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Physicochemical Mechanisms of Trace Metal Bioaccumulation by Microorganisms

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Abstract: Our research group is working towards understanding the fundamental physicochemical mechanisms of trace metal biouptake by aquatic microorganisms in natural systems. Research is currently focused on identifying under what conditions uptake fluxes are limited by physical (*e.g.* diffusion) as opposed to biological (transfer across biological membrane) processes. In addition, the complexation of the trace metals is being examined from both a thermodynamic (stability) and kinetic (lability) perspective in order to elucidate its effect on the overall uptake flux. Up-to-date information on the group, including a current publication list can be found at *http://www.unige.ch/cabe/wilkinson.*

Keywords: Bioaccumulation · Biological limitation · Diffusion limitation · Microorganism · Trace metal

1. Introduction

The work presented here is part of an ongoing initiative to understand some of the important processes controlling the circulation of vital and toxic trace elements in natural aquatic systems. Because trace compounds are often associated with natural colloidal material, a closely related aspect of the work involves the characterization of inorganic particles and biopolymers [1–4], including investigations of their mechanisms of interaction (bridging flocculation, heterocoagulation, *etc.*) [5][6]. These aspects of our work have been recently reviewed in this journal [7] and therefore they will not be discussed here. Instead, this contribution will focus on recent studies of the physicochemical processes controlling the uptake of trace metals by unicellular organisms under environmental conditions.

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The interaction of trace elements with unicellular aquatic organisms generally involves several processes including [8–11]: (1) mass transfer to the biological surface, (2) diffusion through the protective layers around the organism (*e.g.* mucus, cell wall), (3) adsorption at the membrane surface (both to transport sites and biologically inert sites) and (4) transport across the biological membrane (Fig. 1). Any of these steps has the potential to be rate limiting (1: [12][13]); 2: [14][15]; 3: [10][11]; 4: [16][17]) depending on the nature of the organism, the nature and speciation of the accumulated element and the physicochemistry of the external medium.

Our current understanding of the interactions of trace metals with (micro)organisms is based on the underlying assumption that equilibrium is attained among the metal species in solution and those bound to target sites on the membrane surface (*e.g.* transporters, carriers) [16][18]. In this case, biological uptake fluxes, J_{int}, can be related by a first order (linear) relationship to the concentration of any of the metal species in

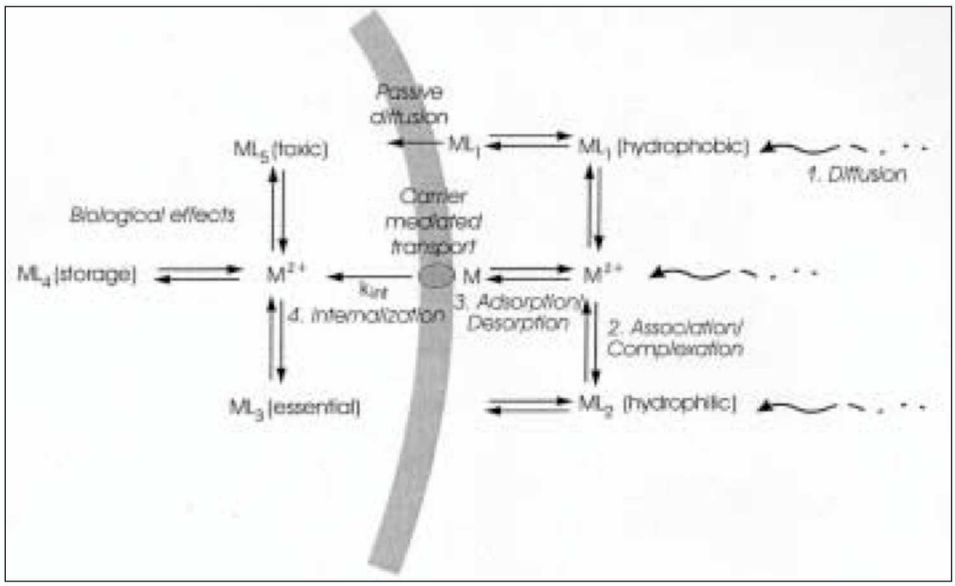

Fig. 1. Schematic representation of the metal uptake process.

equilibrium (*e.g.* the transporter bound metal concentration, $\{M\text{-}L_{tr}\}$, Eqn. 2 or the free metal ion concentration in solution, $[M^{z+}]$, Eqn. 3):

$$M^{z+} + L_{tr} \overset{K}{\leftrightarrow} M\text{–}L_{tr} \overset{k_{int}}{\rightarrow} \quad (1)$$

$$J_{int} = k_{int} \cdot \{M\text{–}L_{tr}\} \quad (2)$$

$$J_{int} = k_{int} \cdot K \cdot \{L_{tr}\}[M^{z+}] \quad (3)$$

where k_{int} represents the internalization rate constant, K the stability constant for the formation of the metal-transporter complex and $\{L_{tr}\}$, the concentration of free cellular metal transporters.

Over the last decade, numerous exceptions to the simple first order uptake predicted above have been observed (for a review, see [17]) but most often without a quantitative understanding of whether the problem was related to the chemical processes in the external media or to the biological transfer of the element across a membrane. Furthermore, recent theoretical advances *e.g.* [19][20] have identified several (environmentally relevant) conditions for which equilibrium (or steady-state) conditions may not hold. It is thus becoming clear that the simple thermodynamic models of trace element bioaccumulation are not sufficiently rigorous to provide a general explanation of observed biological uptake fluxes under environmentally relevant conditions. For example, the relationship between trace metal speciation and bioavailability has only recently been analyzed on the basis of the dynamics of mass transport and the association/dissociation reactions in complexing external media [10][19]. That analysis has revealed that under certain conditions, the diffusive transport flux could be rate limiting in which case labile complexes could contribute to the biouptake flux. A major goal of our work is thus to quantitatively study the major processes that regulate trace element transport and bioaccumulation by a cell. Because the bioavailability of a trace element will depend upon the nature of the rate limiting flux, another important aspect is to quantify, as precisely as possible, each of the important physicochemical processes (internalization fluxes, diffusion, association/dissociation kinetics, *etc.*; Fig. 1) involved in trace element uptake.

2. Biological Limitation of the Uptake Process

In short-term bioaccumulation experiments, the attainment of a steady-state between the organism and the external medium implies that the metal uptake flux is constant and that both dissolved and adsorbed metal concentrations remain unchanged and at equilibrium. In this case, zero order internalization kinetics are predicted for $[M^{z+}]$ above saturation of the metal transporter [9] while a first order metal uptake flux should be observed at lower $[M^{z+}]$ [17].

Pb(II) is an example of a metal with relatively fast association–dissociation kinetics [20][21] for which a steady state between the metal species in the solution and metal bound to the transport sites might be expected [9]. Indeed, short term uptake experiments have typically shown a rapid, steady-state adsorption to the cell wall and a slow, linear internalization (non-EDTA extractable Pb) (*e.g.* Fig. 2; [22]). The observation of a plateau value of adsorbed Pb supports the assumption that equilibrium is attained between the external solution and cell surface. Internalization fluxes, J_{int}, determined from the slope of cellular Pb *versus* time (*e.g.* Fig. 3) have been determined for 5×10^{-9} to 5×10^{-5} M Pb^{2+} in the absence and presence of NTA, IDA, citric and malonic acids. The addition of ligands to the experimental solutions lead to a significant decrease of the Pb uptake flux that was correlated to free $[Pb^{2+}]$ (Fig. 3) but not to the total lead concentration. Maximum internalization fluxes obtained for $[Pb^{2+}]$ larger than about 1×10^{-5} M were equal in the absence or presence of ligand. Furthermore, transporter bound Pb concentrations, $\{Pb\text{-}L_{tr}\}$, were directly related to internal-

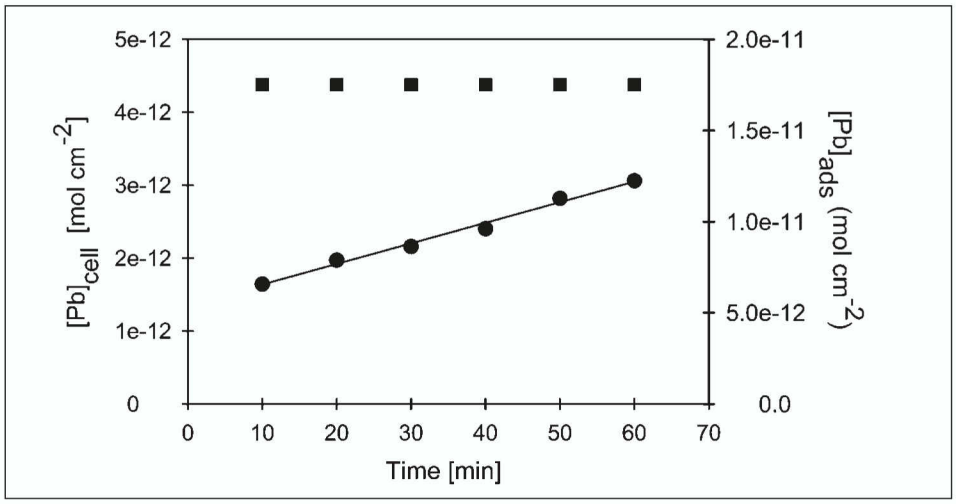

Fig. 2. Adsorbed Pb (*i.e.* removed by 10^{-2} M EDTA wash, ■) and cellular Pb (*i.e.* not removed by 10^{-2} M EDTA wash, ●) as function of accumulation time for the algae, *Chlorella kesslerii*. $[Pb]_{tot} = 10^{-5}$ M; $[Pb^{2+}] = 2.5\times10^{-7}$ M, pH = 6. Taken from [22] with permission.

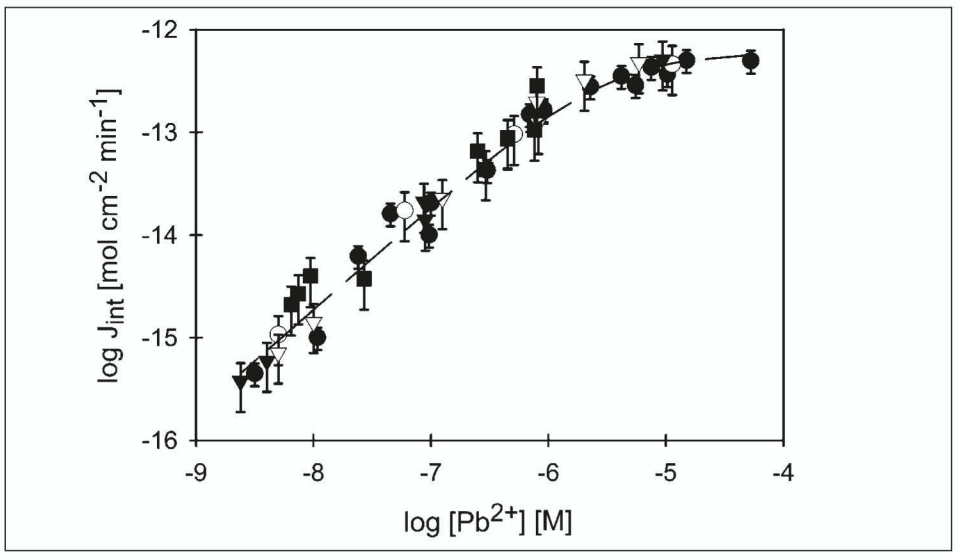

Fig. 3. Logarithmic representation of internalization fluxes as a function of $[Pb^{2+}]$ in the absence (●) or presence of ligand: (■) NTA, (▼) citric acid, (O) IDA and (∇) malonic acid for the algae, *Chlorella kesslerii*. Dashed line represents a Michaelis Menten plot for $K_M = 3\times10^{-6}$ M and $J_{max} = 6\times10^{-13}$ mol cm^{-2} min^{-1}. Standard deviations are given when larger than the symbol size. Taken from [22] with permission.

ization fluxes as would be predicted by the equilibrium models (Fig. 4). The existence of a single saturation plateau (Fig. 3) and the direct relationship between uptake fluxes and carrier-bound Pb (Fig. 4) supports the hypothesis that internalization is predominantly *via* a single carrier.

Complexes are defined as labile when they can form and dissociate many times during their transport through the diffusion layer, with the resulting metal supply to the biological surface potentially determined by both free metal and labile species [19][23]. For a given complex, the lability criterion, L, (Eqn. 4) represents the ratio of flux that would result from a kinetically limited dissociation of the complex to the rate of metal supply by a purely diffusion controlled flux [19–21].

$$L = \frac{k_d \cdot \mu \cdot r}{D_{MY}} \quad \text{with} \quad \mu = \left(\frac{D_{MY}}{k_a \cdot c_Y} \right)^{0.5} \qquad (4)$$

where D_{MY} represents the diffusion coefficient of the complex species, MY, c_Y is the concentration of ligand, Y, μ is the dissociation reaction layer thickness, r is the measured average radius of *Chlorella kesslerii* (*ca.* 1.9 μm) and k_a and k_d are association and dissociation rate constants for ML. Note that Eqn. 4 is valid only in the case of $\delta > r > \mu$, where d is the diffusion layer thickness (*ca.* 20 to 30 μm, [24]). The validity of this equation, the basic assumptions for its derivation, as well as its applicability to microorganisms have been discussed previously [19][20]. Furthermore, the maximum diffusive flux of Pb^{2+} (Eqn. 5; [22]) can be calculated for the free ion concentrations employed here:

$$J_{diff} = \frac{D_M}{r}\left[M^{z+}\right] \qquad (5)$$

Theoretical estimations of L have revealed that labile behavior, (L>>1) could be expected in the presence of citric and malonic acids while NTA complexes were predicted to be non-labile (L<<1), and Pb-IDA complexes could be considered to be a borderline case (L ≈ 1). Nonetheless, for the example examined here, the maximal internalization flux was already much smaller than the limiting supply of free ions, J_{diff}, suggesting that the turnover rate of transport proteins was so slow that the available $[Pb^{2+}]$ was sufficient to satisfy algal demand (in a chemical sense). This observation is not completely unexpected given the lack of known biological demand for Pb.

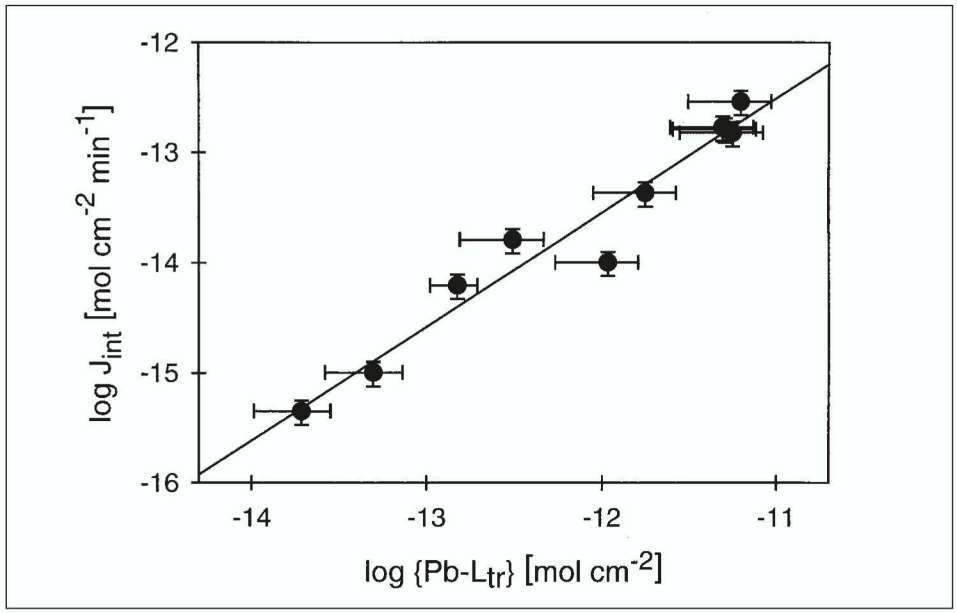

Fig. 4. Logarithmic representation of internalization fluxes as a function of carrier-bound Pb for the algae, *Chlorella kesslerii*. Standard deviations are given in each direction.

3. Physicochemical Limitation of the Uptake Process

Similar to the results observed for Pb, adsorbed Zn rapidly attained a constant value implying that equilibrium had been attained between the cell surface and solution [25]. Furthermore, a small linear increase of cellular zinc *versus* time, suggested that Zn transport across the membrane was rate-limiting with respect to the overall bioaccumulation process. On the other hand, Zn internalization fluxes increased only three to four fold for a solution $[Zn^{2+}]$ ranging from 2×10^{-11} to 1×10^{-3} M (*i.e.* $>10^7$ fold increase) (Fig. 5), demonstrating that Zn uptake was not first order with respect to $[Zn^{2+}]$ in solution. In contrast to Pb, Zn is a micronutrient that is required by the organism. To compensate for low environmental concentrations of Zn, the microorganism can increase its internalization flux [25] to the point where diffusion becomes limiting ($J_{int} > J_{diff}$). This would appear to be the case for $[Zn^{2+}] < 2\times10^{-11}$ M (Fig. 5), concentrations at which Zn uptake fluxes decreased in direct proportion to $[Zn^{2+}]$ in solution ($r^2 = 0.91$). Under these conditions, the flux of free ions alone is insufficient to satisfy the biological uptake requirements

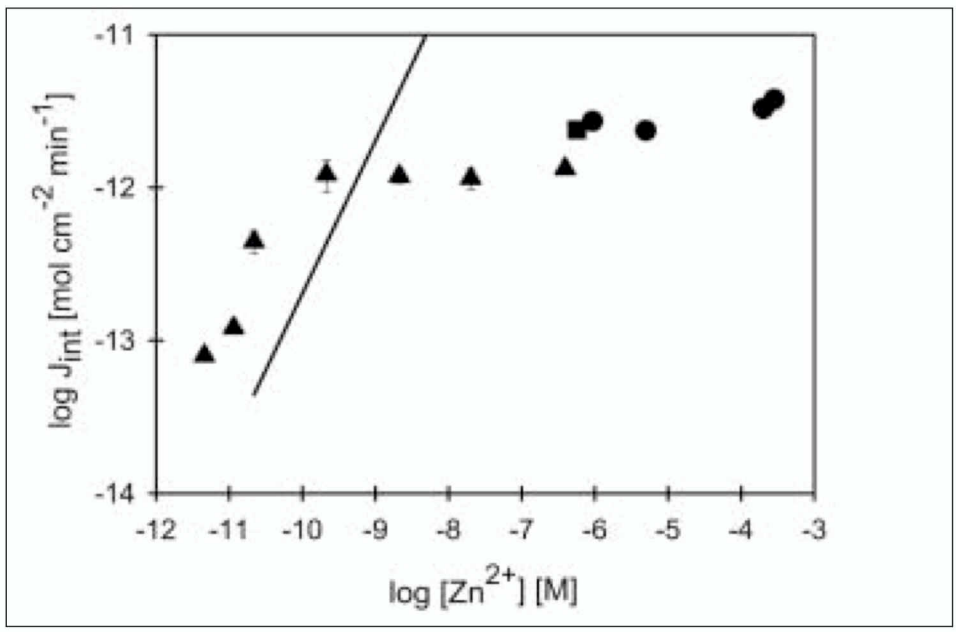

Fig. 5. Relationship between internalization fluxes and $[Zn^{2+}]$ with (▲) or without (●) NTA to buffer the $[Zn^{2+}]$ in solution. Algae (*Chlorella kesslerii*) were preincubated with 10^{-11} M Zn^{2+}. The line represents a calculated diffusion limited flux for Zn^{2+} (see text for further details). Error bars represent standard deviations (n = 4 to 6). Modified from [25] with permission.

of the organism and thus it is relevant to take complex lability arguments into consideration, since labile complexes can provide a potential source of free ion to the transport sites.

4. Influences of the Microorganism

The cell wall or biological membrane of the microorganism is not a model surface that is otherwise inert to its surroundings. The microorganism modifies the physicochemistry of the medium and/or the physicochemical processes in several ways.

(i) *Pre-exposure conditions*

Algae preincubated in slightly limiting conditions of Zn^{2+} (10^{-11} M) are able to generate sufficient numbers of high affinity transporters so as to increase uptake fluxes by up to 100× with respect to algae that are preincubated in 10^{-9} M Zn^{2+} [25]. Due to the relatively larger internalization flux, cells preexposed to 10^{-11} M [Zn^{2+}] were diffusion limited while cells preexposed to 10^{-9} M [Zn^{2+}] were under steady-state (thermodynamic limitation) conditions.

(ii) *Biological internalization is not necessarily passive*

Carrier-facilitated (passive) transport across membranes is most often a first order process [26] that is driven by electrochemical gradients at the biological 'interphase'. This contrasts with the active transport of macronutrients or trace metals [27] that is an energy-dependent process that can be driven against the electrochemical gradient. Zn internalization fluxes by both the green alga, *C. kesslerii* [25] and the gram positive bacterium, *Rhodococcus opacus* [28] were indeed independent of the electrochemical gradient and thus first order uptake was not observed. In this case, it is not possible to relate rigorously chemical speciation in solution to cellular uptake fluxes.

(iii) *Metal efflux is rarely passive*

Most microorganisms are able to modify their efflux rate as a function of metal concentrations inside the cell [29] often as a constitutive phenomenon [25][28]. Although this process will have little effect on the short-term uptake fluxes examined here (especially for algae), net uptake will be reduced over longer term exposures.

(iv) *Production of metal complexing exudates*

In addition to a cellular metal removal *via* efflux, microorganisms may produce metal-complexing ligands that can reduce free metal ion concentrations by several orders of magnitude. This process appears to be especially important (and rapid!) for aquatic bacteria [28–30].

(v) *Competition for trace metal transporters is not necessarily antagonistic*

Chemical thermodynamics would predict that the addition of a second trace metal could either decrease the internalization flux of the first or have no effect. Contrary to expectations, the addition of copper increased Cd bioaccumulation by *Rhodospirillum rubrum* despite the fact that the system was under steady-state conditions [29]. Such an observation cannot be explained by solely chemical reasoning.

5. Summary and Future Research Perspectives

Although it has long been recognized that it is necessary to take chemical speciation into account rather than employing total metal concentrations when predicting ecotoxicological effects, there is currently no scientific consensus as to which chemical species are important under any given conditions. Several early observations, *e.g.* [31], made in the presence of simple ligands such as EDTA and NTA under laboratory conditions have led to the (over)use of thermodynamic (biological limitation) models to predict the effects of trace metals in complex field conditions. The chemical partitioning (chemical speciation) of trace metals in heterogeneous environmental systems is dominated by complexes of variable sizes and chemical lability. The importance of these complexes, and their size [32] and lability distributions will be an important field of future research.

Acknowledgements

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