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Toxic Metals in Aquatic Ecosystems: A Microbiological Perspective

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Microbe–metal interactions in aquatic environments and their exact role in transport and transformations of toxic metals are poorly understood. This paper will briefly review our understanding of these interactions. Ongoing research in Lake Chapala, Mexico, the major water source for the City of Guadalajara, provides an opportunity to study the microbiological aspects of metal-cycling in the water column. Constant resuspension of sediments provides a microbiologically rich aggregate-based system. Data indicate that toxic metals are concentrated on aggregate material and bioaccumulate in the food chain. A provisional model is presented for involvement of microbial aggregates in metal-cycling in Lake Chapala. —Environ Health Perspect 103(Suppl 1):25–28 (1995)

Key words: microorganisms, toxic metals, metal-cycling, Lake Chapala, bioaccumulation, aggregates

Introduction

Understanding the distribution of toxic metals in aquatic ecosystems is important to our assessments of environmental and human health risks from natural waters. It is becoming increasingly apparent that microbial processes may be important and even dominating factors in the distribution of specific metals (1). Our understanding of microbe–metal interactions has been limited by the complexity of both the microbiology and chemistry of natural systems. Laboratory studies, however, indicate the potential for significant interaction, at least within soil and sediment ecosystems. There is considerable information on specific interactions between microorganisms and iron and manganese and on the importance of these interactions in the biogeochemical cycling of these elements (2). The following discussion will focus on the cycling of toxic metals and the potential role of microbe–metal interactions in these processes.

Metal–Microbe Interactions

Interactions between microorganisms and metals can be conveniently divided into

three distinct processes (3), all of which may be important with respect to metal distribution in natural waters: a) intracellular interactions, b) cell–surface interactions, and c) extracellular interactions.

Intracellular Interactions

Assimilation of metals may be important to the microbe in detoxification, enzyme function, and physical characteristics of the cell. Probably the most widely recognized microbial interaction with toxic metals in the aquatic environment, is the microbial methylation of mercury. A considerable number of studies have addressed the importance of this interaction in the volatilization and subsequent bioaccumulation of the lipid-soluble, methylated form of mercury (4,5). However, it is still unclear how significant microbial methylation of mercury is in the bioaccumulation of this metal. Pure-culture experiments have shown that many bacteria and fungi have the capability to methylate mercury (5). However, in the environment, sulfate-reducing bacteria appear to dominate in this process (6). The mechanism is thought to involve intracellular methylation by nonenzymatic transfer of methyl groups from methylcobalamin (vitamin B_{12}) (4). For the microorganism, this is probably a detoxification mechanism, as it results in volatilization of the mercury, and hence removal from the immediate environment of the sulfate-reducing bacteria. The eventual fate of the methyl mercury is then dependent on rates of microbial demethylation, a process that occurs closer to the sediment–water interface.

Although receiving less attention than mercury, methylation of other toxic metals,

with subsequent volatilization, may also occur in the aquatic environment. Methylation has been shown for tin, arsenic, lead, selenium, tellurium, thallium, and antimony (7). Gilmour and co-workers (8,9) correlated production of monomethyl tin in sediment samples with numbers of sulfate-reducing and sulfide-oxidizing bacteria. In addition, they isolated *Desulfovibrio* spp. from the sediments that were able to methylate tin in culture medium at rates similar to those for sediment methylation. Methylation of arsenic by fungi was studied extensively as a result of poisoning from fungal transformations of arsenic in paints (10).

Cell-Surface Interactions

A number of authors have shown that metal binding to cell surfaces is an important factor in the distribution of metals in natural waters (11,12). Algal surfaces contain functional groups (e.g., carboxylic, amino, thio, hydroxo, and hydroxy-carboxylic groups) that can interact with metal ions (12). Gram-negative bacteria possess lipopolysaccharides and phospholipids in their cell walls, with phosphoryl groups as the most abundant electronegative sites available for metal binding (13,14). Gram-positive bacterial cell walls possess teichoic acids and peptidoglycan, providing carboxyl and phosphoryl groups that are potential sites for metal binding (15).

For both gram-negative and positive bacteria, metal binding to cell-surface functional groups is thought to be an important step to intracellular accumulation of trace metals required for enzyme function. In addition, certain bacteria appear capable of using toxic metal species as electron

acceptors, with both selenate and chromate reportedly reduced under anaerobic conditions (*16,17*).

Extracellular Interaction

Extracellular interactions with toxic metals range from the potential to leach metals from sediments by production of acidic metabolites to the formation of colloidal-sized extracellular polysaccharide (EPS) metal complexes implicated in mobilization and transport of toxic metals in soils (*18,19*). Indirectly, toxic metals closely associated with iron oxide (Cd and Zn) have been shown to be solubilized by enzymatic reduction of the ferric iron (*20*). Insoluble complexes may also be formed by the activity of microorganisms. Metal sulfides are extremely insoluble and therefore production of sulfide by the sulfate-reducing bacteria may be instrumental in immobilizing certain metals (*1*).

EPS–metal interactions are of particular interest in their ability to mobilize and transport metals. The ability to essentially bind toxic metals in the colloidal fraction of the organic carbon pool is important in the cycling of metals in any aquatic system. In soilwater, colloidal transport theories suggest that metals bound to colloids may move at faster rates through the soil than other metal species that are more readily adsorbed to soil particles. This may be due to simple size exclusion principles and/or the hydrophilic nature of many bacterial polysaccharides (*21*). Modeling rates of metal contaminant movement to groundwater is therefore complicated by these interactions. Within the water column, dynamics of metal distribution and cycling rates are altered by an association with this colloidal fraction.

Many microorganisms produce EPS (often containing proteins) that strongly binds metals. Interactions between EPS and metal ions are generally considered a direct consequence of negatively charged functional groups on the exopolymer. These include pyruvyl, phosphoryl, hydroxyl, succinyl, and uronyl groups. A pH-dependent binding of positively charged cations can rapidly occur with stability constants in excess of those generally measured for humic substances and other naturally occurring ligands (Table 1).

In addition to the above, we do not fully understand the role of siderophores in toxic metal cycling. Siderophores are low molecular weight organic compounds produced by a number of microorganisms to sequester iron and are thought to be highly specific. However, Cu(II) complexation

Table 1. Conditional stability constants (K_c) of bacterial EPS-metal complexes.

Bacterium	Metal	$K_c, \times 10^8$
Thermus sp.[a]	Cu	0.7
Deleya marina[a]	Cu	24.0
D. marina[a]	Mn	9.0
D. marina[a]	Ni	14.0
Pedomicrobium manganicum[a]	Mn	19.0
Sediment bacterium[b]	Cu	7.3
Humic acids[b]	Cu	6.6
Humic acids[c]	"metals"	0.0032–0.0063
Fulvic acids[c]	"metals"	0.005–0.01

[a]From Ford and Mitchell (*1*). [b]From Mittelman and Geesey (*22*). [c]From ranges given in Thurman (*23*). [Note: differences may partially reflect different measurement techniques used in Thurman (*23*), compared to Ford and Mitchell (*1*) and Mittelman and Geesey (*22*), see original papers.]

with both hydroxamate and catecholate siderophores has been reported and may be important in sequestering copper for production of the tyrosinase enzyme (*24*). In addition, it has been suggested (*25*) that hydroxamate siderophores may play an important role in the reduction of copper toxicity to cyanobacteria. The authors speculated that the geometry of the copper-siderophore complex made it unlikely that it would be assimilated by cyanobacterial cells, as it would not be recognized by the iron transport system.

Ongoing Research

Our research has focused primarily on EPS–metal interactions and the reader is referred to the following publications for more detailed information (*1,3,26–28*). Our laboratory has also been closely involved with metal-transformation studies, with the work of Gilmour and Henry (*5*). Current research is designed to characterize both metal distributions and micro-

bial communities in natural waters. One particular example is our studies on Lake Chapala, Mexico, the principal water source for the Guadalajaran metropolitan area. We have been measuring water column (dissolved and particulate) concentrations of arsenic, lead, cadmium, copper, zinc, nickel, and chromium, throughout the lake, its inlet, and outlet. In addition, we have measured toxic metal concentrations in sediments, plants, and fish. As expected, there is considerable bioaccumulation in fish tissues of certain metals, in particular copper and zinc, which bioaccumulate differently in different fish species (Table 2). From a microbiological perspective, however, association of metals with particulate material may prove to be of greatest interest. Lake Chapala is in many ways limnologically unique. Constant resuspension of sediments results in minimal accumulation of toxic metals in the sediments and an extremely high turbidity [down to 0.2 m secchi transparency (*29*)], which effectively minimizes primary productivity. The extremely high turbidity results from clay-based particulates that consist primarily of $CaCO_3$, adsorbed organic material, and microbiota. Heterotrophic activity associated with this particulate material is thought to be high (OT Lind, personal communication). A significant proportion of toxic metals associated with this fraction. Neutron activation analysis was used to obtain data for a large range of elements; however, of the toxic metals, only chromium and zinc could be directly partitioned between dissolved and aggregate phases, and in some cases selenium and arsenic. Using surficial sediment data, which provides concentrations similar in magnitude to aggregate data due to the constant resuspension, we have also provisionally estimated the potential

Table 2. Trace metal analysis of liver and muscle tissue from Lake Chapala fish (ppm dry weight).

No	Species	Tissue	Cd	Cr	Cu	Pb	Ni	Zn
1	Tilapia	Liver	<3	15	2067	<100	<40	96
		Muscle	NA	0.33	2.8	3.5	0.22	35.4
2	Tilapia	Liver	0.5	4.8	2890	13.5	1.85	102
		Muscle	NA	<0.1	13.6	1.7	0.32	38.1
3	Tilapia	Liver	<1.7	4.04	1820	2.1	1.7	117
		Muscle	NA	0.42	7.5	7.2	0.24	35.2
4	Carp	Liver	2.1	8	163	32	13.9	1443
		Muscle	NA	<0.1	2.23	0.9	<0.3	55.1
5	Carp	Liver	1.4	1.31	104	2.62	0.46	1150
		Muscle	NA	<0.1	3.23	8.8	2.6	186
6	Carp	Liver	<0.5	1.46	126	2.1	0.47	685
		Muscle	NA	<0.1	6.2	<9	3	298

NA, not analyzed.

Table 3. Partitioning of toxic metals between dissolved and aggregate phase (concentrations given as μg l⁻¹ lake water; concentration factors calculated on a ppm basis).

|		As	Cr	Se	Zn	Cu	Cd	Pb	Ni
West Site	Dissolved	34.00	2.00	1.10	36.00	NA	NA	NA	NA
	Aggregate	0.43^a	7.17	0.13^a	10.41				
	Conc fac	121	34487	1100	2779				
	% agg	1.24	78.20	10.27	22.42				
Center Site	Dissolved	36.00	2.30	1.00	37.00	16.60	NA	13.50	12.10
	Aggregate	0.74^a	4.56	0.01^a	6.31	1.86^a		1.90^a	1.68^a
	Conc fac	271	26093	180	2245	1476		1852	1826
	% agg	2.02	66.48	1.35	14.57	10.09		12.34	12.19
East Site	Dissolved	34.00	2.80	1.00	32.00	16.50	2.50	12.80	18.30
	Aggregate	0.50	7.28	0.04^a	13.97	4.10^a	0.05^a	5.30^a	4.88^a
	Conc fac	94	16665	230	2799	1594	120	2656	1710
	% agg	1.45	72.22	3.46	30.39	19.91	1.84	29.30	21.06

Abbreviations: Conc fac, concentration factor; % agg, percent aggregrate associated. ^{a}Surficial sediment data, included for comparison with aggregate data and used for calculating concentration factors and partitioning when aggregate analysis was below detection.

magnitude of partitioning for cadmium, nickel, lead, and copper. These data are summarized in Table 3. For all metals, concentration factors on a part per million basis are high. As would be expected in a high pH lake, metals tend to associate with particulate material. However, on a per-liter basis, only chromium will be greatly underestimated by water column analysis alone. Chromium is concentrated on particles by factors up to 35,000-fold, which is equivalent to 79% of the chromium per liter of water.

Lake Chapala provides a unique system for investigating the potential for interaction between toxic metals and the particulate microbiota. We are interested in the role of bacterial exopolymers within these particulates in retaining toxic metals in suspension. We have isolated a number of bacterial species from unfiltered Lake Chapala water to characterize their exopolymers. Bacterial isolation procedures, exopolymer preparation, and chemical analyses have been described in detail previously by Ford and others (*30*). Exopolymers from six different Lake Chapala isolates were characterized using Pyrolysis-Mass Spectrometry. Specific marker fragments for acetyl groups were found for all isolate exopolymers. These marker fragments have been associated with metal-binding function (*30*). Particulate material may also provide anaerobic microenvironments within the water column, as suggested by a number of authors (*31*), which may provide conditions for metal transformations. These are mechanisms that have all been investigated in the laboratory using pure cultures of bacteria (*26*) or carefully controlled sediment core experiments (*5,18*). We are now designing experiments to compare water column particulate–metal interactions with these laboratory studies using Lake Chapala samples. The aim of this research is to be able to augment current models of metal transport in surface waters [i.e., the model of removal rates of metals by settling particles described by Sigg and co-workers, (*32*)] with a microbiological factor. It may then be possible to insert rates of these important processes into a conceptual model such as that presented for Lake Chapala in Figure 1.
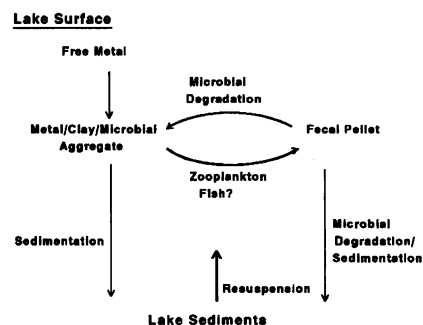
Figure 1. Conceptual model of metal cycling in Lake Chapala, Mexico.

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