BIOGEOCHEMISTRY OF MERCURY IN CONTAMINATED ENVIRONMENT IN THE WIDER IDRIJA REGION AND THE GULF OF TRIESTE

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Abstract

During the reporting period work three main topics have been addressed in order to achieve the objectives of the CRP:

1. *Methyl mercury formation and degradation in sediments of the Gulf of Trieste.*
2. *Preparation of SOIL-1 intercomparison sample.*
3. *Validation of techniques for determination of the rates for methylation and demethylation of mercury in various matrices.*

The present report covers the first two topics, while the third is presented as a separate manuscript in this report, dealing with methylation of mercury in Isopod Porcellio scaber and in lichens.

1. METHYLMERCURY FORMATION AND DEGRADATION IN SEDIMENTS OF THE GULF OF TRIESTE

This work has been done in collaboration with (i) Mark E. Hines from Department of Biological Sciences, Environment and Natural Resources Institute, University of Alaska Anchorage, Alaska, USA and (ii) Jadran Faganeli from the Marine Biological Station, Piran, Slovenia and was jointly published [1].

Activities at mercury (Hg) mines can lead to the mobilization of large quantities of Hg that enter the environment and are transported downstream. Although much of this Hg is deposited near the source, over time much of this Hg can be carried hundreds of kilometers where it can potentially enter and bioaccumulate in distant food webs. Mining activities in the Idrija mining district occurred for 500 years and the legacy of that mining can be seen in high concentrations of Hg throughout the watershed and into the Gulf of Trieste. Mercury concentrations are high in the sediments near the mouth of the Soca/Isonzo River in the Gulf, and the Soca River continues to deliver ~1.5 tons of Hg to the marine environment ~100 km from the mine [2, 3, 4]. Much of the Hg carried to the sea is probably as fine cinnabar particles, and the potential remobilization and further transformation of this Hg is of concern with regard to local environmental and the accumulation of methylmercury (MeHg) in seafood.

Mercury sulfide minerals are subject to dissolution and increased bioavailability when they contact sulfidic environments such as what occurs in coastal marine sediments [5]. This "newly" available Hg can potentially undergo methylation to supply the environment with newly formed MeHg. Indeed, Gulf sediments contain significant concentrations of MeHg and

effluxes of MeHg from Gulf sediments have been observed [6, 7, 8]. However, sediments can also support active demethylation by aerobic and anaerobic bacteria [9]. This demethylation can be due to either oxidative or reductive pathways. The present study was conducted to determine the potential of sediments from the Gulf of Trieste to methylate and demethylate Hg including an assessment of which demethylation pathway is most prevalent.

1.1. Methods

Sediment cores were collected by SCUBA divers at three stations in the Gulf of Trieste. Site D6 is located near the mouth of the Isonzo River, site AA is located southeast of the mouth, and CZ is in the center of the Gulf. These sites were described previously in terms of sedimentology, geochemistry, and biogeochemistry [10, 11]. Site AA is identical to site AA1 in [7,8].

Sediment pore waters were extracted anaerobically by centrifugation [] (HINES et al., 1984). Total mercury was determined using the $SnCl_2$ reduction and cold vapor atomic fluorescence detection (CVAFS) [12]. MeHg was determined following distillation, aqueous phase ethylation, and GC separation using AFS techniques [13, 14]. Pore waters were also analyzed for alkalinity [15], dissolved Fe (AAS), and NH_4^+, NO_3^- and PO_4^{3-} [16].

Total Hg in Gulf of Trieste sediments was determined using cold vapor atomic absorption (CVAAS) after acid digestion [12]. MeHg in sediments was determined using solvent extraction, aqueous phase ethylation, GC separation, pyrolysis and CVAFS detection [13, 14]. Solid phase C, N, and S were determined using a Carlo Erba CHNS analyzer, and organic C similarly after acidification with 1M HCl.

Potential Hg methylation rate constants were determined using ^{203}Hg and a toluene extraction technique [1,6,17,]. MeHg demethylation rate constants were determined using ^{14}C-MeHg[] (HINES et al., 2000). $^{14}CH_4$ liberated from ^{14}C-MeHg was determined using gas proportional counting, whereas $^{14}CO_2$ was measured by gas stripping acidified sediments and trapping of CO_2. SO_4^{2-} reduction rates were determined using ^{35}S and the chromium reduction assay [18]. Schematic presentation if given in Figure 1.

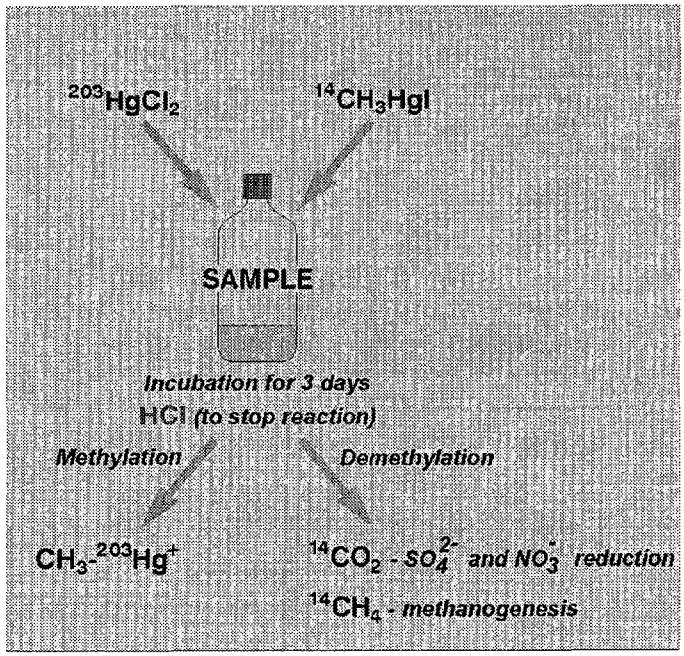

FIG 1: Schematic presentation of the procedure to study methylation/demethylation of Hg [9,17]

1.2. Results

Concentrations of total Hg and MeHg were high near the mouth of the Isonzo River and decreased greatly into the Gulf (Table 1). However, dissolved Hg and MeHg concentrations were similar at all three sites (Table 2). All three sites had similar concentrations of organic C and total N, but site D6 exhibited less total S than the other sites[1, 6].

TABLE I: Hg SPECIES IN THE SEDIMENTS

Depth (cm)	Hg (μg/g)	MeHg (ng/g)
	Site D6	
0.75	31.5	4.86
2.25	35.3	1.18
5.25	39.0	1.76
10.0	47.8	3.98
	Site AA	
0.75	2.38	1.07
2.25	2.26	0.20
3.75	3.69	0.16
5.25	3.23	0.23
7.0	3.42	0.17
10.0	2.74	0.12
	Site CZ	
0.75	0.77	0.39
2.25	0.89	0.28
3.75	0.89	0.13
5.25	0.84	0.16
7.0	0.86	0.09
10.0	0.94	0.09

TABLE II: Hg SPECIES IN PORE WATER

Depth (cm)	Hg (ng/L)	MeHg (ng/L)
	Site D6	
0.75	5.0	0.39
2.25	4.8	0.66
10.0	1.6	-
	Site AA	
0.75	5.9	0.29
2.25	1.2	-
3.75	3.0	1.19
5.25	4.5	0.46
	Site CZ	
0.75	7.8	1.70
2.25	5.1	5.86
3.75	1.5	0.51
5.25	2.1	0.15
10	**6.8**	**0.25**

Hg methylation and MeHg demethylation was active at all three sites (Fig. 2). These data are first order rate constants that are the rates at which the bioavailable pools of Hg and MeHg turnover. If the bioavailable pools did not vary with depth, these depth profiles would indicate that Hg transformations are most rapid near the surface. Concentrations of MeHg decreased greatly with depth (Table 1), suggesting that MeHg demethylation was much more rapid in surficial layers than below ~3.0 cm. Rates of both processes were most rapid in summer by up to 10 fold or more. However, rate constants were not appreciably different between sites on the same dates. The C of MeHg was converted primarily to CO_2.

However, significant proportions of CH_4 were detected in surface sediments in winter (March) at sites AA and CZ, with the highest values noted at CZ. A small, but easily detectable CH_4 fraction was noted in June as well. No CH_4 was detected at any depth in August.

Sulfate reduction (Fig. 3) was measured in March and August, with the highest rates noted at site D6 and during August. In winter, rate maxima occurred at about 2.0 cm, whereas rate maxima in August occurred in the uppermost subsample. All three sites in August displayed a slight subsurface maximum at 7.0 cm, which was probably due to the reworking activities of benthic infauna [6].

1.3. Discussion

The Hg transport legacy of the mining in Idrija is seen at the mouth of the Isonzo River where Hg levels are quite high (Table 1) [4, 7, 8]. Concentrations of Hg decrease quickly offshore, but Hg transformations are active throughout the Gulf. It is likely that Hg is remobilized into bioactive forms near the mouth of the river due to the effects of increased sulfide production as salinity increases [5].

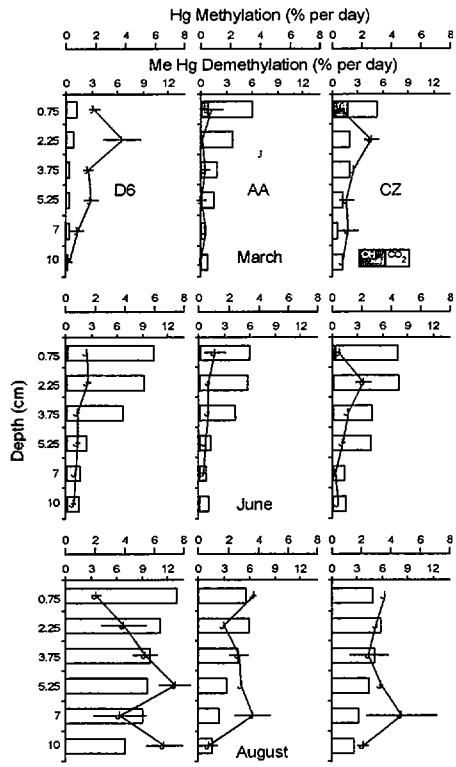

FIG 2: Hg transformation rates in Gulf of Trieste sediments.

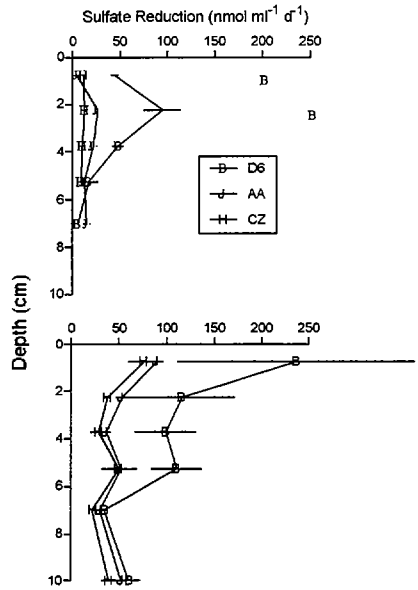

FIG 3: Rates of sulfate reduction in Gulf of Trieste sediments in March (top) and August (bottom)

Although total Hg and MeHg were highest near the river mouth, concentrations of dissolved Hg species were similar (Table 2), suggesting equilibrium between production and flux, or relatively similar rates of production and/or dissolution of Hg species.

Hg transformation rate constants were relatively similar for methylation and demethylation and were highest in summer. The similarity in constants between each site suggested that methylation and demethylation were coupled, i.e., much of the MeHg produced was probably consumed within the sediments. Actual rates of Hg flux can be calculated using pore water Hg species data and activity rate constants. Since all three sites exhibited similar concentrations of dissolved Hg and MeHg, and rate constants were also similar, the rate of production and degradation of MeHg were similar at all sites; again underscoring the potentially tight coupling between the production and degradation of MeHg in Gulf sediments. However, exact flux rates require a knowledge of the concentrations of bioavailable Hg and MeHg, and it is not clear if pore water Hg data represent these pools.

MeHg degradation in Gulf sediments occurred primarily via the oxidative pathway as evidenced by the dominance of the production of CO_2 from MeHg [9,19]. It is likely that the bulk of the MeHg degradation occurred via sulfate-reducing bacteria that are prevalent in these sediments [11]. These same bacteria are responsible for MeHg formation [20]. However, during winter and to a lesser extent in spring, a significant portion of the methyl C of MeHg was converted to CH_4. Since methanogenesis is not expected in these sediments, and would not be found in surface sediments, the formation of CH_4 from MeHg must have occurred by the reductive pathway of the mer operon genetic system in bacteria. The fact that this pathway was only important in surface sediments and during colder periods suggests that the reductive pathway is restricted to oxidizing sediments while the oxidative path is primarily anaerobic. Hence, changes in biogeochemical conditions can affect the pathway of MeHg degradation in sediments.

2. PREPARATION OF THE SOIL REFERENCE MATERIAL SOIL-1 FOR COMPARABILITY STUDIES

Initial plan of the CRP participants was to prepare a reference sediment sample from a tropical region for comparability studies. One of the potential area for sampling was Lake Guri in Venezuela, but the results of total and MeHg revealed that this lake contains low concentration of total Hg, and would, therefore, not be appropriate (Table 3).

The second posibility was to prepare river sediment from the Amazon region, which would be more appropriate as regards the concentration ranges. However, the amount of sample (about 2 kg) sent by Dr. Guimaraes from Brazil to the Jožef Stefan Institute in Ljubljana was not sufficient to prepare a reference material for all the CRP participants.

In order not to delay with the implementation of the work programme of the CRP it was decided to prepare a soil sample from an area close to Idrija mercury mine, Slovenia, that is contaminated with Hg due to continuous deposition of particles enriched with Hg during flood events. Such a sample is representative of areas where mercury transport and deposition is governed by river hydrology, which is typical in a number of mercury contaminated environments.

TABLE III: TOTAL AND MeHg CONCENTRATIONS IN LAKE SEDIMENTS FROM THE RESERVOIR GURI, VENEZUELA

Sample	Total Hg ng/g, DW	MeHg ng/g, DW	% MeHg
EP - 1	49,7 ± 0,7	0,11 ± 0,01	0.22
EP - 2A	87,7 ± 1,0	0,27 ± 0,03	0.31
EP - 2B	82,3 ± 2,1	0,12 0,12	0.15
EM - 1A	84,9 ± 2,9	0.32	0.38
EM - 1B	65,6 ± 2,8	0.08	0.12
EM - 2A	28,6 ± 0,6	0,58 ± 0,03	2.03
EM - 2B	28, 1 ± 0,8	0,97 ± 0,02	3.45

2.1. Sampling and sample preparation of soil-1

Sampling:

About 100 kg of the soil sample will be collected into polyethylene containers in February 2001 from a grassland at Bača which is frequently flooded by the river Idrijca (Figure 4). Surface soil was taken by a plastic shovel. The sample will then be transported to the Department of Environmental Sciences at the Jožef Stefan Institute for further preparation.

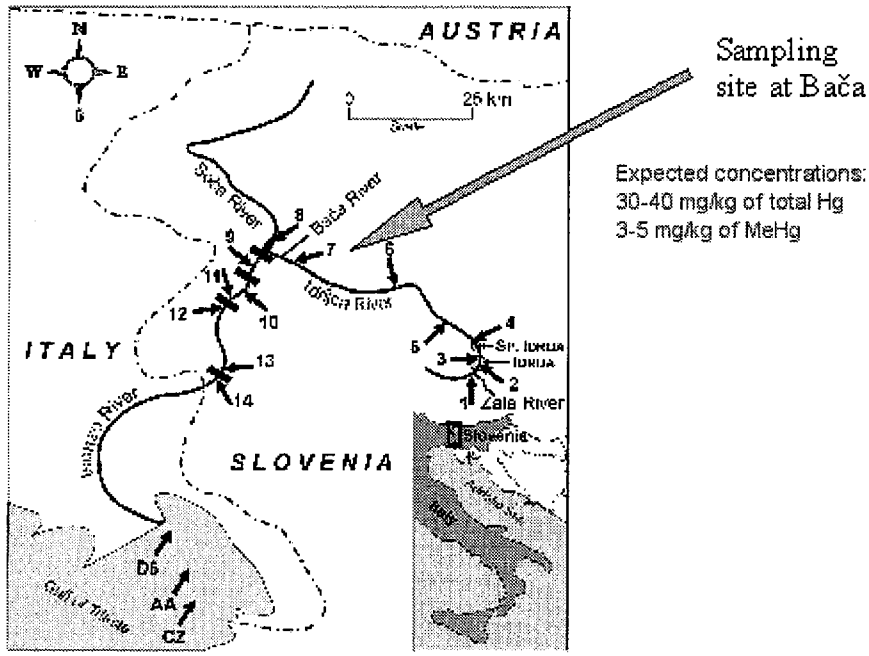

FIG 4: Sampling site for SOIL-1 intercompariosn sample

The same sampling site is regularly monitored since 1995 and the concentrations of total Hg was in the range between 40 to 50 mg/kg dry weight, while MeHg concentrations varied from 3 to 5 μg/kg.

Sample preparation:

After collection, sample will be air dried at 40°C in a drying oven for three days. Samples were ground and homogenised in rotating ceramic mill, sieved through a mesh of 1.4 mm pore size and then through 250 μm. Due to the first set of data on bulk homogeneity, which indicated noticeable non-homogeneities, it was decided to re-homogenize the samples. The results were still not satisfactory, therefore sieving through a sieve with 125 μm had to be conducted The total amount of the sample before bottling was expected to be about 30 kg, however only 5 kg of sample was finally obtained. The whole content of the sample will then be homogenised in a plastic rotating container for 3 days.

2.2. Homogeneity testing

For the bulk homogeneity testing only total Hg was analysed using procedure described below.

2.2.1. Determination of total Hg

Sample aliquots of about 250 and 500 mg were weighed directly in Teflon digestion vessel, and after addition of 4 ml of conc. HNO_3 and 2 ml of H_2SO_4 the vessel was closed and the mixture was left to react at room temperature overnight. Digestion was finished by heating in an Al block at 70°C for 12 hours on a hot plate. The digest diluted with doubly distilled water to the mark (26.8 ml). An aliquot of the digest was added to the reduction vessel and after reduction with $SnCl_2$ mercury was swept from the solution by aeration and concentrated on a gold trap. Mercury was then released from the gold trap by heating and measured on an LDC Milton Roy instrument by cold vapour atomic absorption spectrophotometry (CV AAS). A detailed description of the methods is elsewhere [12]. The detection limit of the procedure is 0.2 ng.ml. The precision varies from 2 to 5 %. Calibration was performed by the Hg standard solution in 5% HNO_3 prepared from pure elemental mercury. The accuracy of this standard solution was verified against the calibration with saturated mercury vapour at known temperature.

The digested samples were also measured by the CV AAS Hg analyzer SANSO SEISHAKUSHO Instrument Model 910, Japan, without amalgamation and comparative results are presented in Figure 5.

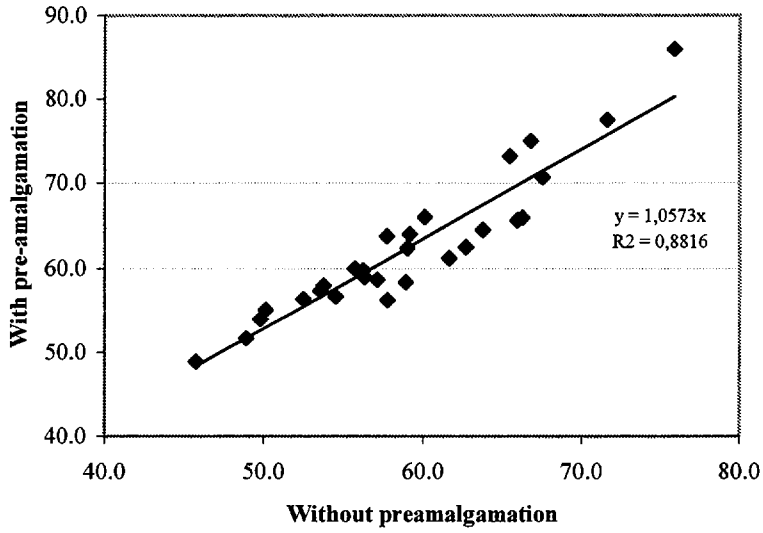

FIG 5: Comparison of two measurement systems (e.g. with and without preamalgamation) using CV AAS detection

2.2.2. Bulk homogeneity testing

10 bottles were taken randomly during rotation from a plastic rotating container for a bulk homogeneity testing. Each bottle was analyzed in three independent aliquots (250 mg sample intake). The results are presented in Table 4.

TABLE IV: THE RESULTS FROM THE FIRST BULK HOMOGENEITY TEST

	1st homogenization 30.5.2001 0.2 g; < 0.25 mm, n=3	**2nd homogenization 10.6.2001** 0.2 g, < 0.25 mm, n=3
1	62.5 ± 4.0	56.5 ± 1.0
2	59.9 ± 6.0	61.2 ± 1.0
3	55.8 ± 9.1	49.9 ± 1.2
4	56.6 ± 2.2	51.9± 2.7
5	59.5 ± 12.7	52.7 ± 1.2
6	60.1 ± 6.3	47.6 ± 0.4
7	54.6 ± 4.5	56.7 ± 0.1
8	57.5 ± 4.2	52.4 ± 3.3
9	60.5 ± 9.8	68.1 ± 0.3
10	59.4 ± 6.5	65.5 ± 0.3
Summary	**58.6 ± 7.0 (11.9%)**	**56.3 ± 6.7 (11.9%)**

Based on the data presented in Table 4 it was decided to sieve the samples again (< 125 μm) and rehomogenize the sample. Only two duplicate analyses of 250 mg sample intake were performed in 10 different bottles taken randomly from the bulk of the sample. The results were not significantly better, threfore a larger sample intake was takem (500 mg). The results are presented in Table 5.

TABLE V: THE RESULTS OF THE SECOND BULK HOMOGENEITY TEST

	23.7.2001, <0.125mm 0.2 g	**24.7.2001, <0.125mm** 0.5g
1	41.2, 43.0	48.8, 52.1
2	51.7, 55.7	48.7, 50.3
3	56.6, 57.0	51.4, 50.0
4	52.0, 50.4	52.8, 53.1
5	44.2, 45.2	52.8, 51.8
6	45.5, 41.8	56.0, 58.3
7	47.7, 46.0	46.3, 46.3
8	45.0, 47.3	47.9, 48.9
9	40.7, 39.8	53.2, 52.5
10	44.2, 46.3	44.1, 44.4
Summary	47.1 ± 5.2 (11%)	50.5 ± 3.7 (7.3%)

Particle size distribution after sieving is presented in Figure 6.

Bottling: Based on demonstrated homogeneity of the bulk material on 500 mg sample weight the material was subsampled into 300 polyethylene bottles (100 ml), each containing about 70 g of the sample.

Within bottle and between bottle homogeneity testing is in progress. 10 bottles will be taken randomly and will be analysed at the Jožef Stefan Institute (JSI) Ljubljana. Three independent sample aliquots (sample 500 mg) will be analysed in each bottle.

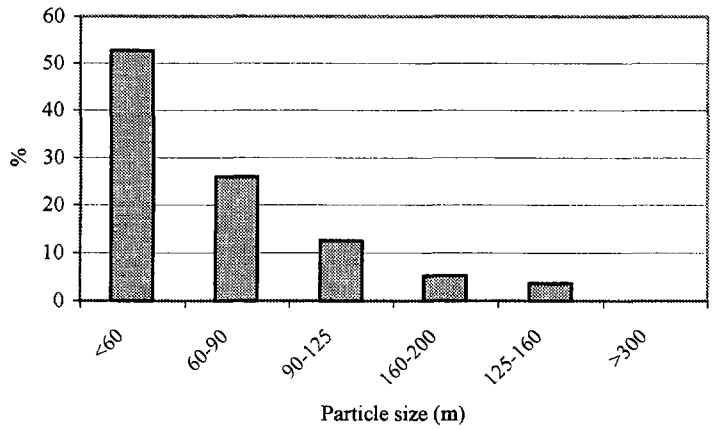

FIG 6: Particle size distribution of SOIL-1 sample

2.3. Determination of Methylmercury

Methylmercury was also determined in two bottles (two indepedent analysis) and the results are shown in Table 6.

The method consists of the following steps:

Approximately 200 mg of the sample + 5 ml of acid solution (18% KBr and 5% sulfuric acid, 1:1) + 1ml 1M $CuSO_4$ shaking for 15 min and extraction into 10 ml CH_2Cl_2, shaking, the aqueous phase discarded

⇓

back-extraction into 20 ml of water by evaporation of CH_2Cl_2 at about 80^0C

⇓

an aliquot of the aqueous sample was then submitted for derivatization by adding 0.2ml of 1% Na-tetraethylborate

⇓

purging of ethylated Hg species on TENAX trap at room temperature

⇓

thermal desorption onto isothermal GC column (60^oC)

⇓

pyrolitic decomposition (600^0C)

⇓

detection by cold vapour atomic fluorescence detector (Tekran 2600)

The method is based on literature references [13, 14, 22, 21].

TABLE VI: DETERMINATION OF MeHg IN SOIL-1, PARTICLE SIZE <0.125MM, SAMPLE INTAKE 0.5 G

Bottle No.	MeHg (ng/g as Hg, d.w.)	Mean ± SD
1	2.56 1.74	2.24 ± 0.35 (15.6%)
2	2.38 2.28	

2.4. Determination of residual water content

About 1g of the sample was dried at 105^{0}C for 48 hours. The water content was 2.225±0.166 %, determined in 10 independent sample aliquots.

2.5. Quality control

Two certified reference materials were used for quality control: (1) CRM 580, Trace Elements in Sewage Sludge of Industrial Origin, obtained from the Community Bureau of Reference (BCR) and (2) IAEA-356, *Trace and major element sand methylmercury in polluted marine sediment* obtained from the International Atomic Energy Agency. The CRMs were analysed in triplicates with each set of analysis. Certified values are given below. These two materials are the only materials that are close to the sample used in terms of matrix and concentration ranges and are therefore suggested to be used a common reference for other laboratories.

TABLE VII: QUALITY CONTROL SAMPLES

Producer[1]	CRM Code No.	Matrix	Certified Value[1]	
			MeHg $mg.kg^{-1}$ as Hg, DW	Total-Hg $mg.kg^{-1}$, DW
BCR	CRM 580	Estuarine Sediment	0.0702±0.0034	132±3
IAEA-MEL	IAEA-356	Polluted Marine Sediment	0.0054±0.00089	7.62±0.65

(1) Certified value ± 95% confidence interval (DW - dry weight) and/or uncertainty

In order to guarantee the stability of the sample during the implementation of the whole CRP a set of three bottles will continoulsy be re-analysed on a three months intervals for total and MeHg using the same analytical procedures as for homogeneity testing.

2.6. Tracer Experiments using ^{203}Hg

Before the sample was distributed at the 2nd RCM meeting in Minmata, October, 2001 some preliminary experiments were conducted using radiotracer ^{203}Hg. The following transformations were checked:

Methylation/demethylation: $^{203}Hg^{2+} \rightleftharpoons CH_3{}^{203}Hg^{+}$

Reduction: $^{203}Hg^{2+} \rightarrow {}^{203}Hg^{0}_{(g)}$

2.6.1. Reduction

Schematic presentation of the reduction vial is shown in Figure 7. Soil sample was transferred to the bottom of the 250 ml impinger and radioactive tracer in aqueous solution was added. The reduced Hg^0 was adsorbed on activated carbon and counted on well type detector HPGe (Ortec, USA).

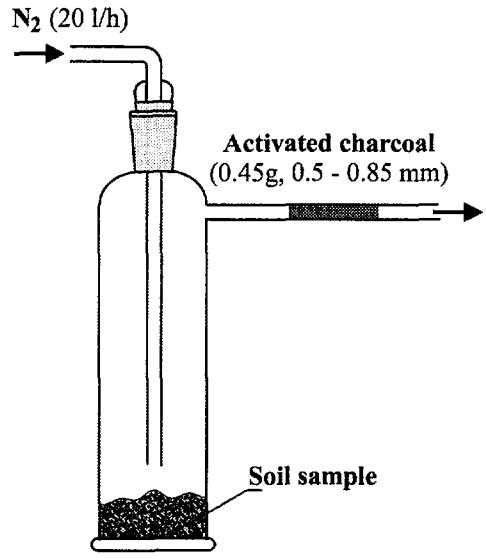

FIG 7: Schematic presentation of the system to study the reduction of $^{203}Hg^{2+}$ in SOIL-1

The first experiment was done with 12 g of SOIL-1, which was carefully transferred into the impinger. The sample was moistened by 6 ml of Milli-Q water sample containing radioactive tracer. Two experiments were initially conducted. One impinger was kept at room T in the dark, while the other was left ambient temperature on under the daylight conditions.

As shown in Table 8, the amount of Hg reduced was significantly higher (7%) at the daylight exposure compared to indoor-dark experiment (up to 3.3%).

In order to compare the ability of processed SOIL-1 sample for further reactions the untreated sample taken from the same experimental area was also spiked with radiotracer. The experiment was conducted under the same experimental conditions as with SOIL1, except that the water sample from the river Idrijca was used instead of Milli-Q water. Interestingly, the amount of spiked Hg reduced was in the range from 4 to 5.5%, which is similar to the first set of experiment, where the SOIL-1 was exposed to daylight No significant difference was observed between day light and dark conditions. This data indicate, that SOIL-1 sample has been altered during drying, grinding and sieving stage, however SOIL-1 sample still has a capacity to reduce Hg and we therefore concluded to be suitable for comparability studies.

TABLE VIII: REDUCTION OF SPIKED $^{203}HG^{2+}$ IN SOIL-1

	OUTDOOR			INDOOR-DARK		
	Preparation	**Incubation**	**Activity (Bq)**	**Preparation**	**Incubation**	**Activity (Bq)**
SOIL-1 (d<0.125 mm)	12 g of SOIL-1 + ^{203}Hg in 6 ml MilliQ (2874 Bq)	38 h 16-27°C N_2 = 2 l/h	202 Bq 200 Bq **(7%)**	6 g of SOIL-1 + ^{203}Hg in 3 ml MilliQ (1437 Bq)	38 h 24°C N_2 = 2 l/h	21.4 Bq 47.2 Bq **(1.3 %; 3.3%)**
Fresh soil sample from the same region	12 g of the soil + ^{203}Hg in 6 ml of the river water (1592 Bq)	42.5 h 14 – 27 °C N_2 = 2l/h	104 Bq **(6.6%)**	6 g of the soil + ^{203}Hg in 3 ml of the river water (795 Bq)	42.5 h 20 – 24 °C N_2 = 2l/h	36.8 Bq **(4.6%)**
	12 g of the soil + ^{203}Hg in 12 ml of the river water (1592 Bq)	42.5 h 14 – 27 °C N_2 = 2l/h	81 Bq **(5.1 %)**	12 g of the soil + ^{203}Hg in 12 ml of the river water (795 Bq)	42.5 h 20 – 24 °C N_2 = 1 l/h	43.4 Bq **(5.5%)**

In addition, an experiment to follow the dynamics of mercury reduction over the first 4 hours after spiking was also implemented. This experiment was conducted under the day light conditions at room temperature. Evidently, the plato was reached in about 4 hours. Further measurements should be done in order to see the dynamics of Hg reduction (Figure 8).

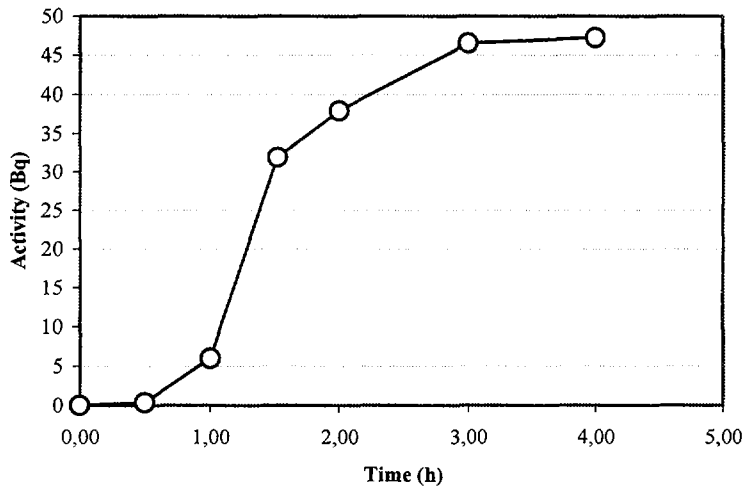

FIG 8: Mercury reduction vs. time of exposure

2.6.2. Methylation

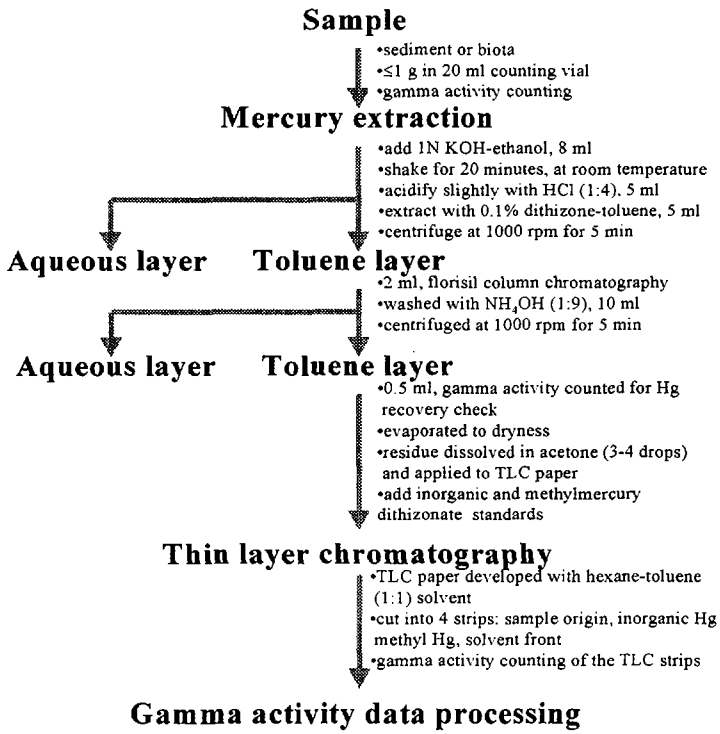

FIG 9: Schematic Diagram for Determining Methylation

Method initially developed by Czuba et al [23] and later modified by Jereb et al. [24] (this issue) was applied. The schematics of the method is presented in Figure 9. The advantage of the method is quantitative extraction of both $^{203}Hg^{2+}$ and $^{203}MeHg^{+}$ and subsequent determination of % of the transformed Hg from the ratio between Hg-Dz and MeHg-Dz thin layer strip.

About 6 g of the sample was spiked with ^{203}Hg in aqueous solution and the sample was incubated at room temperature in closed glass container. Both the original sample from the sampling area and SOIL-1 were taken for the first test. The results are shown in Figure 10. It is clearly shown that a significant difference exist among the two samples. The original, untreated sample methylated spiked inorganic Hg tracer much faster than SOIL-1. However, the methylation rate in SOIL-1 is still clearly evidenced and we concluded that the sample is still suitable for the determination of net methylation rates.

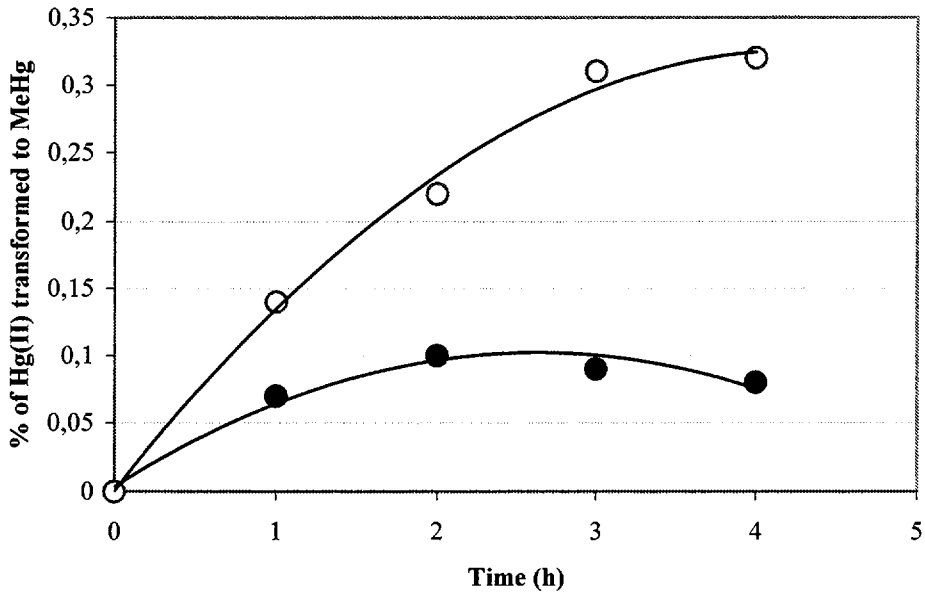

FIG 10: Comparison of net methylation rate in soil samples

2.7. Conclusions and future work

The between and within bottle homogeneity in SOIL-1 will further be checked and reported to the participants of the CRP. Sample will also be analysed for other elements using INAA anfd atomic spectroscopy methods. The participants will be requested to analyse total and MeHg and other trace elements concentrations in the samples by their usual techniques. In addition, laboratories will be asked to determine other important parameters such as DOC, Ph, and Eh. They will be requested to make three separate determinations of each anaylte and to report the results together with a short description of the method used on the report form distributed by the IAEA Technical officer.

During the second CRP it was also agreed that the sample will be used for comparability studies for Hg transformation mechanisms. The protocol agreed are as follows:

Moisture:	- soil:water ratio:	**2:1** w:V (e.g. 6g soil, 3 ml milliQ) **1:1** w:V (e.g. 6 g soil, 6 ml milliQ) Optional: 1:2 w:V use of rain water, river water with different TOC/DOC)

Incubation conditions: Closed glass vials (250 ml) with controlled air flow (10-20 l/h). No rubber to be used. 18 – 25 c in the dark. Optional: exposure to day light.

Incubation time: The soil sample is wetted with milliQ that contain tracer/cold Hg. 48 h of incubation is minimum.

Added tracer/cold Hg: 500 ng each time.

Other data, that are relevant should also be obtained, such as pedological analysis and other physical and chemical parameters.

ACKNOWLEDGMENT

This work was conducted within the programme P531 Biological and Geochemical Cycles funded by the Ministry of Education, Science and Sport (MESS) of the Republic of Slovenia, and through a SLO-USA bilateral agreement funded by the MESS and NSF, USA.

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