Procedure for Examination of Water and Sediment Samples for Total Asbestos Fibre Count by Electron Microscopy

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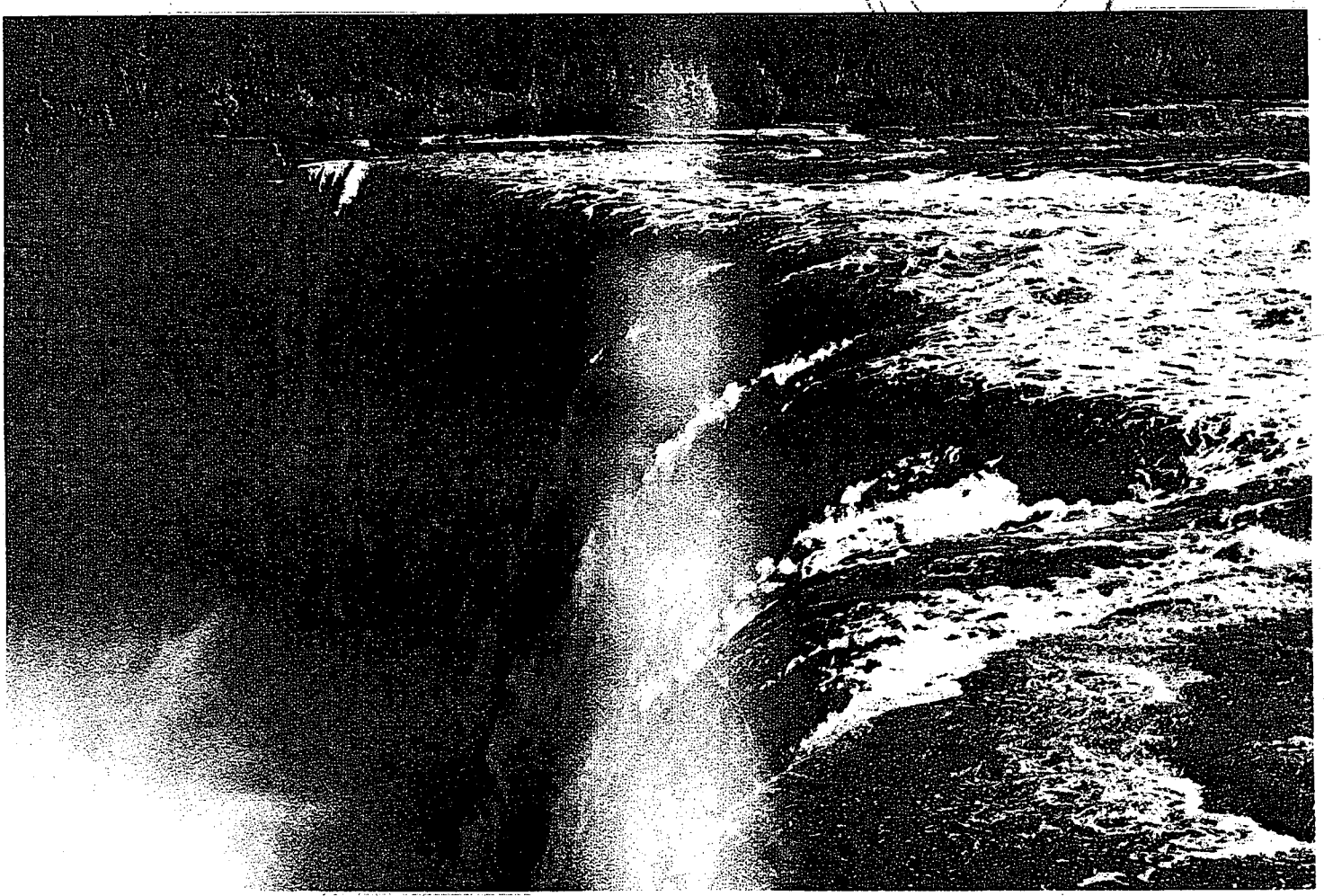

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Abstract

In 1973, a major investigation of asbestos fibres in water and sediment samples of Lake Superior began at Canada Centre for Inland Waters. A procedure for obtaining total fibres per litre of water samples was developed. This report documents that procedure, describing problems encountered and suggested solutions, and outlining the mathematical equations for determining fibres per litre.

Résumé

En 1973, le Centre canadien des eaux intérieures a entrepris une étude importante sur les fibres d'amiante dans des échantillons d'eau et de sédiments du lac Supérieur. On a alors mis au point une technique permettant d'en déterminer le nombre total par litre d'eau. Le présent rapport traite de cette technique, décrit les problèmes rencontrés et les solutions proposées, et donne les équations mathématiques utilisées pour la détermination des fibres.

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INTRODUCTION

The study of asbestos in the environment is important because of the growing concern that asbestos-like fibres may be a cause of cancer. "The discovery of high concentrations of asbestos fibres in the western arm of Lake Superior prompted the monitoring of the whole lake for fibre concentration in order to determine the extent of transboundary movements" (Durham and Pang, 1973). An analytical method using electron microscopy was developed for the enumeration of asbestiform fibres in water (Durham and Pang, 1973). The method has been used successfully by Electron Microscope Laboratory personnel (T. Pang, D. Manolescu and H.W. Zimmermann) in several subsequent studies (Dell, 1975; Durham and Pang, 1975; Lawrence *et al.*, 1974; Lawrence *et al.*, 1975; Lawrence and Zimmermann, 1975a, 1975b).

The purpose of this report is to describe basic modifications to the procedures used to improve overall efficiency and accuracy for specific scientific investigations of the occurrence of asbestos fibres in sediments and water.

ELIMINATION OF CONTAMINANTS

Contamination was the predominant problem when preparing samples for electron microscopic examination. The following methods were devised for obtaining a background count of zero on the carbon-coated copper grids.

Cleaning Equipment for Sample Preparation

The cleaning equipment used for sample preparation is listed in Table 1. Sample water was thoroughly mixed in the storage containers by vigorous shaking and stirring with a stirring rod. After 250 ml of the sample water had been siphoned into a graduated cylinder, it was transferred to centrifuge bottles and capped. Sample bottles were identified with a felt marker which was used to draw a line parallel and approximately 2 cm to the left of the centrifuge bottle seam for centrifuge placement and sample recovery.

Table 1. Cleaning Apparatus for Sample Preparation

Apparatus	Preparation
Stirring rod Plastic siphon tubing	Before and after each use, rinse with distilled water
250-ml graduated cylinder Centrifuge bottle caps	Sonicate using distilled water (5 min)
250-ml centrifuge bottles	Fill with tap water and sonicate (15 min) Flush with distilled water Half fill with distilled water once again and sonicate (15 min) Flush with distilled water again

Samples were balanced, centrifuged at 20,000 rpm for one hour at 4°C, and when removed from centrifuge, the water was poured off. Distilled water (1 ml) was immediately put into centrifuge bottles and sonicated (3 min) at the line parallel to the bottle seam until all material was resuspended in the water.

Preparation of Specimen Grids

The following procedure is based on the work of Bradley (1965).

For electron microscopic examination, a 1-µl drop of sample water was deposited on a copper grid, 3 mm in diameter and made from wire mesh with specific wire spacings of 100 lines, 200 lines or 400 lines per inch. Grids were covered with a support film (evaporated carbon) strong enough to support the sample, but of minimum thickness (200–500Å) to reduce background scattering of the electron beam. The grid preparation procedure follows.

a) Formvar Film

The type of precleaned microscope slides used proved to be an important factor in eliminating problems when preparing the Formvar film. Fisher Brand 12-550A (75 mm x 25 mm) precleaned microscope slides were the most satisfactory.

The procedure may be broken down into the following steps:

1) To prevent grease on slides, disposable vinyl examination gloves were worn to wash four new precleaned microscope slides with soap and water.
2) Slides were sonicated in distilled water for 5 minutes.
3) Slides were then dried under a heat lamp, examined and, if dirty, washed again.
4) Two slides were dipped for 5–10 seconds in the Formvar solution [0.2–0.5 g Monsanto Formvar 15/95 E in 100 ml of 1, 2–dichlorethane (ethylene chloride)], dried for 30 seconds and dipped again.
5) The slides were then dried under the heat lamp for 15 minutes. (If Formvar turned milky, then moisture had been absorbed by Formvar; procedure was repeated with new slides.)
6) Formvar plastic was cut around glass edges (Fig. 1).

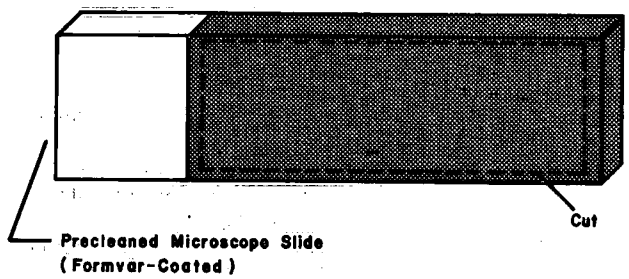

Figure 1. Formvar-coated microscope slide.

7) Distilled water was added to a Buchner funnel (Fig. 2). The slide was inserted into the water very slowly so that the plastic film floated freely. (The film was the correct thickness if it turned a reddish-blue colour. If the film did not leave the slide, it was immersed in distilled water for approximately 15 minutes. The slide was then removed from the water and slowly reinserted at an angle into the water, at which time the film floated freely.)
8) The plastic film was placed on the other precleaned slide and dried under the heat lamp for 2 minutes.

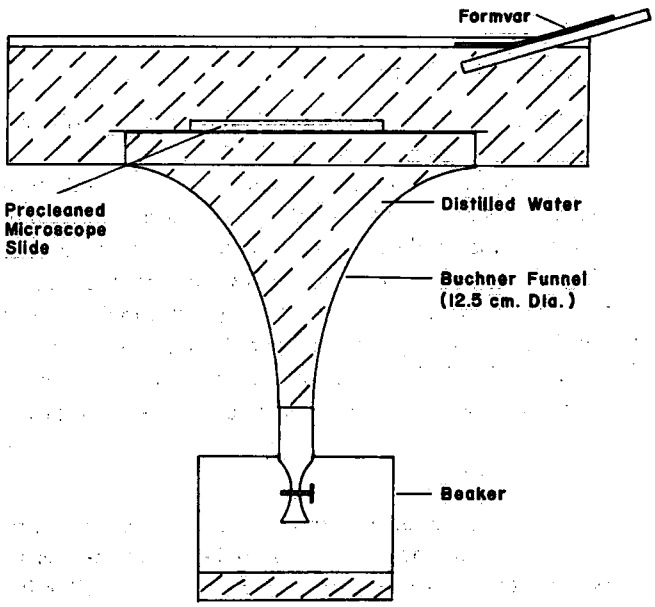

Figure 2. Buchner funnel.

b) Carbon Coating

The glass slide was placed in the C.V.C. evaporation chamber (C.V.C. Instructions Manual, Bendix Corp.). The chamber was vacuumized, and the slide was rotated at speed 3 while 4–5 volts were applied to the carbon rods for 10 seconds. This procedure was found to be the best for producing a good carbon-coated mirror-like finish on slides.

c) Depositing Support Film on Grids

The procedure for depositing support film on grids follows:

1) The Formvar coated with carbon was cut into squares so that the support film covered a grid.
2) The support film glass slide was inserted at an angle into distilled water (in a clean petri dish) so that the support film squares floated individually.
3) Tweezers were used to place a 3-mm copper grid at an angle under the squares while lifting them (support films were mounted on the matt side of the grid for maximum adhesion); the grids were then touched to filter paper for absorption of excess water.
4) Grids were placed on filter paper soaked in chloroform; this dissolved the Formvar and the carbon was left on the grid. After the chloroform had evaporated, the grids were observed under the microscope and, if plastic still remained, were again placed on the chloroformed filter paper. If carbon grids were suitable, 50 squares to 100 squares were counted as a background count. When more than 4 fibres per 50 squares were observed, the grids were used for samples with expected high counts; if 10 fibres or more were observed, the grids were discarded.

d) Specimen Drop Placed on Grids

To deposit a 1-μl drop on the grid, the following steps were taken:

1) *Water Samples*—1 ml distilled water was added to the 250-ml centrifuge container, and sonicated (3 min) until all the material was dispersed in water.
2) *Sediment Samples*—0.1 ml of the <2-μm suspension was added to 9.9 ml distilled water. If too dirty when viewed in microscope, then 1.0 ml (0.1 ml of sample and 9.9 ml distilled water) was added to 9.0 ml distilled water.

3) When high counts were expected or the water was dirty a 2-μl pipette was used (a larger diameter was required to collect the suspended material); if the sample was clear a 1-μl pipette was used. The pipettes were stored in alcohol and kept in a cool place. If the pipettes were believed to be dirty they were cleaned with a solution of one-half 70% nitric acid and the other half hydrochloric acid.
4) When the 1-μl drop had been placed on the specimen grid, it was dried (5 min) in a desiccator. The drop was completely dried to avoid contamination in the microscope. The specimen grid was then placed on filter paper in a petri dish and identified.

EXAMINATION WITH ELECTRON MICROSCOPE

Samples were counted at a magnification of 20,000 X. Some chrysotile identification was obtained using higher magnification, and for proper identification of cummingtonite electron diffraction was required. Due to the similarities in diffraction patterns between cummingtonite and hornblende, it was necessary to measure carefully the spacing between the bright spots and to note other physical characteristics.

TOTAL FIBRE COUNT

Centrifuged Water Samples (250 ml)

$$(a\text{-}m)b \times c_1 \times d \times c_2 \times c_3 = T \text{ fibres/litre}$$

where:

a = number of fibres counted per Y squares

m = background per Y squares (the carbon-coated grid is examined before the 1-μl water sample is deposited on it. [See Section C(4), p. 2.]

$$b = \frac{\text{Number of squares drop covered on grid}}{\text{Y squares}}$$

$$c_1 = \text{constant} = 2.36 = \frac{\text{Total area of drop}}{\text{Visible area of drop}}$$

Due to the thickness of the copper grids, the electron beam cannot penetrate the "lines" which support the carbon. When samples are examined at 20,000 X, these lines (unviewed) cover a larger area than the viewed sample.

$(a\text{-}m)b \times c_1$ = Number of fibres in 1-μl drop

$d = 10^3$

10^3 μl = 1 ml = the volume used to resuspend the material when adhered to the sides of the 250-ml centrifuged bottles after centrifuging.

$(a\text{-}m)b \times c_1 \times d$ = fibres in 1 ml = fibres centrifuged out of 250 ml.

$$c_2 = \text{constant} = \frac{100}{85}$$

The 250 ml of water that was poured off after centrifuging was examined in the electron microscope. It was discovered that 15% of the fibres were still in suspension. Therefore 85% of the total fibres adhered to the side of the centrifuge bottle and were resuspended in the 1 ml.

$(a\text{-}m)b \times c_1 \times d \times c_2$ = Number of fibres in 250-ml water sample

c_3 = constant = 4

250 ml x 4 = 1,000 ml = 1 litre

$(a\text{-}m)b \times c_1 \times d \times c_2 \times c_3$ = Total fibres/litre

Example: $(6\text{-}1)10 \times 2.36 \times 10^3 \times \frac{100}{85} \times 4$

$= 5.55 \times 10^5$ fibres/litre.

Artificially Mixed Samples

Asbestos fibres were suspended in 100 ml distilled water; five batches were combined and diluted to 1 litre with water (Lawrence *et al.*, 1975). Due to high concentration of fibres, it was necessary to dilute certain samples 500 to 1 for examination in the electron microscope. Then

$(a\text{-}m)b \times c_1 \times c_4 \times c_5 = T$ fibres/litre

$(a\text{-}m)b \times c_1$ = Number of fibres in 1 μl (as shown above)

c_4 = constant = 10^6, i.e., 10^6 μl = 1 litre

c_5 = constant = dilution factor

Example: $(443\text{-}1)10 \times 2.36 \times 10^6 \times 500$

$= 5.22 \times 10^{12}$ fibres/litre.

Sediment Samples

"A wet sediment sample was dispersed in distilled water, 5 ml of the <2 μm sediment suspension drawn off after sedimentation of the larger particles and transferred to a preweighed vial" (Dell, 1975).

For examination in the electron microscope, 0.1 ml of the suspension was diluted in 9.9 ml distilled water. If impossible to count, then 1 ml of (0.1 ml of sample + 9.9 ml distilled water) was diluted in 9.0 ml distilled water. Then

$(a\text{-}m)b \times c_1 \times K = T$ fibres/litre

$(a\text{-}m)b \times c_1$ = Number of fibres in 1 μl (as shown above)

If 1-μl drop was taken directly from the <2-μm sediment suspension, then K = 10^6. 10^6 μl = 1 litre.

Example: (43-3)2 x 2.36 x 10^6 = 1.89 x 10^8 fibres/litre.

If 1-μl drop was taken from 0.1 ml + 9.9 ml distilled water solution, then K = 10^8. 10^6 x 10^2 dilution factor of 0.1 ml.

Example: (43-3)2 x 2.36 x 10^8 = 1.89 x 10^{10} fibres/litre.

If 1-μl drop was taken from 1 ml of (0.1 ml + 9.9 ml distilled water) + 9.0 ml distilled water, then K = 10^9. 10^6 x 10^2 x 10 dilution of 1 ml.

Example: (43-3)2 x 2.36 x 10^9 = 1.89 x 10^{11} fibres/litre.

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