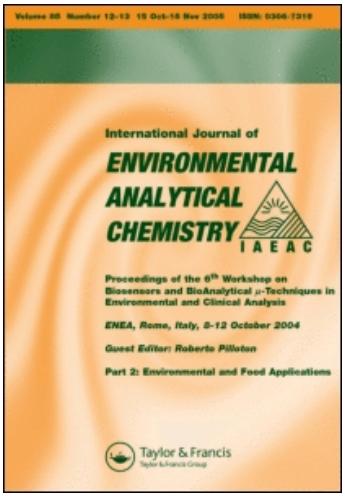

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Studies on Mercury Pollution: Microdetermination of Mercury in Biological Materials by Cold Vapour Atomic Absorption Spectrometry

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A simple, rapid, precise and accurate method for the determination of mercury in biological material is described. Biological samples were digested with nitric acid and acidified potassium permanganate and determined by cold vapour analyser. The proposed method was successfully employed for the determination of mercury in samples of fish, hair and blood.

INTRODUCTION

The discovery of significant concentration of mercury, one of the toxic metallic pollutants in fish and the realization that it plays a vital role in the food chain and ecological balances has considerably stimulated efforts in the direction of improving the analytical methodology of mercury in biological materials.

The analysis of biological material presents a special problem in securing complete sample decomposition and avoiding loss of volatile mercury. Several methods (1–6) have been described for the decomposition of biological samples; they are generally coupled with cold vapour atomic absorption spectrophotometry.

The proposed method incorporates and improves some of the better points of the methods widely employed for the digestion of biological materials. It describes a procedure to decompose the organic matrix of biological materials by acidic potassium permanganate and then its determination by cold vapour analyser.

EXPERIMENTAL

Reagents

All reagents used were of analytical grade of B.D.H. or S.M. Triple glass distilled water was used for solution preparation and other purposes.

Stannous chloride—(10% w/v) 10 g stannous chloride dihydrate was dissolved in 20 cm^3 hydrochloric acid (sp. gr. 1.18) and diluted to 100 cm^3. The resulting solution was heated with metallic tin until the precipitate disappeared and stored over tin in a glass bottle.

Stock mercury solution—1000 mgl^{-1}, 1.00 g mercury metal (Merck, G.R.) was dissolved in 5 ml 5 M nitric acid, made up to 1 l. Working standards were prepared by diluting this solution and kept for the minimum time before use.

Equipment: A Pye Unicam SP 2900 atomic absorption spectrophotometer equipped with a mercury hollow cathode lamp and cold vapour analyser kit was employed for atomic absorption measurements.

Graduated apparatus of standard calibration were used for measurements. Contamination from glassware, stoppers and pipette tips was observed.

Sampling: Fish samples of *Hilsha ilisha* (hilsha, marine fish) and *Labeo rohita* (rohu) were collected from the local fish market and put into numbered sterilized plastic bags. The surface layer of frozen sample was scraped off and discarded, slices were cut off with the help of a sharp razor.

Human hair samples were collected and stored in clean paper bags. The sample was collected from one person on one cutting date.

Human blood was collected with the help of a medical expert. Heparin and E.D.T.A. were added as anticoagulants if the analysis was done after 12 h.

Normal precautions for trace analysis were taken throughout.

Procedure

Washing procedure for hair samples:[9–11] Hair samples were washed for about 2 h in diethyl ether in a soxhlet extractor and allowed to dry in dust-free and mercury-free air.

Analytical Procedure: An accurately weighed biological sample (0.1–0.3 g) was transferred to a 150 cm^3 conical flask. 2–4 ml nitric acid were added to each flask. The flasks were placed in a water bath at 60°C for 10 min with intermittent shaking. The flasks were cooled in an ice bath and 2–3 ml of 8% (v/v) sulphuric acid was added, 3–5 ml oxidising solution was added dropwise. The flasks were removed from the ice bath and

placed in a water bath at 60°C. The oxidation was allowed to proceed for about 10–15 min. The flask was removed from the water bath and allowed to cool at room temperature. Hydroxylamine hydrochloride solution (20% w/v) was added dropwise ($\simeq$5 ml) to reduce the excess potassium permanganate. The flask was shaken slightly to clear the suspension of manganese oxides. To this clear solution sodium chloride ($\simeq$0.05 g) was added. 2 ml of 10% $SnCl_2$ was added immediately before the determination of mercury with the cold vapour analyser kit.

The instrumental conditions were set up for cold vapour analyser (Table I). The spectrophotometer and the pump were warmed up for 30 min. or until the digital display was steady at an absorbance of $\pm$0.001.

TABLE I
INSTRUMENTAL CONDITIONS

Wavelength	—253.7 nm
Slit width	—0.2 mm
Lamp current	—3.5 mA
Filter	—No. 1 (clear)
Integration time	—10 sec

DISCUSSION

Method Development

The method employed incorporates and improves some of the other methods employed for quantitative estimation of mercury in biological materials. The method is superior to Bouchard's method (5) where completion of the digestion cannot be examined due to the brown appearance of the digest and the dark green colour after addition of hydroxylammonium sulphate; in the proposed method the digest is clear and completion of the digestion can easily be seen. The addition of dilute sulphuric acid (8% v/v) prevents the loss of mercury due to heat of mixing. The total time required for complete digestion was 0.5 h compared to 4 h in the Hendzel and Jamieson method (7). The method was successfully employed for the digestion of blood, fish, fish liver and hair samples. Rapidity is an outstanding feature of the method and, therefore, it is especially recommended if large number of samples are to be analysed.

Digestion procedure

Digestion methods include classical destruction of organic matter. Conventional wet ashing, preventing mercury losses during digestion is

very laborious. Autoclave digestion (1) is advantageous due to its speed and elimination of losses of mercury.

Armstrong and Uthe[2] reported that the digestion could be done more rapidly by the addition of nitric acid. Nitric acid alone was also utilised for digestion purpose.[3] Munns and Holland[4] used a boiling mixture of nitric, sulphuric and perchloric acid. Bouchard *et al.*[5] utilised chromic acid for oxidation. The method is disadvantageous due to several practical difficulties such as time requirement to make up the oxidising solution, the brown appearance of the digest, its dark green colour after addition of hydroxylammonium sulphate which prevents to see the completeness of digestion, while the heat of mixing causes the vaporisation of (part of) the mercury. Gardner *et al.*[6] compared chromic acid digestion with the permanganate method and reported the lower mercury recoveries by the former method.

Blood analysis

Much interest has been centered on plasma or serum analysis in connection with health and disease. Determination of mercury in blood samples was done. No measurable amount of mercury was detected in blood samples. Therefore, a standard mercury solution was added to the blood samples and the procedure was then applied. The recovery of added mercury was satisfactory. The results are shown in Table II.

Hair analysis

Determination of mercury in hair has received considerable attention since the Minamata disease in Japan arose from mercury poisining.[8] It is interesting that hair samples of males contain more mercury than females in sexually mature teenagers and adults but not in younger children.[12] Hair was successfully digested and analysed at mercury levels from 0.1 to 1.20 μg (Table II). Standard addition showed an excellent agreement with the actual values.

Fish analysis

Fishes are the main source of dietary mercury intake; they absorb mercury from the water through their gills, skin[13] and digestive tracts.[14]

No measurable amount of mercury was detected in Rohu (**Labeo rohita**). Therefore, a standard solution of mercury was added to the fish samples before digestion and the procedure described earlier was applied. The recovery is illustrated by the results given in Table II. The samples were analysed at mercury levels from 0.08 to 1.0 μg (Table II).

TABLE II
Determination of mercury in blood, hair and fish samples

Mercury added (μg)	Mercury found* (μg)	Relative error (%)
Blood sample:[a]		
0.10	0.105	+5.0
0.20	0.191	−4.5
0.30	0.288	−4.0
0.40	0.415	+3.75
0.50	0.520	+4.0
0.60	0.580	−3.3
0.70	0.715	+2.14
1.00	1.040	+4.0
1.10	1.060	−3.6
Hair sample:[b]		
0.10	0.106	+6.0
0.20	0.208	+4.0
0.25	0.240	−4.0
0.40	0.413	+3.25
0.50	0.490	−2.0
0.80	0.820	+2.5
1.00	1.020	+2.0
1.20	1.160	−3.3
Fish sample: labeo rohita (ROHU)[c]		
0.08	0.086	+7.5
0.09	0.095	+5.5
0.10	0.096	−4.0
0.20	0.205	+2.5
0.25	0.255	+2.0
0.30	0.309	+3.0
0.50	0.492	−1.6
0.70	0.690	−1.4
1.00	1.030	+3.0

*Average of three analyses of the sample. The normal mercury content of the sample was negligible; i.e., the data from Table II verify only the procedure.

[a]Volume of blood sample taken, 1 ml.

[b]Weight of the hair sample, 0.1 g.

[c]Weight of the fish sample, 0.3 g.

Hilsa fish (**Hilsa ilisha**) liver was also analysed. The average mercury concentration was found to be 159 ng/g; the standard deviation was 6.4 ng and the coefficient of variation was 4.03% (Table III).

TABLE III
Determination of mercury in **hilsa ilisha** (hilsa) fish liver

Sample no.	Weight of sample (g)	Mercury added (μg)	(μg)	Mercury found in fish liver (μg)	Mercury concentration (μg/g)
1	0.20	Nil	0.032	0.032	0.160
2	0.22	0.05	0.087	0.037	0.168
3	0.25	0.05	0.090	0.040	0.160
4	0.28	0.05	0.093	0.043	0.154
5	0.30	0.05	0.096	0.046	0.153
6	0.31	0.05	0.099	0.049	0.158

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