



DETERMINATION OF PARAMETERS INFLUENCING METHYLATION AND DEMETHYLATION IN TROPICAL LAKES IN BRAZIL AND NICARAGUA

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Abstract

*Increased awareness about the toxicity of mercury (Hg) has during the latest decades resulted in reduced use of Hg in industrialised countries. Developing countries, on the contrary, have largely increased their anthropogenic Hg emissions caused by its use in gold mining, transfer of Hg emitting factories from developed countries, and increased burning of coal without appropriate flue gas cleaning. The contribution of global Hg sources and the importance of other parameters to increased Hg levels encountered in hydroelectric reservoirs and other areas after flooding is not well understood, especially not in the tropics. The aim of the present study is to increase the knowledge about Hg transformations in tropical areas. Total Hg content in water, biota, and sediment will be determined by atomic absorption and fluorescence spectrophotometry and methyl Hg content in biota by gaschromatography after extraction with acids, hydroxides, and organic solvents. Mercury methylation capacity in sediments, water, and selected biota will be determined with ²⁰³Hg and subsequent radiological measurements of in-situ incubations. Factors affecting the methylation and demethylation rates will be identified with laboratory incubations with ²⁰³Hg at varying environmental conditions such as organic matter, pH, redox potential, conductivity, light, temperature, geochemical factors and populations of bacteria. The populations of bacteria will be determined to quantity by isotope techniques. The first experiments indicate markedly larger methylation capacity as well as bacterial production of incubated samples of *Eichhornia crassipes*, originating from Brazil, compared to *Myriophyllum spicatum* from Sweden. The results are the first step to better understand the importance of environmental parameters and bacterial production for methylation of Hg.*

1. INTRODUCTION

1.1. Scope and aim

The toxicity and human health impacts of methyl Hg are well documented. The transformations between metallic Hg and different organic Hg forms have been extensively studied in temperate areas, but little is known about methylation and demethylation processes under tropical conditions. The present, large anthropogenic Hg emissions in many tropical countries from gold mining, chlor-alkali factories, coal combustion etc. may deteriorate the health of future generations. An increasing global population and subsequent food demand

cannot be at the risk to restrict the consumption of fish, due to eventually increased methyl Hg content. Hence it is necessary to increase the knowledge about factors influencing the net methylation of Hg in tropical as well as temperate environments. Such factors may be organic acids, geochemical factors, and bacteria, in addition to temperature and redox potential [1, 2, 3, 4, 5, 6, 7].

In the present project we aim to determine net methylation capacity of Hg, bacterial production and geochemical parameters. Parameters possibly influencing MeHg-formation during incubation will be studied by incubating parallel samples at different temperatures, different periods (a few hours to four days), in different atmospheres (ordinary air versus nitrogen atmosphere), different pH, conductivity, and light conditions. The influence of micro-organisms and their carbon source on Hg methylation will also be studied.

1.2. Activities related to the CRP

The following activities related to the CRP have been performed since the First Research Co-Ordination Meeting in Ljubljana, Slovenia, 29 November - 3 December 1999:

- April, 2000, submission of a research project entitled "Methylation and demethylation of mercury in tropical freshwaters: studies of controlling factors using nuclear techniques and satellite images", which included planning and presentation of seminars in Brazil and Nicaragua during Feb. - April 2000. Unfortunately the project was not approved by Dept. of Research Cooperation (SAREC) at the Swedish International Development Agency. In addition, Nicaraguan authorities did not approve to submit an application to IAEA. A revised research plan was submitted to SAREC in 2001, "Methylation and demethylation of mercury in tropical freshwaters: studies of controlling factors using nuclear techniques".
- A 4-month guest research stage (Sep. 2000 - Jan. 2001) by Jane Mauro from the Federal University of Rio de Janeiro, Brazil, at the Department of Limnology, Uppsala University, through collaboration with the Institute of Applied Environmental Research (ITM), Stockholm University, Sweden.
- A seminar entitled "Global mercury trade and some theories on reducing mercury in fish from lake Guri", presented by Lars Hylander at the International forum "El problema del mercurio en los embalses: el caso del Reservorio Guri" (The problem of mercury in hydroelectrical reservoirs: the case of Lake Guri), May 17 - 19, 2001, in Estado Bolivar, Venezuela [8].

1.3. Collaboration regarding mercury or nuclear-related methods apart from this CRP

Lars Hylander is in co-operation with researchers from Royal Institute of Technology, Stockholm, Sweden and Kanagawa Environmental Research Centre, Yokohama, Japan, and National Institute of Environmental Sciences, Tsukuba, Japan, using ICP-mass spectrometry and synchrotron radiation X-ray fluorescence (SR-XRF) for studies of isotopes of heavy metals and phosphorus content in waste water and the uptake by plants or deposition on bark. Markus Meili is assessing the ^{137}Cs content in sediment from the Baltic Sea and adjacent freshwater systems in order to study the fate and mobility of ^{137}Cs fallout after the Chernobyl nuclear accident. He has also been studying the bioavailability and turnover of radio-labelled Hg, Se, and Cs and methyl-Hg formation in laboratory microcosms. In addition, he is

involved in European efforts to determine critical atmospheric Hg pollution levels based on Hg balances and predictions for different regions in Sweden.

2. METHODS

Bacterial production is estimated with ^{14}C -leucine incorporation to plant sub samples in scintillation vials, which are then incubated for thirty minutes. Incubations are interrupted with formaldehyde, the samples are sonicated, washed with TCA and ethanol through filters, which are then counted in a LKB Wallack Rackbeta 1217 scintillation counter [9].

The potential net Hg methylation capacity in sediments and selected biota is determined with in-situ and laboratory incubations with ^{203}Hg [10, 11]. Fresh samples of about one gram dry weight in sample volumes of 30-50 mL (2 -3 samples and one acidified control) are incubated with $^{203}\text{Hg}^{2+}$ at concentration of 30-1000 ng total Hg g⁻¹ dry weight. Methylation is stopped by addition of HCl and the samples are frozen until MeHg extraction by toluene containing scintillation salts and measurement by liquid scintillation.

3. RESULTS AND DISCUSSION

Results from experiments on eventual correlation between potential net methylation with bacterial production of incubated macrophytes indicated the importance of several factors. Bacterial production obtained for *Eichhornia crassipes* roots, originating from Brazil, was significantly higher than for *Myriophyllum spicatum* shoots from Sweden. Incubation time had a significant effect on bacterial production, which for *Eichhornia crassipes* increased linearly with time, while for *Myriophyllum spicatum*, bacterial production decreased during the first 25 hours before increasing also there, but to a lower level. There was up to 23% net formation of methylmercury for *Eichhornia crassipes*, while methylmercury production was below the detection limit for *Myriophyllum spicatum*. The accumulated methylmercury production observed for *Eichhornia crassipes* increased with the bacterial production yield, indicating the importance of microbial processes for the mercury methylation [12].

4. PLANS FOR FUTURE WORK

4.1. Plans for 2002 - 2004

Field campaigns in South America are planned to start in 2002, given that financial resources are obtained. Sampling will start at the end of the dry season and be performed once to three times monthly at the onset of the rainy season. This in order to carefully document the load of suspended matter and periphyton, methylation capacity and microbial production when the aquatic system is quite labile due to dust and nutrients washed into the water courses by the rains and oxidising conditions from large quantities of oxygenated water. Sampling continues for another month, but the sampling intensity is reduced when the microbial communities are stabilised in lake and water courses at a high water flux. The transformation to dry season conditions is less dramatic than to rainy season conditions, so a 1-2 weeks sampling campaign is sufficient any time during the end of the rainy season or beginning of the dry season, to complement the data received. The same sampling schedule will be performed in 2004. Laboratory experiments with parameters similar to tropical climate conditions will be

performed in Sweden mainly during 2003, when no field campaigns are planned and personal and laboratory capacity in Sweden is not occupied by field campaigns.

4.2. Expected outcomes

The studies are expected to contribute with valuable information about factors important for methylation and demethylation at different populations of micro-organisms. Bioindicators for methylation might be identified. The importance of environmental factors such as methane production and organic carbon in different forms will be clarified. The project is expected to produce results which can be used when assessing methylation from flooded areas and when assessing risks for public health due to methylation of Hg.

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