Factors Influencing Translocation and Transformation of Mercury in River Sediment

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It is well recognized that most mercury residues in aquatic environments are found in the sediment (1-4). However, the mechanism of release and transfer of mercury from sediment to water, and eventually to living organisms is unknown.

There are, for instance, a number of river systems where mercury levels in fish run high despite low mercury levels in associated sediments (1,2). Recently, Konrad (1) compared the levels of total mercury in sediment against those of fish from the same area in various river systems and found that levels in fish were high whenever the pH of the sediment and water were below 7. Klein and Goldberg (5) found high levels of mercury in ocean sediments near sewer outfalls. They concluded that marine organisms play important roles in dispersing mercury.

Samples of sediment were collected from the Chippewa and Wisconsin River (6) systems along with water from the same site. The Chippewa river sample, used throughout the experiment, was collected in March, 1971, in Chippewa Falls, Wisconsin. Initially, 5 ml of sediment with 5 ml of surface water were transferred in a 30 ml sterile test tube. After the addition of a 10 µl aliquot of 10^{-3} M of $^{203}HgCl_2$ solution (in distilled water) and other test substances, the tube was capped with a sterile cotton plug. The system was shaken once to suspend sediments which precipitated, and was kept at $4^{o}C$ for 30 days. The effects of pH were also studied using the same system. Appropriate aliquots of 0.2 to 1 N-HCl or 0.1 to 1 N-Na_2CO_3 were added to the system and the pH of the water brought to the desired level.

After incubation, the system was centrifuged for 10 min at 3000 g to separate "water" and "sediment". The former was further extracted twice with 5 ml each of toluene. The sediment was twice extracted in 5 ml each of acetone. The acetone was evaporated with a stream of air and the residue partitioned between 5 ml of toluene and water. All toluene extracts were combined and designated as "organic mercury" fraction. The remaining sediment was first dried and then resuspended into 5 ml of distilled water, and a 1/10 portion then directly radio-assayed.

Bulletin of Environmental Contamination & Toxicology,
Vol. 8, No. 5, 1972 © by Springer-Verlag New York Inc.

The combined toluene extracts was also examined, using thin-layer chromatography, for its alkylmercury content (7). The same experimental approach was taken to study the binding and release of $^{203}Hg^{++}$ in several samples of sediments collected from various water systems. The anaerobic condition (Table 1) for sediment samples was provided by maintaining the sample tubes in a N_2 incubator for 30 days at 4°C.

A Sephadex G-25 column was employed to analyze released radioactivity and was identical to the method published previously (7). The pH of the eluents was controlled by either tris-HCl (0.1 M) buffer or addition of an unbuffered HCl-NaOH (0.1 M) solution.

The methods employed to isolate and culture microorganisms from water and sediment samples were identical to ones previously published (7) except that for some anaerobic cultures a NIH-thioglycolate medium (9) was used under CO_2.

To study the effects of various environmental conditions on the rate of mercury release a number of substances were added to the incubation system. A sediment sample from Chippewa Falls was used. It is a sandy sediment with 0.3% of organic matter (0.013% N), 4% of silt, 6% of clay and 90% sand. It contained 22.1 ppm of SO_4-S. The result (Table 1) shows first that Hg^{++} is largely bound to the sediment particles, thus confirming Jernelöv's finding (2). The substances which increased the amount of radioactivity in supernatant were EDTA, FAD, glutathione, and glucose. It is likely that the effects of EDTA addition is due to its chelating action, and that of glutathione addition is due to -SH binding. However, some microbial growth was observed in the glucose incubated system and it is possible that this had some effects on the increase of mercury in the water. On the other hand $SnCl_2$, probably by reducing Hg^{++} to Hg^0, reduced the amount of mercury in water. Other chemicals were also tested. The percentages of Hg^{++} remaining in water for those experiments were: 1.4% (cysteine 2 x 10^{-5} M), 4.5% (ascorbic acid, 10^{-4} M), 5.2% ($FeSO_4$, 10^{-4} M), 5.0% ($KMnO_4$, 10^{-4} M), 3.5% ($FeCl_3$, 10^{-4} M), 9.6% ($CaCl_2$, 10^{-4} M), 2.5% (sucrose, 10^{-3} M), and 7.9% (NADPH, 2 x 10^{-4} M), respectively.

By far the most profound effect, in terms of increased mercury levels in water, was observed when the pH of the system was increased above 7 (Table 1). A general survey was also made on several samples of sediment and water from several sources to correlate the levels of mercury in the water to the pH of the sample. The high mercury sample was collected near a chemical plant. Its mercury level was determined by Iskandar et al. (unpublished data) to be 800 ppm. Total organic matter was 12.23% (0.467% N). Sand, silt, and clay contents were 37, 52, and 11% respectively. It contained 4,666.5 ppm of SO_4-S. The results show a general tendency toward high levels of mercury remaining in water with samples of higher pH.

TABLE 1.

Factors influencing the degrees of absorption of Hg^{++} to the river sediments: Results shown by the percentages of originally added $^{203}Hg^{++}$ (10^{-8} mole) recovered in each fraction.

Sediments	Conditions	Final pH	% Remained in water	% Absorbed to sediment	% Organic** mercury
Natural samples					
Chippewa Falls	control*	6.5	6.0	89.2	4.8
Wisconsin River	low Hg	7.0	8.9	83.7	7.4
	medium Hg	7.25	44.3	49.9	5.8
	high Hg	7.10	40.4	47.6	12.0
Chippewa Lake	low Hg	6.0	19.5	68.1	12.4
Anaerobic condition					
Chippewa Falls	N_2	6.7	13.6	74.4	12.0
pH artificially adjusted					
Chippewa Falls	HCl	3.95	3.0	92.3	4.7
	HCl	4.95	3.0	92.4	4.6
	HCl	6.10	6.6	88.6	4.8
	Na_2CO_3	7.15	2.5	93.1	4.4
	Na_2CO_3	8.05	19.9	76.0	4.1
	Na_2CO_3	8.95	60.0	35.0	4.1
Chemicals added					
Chippewa Falls	EDTA (10^{-3}M)	6.4	44.6	49.2	6.2
	FAD (10^{-3}M)	6.5	22.4	74.9	2.7
	GSH (2×10^{-5}M)	6.1	17.4	78.5	4.1
	Glucose (10^{-2}M)	5.8	13.6	82.7	3.7
	H_2S (gas)	6.3	6.0	79.9	14.1
	$SnCl_2$ (10^{-4}M)	5.8	1.9	92.5	5.6

* The sample served as the control for all subsequent tests.

** Toluene extractable mercury compounds from both water and sediments.

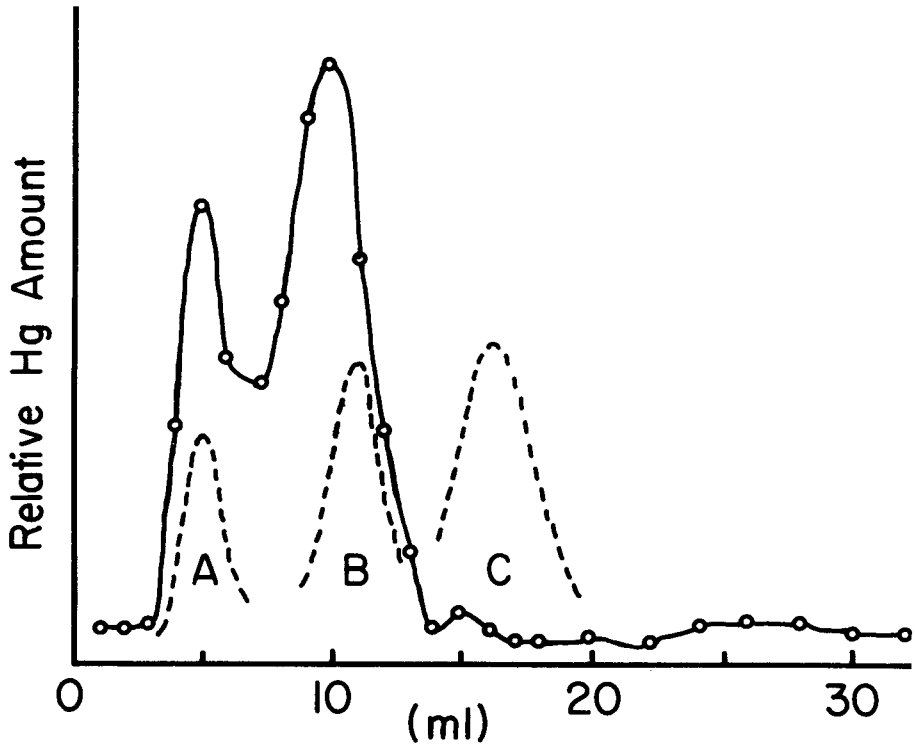

Figure 1.

Sephadex G-25 column elution patterns of (A) ^{203}Hg radioactivity eluted at pH 9 after incubation of $^{203}HgCl_2$ with prewashed (at pH 7) sediment, (B) radioactivity eluted at pH 9 after incubation of $^{203}HgCl_2$ with distilled water, (C) same eluted at pH 3.5 and (D) radioactivity eluted at pH 9 after incubation of $^{203}HgCl_2$ with natural sediment. All sediment samples used were from Chippewa Falls (5 ml of sediment plus 5 ml of surface water per sample). Each sample was incubated with 0.1 μmole of $^{203}HgCl_2$ for 24 hours at room temperature. The ratio between peak A and peak B varies from sample to sample in the case of natural sediments, but always two peaks were observed in the same positions.

To study the mechanisms of such pH effects, efforts were made to study the nature of mercury components in water. First, the sediment (5 ml sediment and 5 ml of tris-buffer) was incubated with 0.1 μmole of $^{203}HgCl_2$ at pH 9 for 24 hours. One ml of the supernatant was then analyzed on a Sephadex G-25 column (Fig. 1). It was found that the radioactive peak was eluted at 5 ml and 10 ml, indicating the presence of mercury in macromolecule form. The first peak aligned with protein macromolecules with molecular weight above 1 million (peak A) eluted from soil, and the second peak coincided with the radioactivity peak of mercury alone at pH 9 (i.e. $^{203}HgCl_2$ incubated with tris-HCl buffer at pH 9, peak B). At pH 3 (adjusted by (HCl) Hg^{++} was found to be eluted at 16 ml (peak C) where substances with M. W. 200 are expected to come out. It appears therefore that release of mercury into water from sediments in this case is controlled by two factors: the first being its relation to organic matter (peak A) at high pH, and the second to changes in the state of mercury itself at high pH (peak B). The magnitude of such a mercury complex at high pH appears to be highly significant. Since the same macromolecule can be produced by adding NaOH to an aqueous solution of HgCl to make pH = 9, it is likely that the state of mercury in the solution is that of a $HgO\text{-}Hg(OH)_2$ complex. Its molecular weight was estimated to be a few thousand by using a 60 cm Sephadex G-25 column.

The above studies established that physico-chemical factors are important in influencing the rate of mercury (including organic matter-bound Hg) release. The degree of influence of microorganisms on the fate of mercury is, however, not clear. For instance, in one of the above experiments considerable microbial growth was found to be associated with high amounts of mercury in water. From these sediments and water, microorganisms were isolated and incubated with $^{203}HgCl_2$ for 10 days. Cells were harvested through centrifugation, and in all cases, microorganisms absorbed almost all of the available mercury. Upon acidification and extraction of the total incubate into toluene in the presence of NaCl (8), only small amounts of radioactivity were found to be convertible into RHgX or R_2Hg (R:alkyl or aryl) forms. Radioautographic examination of the thin-layer chromatograms (7) revealed that almost no methylation had taken place.

To study the relative bonding of mercury with the sediment, a $^{203}HgCl_2$ incubated sample (Chippewa Falls) was first washed three times with potassium phosphate buffer (0.1 M) at pH 7. The sample was then suspended with 5 ml of tris-buffer (0.1 M) at pH 9. The supernatant was analyzed on the same Sephadex column. Only peak A was observed by this treatment, showing that pH 7 washing eliminated all the peak B or peak C material (Fig. 1).

While the phenomenon of mercury release has been partially clarified in these studies, its implications in the environment is far from answered. It is not yet certain that such forms of mercury released into water immediately becomes available to fish and other biological systems, or that they are in forms capable of

being readily taken up by biological systems. For instance, it is difficult to explain the findings of Konrad (1) unless one considers that river systems with high pH readily leach out available mercury. If such is the case, a high accumulation of mercury would be expected in slow exchanging lakes with high pH. Available data are not sufficient to prove or disprove such a possibility.

In summary, from these studies, three factors contribute to the release of mercury into water: alkaline pH, the organic content of sediments, and microorganisms. Such processes are not necessarily directly related to methylmercury formation. The significance of such release is not certain.

Acknowledgements

We thank Dr. J. K. Syers and Dr. D. R. Keeney for supplying some of the sediment samples and the data on mercury levels, and Dr. Thomas W. Chapman for technical suggestions. Supported in part by research grants, EP00809 from the Environmental Protection Agency; and grant number 133-8232 from the Department of Natural Resources, State of Wisconsin.

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