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 Hg use 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. These increased emissions occur mainly in the tropics, where the fate of Hg is not well documented. The aim of the present study is to increase the knowledge about Hg levels and transformations in two tropical areas affected by anthropogenic Hg emissions – the Pantanal wetland in Brazil, housing gold miners using the amalgamation method, and Lake Xolotlán (Managua) in Nicaragua, where a chlor-alkali plant relocated from the USA has emitted much Hg. Actual Hg content in water, biota, and sediment will be determined by atomic fluorescence spectrophotometry and atomic absorption spectrophotometry. Mercury methylation capacity in sediments and selected biota will be determined with in-situ incubations with ^{203}Hg and subsequent radiological measurements. Factors affecting the methylation and demethylation rates will be identified by varying environmental conditions such as pH, redox potential, conductivity, light, temperature, geochemical factors and population of bacteria. Sediment turnover will be studied by determining fallout cesium (^{137}Cs) in sediment profiles. The study is expected to increase the knowledge about Hg-transformations in the tropics and point out proper measures to reduce health hazards due to Hg-exposure.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1. Background

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.

1.2. Studies on mercury and related topics earlier performed by participating researchers

The Hg levels in soil, sediment and biota has been surveyed by Hylander et al. in the Pantanal, Brazil, in 1992 [1] and 1998 [2, 3, 4, 5], and by Guimarães, Malm, and Meili in 1995 [6]. Guimarães et al. showed a large, potential methylation capacity in floating macrophyte roots in Pantanal [7, 8].

The most consumed fish from Lake Xolotlán (Managua), *Cichlasoma managuense,* contained in 1988 to 1989 0.63 ±0.22 μg Hg g^{-1} fresh weight (n=176) [9], which is above WHO guidelines of 0.5 μg Hg g^{-1} in fish for human consumption [10]. The importance of bacterial communities and microbial activity has been studied in sediments by Ahlgren et al. [11] and on water plants by Eriksson, and Törnblom and Søndergaard [12, 13]. Importance of dissolved organic matter on the cycling of environmental mercury in tropical and boreal ecosystems has been studied by Meili [14, 15, 16].

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

Isotope methods (ICP-mass spectrometry, ^{32}P) are used for studies of heavy metal and phosphorus content in wastewater and its uptake by plants. The studies are performed by Lars Hylander in co-operation with researchers from Royal Institute of Technology, Stockholm, Sweden and Kanagawa Environmental Research Centre, Yokohama, Japan.

Markus Meili has a long experience with mercury studies in freshwater ecosystems in Sweden, Canada, and Brazil, supported mainly by SEPA (Swedish Environmental Protection Agency) and SIDA (Swedish Agency for Research in Developing Countries). He is presently involved in European efforts (UN/ECE-LRTAP) to determine critical limits of atmospheric mercury pollution. At the same time he is monitoring and evaluating the remediation of a highly Hg-polluted lake using a novel capping method. Over the years has also conducted a large number of laboratory experiments using radio-labelled tracers to assess the bioavailability and biological turnover of Hg, methyl-Hg, Se, and Cs in microcosms containing dissolved organic matter, microalgae, zooplankton, macroinvertebrates, and fish. His field studies often include the use of ^{13}C and ^{15}N to identify energy sources and actual trophic position of aquatic organisms in various environments, initially a collaboration with the Ecosystems Center in Woods Hole, USA. He has also carried out detailed studies on the distribution of ^{137}Cs in sediments and biota from the Baltic Sea and many adjacent freshwater systems, partly in order to assess the fate of ^{137}Cs deposited after the Chernobyl nuclear accident in 1986, partly to use this nuclide as a tracer of sediment turnover and food web dynamics. This research includes projects supported by SSI (Swedish Radiation Protection Institute) and SKB (Swedish Nuclear Fuel and Waste Management Co). Recently he has started to study the regional and global distribution of ^{129}I in seawater and freshwaters, in particular the fate of the discharges from western Europe in Baltic, Atlantic, and Arctic areas.

Ingemar Ahlgren and Rolf Eriksson are using isotope methods for estimating bacterial activity in Swedish and Nicaraguan lake sediment by using 3H-thymidine incorporation. Isotope methods in studies of bacterial activity are also used by Erik Törnblom in co-operation with the project "Nutrient Transport and Turnover in Littoral Areas and the Subsequent Effects on Water Blooms in Lakes and Coastal Waters" financed by MISTRA

(The Swedish Foundation for Strategic Environmental Research) and with professor Morten Søndergaard from the Freshwater Biological Laboratory, University of Copenhagen, Denmark.

2. METHODS

The coordinates of all sampling sites are determined with a GPS instrument (Global Positioning System). Water parameters such as pH, redox potential, conductivity, turbidity, temperature, etc. are measured in the field when samples are collected or incubations performed. Air temperature, light intensity, wind speed, etc. are also documented. Determinations of Hg in biota and sediment samples are performed using atomic absorption spectrophotometers available at CIRA in Managua, Nicaragua, and in Brazil. The quality of obtained data is assured by certified reference materials.

Determinations of C and Hg in water, ^{137}Cs, C, N, and S in sediment are performed at Uppsala University, at the Department of Earth Sciences and Department of Limnology, equipped with beta- and gamma-counters, and C/N/S-analysers, and at Stockholm University, Dept. of Applied Environmental Research, equipped with an atomic fluorescence spectrophotometer as well as an atomic absorption spectrophotometer for Hg analyses. ^{137}Cs can also be determined at CIRA in Managua. The quality of obtained data is assured by laboratory chemicals or certified reference materials.

Different bacteriological methods will be used in order to determine: i) bacterial production; ii) bacterial abundance and biomass; iii) active/inactive bacteria and respiring bacteria.

i) Isotope methods for quantifying **bacterial production** include: a) the incorporation of ^{3}H-thymidine into bacterial DNA [17, 18] and b) incorporation of ^{3}H-leucine (or ^{14}C-leucine when combined with ^{3}H-thymidine incorporation as a dual label technique) into proteins [19, 13]. Thymidine incorporation into DNA is specific for bacteria, while leucine incorporation into protein is a more general method for measuring bacterial production. The methods can either be used separately or combined in order to obtain an increased amount of ecological information.

ii) **Bacterial abundance and biomass** will be determined on formaldehyde (5%) preserved samples by standard epifluorescence microscopy after appropriate staining. The most suitable stain will be tested and used. Possible stains include DAPI, acridine orange and SYTO 13.

iii) The proportion of **active and inactive** bacterial cells will be determined on live samples using epifluorescence microscopy and Live/Dead staining (Molecular probes). The number and proportion of **respiring** bacteria will be determined on live samples using epifluorescence microscopy and the tetrazolium salt CTC (5-cyano-2,3-ditolyl tetrazolium chloride).

The comparability of bacteriological data is more difficult to assure than data from chemical and isotope analyses. One way to proceed is to divide a homogeneous sample into subsamples for preparation and counting of bacteria cells at the participating laboratories. A library of prepared reference slides is another possibility.

The **potential net Hg methylation** capacity in sediments and selected biota will be determined with in-situ and laboratory incubations with ^{203}Hg [20, 21]. Fresh samples of about one gram dry weight in sample volumes of 30-50 mL (2 -3 samples and one acidified cotrol) are incubated with $^{203}Hg^{2+}$ at concentration of 30-1000 ppb dry weight. Methylation is stopped by addition of HCl and the samples are frozen until MeHg extraction into toluene containing scintillation salts and measurement by liquid scintillation. Samples incubated in Brazil are analysed at the Federal University of Rio de Janeiro, laboratory of radioisotopes, which is equipped with liquid scintillation counter, centrifuges, and chromatography. Samples incubated in Nicaragua are analysed at the Centro para la Investigacion en Recursos Acuaticos de Nicaragua, Managua, which is equipped for commonly performed limnological analyses and a scintillation counter, or brought to Sweden for analysis. The comparability of methylation analyses can be tested by dividing a homogeneous sample into subsamples for analysis at the participating laboratories.

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.

3. RESULTS AND DISCUSSION

In the Pantanal (Fig. 1) fish Hg content was generally below the limit for human consumption in the most intensive gold mining area at the border to Pantanal, as well as in central Pantanal, while fish Hg content was often above 0.5 $\mu g\ Hg\ g^{-1}$ fresh weight at intermediate distances. It is possible that Hg emitted in the mining areas only has a limited residence time in the vicinity of the sources, e. g. due to limited deposition during the dry season of atmospheric emissions and rapid emission or reemission to the atmosphere from Hg in surface water bodies, like lakes and rivers in the actual regions. This could be a result of climatic conditions such as high temperature and seasonal flooding. Eutrophic conditions may contribute to a fast growth and subsequently a lowered bioaccumulation of Hg in fish. It is also possible that the large soil content of oxy-hydroxides of Al, Fe and Mn adsorbs deposited Hg, thus preventing it from reaching the water bodies as long as soil erosion is prevented. This motivates a more detailed study on the influence of dissolved organic matter and oxy-hydroxides of Al, Fe and Mn, as well as of microbial activity on Hg methylation.

Fish Hg concentrations in a carnivorous species in Lake Xolotlán (Managua) (Fig. 2) was ten years ago generally above the limit for human consumption, with highest values in the vicinity of a chlor-alkali plant. Mercury levels in surface sediment were around 0.6 $\mu g\ g^{-1}$ DM. The present Hg-levels in fish and sediment is unknown. Large quantities of Hg have been emitted (totally about 80 tonnes) but the observed impact on the population has not been as severe as in Minamata, Japan, where hundreds died and several thousand people suffered

from nervous disorders, known as "Minamata disease", after emission of an estimated 225 tonnes of Hg [22]. The different impact may be due to different forms of emitted Hg, since part of the Hg emitted from the Minamata factory was MeHg. It is unclear whether also other toxic compounds were emitted in Minamata, contributing to the health impact. Other factors that possibly influence the different impacts between Managua and Minamata are alimentary habits, different climate, and different water recipients (a lake versus the sea). The importance of the eutrophic conditions of Lake Xolotlán (Managua) is not well understood for the methylation of Hg and fish Hg-content.

4. PLANS FOR FUTURE WORK

4.1. Planned experiments

In a co-operative project between Sweden, Uppsala University and Brazil, theFederal Universities in Rio de Janeiro and Cuiabá, we presently study the methylation capacity in floodplain lake sediments and in the root zone of floating macrophytes by incubating samples with ^{203}Hg. The study is performed in the Pantanal wetland near areas where Hg is used in artisanal gold mining. The results will be complemented with bacteriological studies and further studies of methylation as influenced by parameters such as organic acids and geochemical factors. The influence of water pH, conductivity, salinity, organic matter, plant growth stage, plant species, root section, bacterial population etc. on methylation and demethylation rates will be determined under controlled conditions in the laboratory and in the field. ^{137}Cesium will be determined in sediment profiles (0.5-0.8 m length, sliced in 2-cm horizons), collected in floodplain lakes of northern Pantanal (15 profiles), to study sediment turnover.

Methylation studies will also be performed in Lake Xolotlán, Nicaragua, where about 80 t of Hg has been deposited by a chlor-alkali factory. The primary production and bacterial production has been studied in an earlier research co-operation between Uppsala University and Centro para la Investigacion en Recursos Acuaticos de Nicaragua, Managua. The factory is now closed, but climatic conditions, such as frequent and strong winds, have probably caused that Hg in sediment is still exposed to biota of the lake, which will be verified with a study of sediment turnover. A survey of present Hg content in fish and biota will be done and related to earlier studies of total Hg levels and to methylation experiments as described above in the Brazilian part. River Sucio and River Mico in Chontales gold mining area will be included in the survey.

Mercury levels in environmental samples will be compared with Hg levels determined in human hair in the actual study areas. Risk assessment of Hg impact on human health will be performed by calculating a Hg and MeHg mass balance for a tropical region of the Amazons, Rio Negro basin, and compare the results with existing mass balances for temperate regions.

Obtained results will be compared with earlier performed and planned laboratory experiments in Sweden, including water, sediment and biota from temperate environments. Climatic conditions can be manipulated in laboratory. The studies are expected to contribute with valuable information about the importance of methylation and demethylation at different water trophic levels and populations of micro-organisms, which might diverge between temperate and tropical areas.

4.2. Time schedule

Two field campaigns have been performed in Brazil in March 1998 (the end of the rainy season) and in August 1998 (the end of the dry season). Many analyses are concluded and the results will be evaluated until June 2000.

Two field campaigns in Nicaragua are planned for March 2001 (the end of the dry season) and for August 2001 (the end of the rainy season). Parallel to the field campaigns laboratory experiments will be performed in Brazil, Nicaragua and Sweden starting in January 2001.

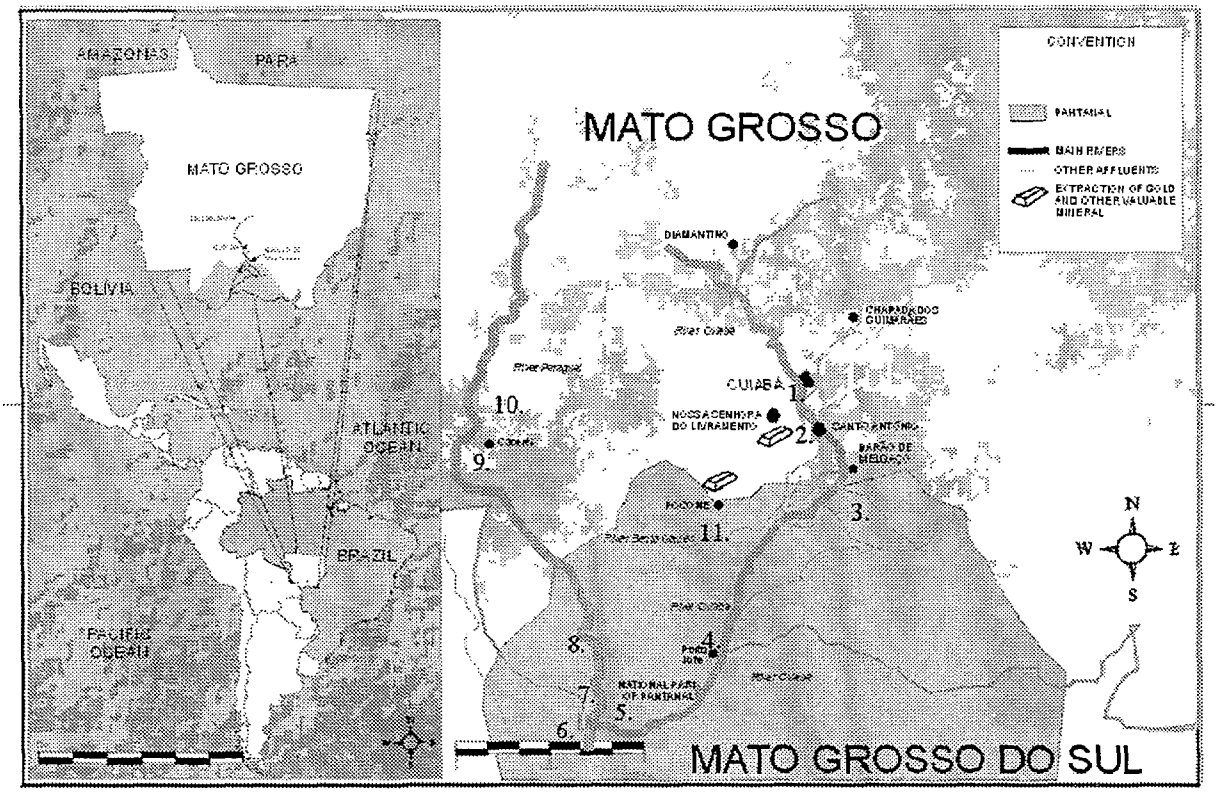

Figure 1. Location of sampling sites in Pantanal (Brazil). 1. Rio Cuiabá at Cuiabá; 2. Volta Grande; 3. Bog stream at Mimoso, Rio Mutum, Baía Sía Mariana, Baía Chacororé, north and south; 4. Rio Cuiabá at Porto Jofre, Baía Jofre; 5. Rio Cuiabá at Baía do Burro, Baía do Burro; 6. Rio Paraguay at Baía Amolar, Baía Amolar; 7. Rio Paraguay at Baía Paraíso and Acurisal, Baía Paraíso; 8. Rio Paraguay at Baía Cachorada, Baía Cachorada; 8. Rio Paraguay South and West of Cáceres, Baía da Cidade Cáceres; 10. Paraguay River at Baía Ximbuva, Baía Ximbuva; 11. Bento Gomes river basin (< 30 km from Poconé).

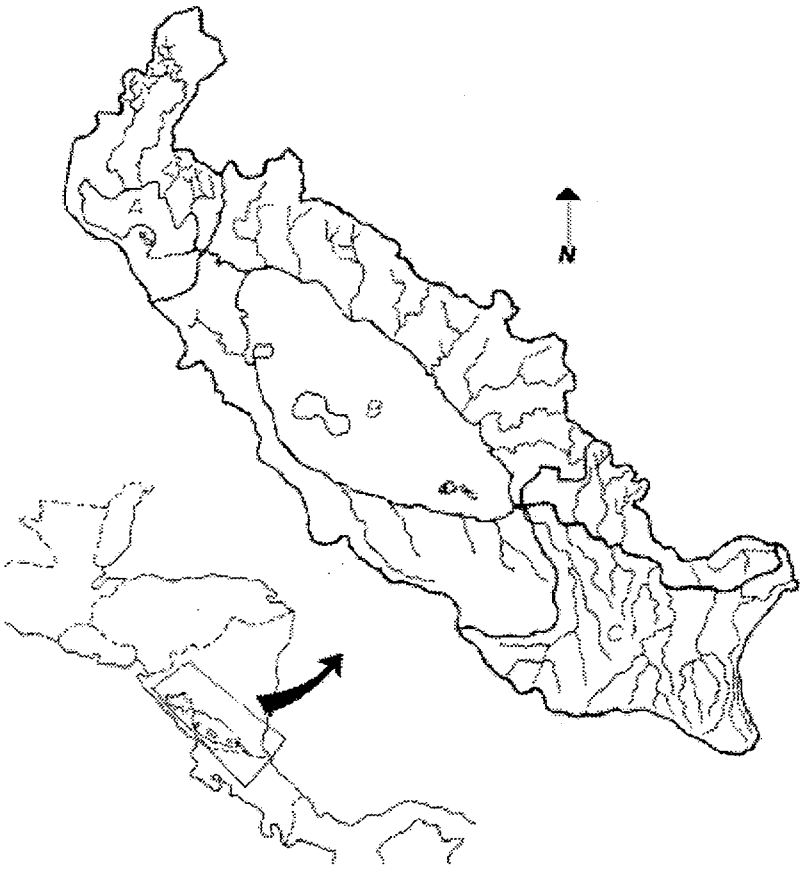

Figure 2. Map of the study area in Nicaragua: A = Lake Xolotlán (Managua); B = Lake Cocibolca.

TABLE I. SCHEDULE FOR ACTIVITIES IN THE COORDINATED RESEARCH PROJECT (CRP) "DETERMINATION OF PARAMETERS INFLUENCING METHYLATION AND DEMETHYLATION IN TROPICAL LAKES IN BRAZIL AND NICARAGUA"

Activity/ Experiment	Start	End	Number of samples	Responsible or actor	Notes
1st field campain in Pantanal	Feb. -98	March-98		Lars/Edinal	Samples described below
2nd field campain in Pantanal	Aug. -98	Sep. -98		Lars/Edinal	Samples described below
3rd field campain in Pantanal	Aug. -99	Aug. -99		Edinaldo	Additional water samples
- Fish: TotHg	March-98	Nov. -98	216	Fernando	
- Fish: MeHg	Nov. -98	?	24	Helena	
- Sediment, roots, biofilm: Methylation	Feb. -98	Dec. -98	26 + replicates	Jean	
- Soil & sediment cores: TotHg	March-98	in process	56 soils&31*≈15 hor.	Edinaldo	
- Soil & sediment cores: 137Cs	Sep. -98	Nov. -99	56 soils&31*≈15 hor.	Markus	
- Soil & sediment cores: C, N, S	Sep. -98	Nov. -99	56 soils&31*≈15 hor.	Markus	
- Soil & sediment cores: Al, Fe, Mn	March-98	in process	56 soils&31*≈15 hor.	Edinaldo	
- Water parameters, susp. matter etc.	Feb. -98	Nov. -98	25*2 + replicates	Lars	
1st field campain in Managua	March -01	April -01		?	Dry season
2nd field campain in Managua	Aug. -01	Sep. -01		?	Rainy season
- Fish: TotHg	March -01		4 species*10*twice	?	
- Fish: MeHg			?	?	
- Sediment, roots, biofilm: Methylation	March -01		20 + replicates	?	
- Soil & sediment cores: TotHg	March -01		20 soils&10*≈15 hor.	?	More if other areas are incl.
- Soil & sediment cores: 137Cs	May -01		20 soils&10*≈15 hor.	?	
- Soil & sediment cores: C, N, S	March -01		20 soils&10*≈15 hor.	?	
- Soil & sediment cores: Al, Fe, Mn	May -01		20 soils&10*≈15 hor.	?	
- Water parameters, susp. matter etc.	March -01		20 soils&10*≈15 hor.	?	

Laboratory experiment, Brazil Methylation exp. with 203Hg in locally sampled sediment and plants, including quantification and identification of bacteria.	?		?	Varying pH, light, org. matter etc.
Laboratory experiment, Nicaragua Methylation exp. with 203Hg in locally sampled sediment and plants, including quantification and identification of bacteria.	March -01	?	?	Varying pH, light, org. matter etc.
Laboratory experiment, Sweden Methylation exp. with 203Hg in locally sampled sediment and plants, including quantification and identification of bacteria.	Jan -01	Dec. -03		PhD-stud. 2 yr experiments. Varying pH, light, org. matter etc.

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