Bull. Environm. Contam. Toxicol. 29, 167–173 (1982)

Effect of Microbial Life Stages on the Fate of Methylmercury in Natural Waters

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It is well established that methylmercury can be produced from inorganic mercury in natural waters by both biological and non-biological means (WOOD et al. 1968; JENSEN AND JERNELOV 1969; LANDNER 1971; AKAGI et al. 1977). The toxicity of methylmercury to aquatic organisms depends, among other things, on its residence time and stability in water systems. The biological half-life of methylmercury in humans has been estimated as 70 days (ABERG et al. 1969; TASK FORCE ON METAL ACCUMULATION 1973). Earlier studies (FURAKAWA et al. 1969; SPANGLER et al. 1973a; BILLEN et al. 1974; BARTLETT et al. 1977) have focussed on determining the types of bacteria involved in the decomposition of methylmercury. We have now studied the transformations of methylmercury in water under a variety of conditions somewhat similar to those found in nature.

These transformations were mainly studied in samples of Ottawa River water, to which had been added a natural organic-rich clay sediment (RAMAMOORTHY AND RUST 1978), living or thermally killed bacteria (Escherichia coli and Pseudomonas fluorescens), or the blue-green alga Anabaena flos-aquae. In addition, transformations of methylmercury caused by growing bacteria were studied.

EXPERIMENTAL

In these experiments, known amounts of labelled ($CH_3{}^{203}HgCl$) and unlabelled methylmercury chloride were added to the systems studied so that the final Hg concentration was 0.1 mg/L. This concentration was intended to simulate the severe mercury contamination conditions of the natural waters. At appropriate times mercury compounds were extracted by dithizone and separated by thin-layer chromatography (TLC). The detailed procedure is shown in Fig. 1.

This procedure led to a very good separation of methylmercury from inorganic mercury (Fig. 2). A quantitative (100%) recovery of the added mercury was noted following this method of separation. In systems containing heterogenous phases, these were separated by filtration (millipore filters, pore size 0.45 µm) and/or centrifugation. Counting of the filters themselves showed that no significant adsorption of mercury on them had taken place.

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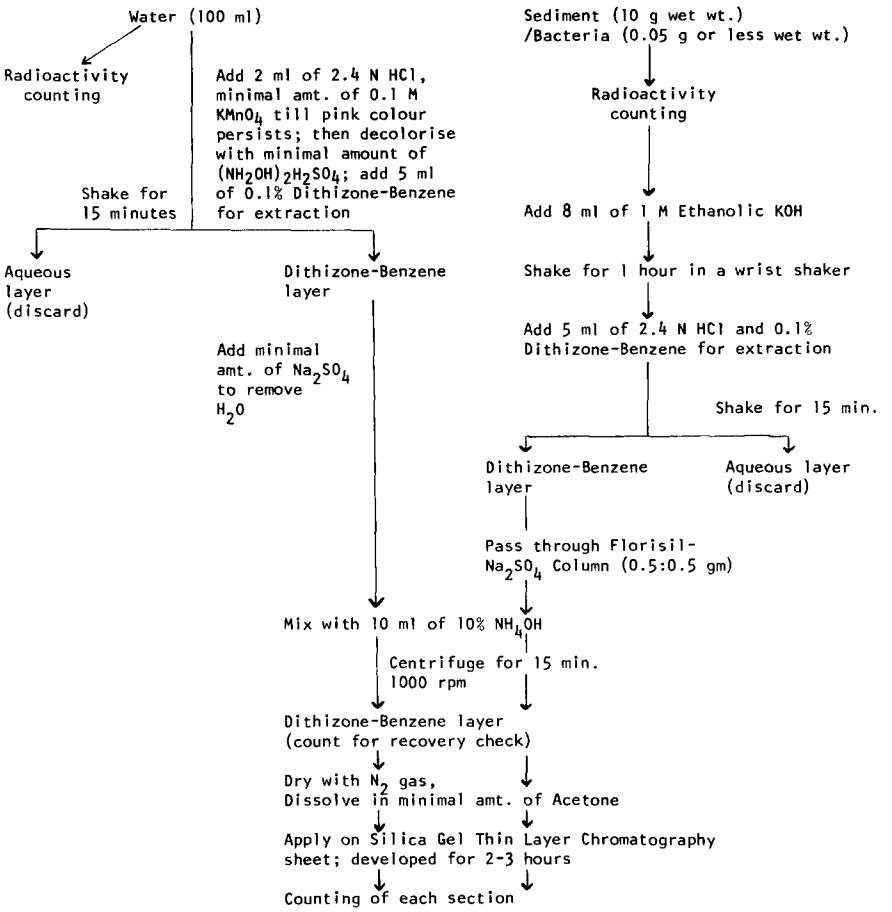

Fig. 1. Flowsheet for the procedures of the analyses of mercury species

The stock solution of methylmercury prepared for the first experiments contained 10% inorganic mercury. This proportion did not change when the stock solution was added to deionized water or to Ottawa River water and incubated for up to 28 days.

RESULTS AND DISCUSSION

When sediment was included in the river water system, the MMC was sorbed onto the sediment, reaching a maximum in 7 days (Fig. 3). At first, no conversion of the MMC occurred, but

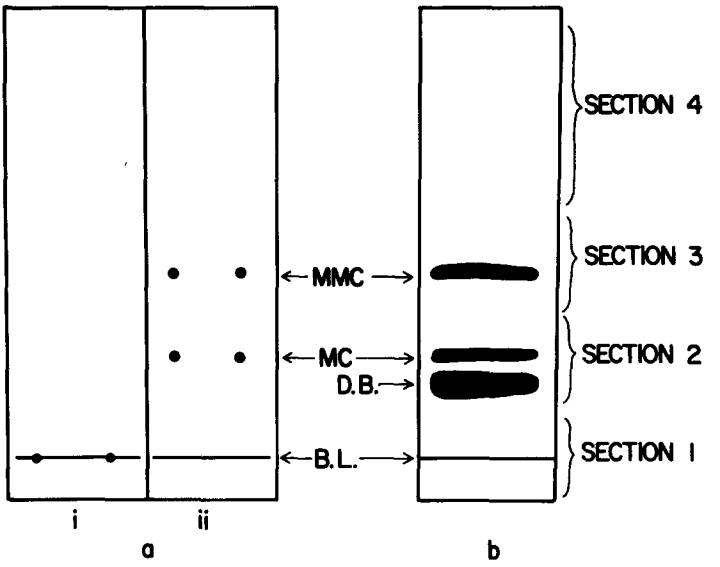

Fig.2. TLC separation of methylmercury and inorganic mercury in standards (a) and in samples (b).
a(i) Dots represent a mixture of standard methylmercury and inorganic mercury dithizonates before developing.
a(ii) After developing with a mixture of benzene-hexane.

B.L. = base line; D.B. = dithizone band; MC = inorganic mercury; MMC = methylmercury.

after 7 days 35% MC was produced, and 55% in 28 days. Almost all of this MC was found bound to the sediment, which has a high affinity for it (RAMAMOORTHY AND RUST 1976).

Living microorganisms had a strong effect on the fate of MMC. A suspension of P. fluorescens sorbed 81.3% of added mercury in the first 12 hr. Most of the added MMC (73%) was converted to MC and was found bound to the cells in 24 hr, and the amount increased to 90% in 72 hr. The water phase contained very small amounts of MC and MMC. E. coli also took up MMC rapidly, but the rate of conversion to MC was slower; 36.4% in 3 days and 50.6% in 7 days. Again, the water phase contained very small amounts of MC or MMC. Experiments with different amounts of bacteria (P. fluorescens) showed that, as expected, the rate of transformation of MMC to MC increased with the concentration of cells.

Anabaena flos-aquae sorbed 81.8% of the added MMC in the first 12 hr; by 3 days, 30% of the added MMC had been converted to MC, and 83.4% by 7 days, with most of the MC being bound to the cells (TABLE 1).

TABLE 1

Distribution of mercury species in different phases of natural water systems at 7th day

Phases	MC(%)	MMC(%)
Deionized water	12.3	86.0
Deionized water†	27.9	71.6
Ottawa River water	11.6	87.2
Ottawa River water†	28.1	71.2
Water column overlying sediment	1.2	0.5
Sediment	34.1	62.8
Water column overlying living *Pseudomonas fluorescens*	4.6	1.5
Living *Pseudomonas fluorescens*	90.0	3.7
Water column overlying thermally killed *Pseudomonas fluorescens*†	1.7	32.4
Thermally killed *Pseudomonas fluorescens*†	14.5	51.4
Water column overlying living *Escherichia coli*	2.0	2.0
Living *Escherichia coli*	50.6	44.5
Water column overlying thermally killed *Escherichia coli*†	1.9	35.2
Thermally killed *Escherichia coli*†	14.2	48.7
Water column overlying living *Anabaena flos-aquae*	4.8	0.6
Living *Anabaena flos-aquae*	83.4	8.7

Amount of sediment = 10.00 gm in wet weight
Amount of biota = 0.45 gm in wet weight
Added mercury concentration = 0.10 ppm(μg/ml)
MC = Inorganic mercury
MMC = Methylmercury
† Experiments done 10 months after

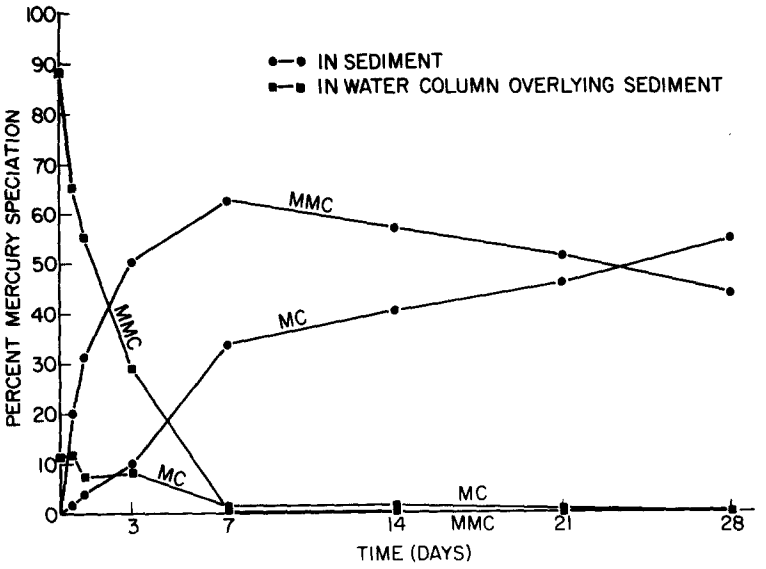

Fig. 3. Stability of methylmercury in Ottawa River water-sediment system.

Viable cell counts of bacterial suspensions incubated in the presence of MMC showed that in the first six hours 30% P. fluorescens or 37% E. coli died; after 7 days about 50% of the P. flourescens and 60% of the E. coli had died. In order to determine if the transformations caused by bacterial suspensions could be ascribed to the dead bacteria, experiments were performed in which the bacteria were killed by boiling (100°C for 30 minutes) before MMC was added.

The stock solution of MMC used for these later experiments contained 28% MC. Almost all of the MC and most of the MMC was bound to the bacterial cells within 12 hours. However, no transformations of these compounds took place for 3 days. After 7 days about half the MC was transformed to MMC; at this time, too, a substantial proportion of the MMC was released into the water column overlying the dead cells (TABLE 1). Transformation of MC to MMC by sterilized sediment (which would contain many dead bacteria) and by sterilized soil (ROGERS 1977) has been previously noted. The failure of the killed bacteria to demethylate MMC shows that such action by bacterial suspensions is due to living cells.

Transformations of MMC and MC were also studied in cultures of bacteria growing in complex media (Peptone-Yeast Extract Broth) (Fig.4). MMC was quickly converted to MC by P. fluorescens. Most of the Hg^{2+} ions were found in the external medium due perhaps to the high binding ability of the latter for Hg^{2+} ions (RAMAMOORTHY AND KUSHNER 1975); the amount in the external medium was less in a more dilute complex medium (not shown).

These experiments were carried out in flasks capped loosely with sterile cotton plugs. The eventual loss of most of the added MMC seems due to its transformation to the volatile HG^{o} form, a reaction that is known to occur with P. fluorescens and with many other bacteria (FURAKAWA et al. 1969; SPANGLER et al. 1973b; NELSON et al. 1973). In growing E. coli, however, there was no transformation of added MMC for 13 hours, although growth was complete by that time.

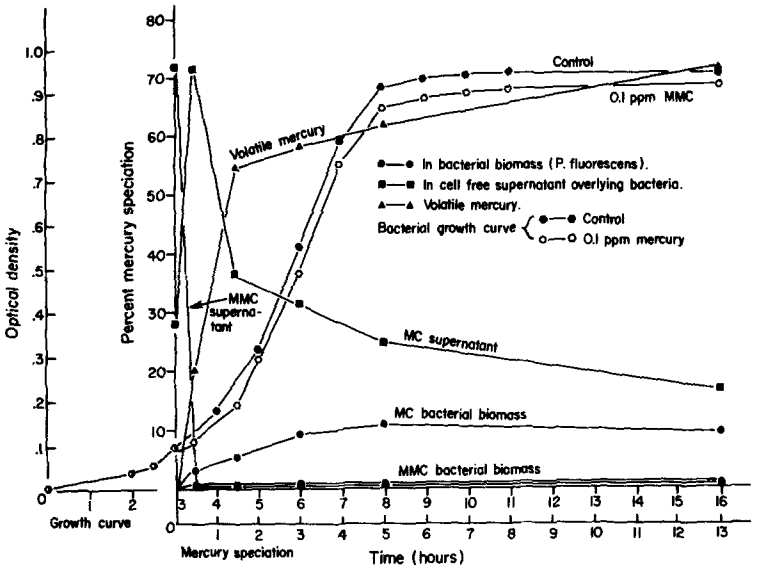

Fig. 4. Transformation of methylmercury in cultures of growing bacteria in Peptone-Yeast Extract Broth medium.

Our results show that growing bacterial cells can transform MC and MMC to the volatile form, readily lost to the aquatic environment. However, living but non-growing bacterial and algal cells cause the demethylation of MMC to MC, and dead bacterial cells can lead to the methylation of MC to MMC. Thus, the physiological state of microorganisms in aquatic systems can greatly affect the way in which these microorganisms deal with Hg compounds, their speciation and hence the amount of the toxic compounds present. Our observations suggest that for meaningful survey of methylmercury the samples should be stored properly and analysed with minimum storage time.

The experimental work was carried out when S. R. was at the National Research Council of Canada, Ottawa.

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Accepted June 8, 1982