1977

Biologically Initiated Auto-Catalytic Mercury Conversion And Its Effect On Elemental Mobility

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INTRODUCTION

Environmental mercury contamination, whether by elemental, inorganic or organometallic compounds, has been recognized recently as a serious water quality problem in many areas of the world. In the past, the general consensus had been that mercury entering lakes or rivers was rapidly removed from the water phase by either chemical or physical interactions with suspended solids and underlying sediments. Furthermore, it was assumed that once contained in the sediments it remained in a relative inert, biologically unavailable form. Recent studies, however, have shown that the aforementioned is not the case; mercury compounds are readily translocated through the water column, concentrated via the food chain, and subsequently reach as well as accumulate in man. Although the interest in mercury as a pollutant is relatively new to the western hemisphere, this has not been the case in other areas of the world. Japan and Sweden have had to cope with the problem for years.

The most notable examples of environmental contamination with mercury occurred in Japan between 1953 and 1970 (1, 2). In Minamata, between 1953 and 1961, 121 fishermen and their families were stricken with a mysterious illness characterized by cerebellar ataxia, constriction of visual fields, and dysarthria. Of these 121 cases, a total of 46 deaths resulted. Additional cases of mercury-induced poisoning, termed "Minamata Disease," were seen in the coastal town of Niigata and in the riverside villages along the Agano River between 1965 and 1970. Six persons died and another forty-one were irreversibly poisoned. In both incidents, the disease broke out mainly among fishermen and their families, and also among other people who fished frequently and/or liked to eat locally caught aquatic produce. Characteristically, the patients in Minamata as well as in Niigata had eaten a great

amount of fish and/or shellfish from contaminated waters.

Even though no deaths were reported in Sweden, the mercury pollution problem became apparent after seed-eating bird populations began to decrease drastically. This conclusion resulted from a study of museum specimens which showed that mercury levels in bird feathers were nearly constant from 1840 and 1965 (3), coincident with the introduction of alkylmercury compounds used as anti-fungal seed dressings (4).

Although mercury and its compounds have long been known to be toxic, it has not been generally recognized that hazards could arise from the disposal of mercurials into aquatic environments nor was it recognized that mercury could undergo a myriad of biochemical transformations. Recent studies (5-8) indicate that many common inorganic and organic mercury compounds which are discharged by industry into public waters, settle in bottom muds and are converted into alkylmercury compounds, i.e.: mono- and dimethylmercury. Even though both inorganic and organic mercury compounds enter natural waters, mono- and dialkyl forms of mercury present the greatest threat to all food chains due to their mobility in water and their solubility in membrane lipids. Mercury present in fish as well as other aquatic organisms is almost entirely in the methylmercury form.

In order to overcome environmental problems caused by mercury, it is essential to understand the fate of mercury in aquatic ecosystems. Several interesting questions have been posed by these observations, and therefore the objectives of this research were: (a) to study the dynamics of inorganic - organic mercury transformation _in situ_, in a model lacustrine environment; (b) to follow the fate of mercury, so mobilized through the various trophic levels of a typical food chain; (c) to elucidate the role of microparticu- lates in the active and/or passive transport of mercury; and (d) to evaluate

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the hypothesis that a mercury cycle, as such, does indeed exist in nature. Utilizing recent advances made in the microbiologist's armamentarius of techniques, the following study was initiated.

LITERATURE REVIEW

Sources of Mercury in the Environment

Prior to the turn of the century (3, 9, 10), mercury release into the
environment was largely accounted for as the result of natural actions, _viz._
via: (a) the weathering of crustal rocks (11-13), and (b) vulcanism and/or
evaporation from deposits (14). The form in which mercury appears in rocks
is not entirely clear, however, it is probably reduced to the metallic form
at magmatic temperatures, vaporized, and eventually combined with residual
sulfur to form the sulfide, cinnabar (15). In weathering reactions, these
sulfides may be oxidized to the metal ($Hg°$) on to the soluble mercuric ions
(H_g^{2+}). Whether released in solution or the solid form, it is clear that
most of the element traverses the natural water systems in association with
the particulates held in suspension (16-20) or in their underlying bed sedi-
ments (8, 21, 22). Mercury in the atmosphere ultimately reaches the earth
either by dry fallout or by precipitation and it is captured by the soil
whereby rainfall-induced erosion and leaching return it, in part, to these
same streams or other waters.

With the advent of civilization, human activities have had a profound
impact on the release of mercury and/or its compounds into the environment.
For centuries, man's contribution was mainly limited to its release through
the atmosphere from fossil fuels such as coal (23), lignite and/or petroleum
(24). As industrialization developed, smelting processes for other metals.
the ores of which contain mercury, added to our contribution. As man devel-
oped sophisticated needs, numerous and varied uses for mercurials were found.
While some are conservative of the metal, others allow leakage, and still
others deliberately introduce mercury compounds into the environment. Among
those promoting leakage, the use of the flow mercury cathode cell, to

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produce chlorine and sodium hydroxide by electrolysis in the chlor-alkali

industry is among the greatest offenders (25). Recognition that metallic

mercury through its oligodynamic action could function as an insecticide (26)

opened the door for the use of mercurials in the agriculture industry. Use of

mercury compounds in the production of fungicides, which are employed as seed

dressings, foliage sprays, and for garden and lawn applications, as well as

slimicides in the pulp and paper industry are among the major deliberately

introduced sources of this element in the environment. In addition to con-

tamination of waterways by the effluents from hospitals, dental facilities,

chemical laboratories and homes, mercury compounds are frequently formed in

side reactions when used as catalysts in the manufacture of other chemical

compounds. Subsequent studies revealed that methylmercury was entering Mina-

mata Bay and the Agano River near Niigata through waste effluents discharged

from vinyl chloride and acetaldehyde manufacturing plants. It was these

sources of mercury that proved to be the etiology of "Minamata Disease" in

Japan.

Since this tragic occurrence of mercury poisoning at Minamata Bay (1)

and Niigata (2) considerable attention has been directed towards an elimina-

tion and understanding of the occurrence of these complexes in the environment.

Almost simultaneously, the Swedes (3, 4, 27) demonstrated that mercury

toxicity was the cause of widespread mortality in seed-eating birds (pheas-

ants, partridges, pigeons, finches) following the introduction of mercurial

fungicides, such as methylmercy dicyandiamide, as seed dressings in their

agrarian programs.

More recently, detection of abnormally high concentrations of mercury

compounds in fish caught in Lake St. Clair and Lake Erie brought the problem

of mercury contamination of natural water systems to public attention in

North America (25).

In retrospect, it appears that the North American continent is no exception to trends seen elsewhere in the world--considerable amounts of mercury and/or its compounds have also been released into the environment and that most has found its way into natural water systems. This has been documented in the United States (28) and is undoubtedly true in other countries.

Mercury Conversion in the Biosphere

Generally speaking one finds mercury being discharged into nature in one of the following forms: (a) as metallic mercury, Hg°; (b) as inorganic divalent mercury, Hg^{2+}; (c) as phenylmercury, $C_6H_5Hg^+$; (d) as methylmercury, CH_3Hg^+; (e) as dimethylmercury, CH_3HgCH_3, or (f) as alkoxi-alkylmercury, $CH_3O-CH_2-CH_2-Hg^+$.

To understand the ecological effects of the different kinds of discharges and the risk factors involved, the transforming reactions between the different compounds of mercury in nature are of central significance. The consequences of these transforming reactions are particularly obvious when it concerns the deposits of mercury in the sediments of lakes and rivers, which can be mobilized through conversion to other, more hard-to-bind forms. These deposits are primarily made up from phenylmercury found in fiber banks, downstream from pulp and paper mills, and inorganic mercury, either metallic or divalent with its high affinity for organic mud, in bottom sediment. Oxidation of metallic mercury (Hg°) to divalent mercury ions (Hg^{2+}) can and has been shown experimentally (29) to occur under conditions present at the bottoms of lakes and rivers.

Despite the fact that most inorganic mercury, as previously mentioned, is found in association with suspended solids (16-20) or immobilized in the

sediment (8, 22) and does not often exist in hazardous concentration in solution, it serves as a ready reservoir for alteration by microorganisms (7, 30-32). Investigations (31) have shown that Hg^{2+}, whether discharged initially in this state or chemically oxidized from metallic mercury via the method described by Jernelöv (29), is methylated in waters and natural sediments by bacteria under anaerobic conditions, be it enzymatically as with the methanogenic bacteria or non-enzymatic via the transfer of methyl groups from Co^{3+} to Hg^{2+} in biological systems. Fagerström and Jernelöv (33) reported that methylation also occurred in the top layer of sediments if they were continuously oxygenated. Furthermore, all microorganisms capable of synthesizing alkyl B-12 type compounds are capable of CH_3Hg^+ synthesis (34, 35). From experimental data, it appears that all forms of mercury may be converted directly or indirectly to either mono- or dimethylmercury (29). An alkaline pH favors a higher proportion of CH_3HgCH_3 as related to CH_3Hg^+ because the former is rapidly degraded to the latter in acid conditions. Additionally, it has been found (36) that mercury methylation rates are influenced by a number of environmental and biological parameters, such as: pH, high organic sediment index (i.e., the product of the percent organic carbon and organic nitrogen in a given sample), increased microbial activity, and elevated mercury concentrations. In addition to their ability to methylate mercury, microorganisms may also degrade organic mercury compounds (37-41). Evidence suggests that microbial decomposition of organomercurials involves cleavage of the $C-Hg^{2+}$ bond, reduction of Hg^{2+} to $Hg°$, and liberation of the corresponding alkanes (42). For example, a member of the genus Pseudomonas isolated from the soil and grown in a medium containing phenylmercuric acetate (PMA) appeared to bind PMA to the cell surface prior to being reduced to metallic mercury (43). It was shown that a reduced

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nicotinamide adenine dinucleotide (NADH) generating system and a sulfhydryl compound were required to form $Hg°$. Thus, the common intracellular reductant NADH may be responsible for mercury metabolism in microorganisms. Similarly, $C_2H_5Hg^+$, $C_6H_5Hg^+$, and CH_3Hg^+ were degraded to $Hg°$ and ethane, benzene, and methane, respectively (42). Relatedly, Spangler et al. (44) isolated 207 bacterial cultures from fish and sediments taken from Lake St. Clair. Thirty cultures were capable of aerobic demethylation with twenty-two and twenty-one of the above thirty being facultative anaerobes and anaerobes, respectively. These authors further showed that the degradation of organomercury was a reductive demethylation reaction resulting in the formation of methane and inorganic ($Hg°$ or Hg^{2+}) mercury.

Microbial conversion of inorganic reserves to organomercury compounds concurrently demonstrate increased solubility in the overlying water, thus improving elemental mobility within the suspending matrix, in addition to increasing their solubility in the lipid components of biological active membranes. Whether the aquatic protists can extract methylated mercury compounds from water in preference to the assimilation of inorganic compounds directly from the surrounding medium (45-47) or not, they are able to concentrate the mercury within themselves to levels considerably higher than those prevailing in their environment. Further up the food chain the mercury concentration in organisms (48-53) increases either by absorption directly from the milieu, with food sources from lower trophic levels (54), or by a combination of both means. Should these organisms, be they macro or micro in nature, remain in the same locale until their demise, the mercury in their cells and/or tissues can be returned to nature in several ways, namely: (a) evapoation as dimethylmercury through decomposition; (b) return to the sediment pool via reductive demethylation, or (c) volatilization of

methylated mercury compounds in sediments and soils.

What then is the ultimate fate of a mercury contaminated aquatic environment? Although a modicum of mercury is no doubt removed from a given locale through vaporization and movement of macroflora and fauna, mobility of the biomass and colloidal particulates--be it voluntary or not--is likely to remove more mercury. Thus if a mercury source is depleted, a body of water theoretically could be expected to cleanse itself of its mercury burden. In fact, however, the likelihood of this occurring has recently been diminished by experiments showing the rate at which microbial methylation in bottom sediments brings about the mobilization of bound mercury (29), the presence of a depot of readily available mercury within the sediment bed, and the replenishment of said depot by reductive demethylation (39, 44).
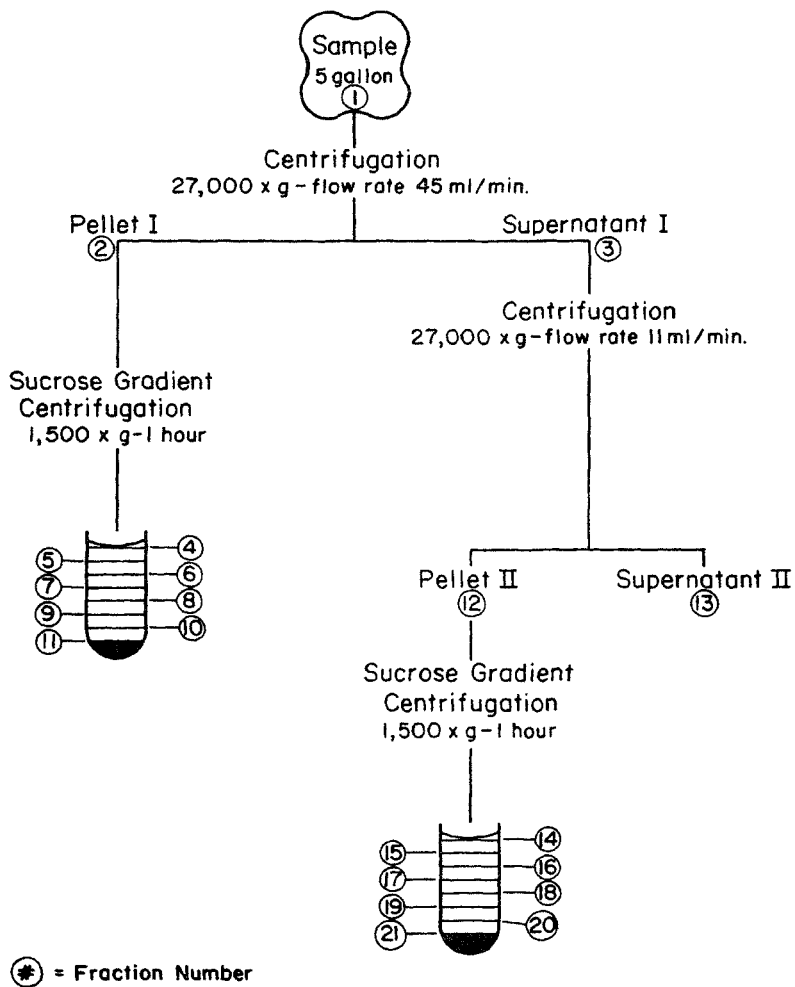
MATERIALS AND METHODS

Sample Collection

Between September, 1967 and June, 1969, 5 gallon water samples were collected by boat at various stations in the western basin of Lake Erie. Each sample was collected using a hand pump from midway between the water surface and the lake bottom (usually 15 to 20 feet in the western basin). All samples were stored in chemically cleaned glassware at 4°C until transportation, processing and/or testing could be accomplished. Many of the 360 samples analyzed were collected prior to and during the major mercury scare in the lake.

Sample Processing (Figure 1)

Upon reaching the laboratory, 100 ml aliquots of each sample were removed for analysis. The remainder of each 5 gallon sample was processed by continuous-flow high-speed centrifugation (Sorvall RC-2B equipped with a Szent-Gorgi continuous-flow attachment) at 4°C with a 45 ml per minute flow rate and a gravitational force of 27,000. This permitted the removal of particles down to 0.3 μm. The supernatant was then passed through the centrifuge at 27,000 x g with a flow rate of 11 ml/min to affect the removal of colloids down to 0.1 μm. Solid residue from these fractionations were subsequently placed on top of gradients constructed of sucrose with a linear density of 1,0765 (19% wt/vol) to 1.2241 (49% wt/vol) and centrifuged at 1,500 x g for 1 hour (55-57). Bands were collected by using a Beckman tube-cutting device and the particulates contained therein were either dialysed (utilizing Union Carbide dialysis tubing) against or washed in "mercury-free diluent" (see below) by high-speed centrifugation (27,000 x g for 30 min).

Figure 1.

Flow Diagram of Procedure Utilized During Experimentation

Sample
5 gallon
①

Centrifugation
27,000 x g – flow rate 45 ml/min.

Pellet I
②

Supernatant I
③

Centrifugation
27,000 x g – flow rate 11 ml/min.

Sucrose Gradient
Centrifugation
1,500 x g – 1 hour

⑤ ④
⑤ ⑥
⑦ ⑧
⑨ ⑩
⑪

Pellet II
⑫

Supernatant II
⑬

Sucrose Gradient
Centrifugation
1,500 x g – 1 hour

⑮ ⑭
⑰ ⑯
⑲ ⑱
㉑ ⑳

✱ = Fraction Number

Total Mercury Determination

Utilizing the technique of Hatch and Ott (58) in conjunction with the apparatus described below (Figure 2) the total mercury content of each fraction was determined by flameless or cold vapor atomic absorption spectrophotometry. Aliquots of each specimen under study were transferred to 250 ml round-bottomed flasks. To the contents of each flask were added 25 ml 18 N sulfuric acid, 10 ml 7 N nitric acid, and enough "mercury-free diluent" to make 100 ml. Treating each reaction flask individually, 20 ml of a sodium chloride-hydroxylamine sulfate solution (60 ml of a 25%, wt/vol, hydroxylamine sulfate and 50 ml of a 30%, wt/vol, sodium chloride solution diluted to 500 ml with "mercury-free diluent") followed by 10 ml of a 10% wt/vol stannous sulfate solution in 0.5 N sulfuric acid was added. Immediately the reaction vessel was attached to the aeration apparatus forming a closed system. The mercury vapor thus produced was analyzed for its absorption at 2535 $\overset{o}{A}$ in a quartz-windowed cell. Absorbance values displayed on the digital readout were recorded for 4 min at 30 sec intervals. These readings were averaged, reduced by that of the reagent control and utilized for calculating the total mercury content of a given sample by comparison with curves prepared from known standards, e.g.: Figure 3.

"Mercury-Free Diluent"

All water utilized to make reagents as well as dilutions was triple distilled, filtered via 0.45 µm membrane filtration (Millipore) and steam sterilized (121°C, 15 psi).

Aquarium (Model Lake)

In an attempt to study a regulated mercury spill in a controlled environment, a model lake was created in a 20 gallon aquarium (Figures 4-6).

Figure 2.

Schematic of Apparatus Employed to Measure Total Mercury

Via the Cold Vapor Atomic Adsorption Spectrophotometric Technique

Perkin Elmer 403
Atomic Absorption
Spectrophotometer

Hollow Cathode Lamp

Digital Readout

Absorption Cell

Air Intake

Exhaust
Hood

Drying
Agent

Reaction
Flask

Varistalic
Pump
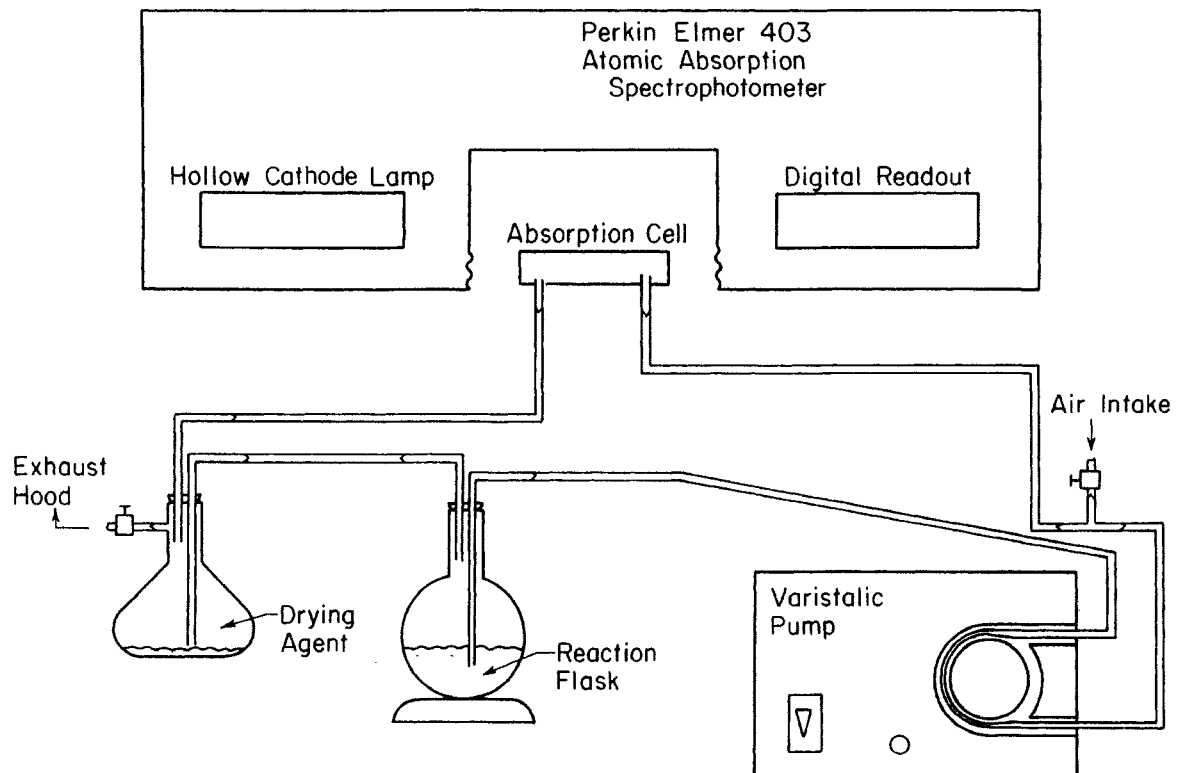
Figure 3.

Typical Standard Curve
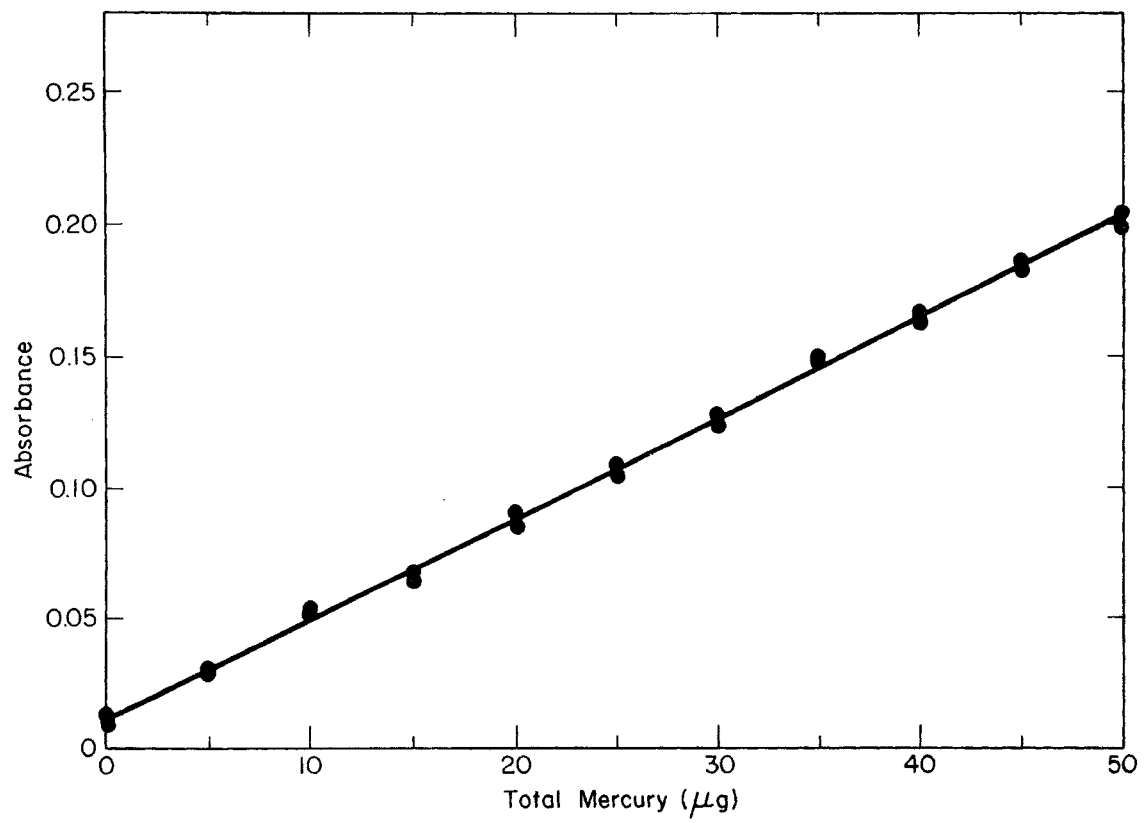
Figure 4.

Diagram of Model Lake

Air →

Affluent
Ⓐ

Suction ←

Effluent
Ⓔ

Air Pump

Ⓕ
Filter

Side View

Ⓐ		Ⓕ
Ⓔ		

Top View
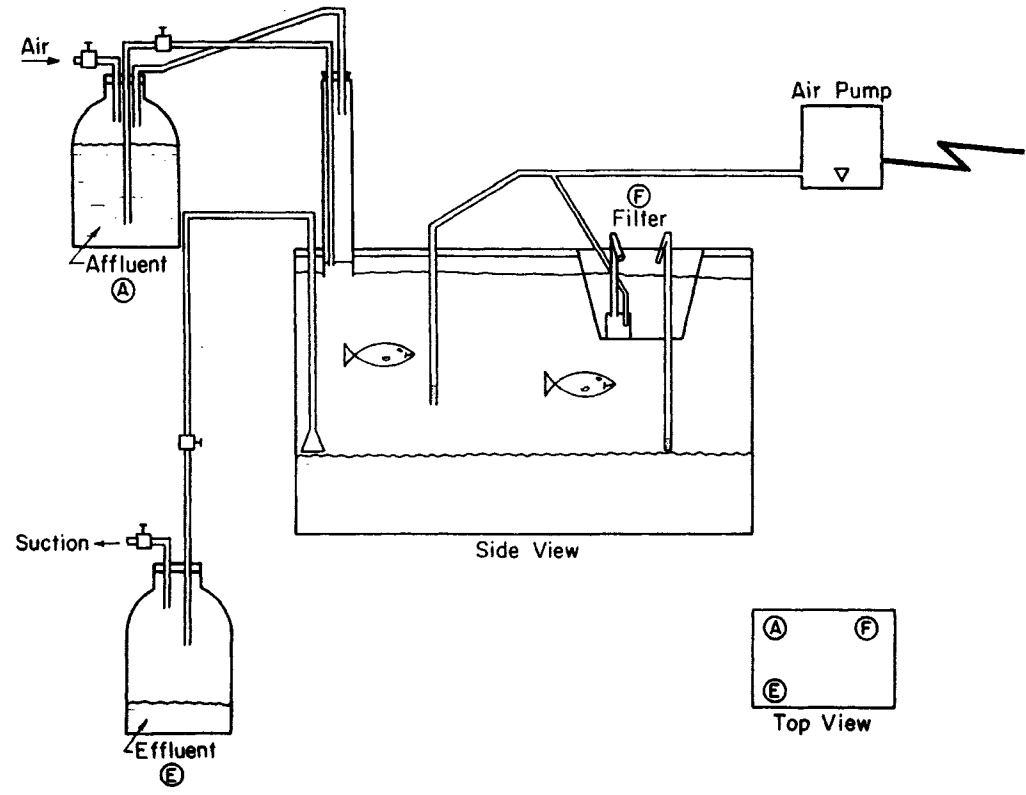
Figure 5.

Cross Sectional Diagram of Stratified Model Lake Bed Sediments

Depth of Layer   Composition of Layer

to 27.0 cm	Water Column
2.0 cm	Aquarium Pebbles
1.5 cm	Aquarium Gravel
1.0 cm	Sand
0.5 cm	Potting Soil
0.5 cm	Olentangy River Mud
0.5 cm	Potting Soil
1.0 cm	Sand
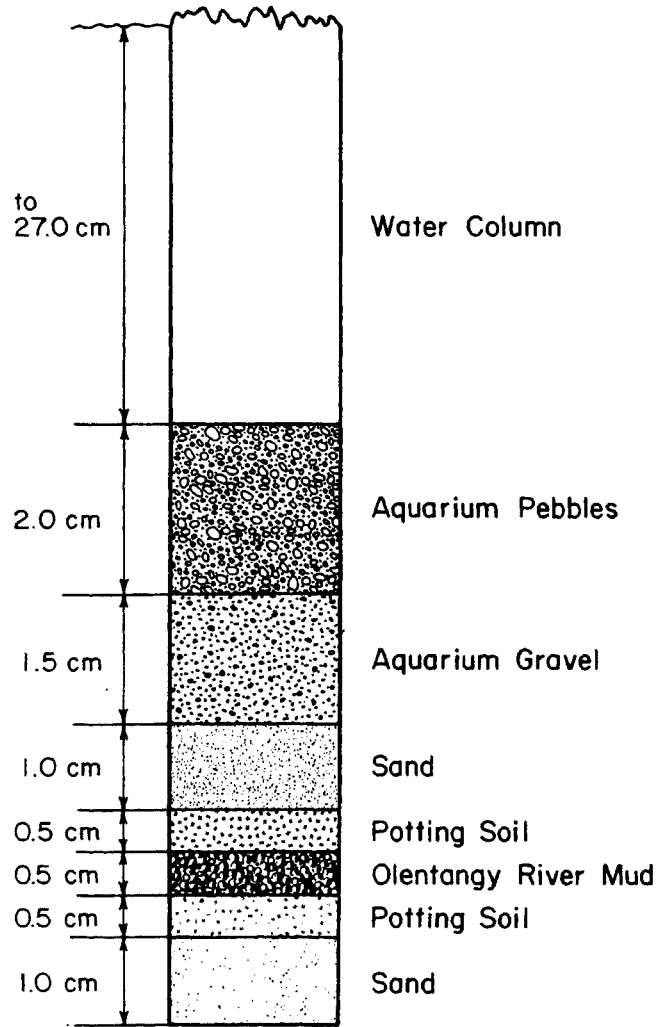
Figure 6.

Aquarium Floor Plan

Aquarium Floor Plan

Site of Regulated Mercury (Hg°) Spill

Fish Feeding Station

Sampling Site for Sediment Cores
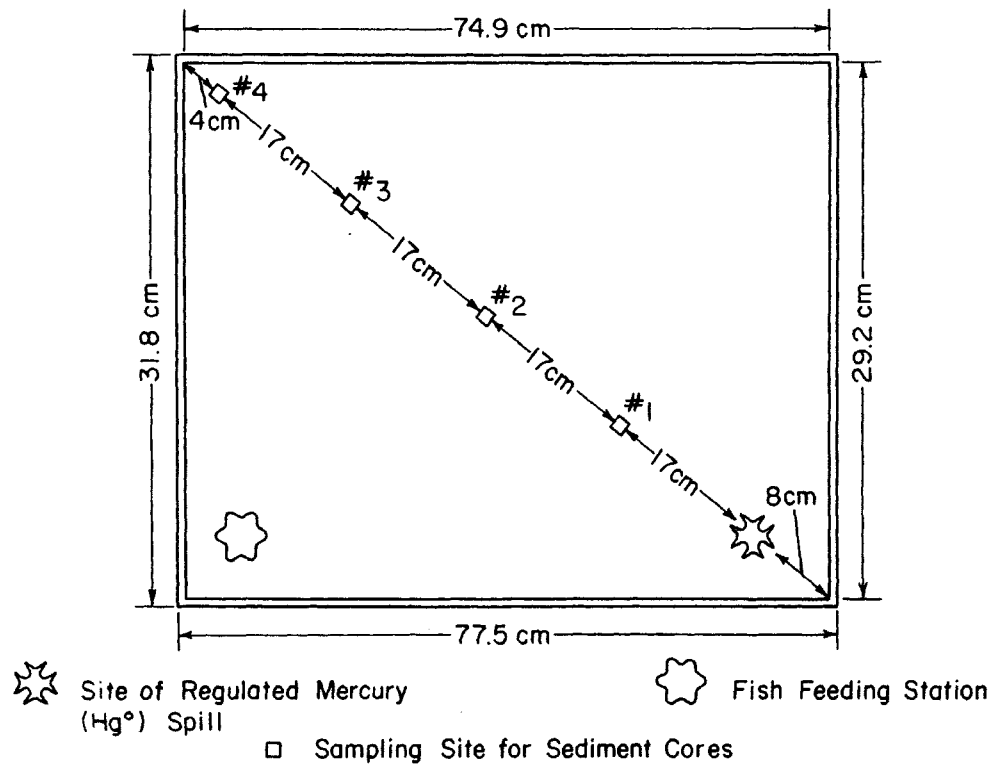
General Layout (Figure 4)

Aeration

Laboratory air, after passing through either the fritted glass sparger or the filtration unit (packed to a depth of 10 cm with Finny Filter Floss - Finney Product, Inc.; 602 Main Street; Cincinnati, Ohio 45202) was adjusted to yield a total flow rate of 500 ml/min.

Filtration

Filter Floss, moistened with "mercury-free diluent" was packed to a depth of 10 cm and replaced every 7 days. Filter entrapped detritus was dislodged from expended floss by gentle washing with "mercury-free diluent." Materials thusly collected were dried at 50°C for 24 hours, weighed, resuspended by vortex action in 65.0 ml of the same diluent and analyzed for total mercury content.

Illumination

Light was provided by means of a plant simulating fluorescent bulb (Sylvania-Enhance) mounted in the aquarium cover. The distance from the bulb surface to the water interface was 6 cm. At weekly intervals, the lamp surface and both sides of its portal were cleaned with commercial window cleaner (Windex) to remove accumulated films.

Temperature

The aquarium and its contents were allowed to equilibrate to ambient laboratory temperatures and kept within that range, i.e., fluctuating between 20°C and 25°C.

Water

All water utilized within the model was double distilled, filtered

(via 0.45 μm Millipore) to remove suspended particulates, and autoclaved (121°C, 15 psi) to eliminate unwanted protists. The affluent to effluent flow rate (Figure 3) was adjusted to 2.0 ml/min.

Sediment Bed (Figure 5)

The sediment bed of the model lake was constructed in the following manner, beginning at the bottom and progressing to the topmost, layers of sand (1.0 cm), potting soil (0.5 cm), Olentangy River mud (0.5 cm), potting soil (0.5 cm), sand (1.0 cm), Aquarium Gravel (1.5 cm), and Aquarium Pebbles (2.0 cm) were stratified.

Aquarium Gravel

Pure natural white Aquarium Gravel (Noah's Ark Pet Center; 1603 West Lane Avenue; Columbus, Ohio 43221) with an average diameter of 2 mm was utilized.

Aquarium Pebbles

Black Decorative Aquarium Pebbles (Melody Brand Products; Maud, Ohio) having a mean diameter of 0.5 cm were employed as the top layer.

Olentangy River Mud

Mud collected immediately after the first Spring thaw (April 5, 1976) was obtained along the bank of the Olentangy River approximately 200 yards south of the Drake Union (The Ohio State University -- Columbus Campus). This layer served as our inoculum in that it contained in addition to mercury methylating microbes found in most sediments (29); water mites of the genus Tyrrellia; several genera of gastropods, i.e., Campeloma and Helisoma; and copious amounts of oligochaete worms, vis.: Tubifex sp.

Potting Soil

Stim-U-Plant Potting Soil (Stim-U-Plant Laboratories, Inc.; Columbus, Ohio 43216) was employed throughout this study.

Sand

Pure silica sand, 20-30 mesh (850-600 μm) was thrice washed in "mercury-free diluent" and dried at 80°C for use.

Ecosystem

One week following the establishment of an equilibrium in the aquarium, 36 goldfish (Carrassius auratus), averaging 4 g in weight, were introduced into the ecosystem. Fish were fed Longlive Shrimp-el-etts Pelleted Fish Food (The Hartz Mountain Co.; Harrison, New Jersey 02029) daily (1 pellet/fish) at the feeding station (Figure 6). The ecosystem was then allowed to re-equilibrate for a one-month period.

Regulated Mercury Spill

Following the removal of base line sediment cores, 1 gram of metallic mercury ($Hg°$) was introduced, at the appropriate site (Figure 6), into the mud layer via a pyrex standpipe. Said glass tube was gently removed by a twisting action to re-stratify the bed sediment.

Sampling

Attached Planktonic Biomass

At 7 day intervals, gelatinous materials attached to the inner glass surface of the aquarium were removed using a single-edge razor blade (Gem). After drying at 50°C for 24 hours and being weighed, specimens were resuspended using a Vortex Mixer in 65 ml of "mercury-free diluent" and analyzed for total mercury content.

Filter Entrapped Detritus

See previous section entitled "Filtration."

Goldfish

At the requisite time intervals, individual fish were sacrificed by placing them in liquid nitrogen (-196°C), dried at 50°C for 48 hours, weighed and suspended in 65 ml of "mercury-free diluent." Total mercury content was determined following digestion of the entire specimen with 25 ml 18 N sulfuric acid and 10 ml 7 N nitric acid. A 48 hour digestion at ambient temperature was employed.

Sediment Cores

Utilizing a truncated 25 ml pipette, sediment cores were taken weekly from pre-selected sites (Figure 6). Following drying at 50°C for 24 hours samples were weighed and analyzed for total mercury content utilizing the technique designed by Hatch and Ott (58) for rock samples.

Snails

Gastropods were processed in the same fashion as goldfish (see previous section), with one exception; the digestion period at ambient temperature was shortened to 24 hours.

Water

Sixty-five ml aliquots were processed in a manner identical to those samples removed from the western basin of Lake Erie.

Volume Measurements

Water suspended particulates were measured following vortex mixing by placing 10.0 ml in a 12 ml graduated (in 0.1 ml subdivisions) conical centrifuge tube and centrifuging (Sorvall GLC-1) in a swinging-bucket head at

1000 rpm for 10 min. When measurements were completed, the sediment was resuspended and the entire 10.0 ml specimen was analyzed for total mercury content.

Weight Measurements

Removal of suspended particulates was accomplished by centrifugation at 1000 rpm for 10 min. Following drying at 50°C for 24 hours each specimen was weighed. Aliquots were resuspended in 65 ml of "mercury-free diluent" and analyzed for mercury.

Weight vs. Volume Measurements

Volume measurements were made according to the aforementioned protocol; upon completion each sample was resuspended, dried at 50°C for 24 hours, and weighed.

RESULTS

Initially the work conducted for this project was concerned with the presence of mercury in the western basin of Lake Erie (Table 1). Previous work has demonstrated: that mercury has been deposited into this area of the lake; that the aqueous environment of this region contains a large number of particulates (59, 60), both inorganic and organic; and that there are micro-organisms capable of methylating the mercury pool present in the sediment. However, little information was available concerning which, if any, of the micro-components of the water column play a significant role in mercury trans-location. Utilizing the protocol delineated in Figure 1 in conjunction with standard curves, mercury levels were determined for water samples and com-ponent fractions from predetermined sites in the western basin of Lake Erie (Tables 2 and 3). The results of these analyses indicated that mercury is: (a) present in varying amounts and locales of the western basin, (b) consis-tently present in higher measurable levels in areas of the lake away from the stronger lake currents, i.e., in bays and/or harbors, (c) readily detectable for long periods of time, (d) particle associated, and (e) present in amounts directly related to particle density.

In an attempt to observe the kinetics of mercury translocation throughout the bed sediment and its overlying water column as well as to study the entry of mercury into the food chain and its concentration via movement from lower to higher trophic levels, a laboratory model (Figures 4 and 6) of a lake was developed. After the bottom sediment was stratified (Figure 5), the flora and fauna added, and lake currents simulated, a 1 month period was allowed for an equilibrium to be established in the ecosystem. Once baseline data was obtained for all model components (Table 4) a regulated mercury (Hg°) spill was introduced into the test system. Utilizing the techniques

TABLE 1

Sample Number	Location*	Date Collected
16	B	9-09-67
39	A	6-10-68
42	B	6-17-68
43	B	6-21-68
51	B	7-02-68
69	B	8-02-68
91	A	8-26-68
105	D	10-15-68
106	A	10-22-68
107	C	10-29-68
115	B	5-16-69
122	B	6-26-69

* A = Rattlesnake Island Area
  B = Middle Island Area
  C = Put-In-Bay and Gibraltar Island Harbor Area
  D = Sandusky Bay Area

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TABLE 2

Fraction Number	Sample Number				
	39	91	106	105	107
	Total Mercury In Parts Per Billion				
1	34	51	63	564	473
2	22	33	42	372	281
3	10	16	19	181	185
4	1	1	1	12	15
5	1	2	2	16	17
6	1	2	2	19	25
7	1	2	2	23	29
8	2	3	3	37	34
9	2	4	4	46	39
10	4	7	8	79	48
11	9	12	19	131	66
12	8	14	16	167	170
13	1	0	2	11	9
14	0	1	1	6	7
15	0	1	1	9	8
16	0	1	1	13	10
17	0	1	1	14	15
18	1	1	2	17	17
19	1	1	2	20	24
20	2	2	3	35	39
21	3	5	5	51	45

TABLE 3

Fraction Number	Sample Number						
	16	42	43	51	69	115	122
	Total Mercury in Parts Per Billion						
1	56	139	200	260	383	398	452
2	42	98	172	182	290	347	388
3	11	39	21	74	91	41	57
4	1	3	3	3	15	16	15
5	2	4	3	5	18	17	16
6	2	7	6	6	20	19	18
7	4	9	14	8	22	21	26
8	4	11	19	11	32	31	33
9	5	14	24	17	36	36	41
10	8	19	39	49	49	73	84
11	15	31	58	81	97	127	147
12	8	28	13	63	79	32	46
13	2	9	5	9	11	4	10
14	0	1	0	3	0	0	1
15	0	1	0	4	3	0	1
16	0	2	0	5	4	1	1
17	0	2	0	6	7	1	1
18	1	3	1	6	9	2	2
19	1	4	2	8	12	3	4
20	3	7	3	13	17	6	8
21	3	8	7	17	26	19	27

previously described, total mercury levels of the various components were monitored over a 10-month period.

As can be seen from the data in Figure 7, mercury is first detectable in sediment cores from Site #1 (Figure 6) 2 weeks post-introduction and shortly thereafter (+4 weeks) at the other sites. Equilibrium is reached throughout the entire sediment after 13 weeks. Upon closer examination, it appeared that mercury moved outward from its initial site in an infinite series of concentric circles and as the distance from said source increased, the time required for mercury to reach any subsequent sampling sites (Figure 6) decreased.

After traversing the entire sediment bed, mercury next appears in the overlying water column (Figure 8) and eventually reaches an equilibrium -- 0.6 of that found in the sediment. Several weeks following the detection of mercury in tank water, we initially detected the accumulation of a planktonic biomass attached to glass surfaces. Subsequent examination of this material showed that it contained mercury (Figure 8) and its mode of accumulation suggested that it was derived from the surrounding water matrix. Approximately 4 weeks after the first appearance of an attached film on glass surfaces, both phytoplankton and zooplankton (containing mercury) were visually detected in tank waters. Mechanical concentration of said suspended particulates occurred via filtration (Figure 8).

The gastropod components of our ecosystem thrived and produced numerous offspring throughout the time span of our experiment. After an initial lag period both species concentrated mercury 1000 fold in relation to their surrounding milieu (Figure 9). Mercury accumulation in both species closely corroborates what is known concerning their feeding habits (61), viz.: mercury appears in Helisoma trivolvis only after it appears in the periphyton film attached to glass, known to be a source of nutriment and in Campeloma

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TABLE 4

Sample	Total Mercury in µg per gram
Aquarium Gravel	0.025
Aquarium Pebbles	0.041
Fish Food Pellets	0.005
Olentangy River Mud	0.013
Potting Soil	0.005
Sand	0.008
Test Sediment Core	0.027
Tubifex sp.	0.016
Water	0.000

Figure 7.

Kinetics of Mercury Translocation Through Model Lake Bed Sediments
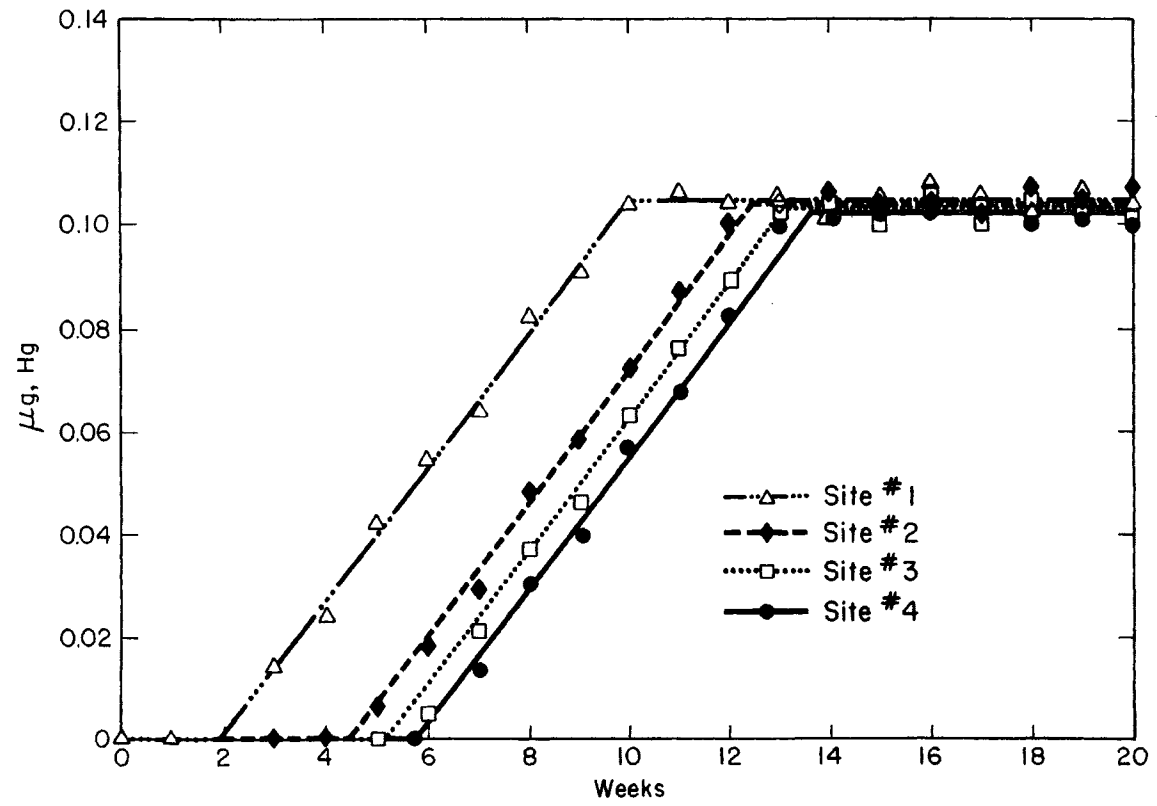
Figure 8.

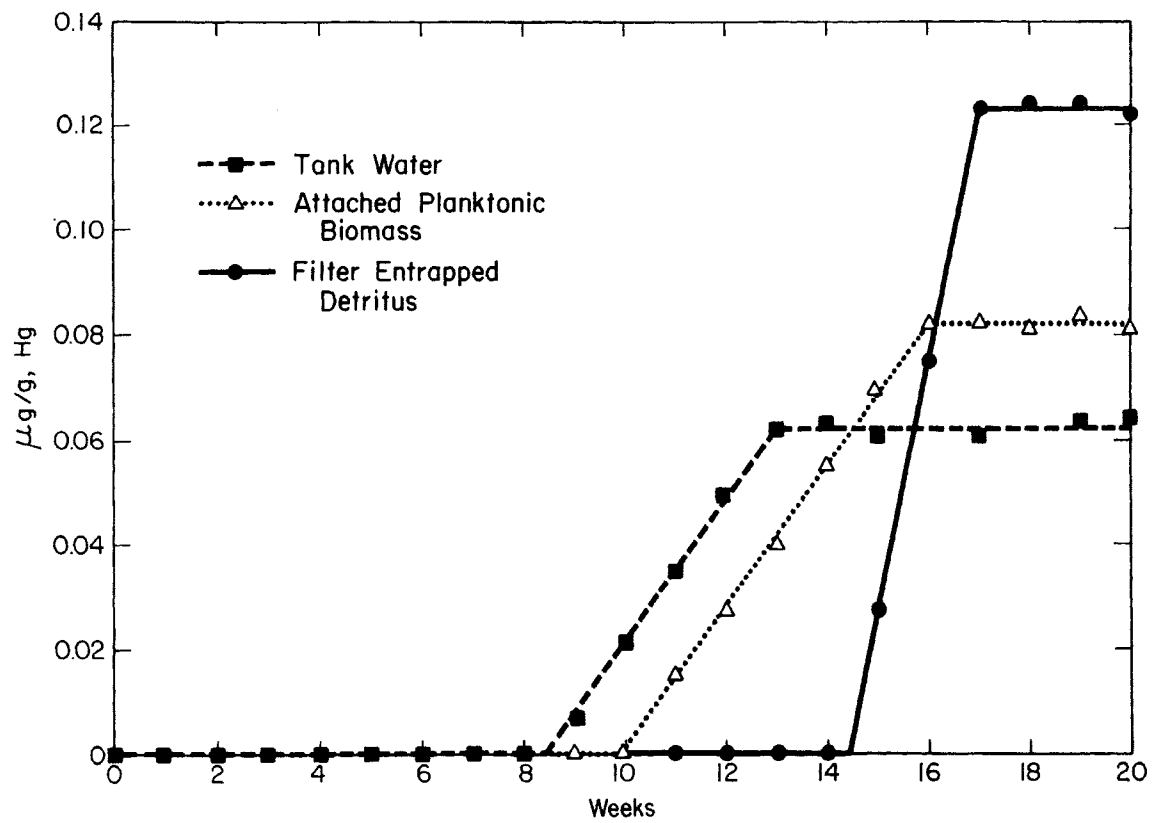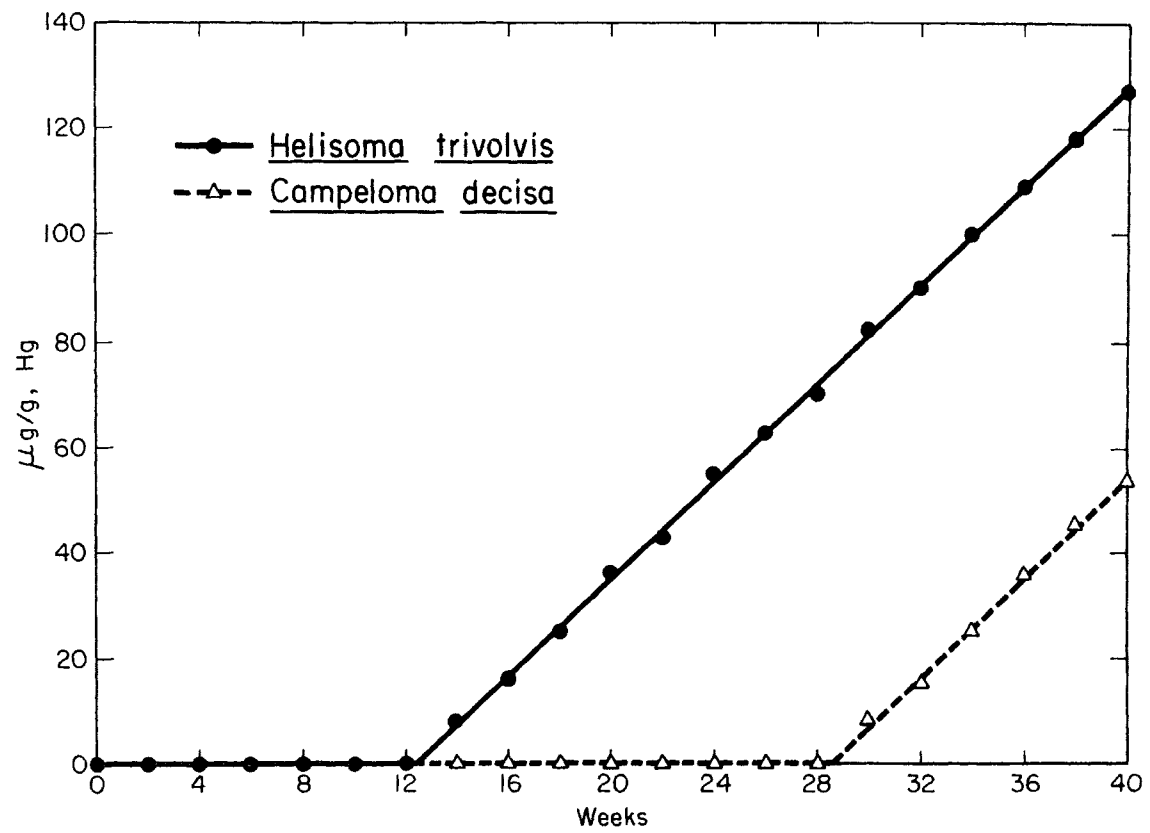Particle Mediated Mercury Mobility in the Water Column

Figure 9.

Mercury Accumulation in Model Lake Gastropods

decisa subsequent to the appearance of a floc of decomposing organic matter deposited between the 22nd and 24th week on the underlying rocks. The latter snail is characteristically found burrowing through soft mud and feeds on decomposing organic material present in or on it (61).

A similar case can be made for the model's ichthyic constituents (Figure 10); once mercury accumulation and concentration (1000 x) is initiated it proceeds at a rate and to levels unaccountable for by externally provided foodstuffs (Table 4).

As mentioned previously, data obtained from the fractionated water samples collected in situ clearly suggested (Tables 2 and 3) that the mercury load of a given sample is present in amounts directly related to particle density. To test this hypothesis, suspended elements (mostly organic plankton) of the model's ecosystem were scrutinized. A linear relationship between both packed biomass volume (Figure 11) and dry particulate weight (Figure 12) in relation to total mercury content was observed. Finally by plotting the volume of suspended particulates against their dry weight (Figure 13) we note that the mercury load carried, either actively or passively, via organic particles is surface associated.

Figure 10.

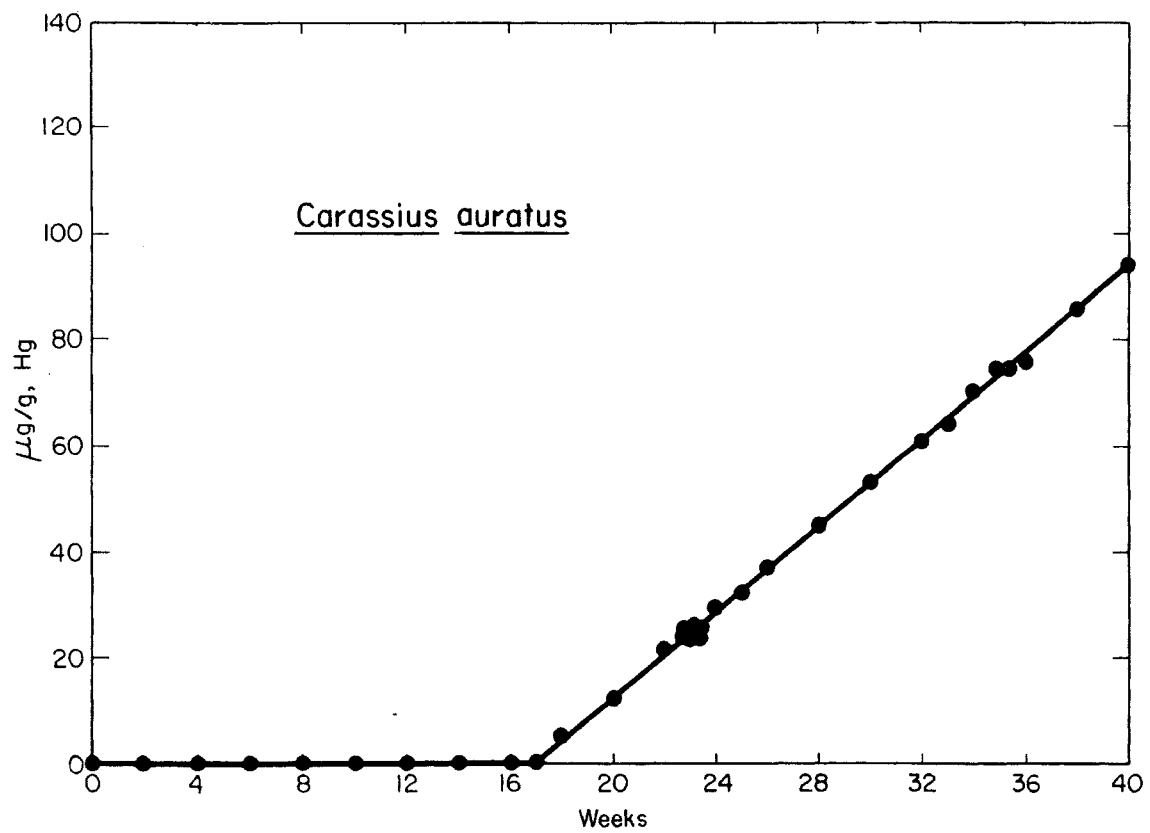Mercury Accumulation in the Model Lake Fish Population

Carassius auratus

Figure 11.

The Effect of Particle Volume on its Associated Mercury Content
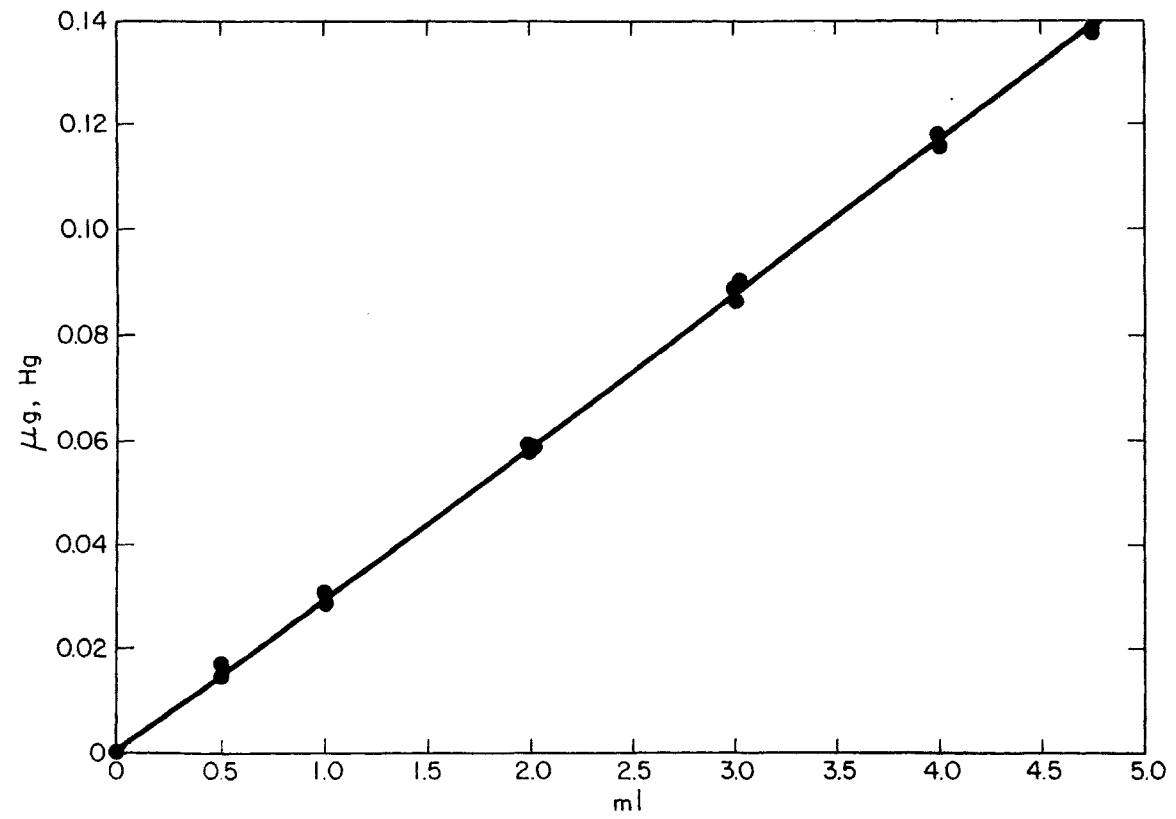
Figure 12.

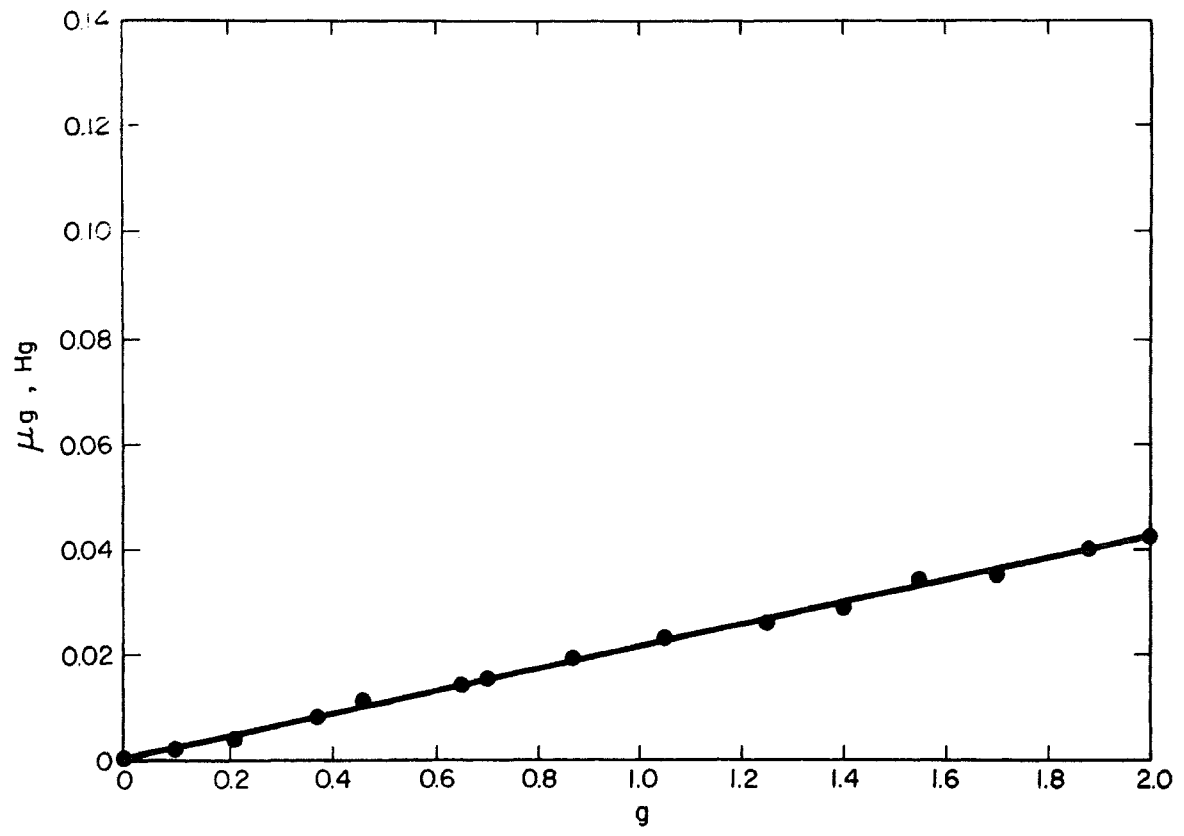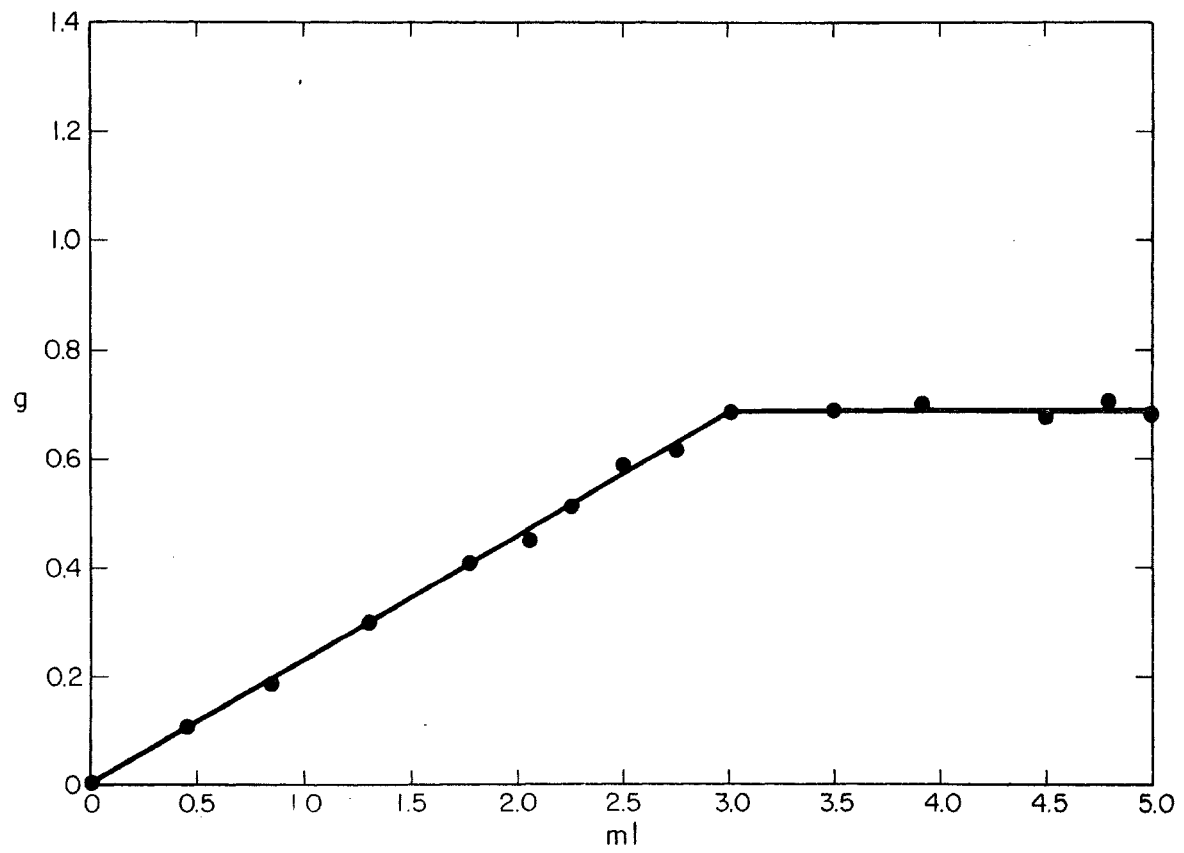The Effect of Particle Weight on its Associated Mercury Content

Figure 13.

Particle Volume Versus Particle Weight for a Given Mercury Burden

DISCUSSION

From the experiments evaluating the role played by particulates in

lacustrine mercury movement (Tables 1, 2 and 3), one readily notes that the

mercury content of the western basin varies with the location from which the

specimen was taken as well as the date. As to be expected, areas of the lake

with diminished water flow, viz.: the bays and/or the harbors have a ten-

dency to show elevated levels of mercury; whether this is due to entrapment of

locally solubilized deposits, accumulation from external sources, or by means

of comparison, depletion of the mercury borne particulates in less quiescent

areas by rapid surface movement is not discernable. Data contained herein is

in concert with the observations of Kovacik and Walters (62) who showed,

through their work with sediment cores taken in the western basin, that

Rattlesnake Island lies within an area showing only background values of

mercury whereas Middle Island is in a province rich in surface mercury pollu-

tion. The latter is due, no doubt in part, to contaminated waters from Lake

St. Clair entering via the Detroit River and traveling long-shoreward along

the northern most or Canadian shore.

Through the use of differential centrifugation it can be readily seen

that the majority of the mercury burden of a given water column lies within

the organic component (planktonic elements > 0.3 μm) while the remaining

amounts are associated with colloidal inorganics (e.g., clay 0.3-0.1 μm). No

matter which of the two distinct components it is found in, mercury has

unequivocally been shown to be associated with suspended particulates; the

charge on any one given particle being directly correlated to its density.

The mechanisms involved in this so called "particle adsorption" can in part

be explained by the affinity of mercury for the sulfhydryl group which can

bind it to suspended organic matter, both living, like plankton, or non-living,

like peat and humus. No doubt, the affinity of zero oxidation state mercury

dissolved in water for lipids and the predilection of mono- and dimethyl

mercury for these very same membrane components, relative to their solubility

in water, facilitates their adsorption by aquatic organisms. Other than

Krauskopf's observations (17) that microcrystalline iron oxides and mont-

morillonite clay absorbed 2+ mercury from water, little is known concerning

the adsorption of mercury on inorganic substrates, their ion-exchange proper-

ties, or differential adsorption for the numerous inorganic species in solu-

tion and/or suspension. The possibility, however, that through microbial

metabolism a zoogloeal mass encases inorganic particulates converting them to

"pseudo-organic particulates" should not be discounted.

Baseline mercury determinations (Table 4) of model lake components

revealed our choice of inoculum, Olentangy River mud, to be low--0.013 μg--

in mercury content. The validity of this information was confirmed by ascer-

taining the level of mercury accumulation in Tubifex species (Table 4) from

their environment. Concentration was shown to be by a factor of 1.23, well

within the range (1.20 $\pm$ 0.26) described by Jernelöv (29).

After the introduction of mercury into the equilibrated ecosystem, one

first detects the appearance of mercury migrating through the bed sediments

(Figure 7). The initial time lag seen between the metal's introduction and

its detection can be accounted for if one considers the possible mechanisms

involved in benthonic mediated mercury translocation. A priori evidence

suggests that a major factor in such mechanisms is the differential solu-

bility exhibited by the various mercury compounds. Although absorption by

organisms may be facilitated by the affinity of zero oxidation state mercury

dissolved in water for lipids, as already noted, it is not likely that this

is an important factor since mercury occurs predominantly in the 2^+ state in

oxygenated water where aquatic organisms must live. On the other hand, however, methylmercury compounds are more soluble in lipids than are 2^+ mercury or metallic mercury in solution; they are also about 100 times more soluble in lipids than in water (63). This allows methylmercury compounds to penetrate more readily than the inorganic forms of mercury into cells, and as a consequence increase the mobility. The key to this theory resides in the conversion of metallic (Hg°) mercury to methylated derivatives and its associated increase in solubility. By combining known facts concerning mercury methylation with the _de facto_ data contained herein, we theorize that the following sequence of events has occurred:

A. Upon exposure to the metallic mercury, microorganisms from the heterogenous population of the benthos are selected that are tolerant of both inorganic and organic mercury compounds, and capable of producing CH_3Hg^+ from either Hg° or Hg^{2+}

B. An increase in numbers of the previously selected microbes occurred with the concurrent establishment of the necessary enzymatic machinery to bring about methylation.

C. Microbially mediated methylation of the inorganic mercury in solution occurred in the top layer of the continuously oxygenated sediment (33).

D. Biologically initiated autocatalytic mobilization of methylated mercury occurs from the rapidly advancing front of multiplying microbes.

E. In the lower layers of the sediment bed and/or areas where microbial metabolism has depleted the oxygen supply, microorganisms are selected that while being refractile to both forms of mercury are

capable of producing Hg° from either CH_3Hg^{+} or CH_3HgCH_3.

F. The increase in numbers of such microbial populations is paralleled with a like increase in their metabolic processes.

G. Reductive demethylation of methyl mercury to methane and inorganic mercury was promoted by the myriad of bacteria thus stimulated (37-44).

By coupling the concomitant methylation of inorganic mercury and demethylation of methylmercury with the selection and growth (or motility) of specialized microbial populations, one can readily visualize the cascade of events necessary to initiate and bring about the translocation of mercury. Let us for a moment consider our original mercury source - a 1 g sphere of metallic mercury. In light of our theory, what is its fate? With each cycle of the previously mentioned series of events the surface area of inorganic mercury increases; subsequent to and in conjunction with this change in availability of mercury we have an ever-increasing population of actively metabolizing microorganisms available to translocate said element thus requiring less time.

Upon the establishment of a mercury equilibrium in the stratified, model-lake sediments, mercury begins to appear in the overlying water column. Microscopic examination of the water matrix showed that the appearance of mercury correlated directly with the migration of the planktonic biomass composed of phytoplankton, protists, and zooplankton into the overlying layer. As these particulates migrate through the fluid medium, they have a predilection for attachment to solid surfaces, mainly the aquarium glass. Once the periplankton film encases all exposed surfaces (approximately 4 weeks after its initial appearance), macroscopic examination of tank waters reveals an

increasing population of mercury bound particulates readily removed by filtration (Figure 8).

At this stage of the experimentation, mercury begins to appear in the brown, ramshorn-shaped gastropod, viz.: Helisoma trivolvis. This flat-coiled snail is characteristically a browsing species which feeds on the algal component of the periplankton film with its own attached periphytic, methylating bacteria. Aquarium enthusiasts employ this species for this very reason, to keep the glass clear of the algal film which otherwise obscures the view of fish or other aquarium animals (61).

Between the 22nd and 24th weeks, a dense floc of organic, decomposing detritus appears on the surface of the underlying stratum. Shortly thereafter, mercury was detected in ovate-conical, green-pigmented -- Campeloma decisa. Dense populations of this species of gastropod are often found in or on mud near wharves and promontories used by fishermen. Here the substrate is often enriched by fish entrails and discarded bait (64). It evidently feeds on decomposing, organic material present.

With the advent of unattached, organic micro-particulates in the water column, one notices the simultaneous accumulation of mercury in the resident goldfish (Carassius auratus). This observation suggested two possible mechanisms for the mercury uptake seen, i.e., (a) mercury containing elements in suspension supplemented the diet of these fish, (b) inorganic mercury in solution was methylated and adsorbed by the fish directly from water via bacteria growing on their slimy bodies. The linear increase of mercury and its concentration in goldfish tissue (Figure 10), may actually continue for years, rather than weeks, as has been reported with other species of fish (49). From our experiments we can only hypothesize the fate of mercury as it climbs one trophic level of the food chain and encounters Homo sapiens.

In an attempt to ascertain how a mercury burden is translocated via these water borne, organic particles, our attention was focused on their physical attributes, namely: volume and weight. Data contained herein (Figures 11-13) shows that while mercury increases with the weight of such particulates; a more profound relationship (increase), however, is demonstrated with their volume. Upon examination of a given sample for these two parameters, it was observed that the weight of a given microbial population reached a plateau whereas the volume of the very same cells contained therein continued to increase. This clearly suggested that mercury translocation mediated via these particulates under study was a surface related phenomenon. Indeed this is not a surprising observation since numerous other activities seen in protists are largely due to their high surface to volume ratio.

In summary, mercury probably moves through the environment in a number of important ways, e.g.: (a) the translocation through the bottom sediments as described herein and which may be considered as a biologically autocatalytic process, (b) the translocation on micro and macro particulates in the water column in a relationship probably bearing upon surface to volume ratio, and/or (c) the mobilization of mercury in association with the motility of the benthonic macrofauna, such as oligochaete worms, gastropods and fish.

SUMMARY

1. The mercury burden found in water columns of Lake Erie's western basin is particle associated; whether organic or inorganic in nature the load carried by individual particles is directly related to their density.

2. Through the use of a model lake, it has been theorized that mercury translocation within underlying bed sediments is cyclical in nature and the result of microbial action.

3. Mercury translocation mediated via organic particulates in the model was a surface related phenomenon.

4. Mercury concentration as it moved from lower to higher trophic levels of the food chain were studied.

5. Observations were made on the fate of mercury entering Lake Erie via the Detroit River from Lake St. Clair and its distribution by currents.

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