UNIVERSITÉ
DE GENÈVE
FACULTÉ DES SCIENCES
Section des sciences de la Terre
et de l'environnement

Symposium celebrating 30 years of the Institut F.-A. Forel

FRONTIERS IN LAKE RESEARCH

September 17th, 2010

Institut des sciences de l'environnement
Site de Battelle, bâtiment D, Carouge, Genève

Workshop 2

Workshop 2. Narrowing the gap between in situ and laboratory approaches to metal availability, bioaccumulation and ecotoxicity.

Moderator: J. Dominik; Reporter D. A. L. Vignati

Workshop goals

In the last decade interdisciplinary research has progressed, but the traditional approaches of the various disciplines still slow down the development of new concepts and research philosophy. This is manifest, for example, in the fields of aquatic geochemistry and ecotoxicology, in which experimental laboratory studies and field-based research do not sufficiently match to produce a generally acceptable vision of interactions between metals and biota that is essential for a sound risk evaluation and associated management of the aquatic systems under anthropogenic stress. To narrow the gap between the results of laboratory experiments and field studies a considerable effort is necessary in developing concepts, tools and designs of both field- and laboratory-oriented research. To stimulate such an effort the major problems have to be recognised and the prospect of how to overcome the difficulties envisaged. The proposed workshop should contribute to identify major existing difficulties in matching field- and laboratory-oriented studies and discuss pragmatic avenues to decrease the disparity between the outcomes of the laboratory and in-situ research on interactions between metals and biota. It should also present new tools for in situ and ex-situ investigations, which may help to improve the environmental relevance of fundamental studies in bioaccumulation and toxicity, providing scientific base for a sound risk assessment procedures.

WORKSHOP PROGRAM

10:45 – 11:35 Introductory plenary lecture

Peter G.C. Campbell – Key factors affecting trace element uptake and toxicity in aquatic organisms.

14:00 – 15:30 Short talks

Mary-Lou Tercier-Waeber – Bioavailable trace metal analysis: from laboratory to in situ measurements. (15 min)

Guillaume Suárez – Novel tools for real-time continuous contaminant effect monitoring (15 min)

Jean-Luc Loizeau and Serge Stoll – Particle-Colloid Size Measurements, Problems Field-Lab. (15 min)

Stéphane Guédron (part a) and Bian Liu, Andrea Garcia-Bravo and Lucie Huguet (part b) – Field versus laboratory approach in the study of a) mercury cycle and b) mercury methylation/demethylation in aquatic ecosystems. (15 + 10 min)

Frédéric Gimbert and Claudia Cosio – Lab-versus-field assessment of metal bioaccumulation: the physicochemical and biological artefacts. (15 min)

15:30 – 15:40 Break – coffee and soft drinks available (for the rest of the workshop)

Overview talks: 15:40 – 16:30

Vera I. Slaveykova – Dissolved organic matter as a key player in metal bioavailability: recent progress and challenges. (20 min)

Benoît J.D. Ferrari and Davide A.L. Vignati – Realism and relevance of the bioaccumulation and ecotoxicological tests. (20 min)

Janusz Dominik – Introduction to the workshop discussion. (10 min)

16:30 –17:30 General discussion (moderator J. Dominik) :

Workshop statement preparation. Reporter D.A.L. Vignati 17:30 –18:15

18:15 Reporting from workshops (plenary) – Workshop 2: 18:25 –18:35

ABSTRACTS

Introductory plenary lecture

Key factors affecting trace element uptake and toxicity in aquatic organisms

Peter G.C. Campbell

INRS Eau, Terre et Environnement, Université du Québec, 490 de la Couronne, Québec (Québec), Canada G1K 9A9 (campbell@ete.inrs.ca)

In the area of trace metal nutrition and toxicology, much qualitative evidence exists to the effect that the total aqueous concentration of a metal is not a good predictor of its "bioavailability", i.e. that the metal's speciation will greatly affect its availability to (aquatic) organisms. Indeed, a convincing body of experimental evidence has accumulated to support the idea that the biological response elicited by a dissolved metal is usually a function of the free-metal ion concentration, $M^{z+}(H_20)_n$. It follows that the complexation of a metal with one or more ligands in solution should lead to a decrease in its bioavailability; this general principle underpins the so-called Free-Ion Model, as well as its more recent derivative, the Biotic Ligand Model (BLM). However, virtually all the experimental evidence in support of the BLM has been obtained in the laboratory in synthetic solutions in the presence of simple inorganic or organic ligands. Natural systems contain additional metal-binding ligands, notably natural dissolved organic matter (DOM), metastable reduced sulphur species (thiols, polysulphides, thiosulphate) and various low molecular weight metabolites. The extension of the BLM to such systems is currently hampered by our relative ignorance of how these diverse ligands affect metal bioavailability. Using silver and cadmium as representative non-essential metals, we have tested the applicability of the BLM in systems that contain some of these natural ligands and thus have a composition that is close to that of natural waters.

A number of key assumptions underlie the BLM, some obvious and others rather more subtle. The aim of this presentation is to examine some of these assumptions and explore the limits of the BLM. The following points will be considered in the platform presentation and in the subsequent workshop:

- Is metal internalization always slow relative to the other steps involved in metal uptake (i.e., is the organism surface in equilibrium with metal species in the exposure solution)?
- Does metal internalization necessarily occur via cation transport (or do other mechanisms exist for metal entry into cells)?
- Must internalization occur for toxicity to appear (or can surface-bond metal exert deleterious effects)?
- How do pH changes affect metal uptake and toxicity?
- How does natural organic matter affect metal uptake and toxicity?

Experiments with algae will be described, demonstrating anomalously high metal toxicity in the presence of common low molecular weight metabolites (citrate and alanine), or in the presence of an assimilable anion (thiosulphate). The demonstration that natural organic matter (DOM) tends to accumulate at biological surfaces, particularly at low pH values, raises a number of intriguing questions about the ambient chemical environment close to the cell surface - the consequences of this accumulation on metal-cell interactions will be discussed.

Funding for our research on metal-organism interactions has been provided by the Natural Sciences and Engineering Research Council of Canada, Quebec *Fonds de Recherche sur la Nature et les Technologies*, the Canada Research Chair Program, and by Kodak Canada.

Short talks

Bioavailable trace metal analysis: from laboratory to in situ measurements.

Mary-Lou Tercier-Waeber

Department of Inorganic and Analytical Chemistry and
F.A. Forel Institute University of Geneva, Sciences II, 30 Quai E.-Ansermet, CH-1211
Geneva 4, Switzerland
E-mail: Marie-Louise.Tercier@unige.ch

The worldwide contamination of ecosystems by anthropogenic and natural releases of metals resulted in the need for understanding their fate and toxicity in aquatic systems. To a large extent, these two issues are related to the chemical speciation of contaminants which may vary continuously in space and time. Detailed measurements of the proportion of specific metal species, or groups of homologous metal species, and their variation as a function of the bio-physicochemical conditions of the natural media in which they are found are thus of prime importance.

Challenges associated with the study of metal speciation in the laboratory and in situ, based on the complexity of trace metal transformations and capabilities of available traditional laboratory and field emerging techniques, will be summarized. Specific conceptual, analytical, and technical criteria that must be considered and/or fulfilled to allow controlled and reliable measurements, at an appropriate time scale, of potentially bioavailable metal species will be discussed. Examples of strategies used to satisfy these criteria will be illustrated by describing advanced voltammetric sensors, mini-/micro-integrated analytical systems, and submersible equipment developed for attended and remote in situ monitoring of relevant metal fractions in term of (eco)toxicity. The significant advantages of such tools, vs. traditional laboratory techniques, in term of spatial and temporal resolution and the promising possibilities that lie ahead for environmental scientists to perform more efficient environmental monitoring of metal biogeochemical cycles and pollution control will be illustrated by selected examples of applications and discussed in term of multidisciplinary approach.

Novel tools for real-time continuous contaminant effect monitoring

Guillaume Suárez

Nanophotonics and Metrology Laboratory, EPFL-STI-NAM, Station 11 (ELG 237),
CH-1015 Lausanne, Switzerland
E-mail: guillaume.suarez@epfl.ch

The collective concern for the preservation of our natural environment from detrimental anthropogenic activities has a need for assessing ecosystems. Generally, it consists of measuring over a period of time a series of targeted contaminants whose presence could be expected. Alternatively, the target is no longer a series of toxicants but indicators that reflect their impact on the biota. In the context of aquatic ecosystems the concentration of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide or nitric oxide, that are naturally encountered in fresh waters can be dramatically enhanced due to the presence of contaminants. This ROS level increase is related to the photochemical degradation of organic pollutants and/or due to their interaction with aquatic organisms. In particular, ROS play a major role in the oxidative stress mechanisms in microorganisms and consequently ROS overproduction in aquatic cells is an indicator of stress/toxicity. Current analytical methods for ROS detection mainly rely on fluorometric measurements. Such end-point bioassays exhibit a low limit of detection (few nanomolar for H_2O_2) but are not well adapted to on-line assessment situations. In this context, novel detection systems based on amperometric and optical (bio)sensors might soon bring new insight. The scaling down of transducing devices due to advancement in micro/nanofabrication tools is offering wide possibilities for highly sensitive biodetection systems. Their integration into microfluidic platforms would hopefully lead to a large variety of instruments ranging from lab-bench oxidative stress on-line detector to in-situ "early sentinel" devices.

Particle-Colloid Size Measurements, Problems Field-Lab

J.-L. Loizeau and S. Stoll
Institute F.-A. Forel, University of Geneva, 10 route de Suisse, CH-1290 Versoix, Switzerland

In aquatic systems the transport and fate of both nutrients and toxic compounds largely depend on their interactions with colloidal particles which are expected to form complex structures through aggregation processes. Owing to the complexity and large number of biophysical and chemical factors influencing these processes, as well as to the fact that natural colloids include many heterogeneous components, several approaches such as field and laboratory measurements of natural samples are necessary to study the complexity of these dynamic processes.

Due to the development of computer modelling and new analytical techniques in the field of colloid science, experimental and computer models have been applied only relatively recently for investigating the dynamic behaviour of simple colloidal suspensions. These models have proved to be valuable and represent convenient tools for the systematic investigation of some of the most important physicochemical factors (pH, temperature, solution ionic strength, particle concentration, and chemical surface properties) influencing not only the morphology but also the kinetics of colloidal aggregate formation.

The choice of one approach (field measurement, laboratory measurement, experimental model or modelling) depends generally upon whether the problem under consideration is defined at a microscopic, mesoscopic or macroscopic level and on the appropriate degree of complexity and level of details that are required.

Two examples will be given: one dealing with the transient nature of the colloid suspension as exemplified by the evolution with time of the colloid size distribution, raising the question of on-site vs. lab measurements; and the other one with modelling-lab models investigation of the interaction processes between colloids and fulvic acids. Advantages, limitations and overall possible connections between the different approaches will be discussed.

Field versus laboratory approach in the study of a) mercury cycle and b) mercury methylation/demethylation in aquatic ecosystems.

part a) Stéphane Guédron and,

part b) Bian Liu, Andrea Garcia-Bravo and Lucie Huguet

Institute F.-A. Forel, University of Geneva, Route de Suisse 10, CP 416, CH - 1290 Versoix, Switzerland

Mercury (Hg) methylation in waters and sediments is highly interdependent of the geochemical cycles of organic matter (OM), iron and sulphides, which affect the bioavailability of inorganic Hg for methylating organisms (i.e., sulfate-reducing and iron-reducing bacteria). Thus, sampling and experimentation performed in the field and laboratory for the study of the Hg cycle needs rigorous methodologies (e.g. ultra clean sampling materials and very sensitive analytical tools) which allow measurements of redox sensitive elements, identification and quantification of main methylating organisms. Indeed, the determination of the methylating activities of natural bacterial assemblages in relation to sediment characteristics, specific environmental conditions and degree of Hg contamination needs to be undertaken to validate laboratory-based measurements and to improve our understanding of Hg cycling in the environment.

In this presentation, advantages and major obstacles for *in situ* experiments and field sampling and/or analysis of previously cited parameters will be presented and discussed. First, general aspects of the sampling strategy and *in situ* analysis will be discussed with regard to environmental settings. Parameters such as spatial heterogeneity and dynamic of the system will determine the time devoted to sampling and analyses in order to get a realistic spatial and temporal resolution for a better understanding of mechanisms involved in Hg methylation. Second, advantages and limitations of *in situ* measurements (redox sensitive elements, ancillary parameter, colloids…) will be discussed and suggestions will be presented for an improvement of these methodologies. Third, we will discuss the use of Hg stable isotopes in studying methylation/demethylation processes, under *in situ* and laboratory conditions (e.g. its advantages, limitations, and gaps) with case studies.

Lab-versus-field assessment of metal bioaccumulation: the physicochemical and biological artifacts.

Frédéric Gimbert[1,2] and Claudia Cosio[1]

[1] Institute F.-A. Forel, University of Geneva, 10, route de Suisse, CH-1290 Versoix, Switzerland
[2] UMR Chrono-Environment, CNRS-University of Franche-Comté, Place Leclerc, F-25030 Besançon Cedex, France (present address)

Bioaccumulation is a good integrative indicator of the chemical exposures of organisms in polluted ecosystems, especially in the case of trace metals which are not metabolized. Recent studies on aquatic organisms [1-3] have shown that several factors have to be taken into account when determining metal bioaccumulation. Geochemical speciation in a complex environment is the important factor; physiology and ecology of the test species are also essential in determining uptake, internal processing and excretion rates of metals. Measured metal body concentrations or bioaccumulation levels are the final result of these factors and can only be correctly addressed when the underlying mechanisms and kinetics are properly understood. For that reason, biodynamic (or biokinetics) has been advocated as a unifying concept [3]. Combining experiments at different levels of organization, biodynamic modeling can therefore be used to address laboratory-versus-field gaps [4-5]. Hence, in this contribution, some fundamental aspects of the biodynamic approach will be emphasized such as the simultaneous consideration of geochemical and biological processes, the distinct integration of various exposure routes and their potential in understanding metal toxicity.

References

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Overview talks

Dissolved organic matter as a key player in metal bioavailability: recent progress and challenges

Vera I. Slaveykova
Institute F.-A. Forel, University of Geneva, Faculty of Sciences, 10, route de Suisse, CP 146,
1290 Versoix, Switzerland
e-mail: vera.slaveykova@unige.ch

Bioavailability is a key concept in bridging aquatic geochemistry of contaminants (*e.g.*, toxic trace metals) to their ecotoxicity. It is currently accepted that the contaminants' bioavailability is controlled by their chemical speciation and in particular, the concentration of the free metal able to bind the sensitive sites of biota. Dissolved organic matter (DOM), a major component of natural waters, can alter the contaminants' speciation; therefore their bioavailability can be expected to be affected by DOM. Nonetheless, the current practice is to "divide" the total contaminant load into a particulate and a dissolved fraction. The latter is used to set regulatory maximum contaminant loads for surface water quality in Europe and Switzerland and also to assess the ecotoxicological hazard, without further consideration of the speciation and bioavailability within the dissolved fraction. In my view this "*paradox*" is related to the existing controversy and gaps in the current knowledge about the role of the DOM on metal bioavailability in surface waters and in particular when coupled to the influence of other environmental stressors.

The recent research efforts and challenges towards the improvement of the understanding of the interaction of DOM with toxic trace metals and biota will be illustrated with examples of our research and the literature. The emphasis will be on the bioavailability of priority toxic trace elements by different phytoplankton species in the presence of model DOM components, DOM isolated from Amazon River basin and waste water treatment plant effluents DOM, and their interplay with increased solar radiance. Different possible avenues for narrowing the existing gap between bioavailability determined in the lab and field will be also discussed.

Realism and relevance of the bioaccumulation and ecotoxicological tests

Benoît J.D. Ferrari[1] and Davide A.L. Vignati[2]
1) Cemagref, UR Maly, 3bis quai Chauveau, CP220, 69336 Lyon cedex 09, France
2) CNR-IRSA, Via del Mulino 19, 20047 Brugherio (MB), Italy

Thanks to the ever-increasing interaction among academia, stakeholders and institutional authorities, achieving an adequate balance between pragmatism (usually resting on the results of controlled, laboratory-based experiments) and environmental realism (as assessed from pertinent field studies) has become a main paradigm and driving force in both environmental research and risk assessment procedures. The development of bioaccumulation and toxicity tests ensuring an adequate balance between pragmatism and realism indeed appears as a cornerstone for building scientifically sound and economically defensible environmental protection strategies. How to obtain such a balance, however, still presents several economical, political, scholarly, scientific, and technical challenges; so that consensus as to the most adequate tools and strategies to develop (or adopt) in the near future is still missing. Starting from the current-state-of-the-art, the speakers will present their view on the most critical challenges towards obtaining such a consensus. Within the framework of the workshop, this intervention is meant to stimulate discussion leading to the definition of an agenda of priorities for both scientists and environmental managers dealing with issues connected with trace metal pollution.

WORSHOP REPORT

Rapporteur: Davide A. L. Vignati

CNR-IRSA, Via del Mulino 19, 20047 Brugherio (MB), Italy

Workshop discussion

At the end of the lectures, the workshop moderator kicked-off the discussion by asking what was most needed to narrow the gap between in situ and laboratory approaches to metal availability, bioaccumulation and ecotoxicity. The moderator suggested *tools*, *concepts*, or *comparative laboratory vs. field studies* as possible main choices. The following discussion articulated on both technical and scientific topics related to the three choices. Besides the conclusions upon which the participants agreed and which are reported below, a couple of critical non-technical aspects arose.

The first point concerned the attitude of the end-users of the scientific information produced by researchers towards research itself. The main users of scientific information (stakeholders, governments and/or environmental authorities) are focused on environmental protection at large with little interest in the specific scientific aspects of any discipline. The second point concerned the extent to which interdisciplinarity is really being achieved in environmental studies. While all workshop participants felt themselves strongly involved in interdisciplinary research, they also recognized that the degree of competence required to answer complex environmental question is such that interdisciplinarity often translates into cooperation between 2 or, at best 3, disciplines. (Incidentally, most of the discussion at the workshop focused on issues related to environmental chemistry and toxicology, thus highlighting how even discerning audiences find it difficult to have a truly interdisciplinary discussion without a good-balance among various disciplines within a given audience).

These points were not subject to in-depth discussion because they were outside the primary scope of the workshop. However, they are mentioned because they are critical to developing socially-relevant science and should guide the non-technical reflections to develop adequate research concepts and tools as well as more effective communication strategies between scientist and other professionals of environmental protection.

Workshop statements

The statements given below were agreed upon by the participants in workshop 2 and presented to the whole public during the closing session of the workshop. They concern three specific scientific areas.

Environmental chemistry

- The importance of the 'free ion' as the main species controlling elements' bioavailability needs to be weighed against the contribution of lipophilic species and other environmentally occurring species which are turning out to be more bioavailable than previously thought.
- Biodynamic and thermodynamic approaches must be better integrated to increase our understanding of how speciation influences bioavailability and how organisms influence metal speciation.
- The development of in situ devices for continuous measurements of an increasing number of environmental variables opens the possibility to verify findings and knowledge based on the 'traditional' collection of discrete samples in the field followed by analysis in the laboratory
- Increasing knowledge on data poor metals must be more actively sought because, at present, abundant database exists for some divalent metals (Cu, Zn, Pb, Cd) but much less for others environmentally important elements (Cr, Hg, Ag, etc).

Ecotoxicology

- More focus must be put on the actual levels of interest for environmental protection (e.g., populations, communities and ecosystems) to overcome the present limits of ecotoxicology in relation to real field conditions. All participants agreed on this statement, but also recognized that most of the ecotoxicological tools will remain based at the individual level.
- Successfully achieving the first statement requires a constant critical evaluation of classical concepts and tools of ecotoxicology in relation to the environmental issues of interest.
- Considering the existence of variable reference conditions and defining the ranges of acceptability as reference conditions for the endpoints of interest are critical steps to the advancement of the science of ecotoxicology and its integration with other disciplines.

General science

- Effective combination of bottom-up and top-down approaches is still far from perfect and must be pursued to further narrow the laboratory vs. field gap.
- Development of tools for in situ measurement must continue, both in chemistry and ecotoxicology.
- The time-scales for any study must be chosen according to the parameters under study and the system being examined. The word ‘system’ can indicate a specific organism, a set of chemical variables, the structure of a given community and so on.
- The choice of the most appropriate tools is often specific to individual studies. Among the available possibilities, scientist must critically choose the most appropriate tools or, should they be lacking or inadequate, invest in the development of new ones.