

Effects of Acidification on the Mobility of Metals and Metalloids: An Overview

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The exchange rates for metals and metalloids between sediments, soils, water and aquatic biota are discussed in terms of normal and acidified ecosystems. Where it is possible, the pathways for a number of toxic elements are presented with special emphasis on the impact of acidification on changing chemical speciation, and on the potential toxicity of such acid-generated chemical species. Concerns for the impact of acidification on the the stability of ecosystems, the safety of drinking water, bioaccumulation in fish and in plants are addressed.

Introduction

Evolution Aspects of Metal Ion Transport

Before examining some of the processes involved in element uptake by living cells, let us consider some of the selection principles involved in the chemistry of life. To accomplish this end several fundamental questions must be asked:

- Which elements are essential for the growth and cell division of microorganisms, plants, and animals?
- Why were these elements selected in the evolution of microorganisms ot almost 4×10^9 years ago?
- What is the role of the geosphere in determining the uptake of essential elements?
- What is the role of the biosphere in the selection of these elements?

Obviously the uptake of elements and their utilization by living cells depends on chemical and physical properties of each element. Of the 92 elements in the periodic table, 30 have been found to be required for microbial life, although not all of these 30 elements are necessary for the growth and cell division of every microbial species. Therefore, in addition to the bulk elements, carbon, nitrogen, hydrogen and oxygen, 26 other elements are required in intermediate to trace amounts. An overabundance of any of these elements can cause an intracellular toxic buildup which often results in death. It is therefore of great significance to understand the impact of large-scale environmental perturbations on the availability of both essential and nonessential elements. The reason for the selection of these 30 elements in the evolution of microorganisms appears to have been determined by two parameters: the abundance of elements in the earth's crust and the solubility of these

elements in water under strictly anaerobic conditions.

Table 1 presents a list of elements in order of their crustal abundances. Of the top 26 elements, 22 are found to be essential for life in higher organisms. Nonessential elements are generally of low abundance in the earth's crust and therefore should not be effective in competing with essential elements in cells through their specific transport systems.

The effect of solubility properties of elements under strictly anaerobic conditions is best illustrated by the biological utilization of iron and the rejection of aluminum. Under anaerobic conditions iron will be present in complexes of lower oxidation state, e.g., Fe(II) salts which would be water-soluble and available for transport into primitive anaerobic bacteria. However, the solubilization of abundant elements such as Al and Pb requires low pH, and so such elements are available to living cells entering the aqueous environment acidified below pH 6.0 (1).

Essentiality Versus Toxicity of Metal Ions

Williams (2,3) has documented those parameters which lead to the uptake of either too little or too much of individual essential elements for the sustenance of cell growth and cell division. For example, the failure of a cell to transport and utilize sufficient essential metal could arise as follows: (1) low availability; (2) excessive competition from other metal ions with similar chemical properties, e.g., Co(II) and Ni(II), Ca(II) and Cd(II), PO_4^{3-} and AsO_4^{3-} , Li^+ and Na^+ , etc.; (3) inadequate synthesis of carrier molecules by the cell; (4) excessive excretion of metal ions by the cell; (5) failure of the energy-driven uptake systems.

Excessive element uptake can occur through the reversal of the above five factors. It is important to recognize that living cells are not at equilibrium with their external environment and therefore a kinetic approach

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Table 1. Crustal abundance of elements in the earth's surface, and a list of some of the elements required to support life.^{a,b}

O, Si, Al,* Fe, Na, Ca, Mg, K, H, Mn, P, S, C, V, Cl, Cr, Zn, Ni, Cu, Co, N, Pb,* Sn,* Br, Be,* As, F, Mo, W, Tl,* I, Sb,* Cd,* Se
All the rest are less than 0.1 µg/g.

^a Elements followed by an asterisk have no known biological function.

^b Range of concentration O = 46.6% to Se which is 0.1 µg/g.

to metal ion transport, binding, toxicity, and resistance to toxicity is much more meaningful than a thermodynamic approach. This biochemical basis for toxicity is complicated by the great variety of reactions at the molecular and cellular levels even in closely related organisms and tissues. Several strategies for resistance to intoxication have been identified (4).

Williams (3) has pointed out that metal ion interactions in biology can be divided into three classes: ions in fast exchange with biological ligands; ions in intermediary exchange with biological ligands; ions in slow exchange with biological ligands.

Examples of those elements in fast exchange include the alkali metals Na⁺ and K⁺, the alkali earth metals Ca²⁺ and Mg²⁺, and of course, H⁺. Those which can sometimes be in intermediary exchange are Fe²⁺ and Mn²⁺. Examples of those in slow exchange are generally in the active sites of metalloenzymes, e.g., Fe³⁺, Zn²⁺, Ni²⁺, Cu²⁺. Metal-to-metal interactions and covalency predominate in the slow-exchange metals and this provides the basis for stability. However, competition for those metal ions in fast exchange is often severe.

As outlined in the previous section, living cells have membranes which cause the initial barrier for metal ion uptake. In prokaryotes the external cell membrane represents the only barrier, but in eukaryotes there are many membranous organelles which can partition metal ions by a variety of mechanisms. A comparative study of metal ion resistance between blue-green algae (prokaryotes) and green algae (eukaryotes) demonstrates how important membranes are to metal ion uptake and toxicity (4). The green algae are much more resistant to high concentrations of toxic metal ions such as Cu²⁺ and Ni²⁺ than are the blue-green algae (5). The external cell membrane of prokaryotes carefully selects those ions which are in fast exchange, e.g., the rejection of Na⁺ and Ca²⁺ and selection of K⁺ and Mg²⁺ (6). In eukaryotes spatial partitioning of metals occurs, even for those in slow exchange, because metal-binding macromolecules can be partitioned in different organelles and in tissues of different cell lines, e.g., metallothionein binding Zn²⁺, Cd²⁺, Cu²⁺, and Hg²⁺ in the kidney cortex. Once the cell-buffering capacity for essential metal ions is exceeded toxicity becomes evident. Toxic effects become evident at much lower concentrations for non-essential metals. In the natural environment nonessential metals seldom reach concentrations in excess of 1 µg/g (i.e., 1 ppm) (Table 1). Examples of circumstances in which natural higher metal concentrations occur are active volcanic regions, such as deep-sea vents, hot springs, and volcanic lakes. However, the combustion of fossil fuels by advanced industrial society has greatly

contributed to acidification of natural waters which has a profound effect on the availability of both essential and non-essential metals and metalloids. In that which follows, I shall attempt to summarize the potential human health effects of acid rain.

Metals and Metalloids of Concern

Mercury

Recent analyses on oil shale show that primitive organisms were actively synthesizing organometals and organometalloids. Therefore biomethylation must have given certain microorganisms selective advantages for the elimination of heavy metals such as mercury and tin, and for metalloids such as arsenic and selenium. The synthesis of less polar organometallic compounds from polar inorganic ions has certain advantages for cellular elimination by diffusion-controlled processes (7,8). The microbial synthesis of organometallic compounds from inorganic precursors is well understood in both the terrestrial environment and in the sea. Mechanisms for B₁₂-dependent synthesis of metal-alkyls have been discovered for the metals Hg, Pb, Tl, Pd, Pt, Au, Sn, Cr and for the metalloids As and Se (8-14). Pathways for the synthesis of organoarsenic compounds have also been shown to occur by a mechanism involving S-adenosyl-methionine as the methylating coenzyme (14).

The ecological significance of B₁₂-dependent biomethylation is best illustrated by B₁₂-dependent and independent strains of *Clostridium cochlearium*. The B₁₂-dependent strain is capable of methylating Hg(II) salts to CH₃Hg(II), whereas the B₁₂-independent is incapable of catalyzing this reaction. Both strains transport Hg(II) into cells at the same rate, but the independent strain is inhibited by at least 40-fold lower concentration of Hg(II) than the dependent strain. This result clearly demonstrates that *Cl. cochlearium* uses biomethylation as a mechanism for detoxification giving the organism a clear advantage in mercury-contaminated systems.

After methylmercury is released from the microbial system, it enters food chains as a consequence of its rapid diffusion rate. In the estuarine environment, the reduction of sulfate by *Desulfovibrio* species to produce hydrogen sulfide is quite important in reducing CH₃Hg(II) concentrations by S²⁻-catalyzed disproportionation to volatile (CH₃)₂Hg and insoluble HgS. It should be pointed out here that there is overwhelming evidence to support the notion that membrane transport of methylmercury is diffusion-controlled. Fluorescence techniques and high-resolution NMR show that diffusion is the key to CH₃Hg(II) accumulation (7). Also, a

of drinking water and concomitant acid corrosion of these metals directly into drinking water supplies. This corrosive aspect of drinking water can be solved, and in fact has been solved for lead in Glasgow, by adjusting the pH of that city water supply with lime, before distribution. Similar measures will understandably have to be taken into consideration in acid-susceptible reservoirs and in private cisterns in regions of the USA where acid rain is severe.

Aluminum

Aluminum is the third most abundant element in the earth's crust. The chemistry of this element under both aerobic and anaerobic conditions makes it unavailable for uptake by living organisms unless those organisms grow at low pH. A detailed study of Swedish lakes shows that the solubilization of aluminum salts becomes increasingly important at the pH falls below 6.0 (1). Similar conclusions are reached by Driscoll in his study of aluminum in acid susceptible lakes in North America (29). The toxicity of aluminum to higher organisms, including man, very much depends on the inorganic chemical speciation of its salts. For example, acute chronic toxicity studies in rats show that $\text{Al}(\text{OH})_3$ is not toxic at exceedingly high doses, but AlCl_3 has low LD_{50} dose (30). The transport and toxicity of aluminum complexes in living organisms including man is not understood, although there appears to be a correlation between Ca^{2+} transport and Al^{3+} transport (30). This is to be expected from the arguments on the exchange rates of elements which I alluded to earlier in this chapter. The connection between aluminum uptake and diseases of the central nervous system has been adequately documented by Wills (31) and Perl (32).

What is missing from this field is a complete understanding of toxic species, and biochemical mode of action of this element. Noninvasive techniques such as *in vivo* Al^{27} NMR should help in unravelling some of these basic questions. However, it is clear that acid precipitation causes dramatic increases in concentrations of aluminum in freshwater systems. The experience in Guam could be repeated in North America unless we address this most serious situation.

At this point I have no ideas on how to prevent the third most abundant element (in the earth's crust) from being solubilized by acidification without the application of broad scale neutralization procedures.

Arsenic and Selenium

There has been very little discussion of the volatilization of the metalloids arsenic and selenium by comparison with sulfur from fossil fuel combustion or from mining and smelting activities. The impact of acidification on the metabolic pathways for arsenic and selenium requires attention primarily because of the differences in reactivity of their oxyanions in acid versus neutral conditions. At acid pH's the standard reduction potential for the $\text{Se}^{\text{VI}}/\text{Se}^{\text{IV}}$ couple is +1.15 V, and for

the $\text{As}^{\text{V}}/\text{As}^{\text{III}}$ couple it is +0.559 V; under these conditions, methylation of arsenic and selenium species to more volatile and less toxic forms should be slowed down considerably which would be expected to lead to a buildup of the more toxic inorganic anions in water. However, at pH 7.0 and above the E_o for $\text{Se}^{\text{V}}/\text{Se}^{\text{IV}}$ changes to +0.05 V and for $\text{As}^{\text{V}}/\text{As}^{\text{III}}$ -0.67 V, this allows efficient methylation by the methylating coenzyme S-adenosylmethionine to produce volatile methylated metabolites (33,34).

This change in reactivity of the oxyanions of arsenic and selenium is likely to be of critical importance to the buildup of concentrations of these elements in surface waters and sediments in the long term. At least this recognition points up the importance of future research efforts in the distribution of these extremely toxic elements.

Effects of Competing Cations and Anions on the Uptake of Toxic Metals and Metalloids

It is clear that inorganic ions compete for transport pathways in living organisms, and that many of the "good ions" such as Mg^{2+} and Ca^{2+} effectively compete with the "bad ions" such as Cd^{2+} and Al^{3+} in biological membranes (35). Clearly one remedial option is to ensure that the concentrations of Ca^{2+} and Mg^{2+} in waters exceed the concentrations of ions such as Al^{3+} and Cd^{2+} . This of course depends very much on the nature of the complexing anions in the water. Much more needs to be done on the actual inorganic speciation of toxic metals in acid-susceptible surface waters. For example, while it is recognized that the concentration of aluminum is very high in the drinking water sources in Guam, to my knowledge there is no information on aluminum speciation in this drinking water. This requires the development of more sophisticated analytical procedures. The first phase of any analytical procedure is the development of appropriate separation techniques. Such separation techniques have been developed using gas chromatography, liquid chromatography, or ion chromatography which are specifically designed for inorganic analysis. However, the connection of these techniques with mass spectrometry for the structural determination of inorganic species, has been slow in developing even though such techniques are well advanced in the determination of organic structures.

Concerns

It is understood that any chemical species in the environment may move from its source through the physical environment and into biota where it may cause either a beneficial or a toxic effect at the cellular level. The pathways taken by each element involve many chemical species, and the distribution of these species is determined by thermodynamic and kinetic considerations. Those species which are stable thermodynamically

cally can often be isolated and quantitated. Ideally the precise chemical structures of each of these stable compounds, or complexes, should be analyzed in that matrix where they exist naturally. Unfortunately, in the stressed system entropy increases and there is a concomitant increase in instability in both the physical and biological context.

The acidification of the aqueous environment has already been shown to cause significant stress on ecosystems. Dr. Stokes presented an example of the total colonization of some acid susceptible lakes in Canada with a single species of a filamentous alga. As diversity of populations decrease to only a few acid tolerant species, then the normal balance for the cycling of elements changes appreciably. This has already been shown to be the case for mercury and aluminum, and may well be the case for the metalloids arsenic and selenium. The net effect of this instability is an increase in the concentration of methylmercury in predatory fish and an increase in the concentration of soluble aluminum species in surface waters. The consumption of methylmercury-contaminated fish has already been the cause of major health problems in Japan and more recently in China, and the consumption of drinking water containing high concentrations of aluminum is implicated in the epidemiology of Alzheimer's disease in Guam.

I wish to acknowledge the support of the NIH AM 18101 for support of some of the research discussed in this review.

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