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New initiatives

Study of mercury pollution near a thermometer factory using lichens and mosses

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"Capsule": *Lichens and mosses were effective as biomonitors of mercury.*

1. Introduction

Mercury has a widespread environmental distribution, originating both from anthropogenic and natural processes. Once in the air, mercury can be widely dispersed and transported to longer distances. In the environment mercury can exist in a number of physical and chemical forms with toxicity is well known to be highly dependent on chemical form (Clarkson, 1997). As a consequence, considerable effort and progress has been made in the development of techniques for the separation and identification of individual mercury species in environmental samples (Sanchez Uria and Sanz-Medel, 1998). Biomonitoring using lichens and mosses is an effective method for assessing the levels of atmospheric trace element pollution including mercury (Merian, 1991; Conti and Cecchetti, 2001; Horvat et al., 2000) as lichens and mosses pick up nutrients directly from ambient air and deposition retaining many trace elements (Ruhling and Tyler, 1968).

In this work, mercury contamination due to a mercury thermometer-making factory situated in the hill station Kodaikkanal (about 2120 m above mean sea level), in a southern state of India, was investigated using lichen (*Parmelia sulcata*) and moss (*Funaria hygrometrica*) samples. The content of mercury in these samples collected from different sites was determined using CV-AAS mercury analyzer and ICP–QMS in order to obtain information on the extent of mercury contamination. As mercury undergoes extensive transformation into various forms as it cycles among the atmosphere, land and water (Ebadian et al., 2001), we have carried out investigations to establish the chemical form of mercury—elemental (Hg^0), inorganic (Hg^{2+}) or organic—in these chosen biomonitors.

2. Materials and methods

2.1. Sampling of lichens and mosses

Samples of the lichen (*P. sulcata*, an epiphytic lichen) were collected from tree trunks and branches 1–1.5 m above the ground. In the case of moss (*F. hygrometrica*) samples, only top portion was collected, discarding the basal portion with adhered soil. In each location, sampling was done at 2–3 individual places and mixed to form a common sample collected in clean polythene bags. These samples were stored in a refrigerator until further use. At the laboratory, the lichen and moss samples were further cleaned of any adhering particles to the greatest extent possible by suspending in water and agitating in an ultrasonic bath for about 30 s, discarding the water and rinsing the samples twice with fresh water. After drying the lichen and moss samples in an oven for 24 h at 40 °C, they were ground to a fine powder in a pre-cleaned agate planetary ball-mill (FRITSCH, Germany) and stored in polythene bottles. Between grinding, the agate vessel and the balls were cleaned thoroughly with dilute acid, rinsed with water and ethanol to prevent any cross contamination.

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2.2. Analytical techniques

A Plasma Quad 3 (VG Elemental, Winsford, UK), Inductively Coupled Plasma Quadrupole Mass Spectrometer (ICP–QMS), located in a class 100 area of the Ultra Trace Analysis Laboratory of our Centre was used for the determination of total mercury in all the lichen and moss samples. Rh and Re were used as internal standards. A cold vapour atomic absorption spectrometer (CVAAS) unit (Mercury Analyser, Model No. MA 5800E, ECIL, Hyderabad, INDIA) was used to determine the concentration of mercury in acid digests as well as in the aqueous extracts.

2.3. Reagents

Sub-boiled hydrochloric acid and nitric acid were prepared using quartz sub-boiling distillation stills located in a class 10 laminar flow workbench. Ultrapure water of ca. 18.2 MΩ cm resistivity was used throughout. Stannous chloride in 10% HCl (w/v) (E Merck, Mumbai, India) and sodium borohydride in 2% NaOH (w/v) (Lancaster, England) were used as reducing agents for reducing Hg^{2+} and total mercury (inorganic and organic mercury), respectively. The reducing agents were prepared afresh daily.

2.4. Collection of mercury in ambient air around the thermometer factory

The most plausible form of mercury contamination around the factory in the ambient air would be elemental mercury (Hg^0). Hence air around the factory was sampled on activated charcoal filters using an air sampler kit (Lawrence and Mayo, Model No. APS2) to trap the mercury vapour. About 200 mg of activated charcoal (SD fine chem., Mumbai, India) was packed in plastic tubes with nozzles at both ends. Both ends of the tube were plugged with glass wool so that air could pass through the activated charcoal bed to trap elemental mercury. Air was sucked through charcoal at a known flow rate (2 l/min) for 15 min thus passing a known volume of air through the charcoal bed.

It was decided to extract elemental mercury trapped in the charcoal samples using sonication with high purity water, as extraction in acid media would convert it into inorganic forms. In order to verify that the trapped elemental mercury could be released under treatment with water alone, trapping of mercury on the activated charcoal and subsequent release in water was simulated in our laboratory. Plastic tubes similar to those used in air sampling, were filled with a weighed amount of activated charcoal (about 1 g) and plugged with glass wool at both ends. Filtered air, at room temperature, passing through a vessel containing metallic mercury, was sucked through these tubes for 2 h. The charcoal powder was taken in a glass vessel and shaken for uniform mixing for 2 h on a mechanical shaker. Aliquots (~200 mg) of this homogenized charcoal powder were weighed accurately and digested in a microwave oven with nitric acid and hydrogen peroxide and mercury content in them was determined.

2.5. Ultrasonic extraction for elemental mercury in lichen/moss samples

Accurately weighed aliquots of lichen and moss samples (300 mg) collected around the factory which exhibited the highest mercury levels of ~8 mg/kg, were taken into glass vessel of the mercury analyzer and sonicated with high purity water in an ultrasonic bath for 15 min. Then the chamber was flushed with mercury free air to determine the content of elemental mercury thus released.

2.6. Microwave digestion of lichen/moss samples for determination of total mercury

Weighed aliquots of lichen and moss samples (200 mg) were transferred to the PTFE digestion vessel (45 ml volume) of Parr Bomb, containing a mixture of HNO_3 (1.5 ml) and H_2O_2 (33 vol.%, 0.5 ml). Digestion was carried out using a domestic microwave oven (650 W) at maximum power for 3 min. The digests were analyzed for total mercury by ICP–QMS. Appropriate blanks prepared similarly were also analyzed.

2.7. Extractions in hydrochloric acid media for studying chemical forms of mercury

In order to study chemical forms of Hg in both the lichen and moss samples, we have adopted the rapid ultrasound assisted extraction method in HCl for mercury speciation in fish tissues reported by Susana and Bendicho (1999). The applicability of the HCl extraction procedure on lichen and moss samples was evaluated by analyzing the reference lichen material (RM) IAEA-336. Accurately weighed aliquots (300 mg) of moss, lichen and the RM were taken in centrifuge tubes and extracted with 5 ml of 2 or 5 mol dm^{-3} HCl using an ultrasonic bath for 15 and 40 min as was necessary. The mercury content in these extracts was determined using CV-AAS using either $NaBH_4$ or $SnCl_2$.

3. Results and discussion

The concentration profiles of mercury on the composite samples of moss and lichen from 12 different locations indicated that lowest concentration of mercury of about 0.2 mg/kg was found in lichen and moss samples collected about 20 km away from the factory, near a

pristine lake area. But as the values prior to the establishment of the factory are not available, it is difficult to say whether these values represent a base line value or whether there has been an increase due to mercury contamination.

3.1. Mercury in activated charcoal samples

Triplicate analysis, after complete microwave digestion of the charcoal powder samples in the simulated experiments and ultrasonic extraction with water for 15 min, gave mean values of 0.77 and 0.73 mg/kg respectively, indicating that more than 95% of the trapped elemental mercury could be released using simple aqueous extraction. Analysis of charcoal filters which were used for the sorption of mercury in air around the factory analyzed through this aqueous extraction indicated that Hg concentration in air near the thermometer factory was around 1.32 μg m^{-3} which is very much higher than the nominal Hg concentration range (0.5–10 ng m^{-3}) reported (Horvat et al., 2000) as typical of non-contaminated areas.

3.2. Non-recovery of elemental mercury from the lichen/moss samples

Analysis of aqueous extracts of the lichen and moss samples by CVAAS indicated that only about 0.1 mg/kg of elemental mercury could be recovered from the lichen sample with ca. 8 mg/kg total mercury collected near the factory. No mercury could be detected in the aqueous extracts of moss samples with similar higher concentrations of total mercury.

3.3. Inorganic and methyl mercury in lichen/moss samples

From Table 1 it is seen that the values obtained for total Hg in the IAEA-336 by microwave digestion procedure as well as the values obtained with 5 mol dm^{-3} HCl extracts, using either $SnCl_2$/$NaBH_4$ as reductants, agree fairly well with certified value for total mercury. It may also be seen that the values obtained for lichen and moss samples, using either of the reducing reagents after 5 mol dm^{-3} HCl extractions are in fair agreement with the total mercury contents. This quantitative recovery of total mercury by extraction with 5 mol dm^{-3} HCl is in agreement with the observations of Susana and Bendicho (1999).

The quantity of mercury found in the 2 mol dm^{-3} HCl extracts of the samples collected near the factory by subsequent reduction with $SnCl_2$ as well as $NaBH_4$ was found to be about 23% of total mercury. Our observations as well as those of Susana and Bendicho (1999) indicated that 2 mol dm^{-3} HCl extracted about 20–30% of the inorganic mercury in these samples. Thus it can be concluded that organic mercury was not present in the lichen and moss samples. These observations establish two facts: (1) mercury in the samples could be totally extracted using the extraction step with 5 mol dm^{-3} HCl and (2) all the mercury was in the form of inorganic mercury as reduction with $SnCl_2$ or $NaBH_4$ produced same values on these acid extracts.

In the case of samples collected around St. Mary's church, ultrasonic extraction for 15 min with 2 mol dm^{-3} HCl showed no mercury but when the extraction time was increased to 40 min, ~20% of the total mercury was found to be extracted. The reasons for the difference in the extent of extraction are not understood.

4. Conclusion and speculation about the meaning of the results

The near complete recovery of elemental mercury from simulated activated charcoal samples, very low recovery of elemental mercury in the lichen and moss samples together tend to suggest that elemental mercury, though the principle form of mercury contamination in this case, is possibly converted into inorganic form (Hg^{2+}) on the lichens and mosses by some means.

Table 1
Mercury concentrations (mg/kg) in lichen and moss samples

Sample	2 M HCl[a]		5 M HCl[a]		Microwave-digestion (ICP-QMS)
	$SnCl_2$	$NaBH_4$	$SnCl_2$	$NaBH_4$	
IAEA-336	ND	ND	0.175±0.006	0.174±0.006	0.177±0.005
St. Mary Church					
L	0.209±0.005	0.204±0.005	1.06±0.02	1.05±0.02	0.93±0.01
M	0.192±0.002	0.197±0.004	0.92±0.01	0.91±0.01	0.86±0.01
Near TM factory					
L	1.52±0.15	1.35±0.14	7.54±0.14	7.42±0.14	7.95±0.12
M	1.65±0.17	1.62±0.15	8.15±0.26	8.32±0.28	8.71±0.15

TM = thermometer, ND = not detected, L = lichen, M = moss, certified mercury value in IAEA-336 = 0.2 mg/kg (confidence interval 0.16–0.24).
[a] Analyzed by CVAAS.

It could also be possible that the elemental mercury, either free or particulate bound, could have been converted into inorganic forms in the atmosphere itself and only this fraction could have been trapped onto the biomonitors during precipitation. Our studies point out that the likely conversion of one form of the contaminant into other chemical forms on the biomonitors should be further studied through speciation approaches before the data on them could be correlated to arrive at the magnitude of atmospheric pollution.

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