1	<0.01
Sewage sludge	S1	30	0.07-0.12
Sewage sludge	S2	6	0.04
Sewage sludge	S3	5	0.2-0.3

Air-dried and then rewetted (to 50% moisture) Rhine sediment R1 showed somewhat more methylmercury, after some weeks of incubation in closed bottles at 15° or 25°C, when 100 ppm Hg as HgCl_2 had been added than without HgCl_2 addition: 0.29 ppm Hg as methylmercury compared to 0.19 ppm on average. In samples that were autoclaved before incubation only 0.09 ppm Hg was found as methylmercury after incubation, although HgCl_2 had been added. In the Rhine polder soil no methylmercury was detectable after incubation, whether HgCl_2 (100 ppm Hg) had been added or not.

From HgCl_2 enriched sediment, bacteria were isolated on a HgCl_2 containing agar medium. Inoculation of sterilized Rhine sediment with these bacteria gave about 0.20 ppm Hg as methylmercury after incubation, whether HgCl_2 (100 ppm Hg) had been added or not. The above results indicate that bacteria may be mediating the mercury methylation.

During constant aeration with 0.5 liter air per hour through air dried and then rewetted samples (150-500 g dry matter) in columns at 29°C, zero up to

TABLE 2

Volatile methylmercury, in $\mu\text{g Hg/kg}$ dry sample, released from aerated columns of soil, sediment and sewage-sludge, previously air dried and rewetted to about 60% of water holding capacity, incubated at 29°C

Sample code	Added mercury compound (mg Hg/kg d.s.)	Methylmercury			Incubation time (days)
		Before incubation	Released	After incubation	
Rp	HgCl ₂	7.4	n.d.*	n.d.	48
R1	—	0	110 ± 10	30 ± 7	60
R1	HgCl ₂	7.4	110 ± 10	38 ± 12	48
R1	HgCl ₂	10	110 ± 10	60 ± 7	32
R1	PMA	10	110 ± 10	50 ± 7	32
Y1	—	0	75 ± 13	n.d.	19
Y1	HgCl ₂	10	75 ± 13	n.d.	19
S1	—	0	70 ± 12	n.d.	30
S1	HgCl ₂	10	70 ± 12	n.d.	30
S3	—	0	250 ± 45	n.d.	42
S3	HgCl ₂	110	250 ± 45	n.d.	65
S3	PMA	100	250 ± 45	n.d.	29
Mixture	HgCl ₂ + PMA	50 + 50	—	0-15	54

* n.d. = not detectable.

60 microgram of mercury as methylmercury was released and volatilized per kg dry sample (see Table 2). Since about the same amount of methylmercury was lost from the samples, methylation of mercury during aeration has not necessarily taken place, perhaps only volatilization occurred. The fact that all the volatilized mercury passed the monomethylmercury trap (carbonate/phosphate solution) and was only captured in the acidified HgCl₂ solution, indicates that it is in the form of dimethylmercury ($\text{CH}_3\text{HgCH}_3 + \text{Hg}^{2+} = 2 \text{CH}_3\text{Hg}^+$). If the trap solutions were used in reversed order methylmercury was also found only in the HgCl₂ solution. If dimethylmercury was released from the samples, the amounts of mercury volatilized as given in Table 2 should be halved. From Table 2 it can also be seen that addition of HgCl₂ of phenylmercuriacetate (PMA) to the samples had, under the chosen conditions, at most a slight effect on the release of methylmercury. Addition of rapidly degradable organic nutrients to sewage sludge S3 plus added HgCl₂ or PMA had no effect. A mixed sample of soil, sediment and sewage sludge released some micrograms of methylmercury only upon simultaneous addition of nutrients and mercury compounds.

We also conducted some experiments under conditions that may be found in underwater sediments. We incubated various amounts of samples at 29°C in 250-ml closed bottles with 40 to 100ml solutions, containing nutrients and/or mercury compounds. Some bottles were put in a sunny place, resulting in the growth of algae. In all cases only very small amounts of methylmercury

TABLE 3

Methylmercury present (in $\mu\text{g Hg}$) after anaerobic incubation of soil, sediment, sewage sludge and compost under nutrient solutions at 29°C

Sample			Added mercury compounds		Methyl-mercury after incubation	Incubation time (days)
Code	Number of samples	Dry matter				
Soil IB 620	1	4.5	HgCl_2 + PMA	500 + 150	n.d.*	9
Soil IB 620**	1	4.5	HgCl_2 + PMA	500 + 150	n.d.	9
R1***	2	5	—	0	1	8-12
R1***	3	5	HgCl_2	500	0.8-1.0	8-12
R1	2	5	HgCl_2	500	1.2-2.8	8-17
R1	4	0.4	HgCl_2	500	0.5-1.7	7-13
R1	3	0.04	HgCl_2	500	0 -0.5	13
R1	1	2.8	—	0	n.d.	8
R1	1	2.8	HgCl_2	500	2.4	8
R1	1	2.8	PMA	150	1.6	8
R1**	1	2.8	HgCl_2	500	0.4	21
R1**	1	2.8	PMA	150	0.4	21
S2	1	2.0	HgCl_2 + PMA	500 + 150	n.d.	10
S4**	1	1.8	HgCl_2 + PMA	500 + 150	1.4	9
S4	1	1.8	HgCl_2 + PMA	500 + 150	1.5	21
S5	1	3.3	HgCl_2 + PMA	500 + 150	n.d.	9
Compost		3.4	HgCl_2 + PMA	500 + 150	n.d.	8

* not detectable amount

** placed in sunlight (growth of algae)

*** no nutrients added

were found after incubation, as is shown in Table 3. The samples were only analysed after incubation. Thus, even in the presence of nutrients and of high concentrations of mercury compounds, rapid methylation did not take place at 'underwater sediment' conditions. Sediment R1 showed more methylmercury upon addition of both nutrients and mercury compounds. The addition of rapidly degradable organic nutrients will enhance microbial activity, possibly also increasing microbial methylation. At the same time the redox potential decreases. Methylation will stop as soon as hydrogen sulfide production gives rise to HgS formation. Mercury sulfide cannot be methylated as such.

Decreasing the amount of sediment did not immediately lead to less methylmercury after incubation. This once more indicates that microbes may be involved in mercury methylation. Where only 0.04 g of sediment was used, all microbial activity may have been inhibited by added HgCl_2 because not all the mercury will be adsorbed by mineral parts of the sediment. Nevertheless in one sample with 0.04 g of sediment, 0.5 μg of methylmercury was found after incubation.

Conclusions

Methylmercury content of soil, sediment and sewage sludge samples is almost always less than 1% of the total mercury present. Total mercury in these samples is not present in an easily extractable form.

Methylation of mercury compounds in these samples was slight or non-existent, under various aerobic and anaerobic conditions, even upon addition of mercury compounds and/or nutrients.

Indications were found that as far as methylation occurs, microbes (bacteria) may be involved.

Some mercury has been released and volatilized from aerated samples, possibly in the form of dimethylmercury.

Since normal agricultural soils have mercury contents of less than 0.1 ppm Hg, methylation is not expected to be a problem.

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Received 5 August 1975

References

- 1 Jernelöv, A. and Jensen, S., *Nature* **223**, 753-754 (1969).
- 2 Hoover, W. L. *et al.*, *Soil Sci. Soc. Am. Proc.* **35**, 850-852 (1971).
- 3 Hatch, W. R. and Ott, W. L., *Anal. Chem.* **40**, 2085-2087 (1968).
- 4 Westöb, G., *Acta Chem. Scand.* **22**, 2277-2280 (1968).
- 5 Poelstra, P. *et al.*, *Neth. J. Agr. Sci.* **21**, 77-84 (1973).