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SHORT COMMUNICATIONS

Experimental Physicochemical Modeling of Interactions between Phototrophic Microorganisms (Anoxiphotobacteria and Cyanobacteria) with Trace Elements in Aqueous Solutions

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INTRODUCTION

One of the most challenging tasks of modern environmental geochemistry is the quantitative modeling of trace-element fluxes (including those of heavy and toxic metals) in the water shell of continents. The major physicochemical factor controlling the migration of trace elements in natural waters is the adsorption of these elements on the surface of solid phases, coprecipitation with newly formed mineral components, and the active or passive consumption by microorganisms. Modern experimental studies and physicochemical descriptions of the interactions of trace elements with inorganic components of natural waters are now at the level of theoretical modeling and have a high prediction potential. At the same time, quantitative physicochemical approaches to the modeling of the influence of a biota on the cycles of chemical elements are developed inadequately. The few past decades witnessed the mushrooming of publications (mostly in English) devoted to studying the reversible (equilibrium) interaction of ecologically significant metals (Cd, Zn, Pb, and U) with the surfaces of bacteria [1, 2]. These studies were centered, first of all, on the experimental measuring of the percentage of adsorbed metals depending on the pH of the environment (pH-dependent adsorption edge) and were conducted with various bacterium strains under laboratory-controlled conditions, in an inert electrolyte, at metal concentrations (15–150 μM) two–three orders of magnitude higher than their concentrations in naturally occurring waters. In spite of the certain limitations of these physicochemical experiments, they facilitated the development of rigorous enough physicochemical models for the complexation of metals at the surfaces of microorganisms, analogously to the modeling of mineral surface equilibria [3]. These models take into account the chemical composition of the surface of the cells and the acid–base equilibria of the functional groups that control adsorption [4, 5] and show certain prediction potential with respect to still-unstudied metals (bi- and trivalent cations) and bacterial species [2]. The thermodynamic approach was still not applied to the description of the interactions of other geochemically important trace elements, such as oxianions (Mo, Ga, and Se), metalloids (As and Sb), or monovalent metals (Tl and Cs), with the surfaces of microorganisms, particularly at low concentrations of the metals, comparable with those in natural waters. This was the primary task of our research.

Another important issue in the application of the physicochemical approach to the quantitative prediction of trace-element migrations in the presence of living organisms is accounting for the active (irreversible) adsorption of these elements during the growth of the cells. The results of laboratory studies of microorganism cultures cannot be directly extended to natural environments because of the significant differences in the composition of the cultural environments with phosphates and organic complex-forming compounds, on the one hand, and naturally occurring aqueous environments, on the other: the speciation of trace elements and, hence, the processes of their active transport through biological membranes are basically different in cultural environments and natural waters. In this context, the most reliable technique for the evaluation of the biological adsorption coefficients is their direct measuring in natural biological communities. Surface hydrothermal systems are characterized by a relatively little varying composition of the fluids and the absence of multilevel biological organization (bacteria, algae, protozoa, and metazoans) that is typical of low-temperature ecosystems. These biological objects, which are often characterized by a monospecific composition of the predominant biomass, provide a unique opportunity for the direct quantitative estimation of the distribution coefficients (K_d) of trace elements between the aqueous solution and microorganisms growing in it. Another task of our research was to measure the K_d of trace ele-

ments in natural environments, namely, in hydrothermal springs in central Kamchatka, and their comparison with the reversible adsorption values obtained in laboratory experiments with cultures of phototrophic microorganisms.

MATERIALS AND METHODS

Cultivation of Bacteria

The cultures of budding purple nonsulfur bacterium *Rhodopseudomonas* sp. KR-95p and *Rhodobacter* sp. f-7bl and the cyanobacterium *Gloeocapsa* sp. f-6gl used in this research are maintained at the Laboratory of Ecology and Geochemical Activity of Microorganisms of the Vinogradskii Institute of Microbiology, Russian Academy of Sciences, in Moscow. The thermophylic green phylomentous bacterium *Chloroflexus aurantiacus* B-3 were provided for this research by the Department of Microbiology of Moscow State University. Cultures of the cyanobacterium *Myxosarcina chroococcoides* CALU-15 and *Aphanocapsa thermales* CALU-307 were received from the collection of microorganisms of the Department of Microbiology of the St. Petersburg State University.

The purple bacteria were cultivated on Pfennig's mineral medium [6] with the addition of 0.1 g/l of yeast extract and 2 g/l of Na acetate (for *Rhodopseudomonas* sp. KR-95p) or malate (for *Rhodobacter* sp. f7bl). The initial pH value was from 7.0 to 7.4. The thermophylic green bacterium *Chloroflexus aurantiacus* B-3 was grown on modified Kastenholtz's medium [6] containing 0.5 g/l of yeast extract, 0.5 g/l of casein hydrolysate, and 2 g/l of Na acetate at pH 7.8–8.2. The cyanobacteria were cultivated in D medium [6] at pH 8.0–8.2.

The anoxiphotobacteria were grown under anaerobic conditions in screw-top flasks completely filled with the medium, and the cyanobacteria were cultivated under aerobic conditions in flasks with cotton-wool stoppers that were one-fourth filled with the medium. All microorganisms were cultivated at an illumination of 2000–3000. lx. The cultivation temperature was 55–60°C for *Chloroflexus aurantiacus* B-3 and 25–30°C for other bacteria. The morphology of the cells was examined under an Axiostar light microscope with phase contrast and a ×100 lens.

Methods of Laboratory Research

The laboratory experiments on the adsorption of trace elements were conducted in hermetically sealed sterile 8-ml polypropylene flasks at continuous stirring in darkness at a temperature of 25 ± 0.5°C. The exposure time was 1 or 3 h, which was sufficient for adsorption equilibria to be established. Preliminary experiments on the absorption kinetics of Zn, Cd, and Pb have demonstrated that a stable metal concentration in the medium was reached already within 10–30 min after its addition to the bacterial suspension and did not change within at least 6 h. All experiments were conducted in 0.01 M solution of $NaNO_3$, at trace-element concentrations of 10^{-7} to 10^{-3} M. The bacterial cultures used in our experiments were living but not growing. The microscopical examination of the bacteria before and after the experiments testified to the absence of the degradation (lysis) of the cells and the deformation of cell walls. As follows from the optical density of the bacterial suspension at a 500 nm wavelength, the number of cells per volume unit also did not change during the experiments. Upon the termination of the adsorption process, the suspension was centrifuged for 15 min at 20°C and 4500 g and filtered through a 0.22-μm membrane filter, and the solution was acidified to pH ~2 by doubly distilled HNO_3 and kept in a refrigerator until the analysis.

The bacterium biomass was weighted after centrifugation for 20 min at 11000 r/min and was also measured spectrophotometrically, by the optical density at 500 nm. The ratio of the wet weight (after centrifugation) to the dry one (after liophylization) was 4.3, 12.3, 9.6, and 13.6 for *Rhodopseudomonas* sp. KR-95p, *Rhodobaster blasticus* f-7bl, *Chloroflexus aurantiacus* B-3, and *Alphanocapsa thermals* CALU-307, respectively.

Materials and Methods of Field Studies

We examined the microbial communities of thermal springs in central Kamchatka, in the area of Karymskii volcano (Akademii Nauk and Goryachii springs). The fieldwork was carried out in August–September of 2002 and August of 2003 [7]. All of the springs are chloride–sodic, sulfide-free, neutral or weakly alkaline (pH 7.5–8.5). Because of the extremale environmental conditions, first of all, the high temperature, the biota of the springs was dominated by prokaryotic organisms. The microbial communities developed in the form of so-called microbial mats, mostly phylamentous phototrophic microorganisms: cyanobacteria and anoxiphotobacteria. The composition and structure of the microbial mats notably varied depending on the physicochemical parameters of the environment, first of all, on its temperature.

The samples for the analysis for element composition were collected from biomats developing in various temperature zones. Samples 38 (Goryachii spring) and 40 (Akademii Nauk springs) were green "mops" (phylamentous aggregates with an average thickness of 1–5 mm, whose one end is attached to the ground), which develop at high flow velocities and a temperature of approximately 55°C. They were dominated by the thermophylic phylamentous cyanobacterium *Mastigocladus laminosus*. Sample 44 is a dense layered biomat of yellow–green color, which grew on the edge of the stream bed, at the water boundary at a moderate and unstable temperature. It was washed by the hot water of the spring on one side and partly dried on the other side. The mat was dominated by mesophilic phylamentous

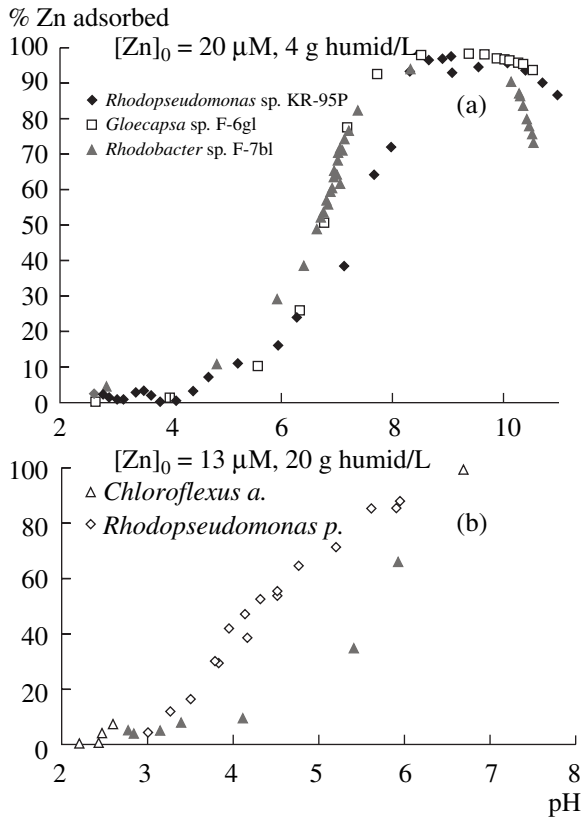

Fig. 1. Zn adsorption on the surface of phototrophic bacteria as a function of pH in 0.01 M $NaNO_3$ solution at 25°C.

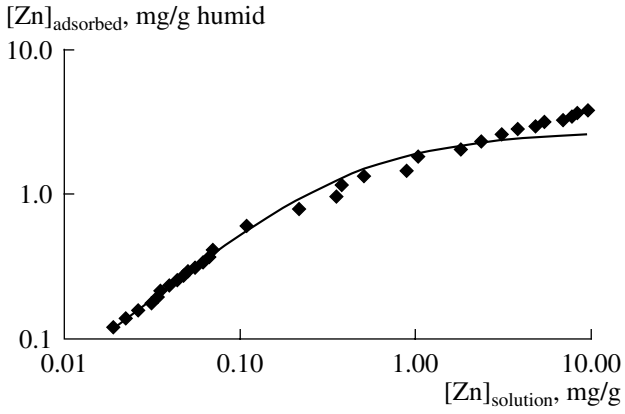

Fig. 2. Isotherm of Zn adsorption on a culture of the cyanobacterium *Aphanocapsa thermals* CALU-307 at a constant pH 8 in 0.01 M $NaNO_3$ solution at 25°C and a biomass concentration of 10 g dry weight per liter.

cyanobacteria, mostly oscillatoriacean. Sample 41 consisted of yellow mops that developed at temperatures of 75–80°C and high flow velocities. The structural basis of these mats was extinct and partly mineralized cells of thermophile phylamentous phototrophic bacteria covered with iron oxides. These mats were evidently formed in winter, at lower temperatures. During the next temperature increase, the phototrophic microorganisms died, but the low destruction rate was favorable for the preservation of their structure. In addition to cyanobacteria, our mat samples (except sample 41) contained anoxiphotobacteria: thermophile green bacterium *Chloroflexus aurantiacus* at 40–65°C and the purple bacteria *Rhodopseudomonas*, *Rhodobacter, Rhodoplanes*, and *Thiocapsa* and heliobacteria.

Solutions for analysis for trace elements were sampled in sterile polyethylene flasks (preliminarily washed with acid), filtered in situ through a 0.22 μm membrane filter, and acidified by doubly distilled HNO_3. The values of pH, Eh, and temperature were determined by an Ekoniks-Ekspert complex ionometer in wells when the samples were taken; the pH was measured by a combined glass electrode, which was calibrated against the NIST standard. Biomat samples were collected by plastic forceps or were cut out by a plastic knife and placed into a preliminarily washed polypropylene flasks, washed by doubly distilled water, and dried in situ at 40°C. The biomass was completely dried at 90°C in the laboratory.

Analytical Procedures

The concentration of trace elements in the filtered samples were determined on a Perkin Elmer 5000 flame atomic absorption spectrometer accurate to ±2% and a detection limit of 10 μg/L (for Zn, Cd, and Pb). Low concentrations (≤100 μg/l) of all trace elements were measured on an Elan-6000 inductively coupled plasma spectrometer accurate to ~5%. High concentrations and activities of Cd and Pb in aqueous solutions were analyzed by chalgogenide solid-membrane ion-selective electrodes (NIKO-Analit). The trace-element composition of the dry biomass and biominerals was determined on an Elan-6000 inductively coupled plasma spectrometer after the complete dissolution of the solid sample in $H_2O_2 + HNO_3$ and then in HNO_3 + HF, evaporation to dry precipitate, and dissolution in HNO_3. All operations on sample preparations were carried out with the use of Teflon materials in a clean room.

RESULTS AND DISCUSSION

The results of the laboratory experiments on determining the dependence of Zn adsorption on pH are shown in Figs. 1a and 1b. In compliance with the general tendency of cations to be adsorbed more actively on the surface of solid bodies with increasing pH, adsorption on cells starts at pH 3–4 and reaches ~100% at pH ~ 7. If the biomass concentration is decreased, the adsorption maximum is reached at higher pH (compare Figs. 1a and 1b). The adsorption of Zn at a constant pH of the solution (Langmuir adsorption isotherm) is presented in Fig. 2. The dependence of the concentration of the adsorbed metal on its concentration in the solu-

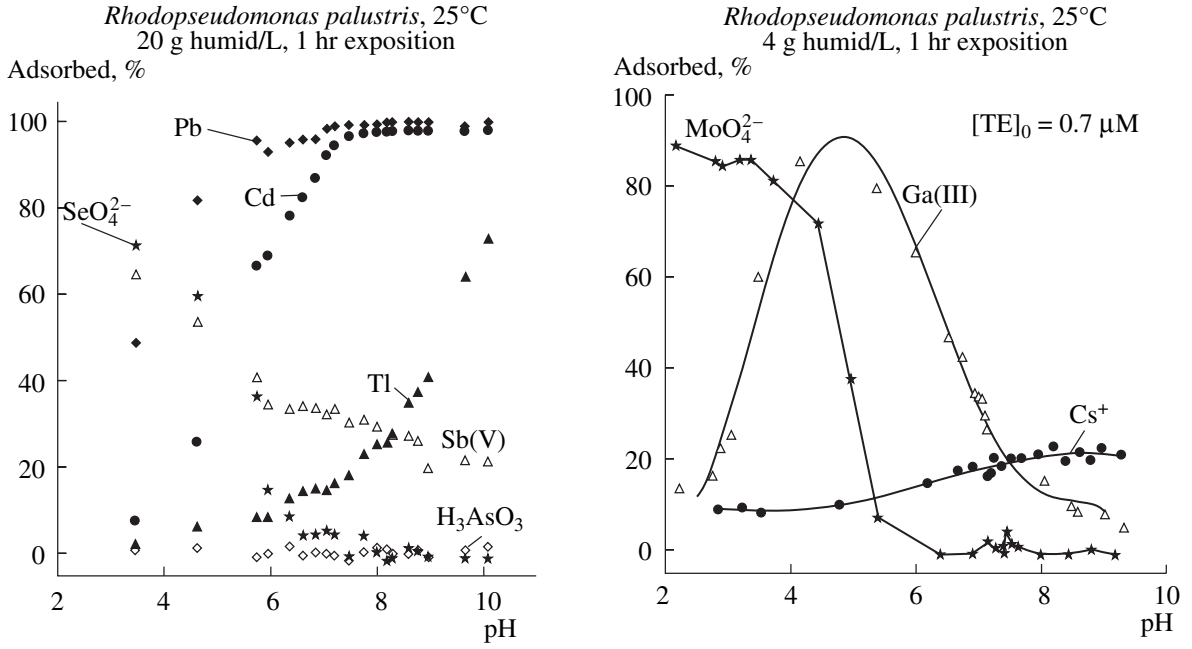

Fig. 3. Trace-element adsorption by the bacterium *Rhodopseudomonas* sp. KR-95p as a function of pH in 0.01 M $NaNO_3$ solution at 25°C. Initial trace element concentrations: $[H_3AsO_3] = 3.27$ μM, $[SeO_4^{2-}] = 6.1$ μM, $[Cd^{2+}] = 0.427$ μM, $[Tl^+] = 1.13$ μM, $[Pb^{2+}] = 1.16$ μM, [Sb(V)] = 3.86 μM.

tion is approximated by the Langmuir equation and is illustrated by the solid line in Fig. 2:

$$\Gamma_{ads} = (\Gamma_{max} K_L [Zn]_{solution})/(1 + K_L [Zn]_{solution}), \quad (1)$$

where $\Gamma_{max} = 2.65$ mg/g wet weight, and $K_L = 2.43$ l/mg. At low metal concentrations (≤1.5–15 μM), which are the closest to those in natural waters, the concentration of adsorbed metal shows a linear dependence on its concentration in the solution. This dependence can be expressed as the distribution coefficient:

$$K_d = [Zn]_{ads}/[Zn]_{solution}. \quad (2)$$

The values of the experimentally determined Zn distribution coefficients between biomass and aqueous solutions depend on the pH and bacterial species (table). Nevertheless, among the microorganisms considered here, cyanobacteria (of the genera *Gloeocapsa*, *Aphanocapsa*, and *Myxosarcina)* always displayed higher adsorption capacities compared to anoxigenic phototrophic bacteria. The most plausible explanation of this is the higher concentration of carboxyl groups in the upper polysaccharide sheaths of cyanobacteria.

The speciation of elements in aqueous solutions and the charges of the complexes are the main factors controlling their affinity to the surface of bacterial cells. The pH dependences of the adsorption of cations (Pb^{2+}, Cd^{2+}, Tl^+, and Cs^+) and anions (SeO_4^{2-}, MoO_4^{2-}, and $Sb_2O_5^{2-}$), hydrolysate elements (Ga), and neutral molecules (H_3AsO_3) shown in Fig. 3 imply the interaction of positively charged ions (Me^{n+}) with carboxyl (>COO^-) or phosphate (>PO^-) groups of the external polycaccharide sheaths, peptidoglycan, and teichoic acids, whereas the amine groups of proteins (>NNH_3^+) are the most active in adsorbing anions (An^{m-})

$$>COO^- + Me^{n+} = >COOMe^{n-1}, \quad (3)$$

$$>NH_3^+ + An^{m-} = NH_3^{-(m-1)}, \quad (4)$$

where Me^{n+} is Cd, Pb, Tl, or Cs and An^{m-} is MoO_4^{2-} or $Sb_2O_5^{2-}$. An exception is Ga: it is adsorbed as the Ga^{3+} trivalent cation in acid solutions but is desorbed in the form of the $Ga(OH)_4^-$ anion in neutral and alkaline

Distribution coefficients (liter per gram of dry weight) for Zn between 0.01 M solution of $NaNO_3$ and the biomass of living but not growing phototrophic bacteria at 25°C. Experimental conditions: $[Zn]_{aq}$ = 0.15–15 μM, biomass concentration = 4–10 g of wet weight per liter

Bacteria	pH	K_d [L/g dry]
Myxosarcina chroococcoides CALU-15	8.0	82 ± 10
Gloeocapsa sp. f–6gl	7.85	100 ± 10
Aphanocapsa thermales CALU-307	7.65	27
Rhodopseudomonas sp. KR-95p	7.95	24
Chloroflexus aurantiacus B-3	6.80	8.6
Rhodobacter sp. f-7bl	7.36	7.4

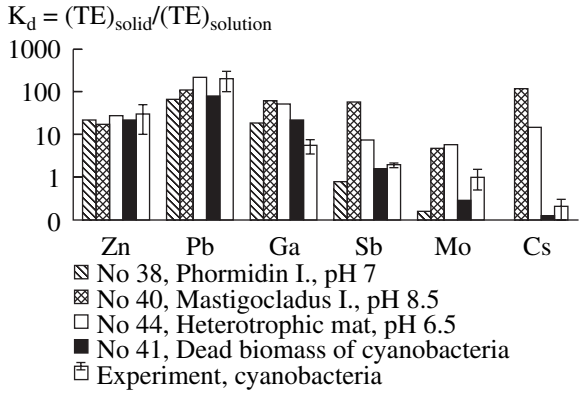

Fig. 4. Comparison of the distribution coefficients (liter per gram of dry weight) of some trace elements in natural cyanobacterium communities (active consumption at 40 ± 10°C) and laboratory experiments on reversible adsorption on sorption on cell surface at 25°C. See section "Materials and Methods of Field Studies" for the description of the samples of natural microbial communities (nos. 38, 40, 41, and 44). The laboratory cultures of phototrophic bacteria were cyanobacteria at 25°C.

solutions, analogously to the behavior of this element on mineral surfaces and complexation with ligands in aqueous solutions [8, 9].

The strong dependence of trace element adsorption on the pH of the aqueous solution (Fig. 3) is very significant for the analysis of interactions of phototrophic microorganisms, particularly, cyanobacteria, with dissolved trace elements during the diurnal cycle of photosynthesis. As is well known from natural measurements by microelectrodes [10] and from model simulations [11, 12], local pH variations in the microlayer near the cell walls of microorganisms amount to ±3–4 units depending on the primary production intensity. These variations are obviously able to significantly modify adsorption and the adsorption flux of trace elements in the course of photosynthesis. In particular, the prevailing tendency of cations (Zn, Cd, and Pb) should be their preferable accommodation in cell walls in daytime, whereas anions (Mo and Se) should be more significantly adsorbed by microorganisms at night, when the pH in the near-wall layer decreases. These tendencies should be taken into account in the quantitative physicochemical modeling of the adsorption of trace elements by photosynthesizing microorganisms.

It is now pertinent to consider more closely the adsorption coefficients of trace elements by the biomass of natural microbial communities that live in neutral thermal springs in central Kamchatka [7]. The distribution coefficients

$$K_d = [TE]_{dry\ biomass}/[TE]_{solution}, \quad (5)$$

reported above are average values that generalize our results obtained over the past two years. The concentrations of trace and major elements and the pH of the discharged water measured in the same spring with a time interval of two weeks and one year differed by no more than ±20–30% and ±0.2–0.3 pH units. This allowed us to regard the adsorption of trace elements by the growing biomass as a stationary process, which occurs at a constant (specified from outside) potential of trace elements. The distribution coefficient obtained for some trace elements and various types of microbial communities are shown in Fig. 4. This plot also displays the experimental distribution coefficients of trace elements obtained in the laboratory with the use of cultures of phototrophic microorganisms in the course of reversible adsorption on the surface of cells at near-neutral pH. The distribution coefficients of metals experimentally determined for adsorption on the surface of cells of bacterial cultures are of the same order as the values measured in natural systems. The greatest K_d variations are typical of elements weakly interacting with cell surfaces (Mo, Cs, As, and Sb). For these elements, the discrepancies between the "adsorption" and "consumption" values reach one to two orders of magnitude. The variations in the K_d depending on the sources and the types of the microbial communities are also significant.

CONCLUSIONS

Our research was aimed at the systematic analysis of the parameters of interaction of heavy metals and trace elements with aquatic phototrophic microorganisms. The Zn distribution coefficients between the biomass and aqueous solution vary from 10–30 l per gram of dry weight for anoxiphotobacteria to 80–100 for cyanobacteria and reflect the interaction of a bivalent cation with the carboxyl groups of polysaccharides and peptodoglycan of the cell wall and sheaths.

The speciation of trace elements in aqueous solutions and the charges of their complexes are the main factors controlling the character of the dependences of metal adsorption on the physicochemical parameters of the solutions. The pH values of the solutions are one of the main parameters controlling adsorption and, perhaps, also the consumption of chemical elements by microorganisms. The distribution coefficients obtained in laboratory experiments for trace-element partitioning between bacterial cultures and aqueous solutions are of the same order as the values obtained for natural microbial communities.

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