Biodegradation **3**: 147–159, 1992.

Mercury biotransformations and their potential for remediation of mercury contamination

Tamar Barkay[1,*], Ralph Turner[2], Erwan Saouter[3] & Joanne Horn[3]
[1] *Microbial Ecology and Biotechnology Branch, U.S. Environmental Research Laboratory, Gulf Breeze, FL 32561, USA;* [2] *Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA;* [3] *Center for Environmental Diagnostics and Bioremediation, University of West Florida, Pensacola, FL 32514, USA (* Address for correspondence: US EPA, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA)*

Key words: bioremediation, inorganic mercury, organomercury

Abstract

Bacterially mediated ionic mercury reduction to volatile Hg^0 was shown to play an important role in the geochemical cycling of mercury in a contaminated freshwater pond. This process, and the degradation of methylmercury, could be stimulated to reduce the concentration of methylmercury that is available for accumulation by biota. A study testing the utility of this approach is described.

Abbreviations: Hg^R – inorganic mercury resistance; Org-Hg – organomercury; Org-Hg^R – organomercury resistance; SRB – sulfate reducing bacteria; Methyl-B12 – methylcobalamine

Introduction

I. Metals and metaloids – general

i. Metal resistance

Metals are crustal elements in whose presence living organisms have evolved. Many metals are essential for the metabolism of living organisms: as prosthetic groups in metalloproteins (e.g. coenzymes), regulators of ionic environments, and in maintenance of membrane potentials. Yet, all metals are toxic at elevated concentrations, and mechanisms enabling life at high metal concentrations exist in all organisms. Eukaryotes and prokaryotes have inducible metallothioniens. These are small proteins with a high cysteine content that sequester metals and thus control metal toxicity and homeostasis (Hamer 1988; Olafson 1984). Phytochelatins are small peptides (5 to 17 residues) consisting of repetitive τ-glutamylcysteine residues with a carboxyl-terminal glycine that are produced by plants in addition to metallothioniens, and serve an analogous function (Rauser 1990).

A variety of mechanisms to combat toxic metals exist in bacteria (Table 1, Silver 1991). For a detailed description of metal resistance mechanisms see the recent review by Silver & Walderhaug (1992). Most, but not all mechanisms, are plasmid encoded, and the majority involve transport phenomena. Transport-related metal resistance mechanisms include efflux, the export of metal ions, following their 'erroneous' uptake by essential ion transport systems (e.g. Cd^{2+} in *Staphylococcus aureus* by the Mn^+ transport system; Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} in *Alcaligenes eutrophus* by a transport system that has a broad specificity range; AsO_4^{3-} (arsenate); AsO_2^- (arsenite), Sb^{3+}, and TeO_3^{2-} (Turner et al. 1992) via a PO_4 transport system in both gram positive and negative bacte-

Table 1. Genetically defined, metal resistance mechanisms in bacteria.[1]

Metal	Resistance mechanism	Genetic system/regulation
Cadmium	Gram positive (*S. aureus*): i. ATPase mediated efflux of Cd^{2+} that enters the cell *via* the Mn^{2+} transport system. Also confers resistance to Zn^{2+}	Plasmid encoded *cadA* (specifying the ATPase) and *cadC* (encoding a soluble protein). Induced by Cd^{2+}, Bi^{3+} and Pb^{2+}
	ii. Enhanced Cd^{2+} binding. Also confers resistance to Zn^{2+}	Plasmid encoded *cadB*
	iii. Efflux system. No additional metal resistances	Chromosomally encoded. Not related to *cadA*
	Gram negative (*A. eutrophus*): Efflux system of Cd^{2+} (and Co^{2+} and Zn^{2+}) that are taken-up by a nonspecific cation transport system	*czcABCD*. Induced by Cd^{2+}, Zn^{2+} and Co^{2+}. *czcR*, *czcS* and possibly *czcD* have been identified as regulatory elements
Arsenate, Arsenite, Antimony, Tellurite	i. Arsenite and Sb^{3+} stimulated ATPase. Specific to AsO_4^{3-}, AsO_2^- and Sb^{3+}, in both Gram positive and negative bacteria	*arsABCD*. *arsA* – ATPase; *arsB* – trans-membrane protein (anchors the ATPase, anion channel (?)[2]); *arsC* – specifies arsenate resistance (involved in arsenate reduction). *ars* is negatively regulated by the *arsR* gene product. AsO_4^{3-}, AsO_2^-, Sb^{3+} and Bi^{3+} are inducers
	ii. Oxidation of AsO_2^- to the less toxic AsO_4^{3-} in Gram negative bacteria	
Chromium	Reduced accumulation of Cr^{6+} (by either reduced uptake or an efflux system) by resistant cells of *P. aeruginosa* and *A. eutrophus*	*chrA* specifying a hydrophobic polypeptide. *chrB* (found only in *A. eutrophus*) – role in regulation (?). Inducible by Cr^{6+} in *A. eutrophus*. Constitutive in *P. aeruginosa*
Copper	i. *P. syringae*: Periplasmic binding and extracellular sequestration	Plasmid encoded *copA* and *copC* – periplasmic proteins (Cu^{2+} accumulation (?)); *copB* – an outer membrane protein (Cu^{2+} sequestration (?)). Repression by the *copR* (chromosomal location) gene product and induced by Cu^{2+} and the *copI* (plasmid location) gene product
	ii. *E. coli*: Efflux of Cu^{2+} that is taken up by a Cu^{2+} specific transport system. Resistance requires interaction between components of the transport system and an efflux system (?)	Chromosomally located *cut* system encodes transport, intracellular binding and efflux proteins. Plasmid located *pco* system specifies intracellular binding and efflux
Mercury	In a wide variety of Gram positive and negative bacteria, intracellular reduction of Hg^{2+} to the volatile Hg^0 and the degradation of organomercury to Hg^0 and an organic moiety.	Specified by the *mer* system (plasmid or chromosomal location). *merTP(C)* – transport of Hg^{2+}; *merA* – subunit of mercuric reductase; *merB* – organomercurial lyase. Positively/negatively regulated by the *merR* gene product, Hg^{2+} and organomercury compounds, act as inducers

[1] Prepared after Silver (1991) and Silver & Walderhaug (1992).

[2] (?) – Hypothesized by considering available data.

ria). In contrast, sequestration and periplasmic accumulation of Cu^{2+} was found to be the resistance mechanism in plant-pathogenic *Pseudomonas syringae*. Chemical transformation and detoxification of metals as the basis for resistance are well characterized for Hg^{2+} and organomercury compounds (see below). In addition, AsO_4^{3-} resistance involves reduction prior to its efflux as AsO_2^-. Microbes mediate numerous metal biotransformation reactions that are unrelated to metal tolerance. They reduce Cr^{6+} to Cr^{3+} (Bopp & Ehrlich 1988), methylate inorganic- (Hallas et al. 1982) and organo- (Guard et al. 1981) tin, and reduce (Steinberg & Ormland 1990) and methylate (Karlson & Frankenberger 1989) inorganic selenium.

ii. Metal pollution and remediation

The unique physical-chemical and toxic properties of metals have promoted their wide use in industrial processes and as biocides. As a result, higher concentrations than those naturally present in the biosphere have accumulated in the environment, causing public health hazards and ecological perturbations. The removal of metals is, therefore, a challenge to environmental management. Common methods of metal removal are based on concentration of metals mostly by ion exchange and sorption to a variety of resins, and precipitation as metal sulfides. Recovery and recycling are sometimes possible (Patterson & Passino 1987).

Biodegradation, the microbial-mediated transformation of hazardous material, is not applicable to metals, because, unlike organic pollutants, metals as elements cannot be mineralized to ubiquitous nontoxic compounds, such as H_2O and CO_2. However, biomobilization is a valid concept in the management of metal pollution. Biomobilization contributes to metal remediation by:

- transforming metals to less toxic chemical forms
- compartmentalizing metals in those parts of the environment where they are of less public health concern, and
- concentrating metals to facilitate disposal and/or recycling.

The reduction of uranium by the activity of anaerobic bacteria that use U^{6+} as an electron acceptor (Lovley & Phillips 1992) is an example of metal concentration, because the resulting U^{4+} precipitates and can be easily removed from polluted waters. This process is currently being tested for its efficacy in remediating contaminated groundwater (Lovley et al. 1992). Detoxification and compartmentilization of toxic selenium has been achieved (Frankenburger Jr. 1992) by stimulating soil fungi that methylate Se^{4+} to volatile and less toxic dimethylselenide (Karlson & Frankenberger Jr. 1989). Methylation was stimulated by amendments with nutrients and maintenance of oxic and moisture conditions (Frankenburger Jr. 1992). Microbial transformations of mercurial compounds to volatile Hg^0 are well understood (Silver & Walderhaug 1992). These transformations and their potential for the remediation of mercury contaminated environments are the topic of this publication.

II. Mercury

i. Mercury as an environmental contaminant

The sources of mercury in the environment are both natural and anthropogenic. Natural sources result from the geochemical cycle of mercury (Fig. 1). Anthropogenic sources contribute up to two-thirds of the current input of mercury into the environment. Due to its unique chemical and phys-

Table 2. Physical-chemical properties of mercury and their exploitation in industrial products.[1]

Property	Industrial use
Liquid at ordinary temperature, uniform volume expansion and high surface tension	Measuring devices
Low electrical resistance	Conductors
Ability to form amalgams with other metals	Recovery of metals, dentistry
Toxicity to biological systems	Biocides
Brilliant colors	Paints

---

[1] Prepared after Nriagu 1979.

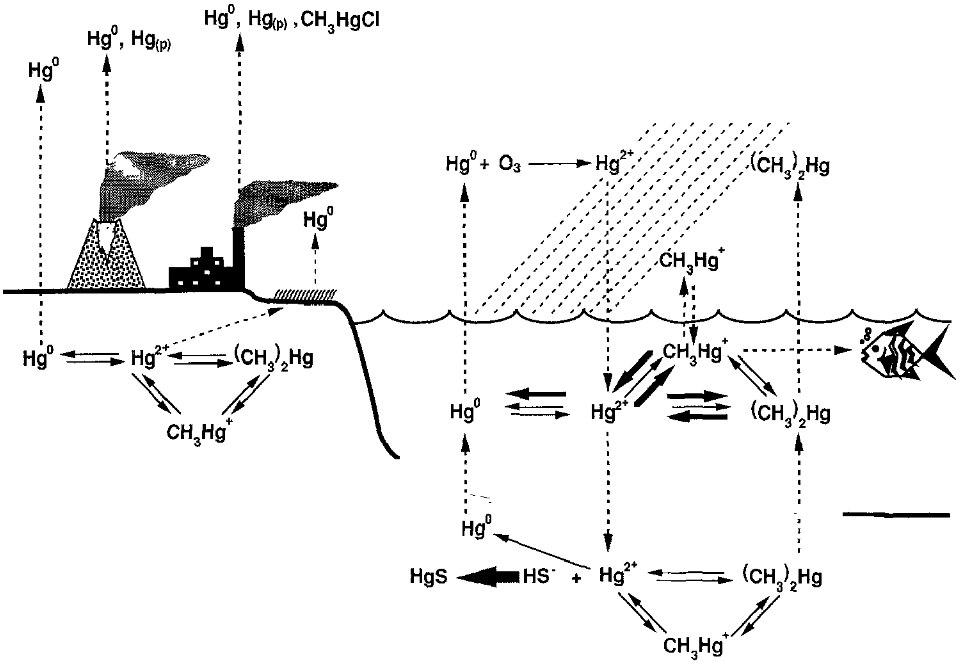

Fig. 1. The mercury cycle in the biosphere. Bold arrows – microbially mediated processes; full line arrows – transformations; broken line arrows – transport processes; a very bold arrow depicts the magnitude of the precipitation reaction between HS$^-$ and Hg^{2+}. (Reproduced with permission from Barkay 1992.)

ical properties, mercury has been an attractive resource for several industries (Table 2). Historically, mercury was used as a pigment in paint production and in medicine (a use that is curiously being continued to this day, mostly as dental amalgams). Applications in metal recovery and felt production were introduced around the turn of the century. Mercury is currently used as an electrode in the chloralkali process, it is an important resource in electrical industries (in fluorescence lights, light switches, thermostats and batteries) (Nriagu 1979). Today, since regulatory restrictions on the disposal of mercury have been enacted, the burning of fossil fuels, the use of mercury in gold mining (mostly in third world countries), and possibly the incineration of solid wastes, remain the major anthropogenic sources of mercury to the environment.

The most common route for mercury toxicosis is by the consumption of tainted food stuff, particularly fish and shellfish (D'Itri & D'Itri 1978). Mercury is a neurotoxin interfering with the normal development and functioning of nerve and muscle systems. Toxicity is manifested as various neuromuscular malfunctions ranging from loss of sensation in mild cases to blindness, paralysis and death in severe cases (Nriagu 1979).

In recent years hazardous mercury concentrations have been discovered in freshwater fish from a broad range of geographic areas. Essentially all tissue-associated mercury in fish is CH$_3$Hg$^+$, and the high concentrations observed are the result of biomagnification through trophic level interactions (Watras & Bloom, in press). The amount of CH$_3$Hg$^+$ that is available for accumulation by aquatic biota is directly dependent on the various reactions that constitute the geochemical cycle of mercury in aquatic environments.

ii. The role of microorganisms in the geochemical cycling of mercury

a. Degradation of organomercury and reduction of Hg^{2+}

Bioremediation can be viewed as the recruitment of pollutants by geochemical cycles. Thus, mercury biomobilization makes use of the principles that govern its geochemistry (Fig. 1). Microorganisms

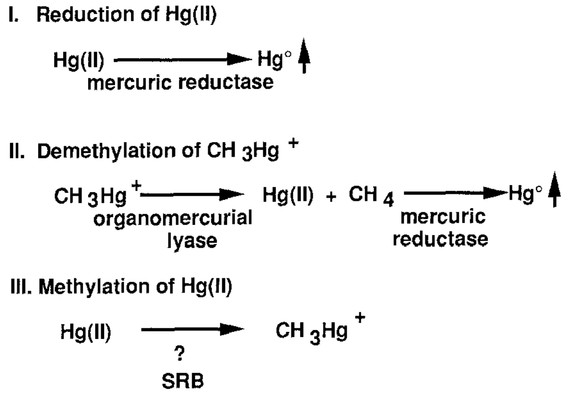

Fig. 2. Microbial transformations of mercury.

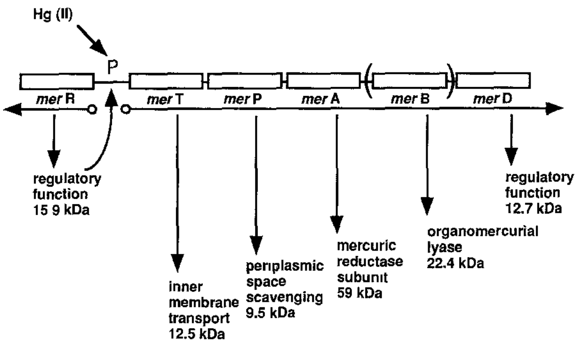

Fig. 3. The *mer* operon. Horizontal arrows – direction of transcription; vertical arrows – polypeptides translated from the corresponding genes. *merTPAD* are from Tn501; *merD* is from the broad spectrum mercury resistance plasmid R381. (Reproduced with permission from Baraky 1992.)

carry out reactions that transform mercury between its three major chemical forms, Hg^{2+}, Hg^0, and organomercury (Fig. 2). They reduce Hg^{2+} to Hg^0, they degrade organomercury (Org-Hg), and they methylate Hg^{2+}. These biological transformations, together with chemical reactions, constitute the mercury cycle in the biosphere (Fig. 1; for a detailed discussion see Barkay 1992). The relative contribution of biological *vs* chemical transformations of mercurial compounds is unknown and probably depends on the concentration of mercury and specific environmental conditions.

The reduction of Hg^{2+} and degradation of Org-Hg are detoxification mechanisms allowing bacterial growth in the presence of Hg^{2+} and Org-Hg, respectively. Bacteria resistant to Hg^{2+} produce mercuric reductase (MR), a flavin-containing disulfide oxidoreductase that catalyzes the reaction:

$$\begin{aligned} &Hg(SR)_2 + NADPH + H^+ \rightarrow \\ &\quad Hg^0 + NADP^+ + 2RSH \end{aligned}$$

The resulting Hg^0 is subsequently volatilized due to its low aqueous solubility (6×10^{-6} g/100 ml water at 25 °C) and high vapor pressure [Henry's constant (H) = 0.3], leaving the immediate environment of the organism, which can then resume growth. A small proportion of Hg^{2+}-resistant (Hg^R) bacteria are also resistant to Org-Hg due to the activity of an additional enzyme, the organomercurial lyase (OL). The OL cleaves the C-Hg bond by a protonolytic attack, as follows:

$$R'HgSR \xrightarrow[\text{Excess RSH}]{} Hg\,(SR)_2 + R'H$$

The product of this reaction, $Hg(SR)_2$, is then reduced by the MR to Hg^0 (Walsh et al. 1988). Thus, resistance to organomercury is mediated by the sequential activity of OL and MR, and all org-Hg^R bacteria are therefore resistant to Hg^{2+} (broad-spectrum mercury resistance). On the other hand, the majority of Hg^R bacteria do not produce OL and are therefore sensitive to org-Hg (narrow-spectrum mercury resistance).

The genes specifying the various functions needed for reduction of Hg^{2+} and degradation of org-Hg are organized in the mercury resistance (*mer*) operon (Fig. 3). The *mer* operons from several organisms have now been analyzed (Silver & Walderhaug 1992), and were all found to specify functions that transport Hg^{2+} through the cell wall (*merT*, *merP*, and *merC*), the MR (*merA*), the OL (*merB*; in broad-spectrum resistance) and regulatory functions (*merR* and *merD*). Some *mer* operons have only one of the regulatory, or two of the transport, genes. Expression of the *mer* operon is negatively regulated (in the absence of Hg^{2+}), and positively regulated (in presence of Hg^{2+} and some organomercury compounds) by the interaction between a dimmer of MerR (the gene product of *merR*), the DNA at the operator/promoter region, and Hg^{2+}. *In vitro* studies employing DNA fingerprinting and restriction site protection analysis have shown that MerR and RNA polymerase are bound to the

DNA in the absence and the presence of Hg^{2+} (Summers 1992). In the absence of Hg^{2+}, this interaction forms a complex that prevents transcription. A conformational change that occurs upon binding of Hg^{2+} (through a tri-coordinate bond with three cysteine residues of the MerR dimmer; O'Halloran et al. 1989), allows subsequent transcription of the *mer*TPA(B)D operon. In addition, MerR is a negative regulator of *merR* transcription both in the absence or the presence of Hg^{2+} (Summers 1992).

When considering the contribution of *mer*-encoded reactions to the geochemical cycling of mercury, it should be noted that chemical reactions are known to reduce Hg^{2+} to Hg^0 in the absence of biological activities (but they may be indirectly mediated by living organisms). In contrast, the purely chemical degradation of org-Hg proceeds so slowly that it is considered negligible (Walsh et al. 1988). Chemical reactions resulting in Hg^0 volatilization, include reduction of Hg^{2+} to $2Hg^+$ followed by disproportionation to Hg^0 and Hg^{2+} (Baltisburger et al. 1979), and reduction by the interaction of Hg^{2+} with free radical electrons of humic acids (Alberts et al. 1974). Observations indicating evolution of Hg^0 by algae grown in pure cultures (Ben-Bassat & Mayer 1978), and possibly by marine phytoplankton (Kim & Fitzgerald 1986), may be explained as an indirect reduction of Hg^{2+} to $2Hg^+$ by organic acids that are produced during photosynthesis.

b. Methylation of Hg^{2+}

The first report demonstrating that microbes in anaerobic freshwater sediments methylated Hg^{2+} was published in 1969 (Jensen & Jerenelöv 1969). It has since been shown to occur in marine and estuarine sediments (Blum & Bartha 1980), in aerobic and anaerobic waters, and in the fish intestine (see review by Winfrey & Rudd 1990). Whereas many microbes can methylate Hg^{2+} in laboratory cultures (Vonk & Sifpesteijn 1973), the organisms responsible for this activity *in situ* have not been clearly identified. Early suggestions that methanogens could methylate Hg^{2+} (Wood 1974) were rejected when experiments were performed employing specific inhibitors of methanogenesis and sulfate reduction. The results of these experiments implicated sulfate reducing bacteria (SRB) as the principal Hg^{2+} methylators in estuarine (Compeau & Bartha 1985) and possibly also in freshwater (Winfrey & Rudd 1990) sediments.

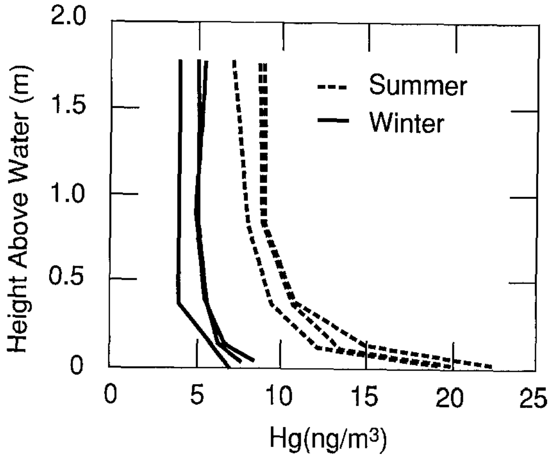

Fig. 4. Elemental mercury gradients formed by the evasion of Hg^0 from RL to the air.

A proposed mechanism of methylation involves the non-enzymatic transfer of a negatively charged methyl group, CH_3^-, from methylcorrinoids, such as methylcobalamine (methyl-B12), to Hg^{2+}. Microbial metabolism facilitates this reaction by the enzymatic transfer of CH_3^- from methyltetrahydrofolate to B12. This proposal is supported by the isolation of mutants that concomitantly lost the ability to synthesize methyl-B12 and CH_3Hg^+ (Pan-Hou & Imura 1982). A novel methylcobalamine-like corrinoid (Berman et al. 1990; Choi et al. 1991) was isolated from a methylating SRB, *Desulfovibrio desulfuricans*. Although information regarding the exact mechanism of bacterial Hg^{2+} methylation is scarce, it is generally thought to involve methyl transfer reactions.

Abiotic sources of methylmercury include Hg^{2+} interactions with humic and fulvic substances (Nagase 1984), and methyl transfer from dissolved organic matter to Hg^{2+} may be mediated photochemically in the presence of sulfur (Akagi et al. 1974). Volatile $(CH_3)_2Hg$ may be formed by a disproportionation reaction between $2CH_3Hg^+$ and H_2S (Deacon 1978).

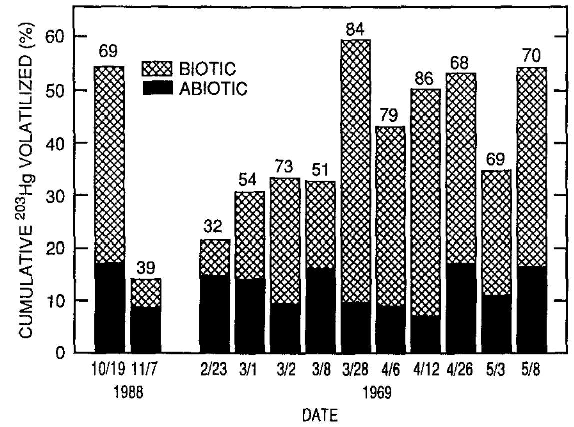

Fig. 5. Biotic and abiotic volatilization of Hg^0 by RL water samples. Numbers above bars indicate percent of volatilization due to biotic activities.

Microbially mediated removal of mercury from a contaminated freshwater stream

East Fork Poplar Creek is a freshwater stream in the vicinity of Oak Ridge, TN. It is a low-order stream-pond ecosystem, contaminated by inorganic mercury released from a nuclear weapons plant located at the headwaters of the stream. Drainage water from the plant is routed through a plastic-lined pond, Reality Lake (RL), with an 8-h retention time prior to discharge into the creek. In recent years, we have been studying the role of microorganisms in the cycling of mercury in RL (Turner et al. 1989; Barkay et al. 1991; Liebert et al. 1991). Control (uncontaminated) samples were collected at a high order river-reservoir system with no known mercury contamination. The results of these studies have encouraged us to propose bioremediation for the management of mercury in aquatic systems.

The role of microbial reactions in the evolution of Hg^0 from RL

Field observations, laboratory experiments with samples taken from RL, and analyses of the indigenous microbial community, have shown that microbes remove mercury, as Hg^0, from RL. A gradient in Hg^0 concentration was detected in air above the pond, surface (Fig. 4), suggesting evasion from the water to the atmosphere. Gradients were measured by pumping air through iodated activated charcoal tubes (to strip Hg^0) that were placed at variable heights above the pond, using a buoy to which a scaffold carrying five air sampling devices was attached. Aerial concentrations of Hg^0 decreased rapidly with height above the water, probably by dilution. Air in close proximity to the surface of the pond contained at least twice as much Hg^0 during summer months as compared to winter months, and as a result stronger gradients were obtained during the summer as compared with winter. The larger flux of Hg^0 during the warmer part

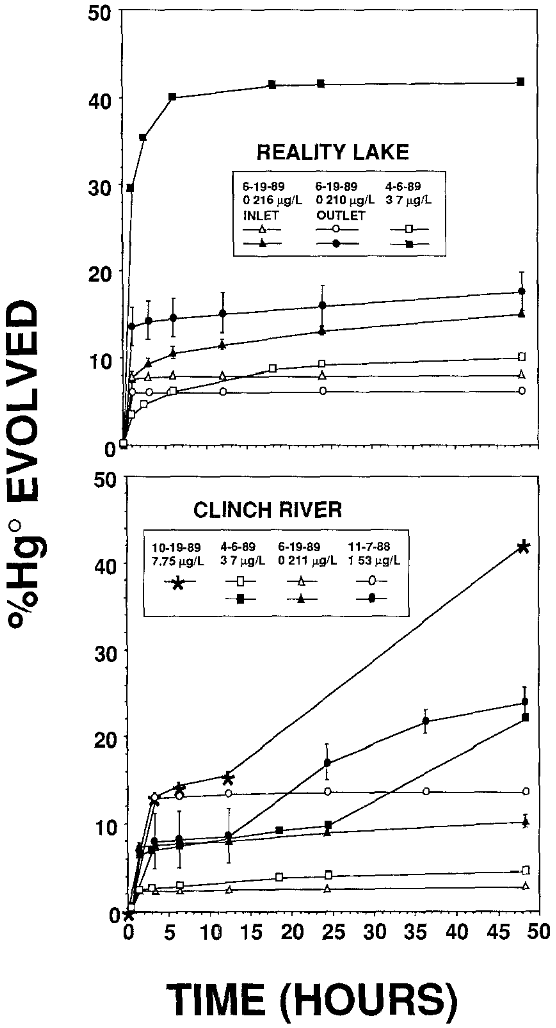

Fig. 6. Evolution of Hg^0 from water samples. Data are presented for each sampling site. Assay dates and total substrate concentrations (indigenous and $^{203}Hg(II)$) are indicated. Full and empty symbols indicate live and heat-killed samples, respectively. (Reproduced with permission from Barkay et al. 1991.)

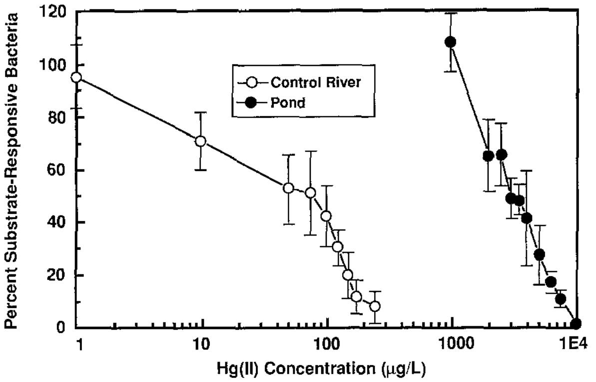

Fig. 7. Tolerance to Hg^{2+} of RL and river microbial communities. Dose response curves are based on direct counts. Percent cells that utilize yeast extract in the presence of increasing concentrations of Hg^{2+}, of cells utilizing yeast extract in the absence of Hg^{2+}, were plotted against test Hg^{2+} concentrations. (Reproduced with permission from Liebert et al. 1991.)

of the year may be due to biological activities and to the effect of temperature on partition of Hg^0 between water and air.

Comparisons of biotic with abiotic Hg^0 reduction/volatilization from RL water samples, measured using a $^{203}Hg^{2+}$, showed that biotic processes were relatively more significant during spring as compared to winter months (Fig. 5). Abiotic Hg^0 evolution was measured in autoclaved samples. Less than 40% of Hg^0 was generated by biotic processes in Nov. 88 and Feb. 89, and this proportion gradually increased to 70–80% through March–May 89. These results suggested that an active microbial community in the pond reduced Hg^{2+} to Hg^0. This conclusion was further supported by studies that compared the microbial communities of the pond with those of communities from the control river site. The pond community was more tolerant to Hg^{2+} (Liebert et al. 1991), which correlated with an enrichment of DNA sequences with homology to *mer* genes, and the biological reduction of Hg^{2+} to Hg^0 (Barkay et al. 1991).

Different kinetics of Hg^0 evolution by active pond and control samples were observed (Fig. 6). Elemental mercury was immediately evolved from pond samples after spiking with $^{203}Hg^{2+}$. On the other hand, Hg^0 from biologically active river samples was evolved in two distinct phases. An initial burst of Hg^0, also present in abiotic samples, was followed by a second increase in activity 12 to 30 h after spiking. In all cases, we did not observe further increase in Hg^0 in autoclaved samples following initial Hg^0 evolution. Thus, we hypothesized that initial activity in river samples was due to abiological processes that reduce Hg^{2+}, whereas reduction of Hg^{2+} by biological activities began after a lag period of 12 to 24 h. A lag period prior to the initiation of Hg^{2+} reduction is considered an acclimation period, and is commonly observed in samples that have not been previously exposed to Hg^{2+} (Barkay 1987). According to this hypothesis, the instantaneous $^{203}Hg^0$ evolution observed in biologically active pond samples strongly suggests that the microbial community of the pond was acclimated to Hg^{2+} and that a substantial proportion of the Hg^0 formed *in situ* was due to microbial activities.

Enrichment of active populations in response to the toxicity of Hg^{2+} is the most likely mechanism of acclimation (Barkay 1987), although induction of enzymatic activities and gene transfer between populations (Spain et al. 1980) cannot be ruled out. Indeed, the pond community had a greater number of organisms resistant to Hg^{2+} than the control river community (Fig. 7). Resistance was quantitated by a direct counting method (Liebert & Barkay 1988) that is based on a modification of methods for enumeration of viable bacteria in aquatic samples (Kogure et al. 1979). Water samples were incubated with yeast extract as a growth substrate, nalidixic acid to inhibit cell division, and variable Hg^{2+} concentrations. Under these conditions, only Hg^{2+}-resistant cells can utilize the available substrate and form large elongated cells. The percent of large cells formed in the presence of Hg^{2+}, of those formed in control samples without Hg^{2+}, is a function of the tolerance of viable bacteria to Hg^{2+}. A dose response curve obtained when these percents are plotted against test Hg^{2+} concentrations (Fig. 7) is used to calculate IC_{50} (concentration that inhibits 50% of elongated cells). Thus, IC_{50} values for the pond and river communities were 3,582 ± 160 and 69.97 ± 4.66 µg Hg^{2+}/L, respectively (see Liebert et al. 1991 for a description of calculations and statistical analyses). The 50-fold difference in IC_{50} values indicates that Hg^{2+}-tolerant popula-

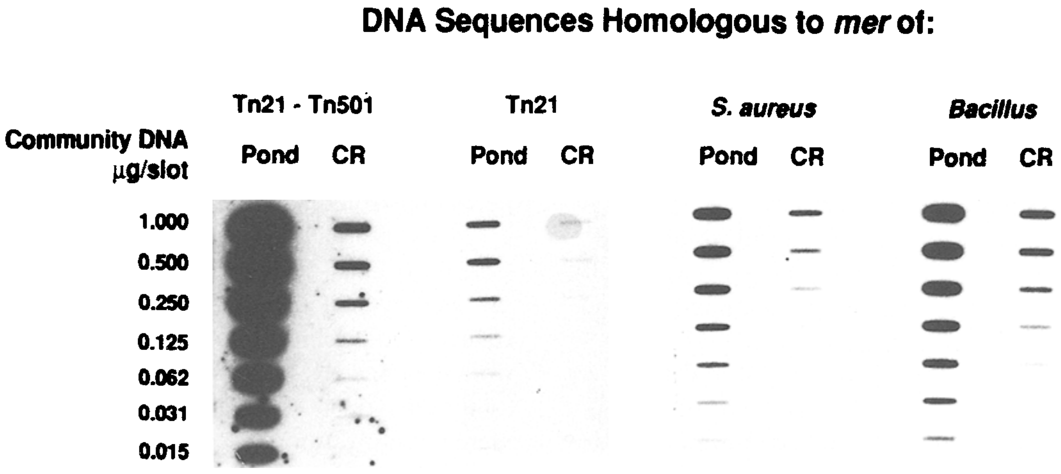

Fig. 8. Abundance of *mer* genes in RL and river microbial communities. Autoradiogram of results obtained by hybridizing DNA that was extracted from pond (Pond) and river (CR) water samples, with *mer* probes. Intensity of hybridization signals is directly related to the abundance of *mer* in DNA extracts. (Reproduced with permission from Barkay et al. 1991.)

tions are enriched in the microbial community of the contaminated pond.

Enriched Hg^R bacteria in the pond most likely carried *mer* genes because DNA-DNA hybridizations with four *mer* probes (Barkay et al. 1989) showed a higher abundance of these genes in DNA extracted from pond, as compared to river, biomass (Fig. 8). Autoradiographic signals were quantitated by densitometry using a calibration curve prepared with known amounts of the various *mer* operons. The abundance of all *mer* systems (per µg community DNA) was higher in the pond community as compared to the control river community. Increase in abundance was as high as 72-fold for DNA homologous to *mer* of transposons 501 (Tn501) and 21 (Tn21) (18.121 ± 4.059 and 0.251 ± 0.023 ng target DNA/µg community DNA for pond and river communities, respectively).

Compilation of the data from RL strongly suggests that *mer*-specified bacterial activities reduce Hg^{2+} to Hg^0, thereby contributing to the volatilization of Hg^{2+} from a mercury-contaminated freshwater pond. This study therefore demonstrates the role of a specific bacterial reaction in the geochemical cycling of mercury in the environment. Could this reaction be stimulated to further remove Hg^{2+} from the pond? A decrease in Hg^{2+} concentration would indirectly affect bioavailable CH_3Hg^+ concentration by substrate limitation, according to the following equation:

$$Hg^0 \longleftarrow Hg^{2+} \rightleftharpoons CH_3Hg^+$$

The feasibility of this approach to the remediation of mercury is currently being tested in our laboratory.

An experimental approach to test the feasibility of mer*-mediated reactions for the remediation of contaminated sites*

The effect of manipulations on the rate of Hg^0 volatilization and CH_3Hg^+ degradation is tested using an experimental approach that progresses from a simple laboratory test system to one incorporating more of the known elements involved in total ecosystem mercury cycling. Analysis is based on quantitation and speciation of mercury by the methods of Bloom & Fitzgerald (1988), and Bloom (1989) in the various compartments of each test system.

At present we do not know whether mercury transformations in the pond are limited by the availability of nutrients for microbial activities, the size of the Hg^{2+} reducing and CH_3Hg^+ demethylating populations, or the availability of substrates for transformation reactions. The following treatments will therefore, be attempted:

(i) stimulation of general microbial activities, among them mercury-specific reactions, by the addition of limiting growth substrates,

(ii) stimulating only Hg^{2+} reduction and CH_3Hg^+ demethylation by the addition of bacterial strains capable of these activities, and

(iii) increasing availability of the substrates, Hg^{2+} and CH_3Hg^+.

Rationale and procedures for treatments (i) and (ii) are described below.

Addition of growth substrates to stimulate activities of indigenous microorganisms has been demonstrated as a successful approach to remediation of contaminated sites (Thomas & Ward 1989). This treatment may stimulate general microbial activities in RL because concentrations of essential nutrients suggest that carbon and phosphorous may be limiting (Table 3). Typical concentrations of available carbon (as DOC), nitrogen (as $NH_4 + NO_3$), and phosphorous (as total PO_4) in RL are 3.67, 4.83, and 0.28 mg/L, respectively. Not only that DOC is low, approaching values found in oligotrophic waters (Morita 1982), but these values constitute an imbalanced C:N:P ratio of 13:17:1. The bacterial cell composition has C:N:P of 17:5:1 (%) (Stanier et al. 1971), and natural planktonic bacterial assemblages have a C:N ratio of 4:1 (on a weight/volume basis) (Nagata 1986). Assuming that approximately 50% of the carbon consumed by microbes is assimilated into cellular material, a C:N:P ratio of available nutrients that deviates from 34:5:1 indicates that bacterial growth and activities are limited. Thus, microbial activities in RL could be stimulated by the addition of carbon (added as glutamate, glucose or as a mixture of amino acids) and/or phosphorous (added as PO_4).

Table 3. Concentrations of essential microbial growth substrates in Reality Lake.

Element	Mg/L	
	Range	Mean ± SD
Carbon (DOC)	1 - 4	3.67 ± 0.82
Nitrogen	3.5 - 6.0	4.83 ± 1.25
Phosphorous	0.2 - 0.4	0.278 ± 0.067

Values are typical to RL, measurements of organic and inorganic nutrient concentrations in RL are performed routinely with samples collected weekly.

Increasing the population size of active microorganisms by application of genetically engineered or native microbes has been a much discussed prospect in environmental management (Halvorson et al. 1985). We have demonstrated Hg^{2+} reduction (see above) and CH_3Hg^+ degradation (Temple et al. 1989) by biologically active water samples; and reducing and demethylating strains were isolated from the pond (Rochelle et al. 1991). The possibility that the abundance of active populations limits mercury transformations will be investigated by introducing active bacterial strains to water samples collected in RL and following their effect on rates of Hg^{2+} reduction/volatilization and CH_3Hg^+ demethylation. Strains with high levels of MR and OL will be selected for testing, from a collection of Hg^R and org-Hg^R bacteria, that was isolated by enrichment from RL water. In addition, genetically engineered *Pseudomonas putida* strains harboring a constitutively expressed broad-spectrum *mer* operon will be employed. The broad-spectrum pDU1358-derived *mer* operon (Griffin et al. 1987) deleted of regulatory elements (*merR* and *merD*) was transposed into *P. putida* using the Tn5-based delivery system of Herrero et al. (1990), and Hg^{2+} and phenylmercuric acetate (PMA) resistant derivatives were selected. Some of these strains, constitutively expressing *mer* functions from *P. putida* promoters, were subsequently shown to be highly tolerant to Hg^{2+} and PMA and to hyper-produce MR (Horn et al. submitted for publication). Furthermore, they effectively removed Hg^{2+} from wastewaters in an experimental, closed, treatment system (Horn, in preparation). Because of concerns regarding release of genetically engineered microorganisms, testing of these engineered *P. putida* strains will be performed in contained systems, shake flasks, and microcosms; field applications (see below) will only employ native non-engineered organisms.

Evaluation of the success of remedial treatments

Our approach to test the effectiveness of remedial treatments is based on evaluating how the geochemical cycle of mercury is affected by accelerat-

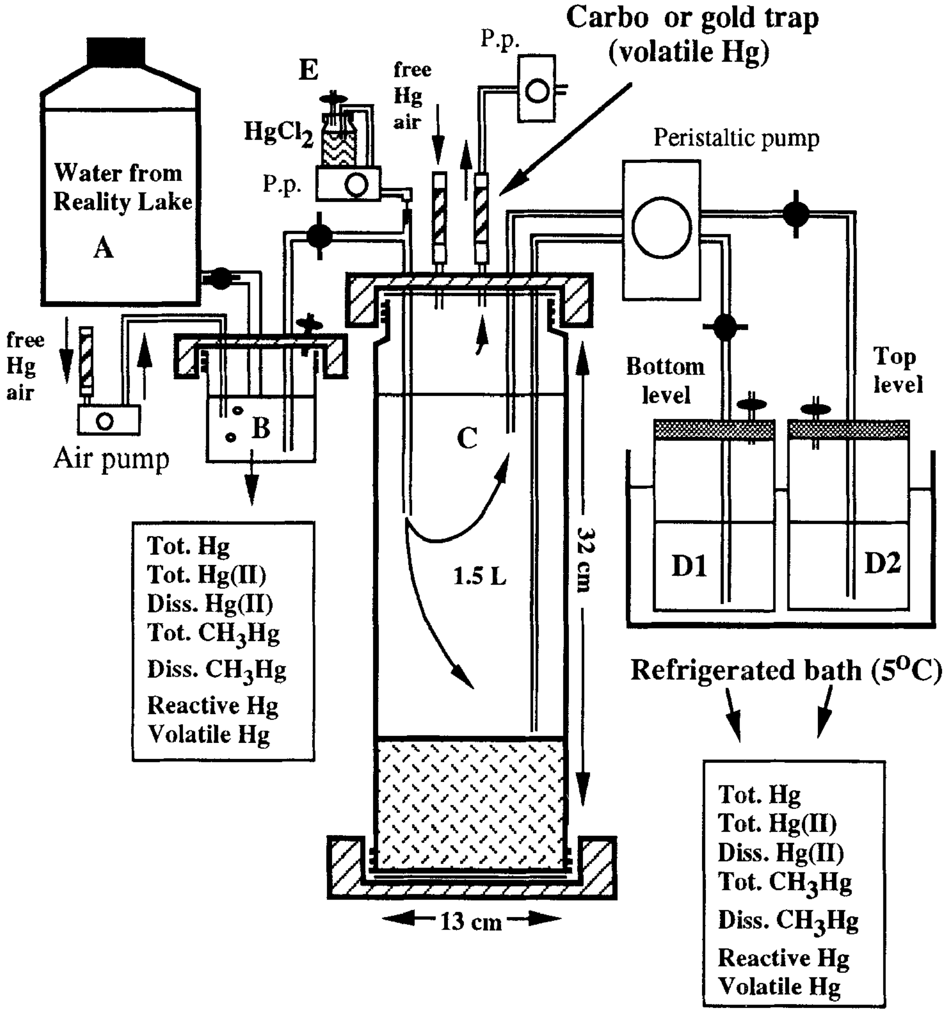

Fig. 9. A microcosm system for the study of geochemical cycling of mercury in aquatic environments. Various species shown to play a role in the geochemical cycling of mercury, are monitored in inlet water, outlet water and air leaving the microcosm. In addition, mercury in sediment and microbiota is quantitated and speciated when microcosm experiments are terminated.

ing specific mercury biotransformations. The approach integrates analyses of mercury pools and fluxes into a mathematical model that describes the mass balance of mercury in aquatic ecosystems. This mercury cycling model (MCM) can be used to predict the pool size of different mercury species using known rates of transformations (Hudson et al. 1991). Thus, once the MCM is shown to describe the cycling of mercury in RL, it can predict the magnitude of increase in Hg^{2+} reduction and CH_3Hg^+ degradation rates that is needed to effectively reduce the concentration of CH_3Hg^+ available for accumulation by fish.

Effect of remedial treatments on rates of Hg^{2+} reduction and CH_3Hg^+ degradation will initially be measured in flasks (Turner et al. 1989) by following evolution of Hg^0. Effective treatments (i.e. with a potential to significantly decrease concentration of CH_3Hg^+) will then be tested in microcosms. A mercury geochemical cycling microcosm has been constructed to allow speciation and quantitation of the major chemical species of mercury that are present in RL (Fig. 9). Inventories of the various species of mercury in air, and inlet and outlet waters, enable determinations of pool size, fluxes between pools, and rates of transformations. This microcosm system utilizes state-of-the-art methods for mercury analyses (Bloom 1989; Bloom & Fitzgerald 1988), therefore allowing evaluation of promising remedial treatments in terms of their effect on the geochemical cycle of mercury in RL. In addition, various aspects of the geochemical cycling of mercury in aquatic environments can be studied using this microcosm because sensitivity of analyses allows for accurate measurements of mercury at background concentrations (10^{-12}–10^{-15} M).

Treatments shown to be effective in microcosms will then be tested in enclosures placed in RL. Field enclosures have been constructed and their utility tested. Our objective is to demonstrate a decrease in CH_3Hg^+ concentration in the water and in fish that are placed in these enclosures.

Acknowledgements

Thanks are extended to Mary Anna Bogle, Cynthia Liebert and Mark Gillman for their assistance. The work reported here was partially supported by Electric Power Research Institute agreement RP3015–03.

References

Akagi H, Takabatake E & Fujita Y (1974) Photochemical methylation of inorganic mercury in the presence of solid sulfur. Chem. Let. 761–764

Alberts JJ, Schindler JE, Miller RW & Nutter Jr. DE (1974) Elemental mercury evolution mediated by humic acid. Science 184: 895–897

Baltisberger RJ, Hildebrand DA, Grieble D & Ballintine TA (1979) A study of the disproportionation of mercury (I) induced by gas sparging in acidic aqueous solutions for cold-vapor atomic absorption spectrometry. Anal. Chim. Acta 111: 111–122

Barkay T (1987) Adaptation of aquatic microbial communities to Hg^{2+} stress. Appl. Environ. Microbiol. 53: 2725–2732

Barkay T (1992) The mercury cycle. Encyclopedia of Microbiology, Vol 3. Academic Press Inc. San Diego

Barkay T, Liebert C & Gillman M (1989) Hybridization of DNA probes with whole-community genome for detection of genes that encode microbial responses to pollutants: *mer* genes and Hg^{2+} resistance. Appl. Environ. Microbiol. 55: 1574–1577

Barkay T, Turner RR, VandenBrook A & Liebert C (1991) The relationships of Hg(II) volatilization from a freshwater pond to the abundance of *mer* genes in the gene pool of the indigenous microbial community. Microb. Ecol. 21: 151–161

Ben-Bassat D & Mayer AM (1978) Light-induced Hg volatilization and O_2 evolution in *Chlorella* and the effect of DCMU and methylamine. Physiol. Plant. 42: 33–38

Berman MT, Chase Jr T & Bartha R (1990) Carbon flow in mercury biomethylation by *Desulfovibrio desulfuricans*. Appl. Environ. Microbiol. 56: 298–300

Bloom N (1989) Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence. Can. J. Fish. Aquat. Sci. 46: 1131–1140

Bloom NS & Fitzgerald (1988) Determination of volatile mercury species at the picogram level by low temperature gas chromatography with cold-vapor atomic fluorescence detection. Anal. Chim. Acta 209: 151–161

Blum JE & Bartha R (1980) Effect of salinity on methylation of mercury. Bull. Environm. Contam. Toxicol. 25: 404–408

Bopp LH & Ehrlich HL (1988) Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. Arch. Microbiol. 150: 426–431

Choi S-C, Berman M & Bartha R (1991) Evidence for a novel Hg^{2+}-methylating corrinoid in *Desulfovibrio* desulfuricans. Abst. 91th Annu. Meet. Am. Soc. Microbiol., Q-261, (p 320)

Compeau GC & Bartha R (1985) Sulfate-reducing bacteria: principle methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. 50: 498–502

Deacon GB (1978) Volatilization of methyl-mercuric chloride by hydrogen sulphide. Nature (London) 275: 344

D'Itri PA & D'Itri FM (1978) Mercury contamination: a human tragedy. Environ. Manag. 2: 3–16

Frankenberger Jr. WT (1992) Dissipation of soil selenium by microbial volatilization at Kesterson reservoir. Abst. 203rd Am. Soc. Chem. Nat. Meet. San Francisco, CA April 5–10, 1992

Griffin HG, Foster TJ, Silver S & Misra TK (1987) Cloning and DNA sequence of the mercuric- and organomercurial-resistance determinants of plasmid pDU1358. Proc. Natl. Acad. Sci. U.S.A. 84: 3112–3116

Guard HE, Cobet AB & Coleman III WM (1981) Methylation of trimethyltin compounds by estuarine sediments. Science 213: 770–771

Hallas LE, Means JC & Cooney JJ (1982) Methylation of tin by estuarine microorganisms. Science 215: 1505–1507

Halvorson HO, Pramer D & Rogul M (1985) Engineered Organisms in the Environment: Scientific Issues. American Society for Microbiology, Washington DC

Hamer DH (1988) Metallothionein. Annu. Rev. Biochem. 55: 913–951

Herrero M, de Lorenzo V & Timmis KN (1990) Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. J. Bacteriol. 172: 6557–6567

Horn J, Brunke M, Deckwer W-D & Timmis KN (1993) *Pseudomonas putida* strains which constitutively hyperexpress mercury resistance for biodetoxification of organomercurial pollutants. Appl. Environ. Microbiol. (in press)

Hudson RJM, Gherini SA & Munson RK (1991) The MTL mercury model: a description of the model, discussion of scientific issues, and presentation of preliminary results. In: Mercury in Temperate Lakes – 1990 Annual Report

Jensen S & Jernelöv A (1969) Biological methylation of mercury in aquatic organisms. Nature 223: 753–754

Karlson U & Frankenberger Jr. WT (1989) Accelerated rates of selenium volatilization from California soils. Soil Sci. Soc. Am. J. 53: 749–753

Kim JP & Fitzgerald WF (1986) Sea-air partitioning of mercury in the equatorial Pacific ocean. Science 231: 1131–1133

Kogure K, Simidu U & Taga N (1979) A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. 25: 415–420

Liebert C & Barkay T (1988) A direct viable counting method for measuring tolerance of aquatic microbial communities to Hg^{2+}. Can. J. Microbiol. 34: 1090–1095

Liebert C, Barkay T & Turner RR (1991) Acclimation of aquatic microbial communities to Hg(II) and CH_3HgCl in polluted freshwater ponds. Microb. Ecol. 21: 139–149

Lovley DR & Phillips EJP (1992) Reduction of uranium by *Desulfovibrio desulfuricans*. Appl. Environ. Microbiol. 58: 850–856

Lovley DR, Landa ER, Phillips EJP & Woodward JC (1992) Remediation of uranium-contaminated soils using uranium extractants and microbial uranium reduction. Abst. 203rd Am. Chem. Soc. Nat. Meeting, San Francisco April 5–10, 1992

Morita RY (1984) Starvation-survival of heterotrophs in the marine environment. Adv. Microb. Ecol. 6: 171–198

Nagase H (1984) Mercury methylation by compounds in humic material. Sci. Tot. Environ. 32: 147–156

Nagata T (1986) Carbon and nitrogen content of natural planktonic bacteria. Appl. Environ. Microbiol. 52: 28–32

Nriagu JO (1979) The Biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Biomedical Press, Amsterdam

Pan-Hou HS & Imura N (1982) Involvement of mercury methylation in microbial mercury detoxication. Arch. Microbiol. 131: 176–177

Patterson JM & Passino R (1987) Metals Speciation, Separation, and Recovery. Lewis Publishers, Inc., Chelsea, MI

O'Halloran TV, Frantz B, Myung KS, Ralston DM & Wright JG (1989) The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex. Cell 56: 119–129

Olafson RW (1984) Prokaryotic metallothionein. Internat. J. Peptide Protein Res. 24: 303–308

Rauser WE (1990) Phytochelatins. Annu. Rev. Biochem. 59: 61–86

Rochelle PA, Wetherbee MK & Olson BH (1991) Distribution of DNA sequences encoding narrow- and broad-spectrum mercury resistance. Appl. Environ. Microbiol. 57: 1581–1589

Silver S (1991) Resistance systems and detoxification of toxic heavy metals. In: Rossmoore H (Ed) Proceedings of the Eighth International Biodeterioration Symposium (pp 308–339). Elsevier Applied Science, London

Silver S & Walderhaug M (1992) Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol Rev. 56: 195–228

Spain JC, Pritchard PH & Bourquin AW (1980) Effects of adaptation on biodegradation rates in sediment/water cores from estuarine and freshwater environments. Appl. Environ. Microbiol. 40: 726–734

Stanier RY, Doudoroff M & Adelberg EF (1971) General Microbiology. The Macmillan Press Ltd., London

Steinberg NA & Ormland RS (1990) Dissimilatory selenate reduction potentials in a diversity of sediment types. Appl. Environ. Microbiol. 56: 3550–3557

Summers AO (1992) Untwist and shout: a heavy metal-responsive transcriptional regulator. J Bacteriol. 174: 3097–3101

Temple KA, Turner RR & Barkay T (1989) Volatilization of divalent mercury and demethylation of methylmercury: microbial adaptation in a mercury-contaminated site. Abst. Q-186, 89th Annu. Meet. Am. Soc. Microbiol. (p 361)

Thomas JM & Ward CH (1989) In situ biorestoration of organic contaminants in the subsurface. Environ. Sci. Technol. 23: 760–766

Turner RJ, Hou Y, Weiner JH & Taylor DE (1992) The arsenical ATPase efflux pump mediates tellurite resistance. J. Bacteriol. 174: 3092–3094

Turner RR, VandenBrook AJ, Barkay T & Elwood JW (1989) Volatilization, methylation, and demethylation of mercury in a mercury-contaminated stream. In: Vernet JP (Ed) Proc. Int. Conf. Heavy Metals in the Environment (pp 353–356). CEP Consultants Ltd., Edinburgh, UK

Vonk JW & Sijpesteijn AK (1973) Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. Antonie van Leeuwenhoek 39: 505–513

Walsh CT, Distefano MD, Moore MJ, Shewchuk LM & Verdine GL (1988) Molecular basis of bacterial resistance to organomercurial and inorganic mercuric salts FASEB J. 2: 124–130

Watras CJ & Bloom SN (1992) Mercury and methylmercury in individual zooplankton: implications for bioaccumulation. Limnol. Oceanogr. 37: 1313–1318

Winfrey MR & Rudd JWM (1990) Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. 9: 853–869

Wood J (1974) Biological cycles for toxic elements in the environment. Science 183: 1049–1052