Bull. Environm. Contam. Toxicol. 25, 456-464 (1980)

Translocation of Mercury and Microbial Adaptation in a Model Aquatic System

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Mercury in aquatic sediments is a major pollution problem. High levels of mercury in the fish of Lake Erie led to a ban on commercial fishing there. Mercury added to an aquatic system is known to settle into and remain in bottom sediments (GARDNER 1974). Much lower levels of mercury have been detected in the overlying water than in the sediments of aquatic bodies (MILLS and COLWELL 1977, NELSON and COLWELL 1975). Accumulation of mercury and other heavy metals from sediments has been reported for bacteria and algae (LAUBE et al. 1979, RAMAMOORTHY et al. 1977). HAMDY and PRABHU (1979) reported the transferance of mercury through a model food chain from bacteria to Chilids.

The addition of mercury to an aquatic system may come from natural sources or human activities. Natural release of mercury is mainly due to weathering of rock which contains mercury (CADIGAN 1970, JOVANOVIC and REED 1968) and it may be found adsorbed to bottom sediments and suspended particulate material (DALL'AGLIO 1968, KUDO 1976, THOMAS 1973). The activities of man which contribute to the addition of mercury to the environment have been either agricultural or industrial. Both types of usage have been greatly reduced in recent years (VAN DEN BERG 1971). GRANT (1971) reported that the quantity of mercury added to the environment by natural sources is more than is added by industrial and agricultural combined.

The transformations which mercury may undergo in an aquatic system have been described (BRINKMAN and IVERSON 1975, GAVIS and FERGUSON 1972, JERNELÖV 1972, PEAKALL and LOVETT 1972). It has been found that bacteria in sediments play an important role in the mercury cycle. Bacterial populations posses the ability to form methylmercury from the mercuric ion (HAMDY and NOYES 1975, JENSEN and JERNELÖV 1969, JERNELÖV and MARTIN 1975, VONK and SIJPESTTEIJN 1973), to reduce mercuric ion to the elemental state (MAGOS et al. 1964, NELSON and COLWELL 1975, SUMMERS and SILVER 1972), and to degrade organomercurials (BILLEN et al. 1974, CLARK et al. 1977, NELSON et al. 1973, SPANGLER et al. 1973). Higher levels of mercury-resistant bacteria have been found in mercury-contaminated sediments than in sediments containing low levels of mercury (NELSON and COLWELL 1975, TIMONEY et al. 1978).

The work described in this report was undertaken to investigate the movement of mercury (initially as Hg^0) through components of an aquarium model aquatic system. Changes in mercury-resistant levels of bacterial populations were followed with increasing sediment mercury load.

0007-4861/80/0025-0456 $01.80
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MATERIALS AND METHODS

Model Lake System

The layout of the 20 gallon model lake system is shown in Figure 1. The system was aerated with filtered laboratory air supply. Tank water was filtered through Finny Filter Floss (Finny Products, Inc., Cincinnati, Ohio). The filter material was changed every 7 days. The model system was illuminated by means of a plant-stimulating fluorescent bulb which was mounted in the aquarium cover. The water utilized was double distilled and adjusted to a flow rate of 2.9 liters per day in the translocation experiments and to 2.0 liters per day in the bacterial resistance experiments.

The sediment bed was stratified as shown in Figure 2. Natural white aquarium gravel had an average diameter of 2 mm. Black Decorative Aquarium Pebbles (Melody Brand Products, Maud, Ohio) averaged 5.5 mm in diameter. The pure silica sand was 20-30 mesh. Gravel, pebbles, and sand were washed three times with double distilled water and dried prior to use. Potting soil was a product of Stim-U-Plant Laboratories (Columbus, Ohio). Olentangy River mud was collected from a site approximately 180 meters south of the Drake Union on the Columbus campus of The Ohio State University. Mud was placed in the tank immediately after collection and served as the source of diversified macro- and microorganisms.

One week after the model lake system was assembled, 36 goldfish (*Carassius auratus*) averaging 4 g in weight were added. The fish were fed Longlive Shrimp-el-etts Pelleted Fish Food (Hartz Mountain Corp., Harrison, New Jersey) at a rate of one pellet per fish per day. The system was allowed to equilibrate for one month after adding the fish.

Mercury Addition

Metallic mercury was added to the model lake system in the translocation studies. A 1 g globule of Hg^0 was placed into the mud layer of the bed sediments via a glass tube. The tube was withdrawn with a twisting motion to restratify the sediments.

In the study of bacterial populations, mercuric mercury (Hg^{2+}) was added to the model system. The affluent water was amended with 0.5 mg Hg^{2+}/liter (as $HgCl_2$) daily. After nine weeks the addition was increased to 5.0 mg Hg^{2+}/liter.

Mercury Analysis of Components

Attached planktonic biomass was scraped from the sides of the tank, dried at 50°C for 24 h, weighed, and resuspended in 65 ml of triple distilled water. Filter entrapped detritus was dislodged by gentle washing and treated as above. Goldfish and snails were sacrificed by placing them in liquid nitrogen, dried at 50°C for 24 h, weighed, and resuspended in 65 ml of triple distilled water. Acid digestion with 25 ml 18N sulfuric acid and 10 ml 7 N nitric acid was carried out for 48 and 24 h respectively. Sediment cores were removed from preselected sites (Fig. 3) in the bed sediments, dried at 50°C for 24 h, pulverized, and weighed. All components were analyzed for total mercury content by the methods of HATCH and OTT (1968).

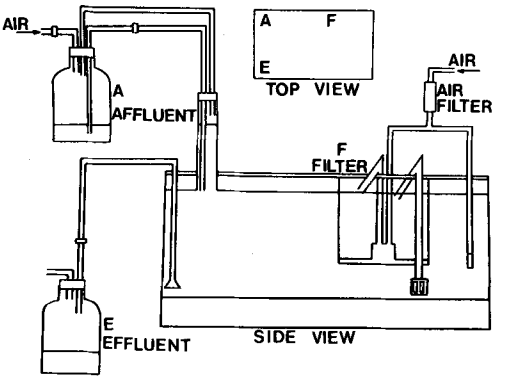

Figure 1. General design of the model aquatic system.

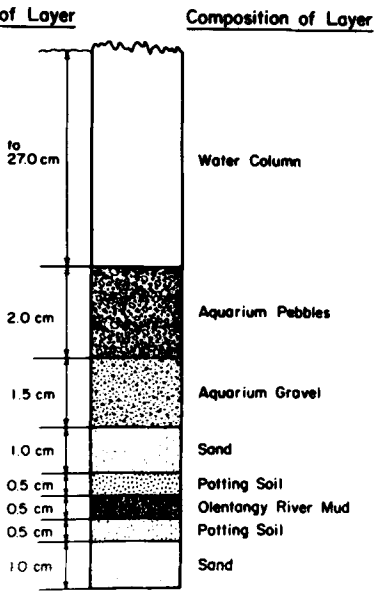

Figure 2. Stratification of bed sediments. Water column depth refers to the height above the aquarium bottom.

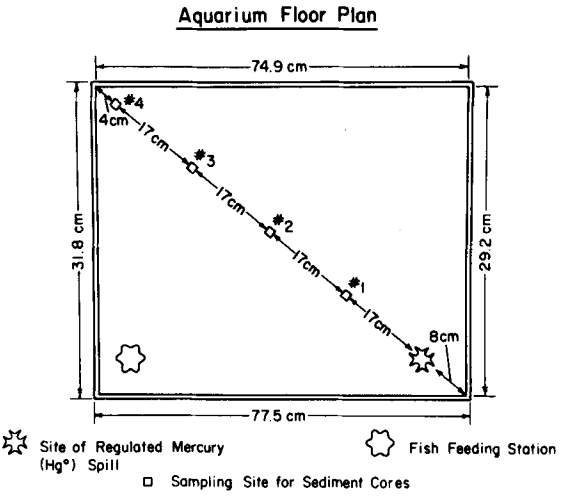

Figure 3. Floor plan of the model aquatic system showing the sites of Hg addition and sampling

Mercury Resistance of Bacterial Populations

Total viable counts (TVC) of aerobic heterotrophic bacterial populations were determined by plating serial dilutions of sediment cores onto Tryptone Glucose Extract Agar (Difco Laboratories Inc., Detroit Mich.) which was used without amendment or was amended with 6 µg Hg^{2+}/ml (as $HgCl_2$). The plates were incubated for 5 days at 25°C. Percentages of mercury(II)-resistant bacteria were determined relative to the total viable count in the absence of mercury in the medium.

RESULTS

Mercury was first detected at Site 1 (Fig. 3) three weeks after its introduction into the system (Fig. 4). Mercury was detected at all other sites within seven weeks after deposition. The mercury appeared to travel through the sediments at an increasing rate as it moved away from the spill site. The first 17 cm were traversed in 3 weeks, the second 17 cm in 5 weeks (post addition of mercury), the third 17 cm in 6 weeks, and the final 17 cm in less than 7 weeks. Thus, the mercury moved through the sediments at an ever increasing rate. Equilibrium was reached after 13 weeks at a mercury load of about 0.1 µg per g sediment.

Tank water contained less than detectable levels of mercury until the bottom sediments had been completely traversed (Fig. 5). After twelve weeks the tank water reached an equilibrium value that was 0.6 times that of the bottom sediments. Mercury was detected and concentrated in the attached planktonic biomass after 10 weeks. Mercury levels in the filter entrapped material did not reach detectable levels until the fifteenth week.

The gastropods *Helisoma trivolvis* and *Campeloma decisa* accumulated Hg to a level 1000 fold over the tank water (Fig. 6). The former snail is known to feed on periphyton film on surfaces and therefore it accumulated mercury only after the attached biomass on the sides of the aquarium glass had concentrated Hg. Mercury appeared in *C. decisa* subsequent to the formation of decomposing organic matter between the 22nd and 24th weeks on the bottom of the tank. This snail is known to inhabit soft muds and to feed on decomposing organic material.

The fish in the model system also accumulated high concentrations of Hg (Fig. 6). Like the snails, the mercury content did not reach equilibrium in the time alloted for the experiment.

The rapid passage of Hg throughout the bottom sediments suggested that the bacterial population may have undergone changes in its sensitivity to mercury. The model lake was reassembled as before. Mercuric ion was added to the incoming water and passed through the system at a rate of 1 mg Hg^{2+} per day.

The mercury content of the sediments rose to approximately 0.25 µg Hg/g after 5 weeks (Fig. 7). The total viable count (TVC) changed less than one log throughout the experiment. Fluctuations in the population occurred early in the experiment, but the TVC only decreased slightly at the end. The percentage of the of the TVC that was resistant to 6 µg Hg^{2+}/ml remained nearly the same as the original level when the flow rate was 1 mg Hg^{2+} per

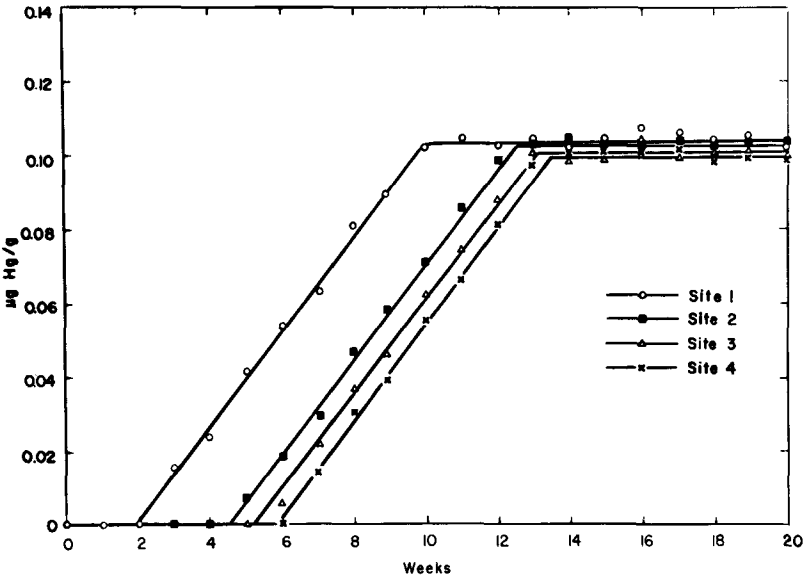

Figure 4. Translocation of Hg through the bed sediments.

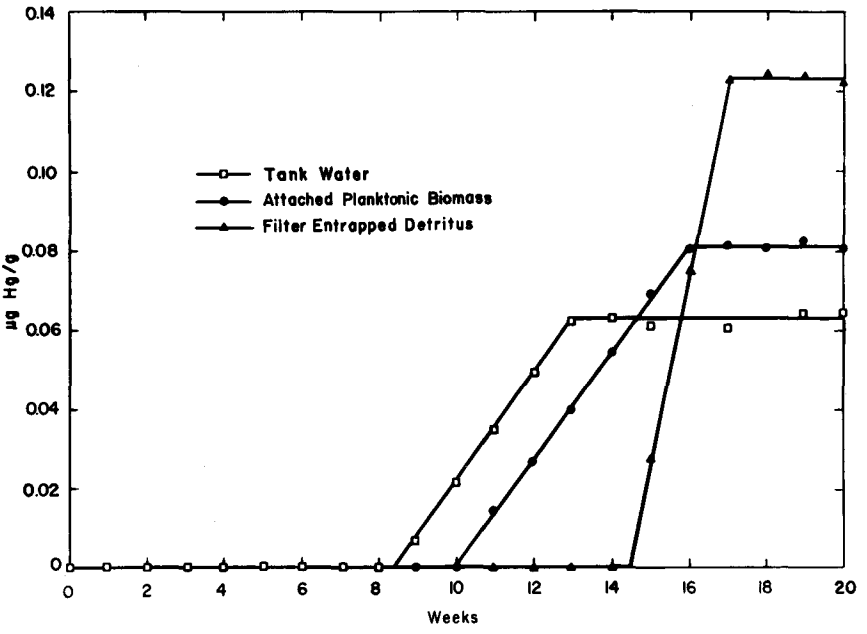

Figure 5. Mercury levels in water and particulate matter.

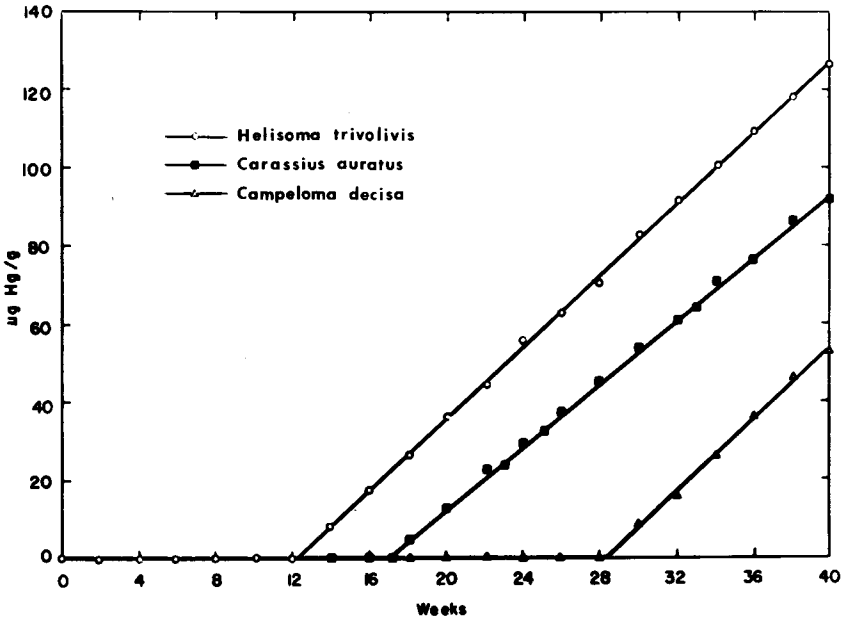

Figure 6. Mercury accumulation by fish and gastropods.

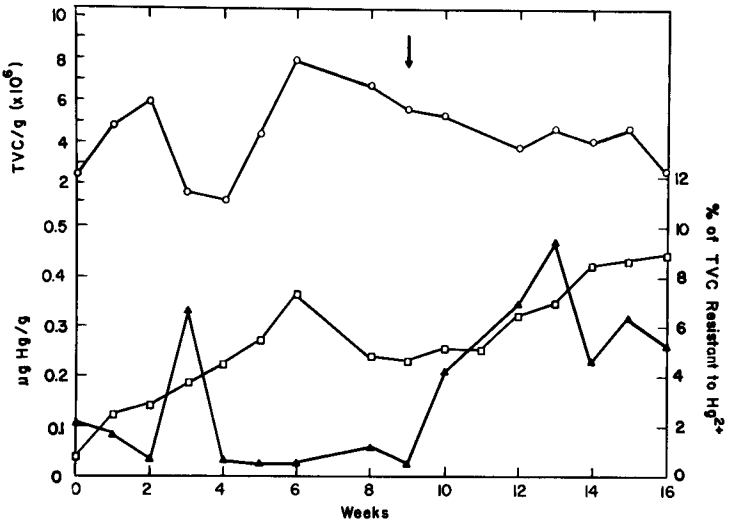

Figure 7. Affects of Hg addition on sediment bacterial populations. Total viable count (○); % of TVC resistant to 6 µg Hg^{2+}/ml (▲); µg Hg/g sediment (□). Arrow represents the point at which the Hg flow was increased to 10 mg Hg^{2+} per day.

day. A large increase was noted at Week 3 when there was a sharp decline in the TVC.

At Week 9 the mercury flow rate was increased to 10 mg Hg^{2+} per day (Arrow, Fig. 7). The mercury content of the sediments again rose, while the total viable count declined slightly. The percentage of the TVC resistant to 6 µg Hg^{2+}/ml increased markedly from around 1% up to 10% and then fell to 5% after 16 weeks.

DISCUSSION

Mercury translocation was shown to occur when Hg^0 was added to a model aquatic system. The mercury must first pass through the bed sediments before reaching the water column. The initial step in the translocation must be the oxidation of Hg^0 to Hg^{2+}. JERNELÖV and MARTIN (1975) reported that the oxidation of Hg^0 may occur in aerobic sediments such as the overlying sediments in aquarium models. Several bacterial genera have been implicated in elemental mercury oxidation, however, the direct action of living microbial cells may not be required (HOLM and COX 1975). Further action on the mercuric ion could result in the methylation of mercury by bacteria present. The formation of these highly soluble mercury derivatives would lead to an increased migration rate for mercury.

Mercury appeared in the water column only after the establishment of an equilibrium in the bed sediments. The presence of Hg in the water column was followed closely by the appearance of mercury in the periplankton film which adhered to the aquarium glass. LAUBE et al. (1979) and RAMAMOORTHY et al. (1977) showed that heavy metals could be concentrated from either water-borne or sediment-bound sources by both algae and bacteria. The gastropods accumulated mercury related to their feeding habits (Fig. 5 and 6). *Helisoma trivolivis* concentrated mercury soon after its appearance in the attached biomass. *Campeloma decisa*, a bottom feeder, showed increased mercury levels after the appearance of the organic floc on the bottom which was deposited by the fish. Mercury in fish was first detected at about the same time as the mercury level in the suspended particulate matter reached equilibrium. The mercury levels in the fish and snails showed no tendency to reach equilibrium within 40 weeks (Fig. 6). This observation suggested two possible mechanisms for Hg uptake by fish. *(1)* Particulates with adsorbed Hg supplemented the diet of the fish and *(2)* inorganic mercury was methylated by bacteria in the slime layer of the fish and was adsorbed directly by the fish.

Addition of mercury in the form of the mercuric ion also increased the mercury load of the bed sediments. At a mercury flow rate of 1 mg Hg^{2+} per day, the equilibrium mercury level reached was about 0.25 µg Hg per gram. This level was higher than that reached in the studies in which Hg^0 was used as the source of mercury content of the sediments to approximately 0.43 µg Hg/gram.

Total viable counts (TVC) of aerobic heterotrophic bacteria changed less than one log throughout the study. The percentage of the TVC resistant to 6 µg Hg^{2+}/ml of medium did not increase while the mercury flow rate was 1 mg Hg^{2+} per day. A peak was ob-

served at Week 3 (Fig. 7) that occurred at the same time as a sharp drop in the TVC. This small change in the % TVC resistant to 6 μg Hg^{2+}/ml may be due to the sorption of mercury by the sediments. When the mercury flow was increased to 10 mg per day, the % TVC resistant to Hg^{2+} rose rapidly. This may be due to an increased mercury content in the interstitial water of the sediment. A threshold mercury level may have been exceeded which caused an increase in mercury-resistant bacteria.

The mechanisms by which the bacterial population increased its Hg tolerance in this study is not known. Mercury-resistance in microorganisms can be related to increased cellular pools of thiol containing compounds (ROSS and OLD 1973), to the presence of increased levels of extracellular complexing molecules such as proteins and amino acids (NUZZI 1972), and to formation of volatile forms of mercury such as Hg^0 and methylmercury (CLARK et al. 1977, VONK and SIJPESTEIJN 1973). Acquisition of plasmids may confer resistance to mercury in members of the *Enterobacteriacae* (NAHAHARA et al. 1977) and in *Pseudomonas* sp. (LOUTIT 1970).

Studies of natural environments which have been contaminated by mercury show that there are greater percentages of mercury-resistant bacteria in these areas than in less contaminated regions (NELSON and COLWELL 1975, TIMONEY et al. 1978). TIMONEY et al. (1978) showed that there was a positive corelation of increased mercury and other heavy metal resistance due to the presence of plasmids. They also found there was an increase in antibiotic resistance in mercury-resistant bacteria which contained plasmids. Thus, an increased Hg load in bottom sediments may not only lead to more mercury-resistant organisms, but may also cause an increased pool of antibiotic bacteria as well.

In summary, mercury is probably translocated through the environment in several ways. *(1)* Translocation through the bottom sediments occurs and is due in part to biological processes. *(2)* Translocation of mercury onto micro- and macroparticulates in the water column leads to dispersal of mercury and is probably related to the surface to volume ratio of the particulates. *(3)* Mercury ia also mobilized by association with benthic macrofauna such as oligochaete worms, gastropods, and fish.

ACKNOWLEDGEMENTS

The work in this report was supported in part by a grant from the U.S. Department of the Interior, OWWR Project 501X. We wish to thank Barbara M. Witte for technical assistance and Carol B. Stein for identification of snails.

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