Supplement 2

SPECIATION OF Hg IN LICHENS

VESNA JEREB, MILENA HORVAT

Department of Environmental Sciences, Jozef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

1. INTRODUCTION

Lichens have long been regarded as a suitable tool for monitoring the relative levels of atmospheric pollutants (1). Lichens have neither roots, a waxy cuticule nor stomata: hence, for mineral nutrition they are largerly dependent on wet and dry deposition from the atmosphere. Moreover, lichens are perennial and can accumulate elements over long periods of time. Therefore, concentrations of elements in lichens represent the average levels of elements in the atmosphere for a long period of time.

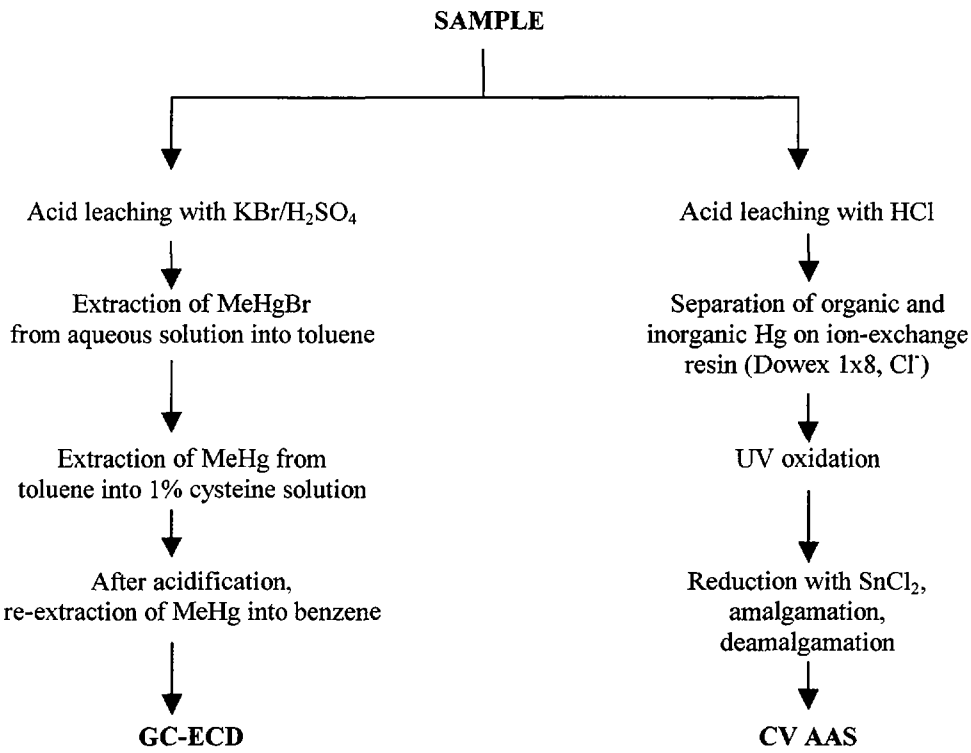
The epiphytic lichen *Hypogymnia physodes*, shown in Figure 1, is a good bioindicator of air pollution with total mercury (THg). In addition, it contains small amounts of methylmercury ($MeHg^{+}$). The first aim of our work was to test analytical techniques for determination of MeHg in lichens taken from different locations in Idrija and reference locations.

FIG 1: Hypogymnia physodes

Further, it is not clear, so far, whether MeHg in lichens comes from air via wet and dry deposition or inorganic Hg is methylated in the lichens themselves. To answer the question of the origin of MeHg in lichens, a radioactive tracer ($^{203}Hg^{2+}$) was used.

2. DETERMINATION OF MeHg IN LICHENS

Methylmercury in lichens is determined by two different techniques. They include diffferent isolation steps (ion-exchange and solvent extraction) and diffferent detection systems (CV AAS and GC ECD) (2) as schematically shown:



Results obtained for MeHg with two independent methods are shown in the table below. Lichens were also analysed for THg by the use of validated method (acid digestion folllowed by CV AAS):

TABLE I: TOTAL AND METHYL MERCURY IN LICHEN SAMPLES OBTAINED BY USE OF DIFFERENT TECHNIQUES

Lichen sample	**THg** (ng/g, d.w.)	**MeHg** (ng/g, d.w.)			% MeHg
		Ion-exchange CV AAS	Extraction GC ECD	*difference*	
1	105 ± 4 (3)	4,4 ± 1,1 (9)	3,64 ± 0,04 (4)	0,8 ± 1,1	3,5
2	209 ± 6 (8)	4,2 ± 0,2 (9)	2,67 ; 2,67	1,5 ± 0,2	1,3
3	286 ± 5 (3)	5 ± 1 (6)	3,56 ; 3,56	1,4 ± 1,1	1,2
4	864 ± 34 (4)	22,4 ± 0,7 (11)	19 ± 1 (4)	3,4 ± 1,2	2,2
5	1360 ± 110 (9)	13,7 ± 0,9 (8)	5,6 ± 0,1 (4)	8,1 ± 0,9	0,4
6	1450 ± 50 (9)	19 ± 2 (9)	9,4 ± 0,1 (4)	9,6 ±2,3	0,6
7	2060 ± 180 (9)	17 ± 1 (9)	10,52 ± 0,01 (4)	6,7 ± 1,1	0,5
8	6100 ± 100 (3)	50 ± 5 (19)	23,1 ± 0,7 (4)	26,4 ± 5,4	0,4

( ) number of determinations

The results show that concentration of MeHg from the two techniques are very different. In all cases results are much higher when ion-exchange/CV AAS technique was applied. This is due to its non-specific separation of organic and inorganic Hg on the ion exchange resin. In order to obtain accurate results for MeHg in lichens, the solvent extraction/GC ECD technique should be applied. The data obtained also show a positive correlation between total and methyl mercury in lichens.

3. ORIGIN OF MeHg IN LICHENS

To answer the question about the origin of MeHg in lichens, the radioactive tracer $^{203}Hg^{2+}$ was used. The epiphytic lichen *Hypogymnia physodes* was sampled in a Hg unpolluted area in Slovenia. They were spiked with inorganic form of ^{203}Hg. Some tree branches with lichens were exposed to the atmosphere (i.e. hung on tree). Two samples were kept in the laboratory in the dark. After 52 days lichens were carefully separated from the bark, then crushed and ground with the addition of liquid nitrogen.

Before separation of inorganic and methyl Hg we checked the activity of samples which were kept outside on the tree and in the laboratory and results are presented on Figure 2.

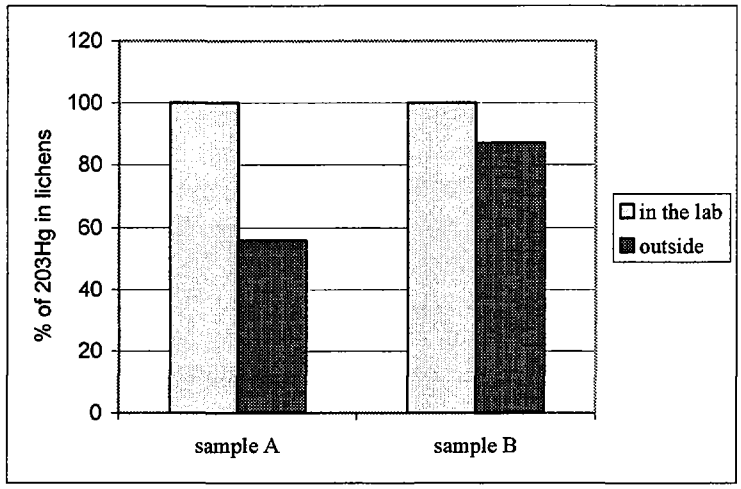


FIG 2: Activity of lichen samples exposed to the atmosphere relative to activity of samples kept in laboratory.

Lichens which were kept outside contained less ^{203}Hg after 52 days than lichens which were kept in the lab. Loss of ^{203}Hg could be due to washing with rain, reduction of Hg on sunlight and evaporation.

For isolation and separation of Hg species in lichens, we adopted radiochemical technique of Akagi et al. (3)

Quantitative isolation of all Hg species from lichens is not easy. Results of our work are shown in the Figure 3.

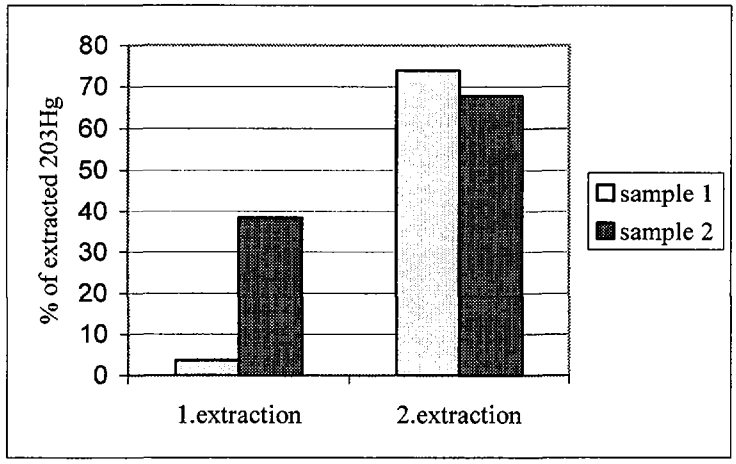


FIG 3: Effectivness of Hg-leaching from lichens at different leaching conditions

Sample 1 was first shaken for half an hour at room temperature. Results show that the extraction efficiency was very poor. On the other hand, sample 2 was leached for 0.5h at elevated temperature and its extraction efficiency was relatively much better. Therefore, leaching at elevated temperature was repeated for both samples for 1.5h, but total extraction effficiency was still around 70%. This extraction efficiency should be also kept in mind when interpreting the final results about the origin of MeHg in lichens.

After extraction of Hg species into 0.1% dithizone-toluene, extracts were cleaned-up (washing the excess of dithizone) and dried by purging with nitrogen. Dried extracts were dissolved in acetone and applied to thin-layer chromatographic strips. Mobile phase was the mixture of hexane and toluene in mixing ratio 1:1. After separation, TLC strips were cut in 4 smaller strips according to sample application line, Hg^{2+}-line, $MeHg^{+}$-line and solvent front. Activities of all strips were measured on coaxial HPGe detector. Results are shown as follows in Table 2.

TABLE II: TRANSFORMATION OF $^{203}HG^{2+}$ INTO $ME^{203}HG^{+}$ IN LICHENS *HYPOGYMNIA PHYSODES*

Lichen	% of methylated $^{203}Hg^{2+}$
Sample 1	0,54 ± 0,07
Sample 2	0,79 ± 0,09

Results show that a small fraction of added $^{203}Hg^{2+}$ is methylated in lichens. However, it must be kept in mind that the percentage of $^{203}Hg^{2+}$ that was transformed into $Me^{203}Hg^{+}$, as shown in the Table 2, is reffered to extraction efficiency which was approximatelly 70%. Therefore, these data only indicate the potential of lichens for methylation of Hg^{2+}.

REFERENCES

[1] Lupšina, V., Horvat, M., Jeran, Z., Stegnar, P.,. Investigation of Mercury Speciation in Lichens, Analyst, 117, 1992, 125-127

[2] Horvat, M., May, K., Stoepller, M., Byrne, A.R., Comparative studies of methylmercury determination in biological and environmental samples. Appl. Organomet. Chem., 1988, 2, 515-524

[3] Akagi, H., Ikingura, J.R., Radiochemical technique for rapid assessment of mercury transformation and distribution in the aquatic environments,