CHAPTER II

METHODOLOGY

ENVIRONMENTAL SAMPLING METHODS AND INSTRUMENTS
SAMPLE PREPARATION AND ANALYTICAL TECHNIQUES

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1. **General**

Exposure to metals may be occupational as well as non-occupational. The latter refers to exposure of common man by way of metallic contamination of community environment from various sources. Occupationally speaking, a vast array of metals and metallic salts, directly or indirectly, are used in many industrial processes. Many of these are toxic to varying degrees and hence pose hazards particularly to the occupationally exposed population. In industries, exposure occurs principally through the respiratory system in the form of dust, fumes and vapour. Also harmful substances can be absorbed through the skin and ingestion along with food if hands are contaminated and not properly washed since bare hands are used while eating in India. The amount of toxicant absorbed depends on its concentration in the air of working environment, physical and chemical
characteristics of the toxicant and the worker's breathing capacity. The first prerequisite for assessing occupational exposure to any toxicant obviously is monitoring the air of the working environment followed by assay of the toxicant and/or its metabolites in the blood, urine, hair or other biological specimen wherever possible. The first two combined together can be useful for calculating the degree of the exposure hazard and eventual absorption by the body.

Thus, the need for proper reliable and adequate sampling procedures both for air and biological monitoring can hardly be overemphasised. Sampling forms an integral and crucial part of industrial hygiene and air pollution programmes, but, which, unfortunately, is not always ascribed adequate attention in such studies.

A proper and reliable sampling in itself, is, however, not the end. It must always be followed by equally reliable and accurate steps for processing the sample into analyzable form and final identification and determination. Therefore, the success of such studies partly depends on the sampling techniques and partly on the procedures adopted for the sample preparation and techniques applied for the determination.
2. Sample collection

2.1. Air samples

2.1.1. Theoretical: Sampling and analysing air for contaminants in community and at working place is a problem which has been refined through the last 5 decades by several hundred industrial hygienists. Historically, air sampling in workrooms perhaps goes back to 10th century. However, it is fair to assume that most sampling in workrooms, especially routine and representative, is principally a 20th century innovation; even now it is mandatory only in few countries.

The need for sampling air in workrooms and industrial environments, of course, is apparent because of the desirability of providing a safe working atmosphere which will not cause any occupational disease or disability. In industrial hygiene practice sampling and analyses are useful means of evaluating the workroom atmospheres conveniently in order to provide data for designing the necessary engineering control and often to correlate with clinical manifestations in the exposed persons. Besides, air sampling and analyses are useful for evaluating new types of control equipment for
performance, leakage and critical reactions during the operations cycle.

The nature of contaminants is one of the deciding factors in choosing the proper method for sampling the air of work environment. The type of contaminant which occurs in a work place will, in turn, be dependent upon the industrial operations employed. These air contaminants, encountered in the industrial environment, can be divided into three groups depending upon their physical characteristics, viz. gases and vapours; particulate matter or aerosols and the miscellaneous forms of contamination. The particulate matter or aerosols may be in the form of solids (dust, fumes or smokes) or in the form of liquid (mist or fog).

If sampling is to be intelligently conducted, prior knowledge of the physical state in which a substance exists must be available or else a judgement must be made. Devices which are only meant for collecting particulate matter such as filter paper samplers, do not usually collect gases or vapours and hence, an incorrect selection of sampling method may lead to erroneous results. In order to make a correct analysis, a proper, representative and adequate amount and number of samples
of the material to be analysed must be obtained.

After choosing the appropriate sampling device, an analyst has to think of sampling places and number of samples, sampling time, sampling rate, the volume of the sample to be collected, etc. Watson (1954), Keenan (1958), Mossy and Keenan (1958), Jacobs (1960) have discussed in detail the procedures for the various type of air sampling and other relevant parameters for sampling. The number of samples to be collected depends on the information sought. The complexity of the problem of choosing the number of samples is emphasized by Mina (1941). With respect to obtaining information concerning a given working environment, it has been recommended that samples be collected from at least three general sampling locations (American Public Health Assoc., 1939-40) in order to get a fairly complete sample of the workers environment. These are:

(i) In the immediate vicinity of the workers in a particular environment (preferably from the breathing zone).

(ii) Near the source of the contaminant entering the general atmosphere.

(iii) From the general work room atmosphere.
The samples of air taken for industrial purposes can be divided into two major groups, one called "Instantaneous", "spot", "snap" or "grab" samples and the other "integrated or continuous" samples. For the study of occupational exposure, integrated or continuous samples give a measure of the average exposure of a worker than grab samples, whereas grab samples provide information on the fluctuation of contaminant concentration.

For collecting samples of particulate matter, the following categories of devices can be employed:

(a) settling chambers/sedimentation cells
(b) centrifugal devices
(c) impingers and impactors
(d) scrubbers
(e) filter and filter media
(f) electrostatic precipitators
(g) thermal precipitators.

Filters and filter media are one of the ideal methods of sampling particulate matter since (i) they now can be produced to various specifications and will collect particulates with minimal auxiliary equipment, (ii) are easier to handle and (iii) are less expensive.
2.1.2. **Instrumental:** Basically an air sampling instrument is a system comprising:

- A collection or filtering device.
- A flow meter or orifice meter to indicate the flow of air through the collecting device, or a gas meter to measure the volume of air collected.
- A suction device or pump.

Various types of instruments are commercially available. Home-assembled instruments based on the above principles are equally good. The various types of instruments used for air sampling, in the present studies are described below:

**Hi-volume Air Sampler:** (The Staplex Company - Figure-3a)

This instrument is used to collect airborne particulate matter on membrane, whatman or cellulose acetate filter paper at a high sampling rate. This sampler has good efficiency for industrial dusts and for particulates ranging from 0.01 to 10 microns in diameter. Air to be sampled for particulate contaminants is drawn through either a pleated or a flat circular filter, or a flat rectangular filter by means of an electrically driven 2-stage centrifugal fan. In the present studies, high volume sampler with pleated filter
paper was used. A large volume of air was drawn through the filter paper in order to get the total (gravimetric) dust concentration as well as for ovulating the dust for chemical analysis of the metallic content. To find out the average exposure to metals in the community, sampling was performed from 0000 hrs to midnight (18 hours), at a rate of 1 m$^3$/min. In case of working shop general environment, high volume samplers was used for the entire shift of 8 hours.

Low volume sampler: (Model LV-1, Steplex Company - Figure-3b)

It is handy and light weight instrument (3 kg), equipped with the motor, blower and flowmeter. It can be used with 1½" or 2" diameter filter paper or adapted for use with other collection methods. The pump draws 23.32 L/min, with a resistance pressure of 7.62 cm. of mercury or upto 30 L/min. Air flow is measured through a rotameter calibrated from 15 to 35 L/min. and equipped with an integrate control valve. In the present studies, the low volume samplers were used in various industries like welding shop, foundry operations and glass work in ceramic industries. Glass fibre filter discs were used as collection media.
Herchlet: (C.F. Casella & Co. Ltd. - Figure-3c)

The herchlet collects a large sample of respirable dust while it is airborne. Air is drawn first through an elutriator made by providing a number of horizontal plates at designed inter plate distances and length for providing the appropriate size cut off. The air is then passed through a critical orifice and dust is trapped in a filter paper attachment. The herchlet was used in ceramic glaze industries to assess the respirable dust concentrations in the workplace. The glass fibre filter papers (4.1 cm diameter) were used as the collection media and air was drawn through the filter paper at the rate of 450 L/min. and the cut off size of the particulates was up to 7 micron.

Greenburg-Smith Impinger: (Figure-3d)

It is the standard instrument for the collection of dust, fume and mist. It has a varying efficiency in the collection of lead fumes, which depends on the sampling flow rate. It was shown by Keenan and Fairhall (1944) that the efficiency of a large impinger could be brought to nearly 100% at a sampling rate of 42.5 L/min. Efficiency can be increased by attaching the two bubblers in series.
Locally fabricated aluminium cone filters, a good vacuum pump and rotameter or any device, which records the volume of air sampled - are used to form a device for the sampling. In the present study, this home assembly with glass fibre disc were used very often for the collection of air samples from the work place.

2.2. Biological samples

In any community and industrial health conservation programme, biological monitoring and analyses provide valid information on population exposure to toxic materials, though it is very difficult to undertake as a large scale of a routine basis. Biological analysis indicates whether the exposed group of personnel shows an excessive absorption or excretion rate for the airborne substances or one of its metabolites. Many investigators, therefore, have recommended biological monitoring, as a part of occupational health studies and safety control (Linch, 1972; Stokinger, 1972).

Harmful materials can enter into human system
by any of the routes of inhalation, injection and/or skin absorption. These materials may get absorbed into the blood stream and carried to other centres where they may accumulate. The excess and unwanted materials are removed from the blood by the help of kidney and then they come out as a fluid - urine. They may also be excreted through faeces and sweat. It contains waste products from metabolism as well as any other substances present in excess amount that must be eliminated. Increased level of toxicant in blood and urine is related to the level of exposure to that toxicant. As a reason, blood and urine are used widely for evaluating the exposure risks.

There were practical difficulties to get the samples of faeces, sweat, hair, etc. The main difficulties were: (i) reluctance of workers to permit collection of these samples, (ii) cooperation of management in sparing the workers for long period needed for the collection of these samples was not available, (iii) large size of sample needed and (iv) higher probability of contamination of the samples, therefore, in the present studies, blood and urine of the exposed group and control group were used to evaluate the degree of exposure to various heavy
metals. Details of the blood and urine sample collection procedures are given below.

2.2.1. Blood samples: Blood was drawn from the cubital vein of the subject between 1100 and 1200 hrs during the duty hours and transferred into heparinized tubes. Samples were brought to the laboratory in an ice-box and kept in the refrigerator till analysis.

2.2.2. Urine samples: About 100 ml of urine was collected at the same time when the blood was collected. 1 ml of concentrated HCl was added in each sample as a preservative to avoid any precipitation during the time lapse between sampling and processing for analysis.

2.3. Water, food and other samples:

Man living in the general community is exposed to heavy metals through air, water and food. Therefore, typical samples of various food grains, pulses, oils, vegetables, fruits, meat, eggs and fish; total composite diet, drinking water and cigarettes consumed by the population were analysed for trace heavy metal content. The samples were collected in the manner described below.

Water samples: Water samples were collected from
the various locations of the city and villages in 1.5 litre glass bottles, which had been cleaned with concentrated HCl, rinsed with tap water followed by distilled water. Prior to filling the sample, bottles were again rinsed two or three times with the sample being collected. 2.0 ml of concentrated HCl was added to each sample to avoid precipitation.

Raw food stuffs like food grains, pulses, vegetables, fruits, oil, milk, fish, eggs, cotton were purchased from the local market or brought from the consumers' house. Prepared food dishes were collected from the lodges, hostels, caffees and various households. Cigarettes and beedis of various brands were purchased from the local market.

3. Sample preparation:

After proper, adequate and reliable sample in required amounts has been obtained, it was subjected to further processing for eventual analysis. Methods used for preparing the different samples are described below.

3.1. Air sampling:

The total dust (gravimetric) concentration for unit volume air sampled was computed by calculating the
weight differences of the filters before and after the sampling and the total volume of air sampled for the location. Afterward, the particulate laden filter papers were cut into small pieces, and digested for one hour in 100 ml of concentrated HCl over low heat. The solution was filtered through metal free cotton and washed down thrice with distilled water. The combined extract was evaporated nearly to dryness. The residue was redissolved in 1 ml HCl and 5-6 drops of HNO₃ and made up to 10 ml with distilled water. The solution was filtered again. The same amount of acid was used for a blank filter paper and a 10 ml blank solution was prepared in the same manner. The solutions obtained as above were directly used for analyses in the AMS.

Air samples collected, using 1% nitric acid solution as an absorbent, by means of an impinger, were evaporated nearly to dryness and residue was dissolved in 10 ml of diluted acid solution (1% HCl solution).

3.2. Water, food and biological samples:

All organic matter in biomaterials have to be destroyed before determination of trace metal concentration. This may be done either by dry ashing
or wet oxidation. As dry ashing in a muffle furnace at a temperature of 500-900°C may introduce doubts due to loss of elements by volatization in case of certain elements or by reaction with the material of the container, or contamination of samples by atmospheric dust or substances volatized from the interior of the furnace, the wet oxidation method is preferred by most analysts. There are a very large number of papers describing methods of wet-oxidation that have apparently proved satisfactory (Middleton and Stuckey, 1966; Cornish, 1959, 1970; Analytical Method Committee Reports, 1965, 1976; Hanson, 1973). The method adopted in these studies is the simple wet oxidation procedure for biological materials described by Roits et al (1969).

The amounts of various samples taken and of acid added for the digestion were as follows:

1. 10 ml of whole blood + 60 ml HNO₂
2. 50 ml of urine + 10 ml HNO₃
3. 5 gm of food samples (dry weight) + 50 ml HNO₃
4. 1 cigarette/food + 40 ml HNO₃
5. 1000 ml water + 10 ml HNO₃

Utmost care was taken during the addition of acid to avoid the frothing of the sample.
The sample with nitric acid in a Erlenmeyer flask was set on a sand bath and heated continuously for 3-4 hours at low heat till a clear yellowish solution resulted. The sample was cooled and filtered through a pad of natural free cotton. The residue was washed with diluted nitric acid. 1 ml of concentrated sulphuric acid was added to the filtrate and again heated slowly in the beginning and rapidly afterwards.

To oxidize the material completely, digestion mixture of 72% perchloric acid and concentrated nitric acid (2:1) was added, as and when the filtrate began to char. At the end of the oxidation, the solution was evaporated to nearly dryness. The residue was cooled, and redissolved in 1 ml of concentrated hydrochloric acid, 5-6 drops of concentrated nitric acid and about 5 ml of distilled water was added. The solution was boiled, cooled and made upto 10 ml with triple glass distilled water.

In the same manner, fixed amount of acids were used for digestion for preparing a 10 ml acid blank solution. The solutions prepared as above were directly used for analyses.

Metals: The metallic elements determined in these
samples were in ascending order of atomic number, chromium, manganese, nickel, copper, zinc, calcium and lead. These metals commonly occur in industrial environment and body fluids.

4. **Analytical procedures**

4.1. **Reagents and Glassware**

(a) Standard stock solutions of pure metal were prepared from ultra-pure metals powders (VARILON CORPH. ALFA Products). Stock solutions were made by dissolving exactly 1 mg of the metal powder in a minimum volume of acid and made up to 100 ml with triple glass distilled water. Required working solutions of 0 to 10 μg/L range were prepared from the stock solution by proper dilution.

(b) Analar quality (BDH Products) acids were used in processing the digestion of various samples.

(c) Triple glass distilled water was used for making solutions and final rinse of glasswares.

(d) All glasswares were kept overnight in hot dilute nitric acid and washed repeatedly with triple
distilled water.

(e) Metal free cotton: The sterilized surgical cotton was leached in the hot diluted acid solution (HCl) for 4-5 hours. Cotton was then washed with distilled water till acid was removed. Again this treated cotton was washed with triple glass distilled water, and excess water was removed by vacuum suction. Finally, it was dried in an oven at 100°C till moisture was completely removed.

4.2. End determination:

Many analytical techniques are available for the determination of metals and most of them have been applied successfully for analysing the environmental samples and biomaterials. Spectrophotometric and polarographic methods were used almost exclusively for the determination of metals in food and various biological samples up to 1960. Currently, neutron activation analysis, electrochemical methods, viz; oven techniques and atomic absorption spectrophotometry are more specific methods for determination and therefore these methods are used widely. The relative merits and demerits of different analytical techniques are given in Table-9. Neutron activation analysis is costly
and not generally available and hence can only be applied for a limited number of samples. Research grade ionizer and electrodes for the specific metal ions were not available. Ring oven techniques are used only for semi-quantitative determination at present. The recently developed techniques of AAS are most suitable for the rapid and reliable analysis of trace metals, especially for analysing the trace metals in environmental and biological samples (Soman et al., 1968, 1969; Joseph et al., 1968).

In the present study, a Perkin Elmer Model 305A Atomic Absorption Spectrophotometer was used for the analysis of trace metals in the various samples. Instrumental settings, i.e. wavelength and slit band, type of flame, sensitivity and working range of concentration for the metallic elements studied in the present study are shown in Table-10.

A typical output of the recorder of AAS used in the studies showing the nature of the peaks obtained for metal concentrations in different samples is shown in Figure-4.
5. **Experimental procedure: types and number of samples**

To evaluate the occupational exposure to trace heavy metals in different occupations, the experimental work was divided into two parts:

**A. Evaluation of background exposure to trace heavy metals in community.**

**B. Evaluation of occupational exposure to trace heavy metals in different industries.**

The details of various types and number of samples analysed are given in Table-11. It will be appreciated that the evaluation of occupational exposures needs analysis of a large number of samples to give meaningful results. More than 4000 determinations have been made for the present studies.

6. **Interpretation of results**

The concentration of trace metals have been expressed in suitable units as $\mu g/m^3, ng/m^3, \mu g/gm, \mu g/100 ml, \mu g/l$, etc. depending upon the substrate. Wherever possible, the observed values were compared with TLV and MAC values. Mean and standard deviations have been calculated and test of significance (t-test) were applied wherever necessary.
### Table-2

**Relative merits and demerits of currently used analytical techniques**

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>mg-ng</td>
<td>Versatile, Simple, Selective, Specific, Good reliability of results, Instrument generally available.</td>
<td>Time consuming and tedious, Problem of interferences, Loss of metals during processing i.e. digestion, extraction, etc.</td>
</tr>
<tr>
<td>Colorimetry</td>
<td>mg-ng</td>
<td>Quito accurate, More than one metal determination in single polarogram, Problem of interference rare.</td>
<td>Requires skillful operation, Limited to electroactive metals.</td>
</tr>
<tr>
<td>Polarography and Anodic Stripping</td>
<td>mg-ng</td>
<td>Specific, most reliable, rapid, Requires small sample size.</td>
<td>Requirement of &quot;Neutron&quot; flux source, cost per sample is high.</td>
</tr>
<tr>
<td>Neutron activation</td>
<td>mg-pg</td>
<td>Specific, reliable, rapid, Requires small sample size.</td>
<td>Addition of ISA/pH adjuster, Frequent cleaning of electrodes, Research grade ions and specific ion electrodes required.</td>
</tr>
<tr>
<td>Specific ion electrode</td>
<td>ug</td>
<td>Used in field work, Simple and fast accurate analysis.</td>
<td>Skillful operation, and wide experience needed.</td>
</tr>
<tr>
<td>Ring oven technique</td>
<td>ug-ng</td>
<td>Simple and fast, More than one metal can be determined in a single operational ring.</td>
<td>Not easily available, Separate hollow cathed lamps for the individual metal, Loss of metal during digestion, extraction.</td>
</tr>
<tr>
<td>Atomic absorption/ emission spectro-</td>
<td>mg-ng</td>
<td>Versatile, specific, reliable, sensitive and fast. Problem of interference are easily overcome.</td>
<td>Not yet fully developed.</td>
</tr>
<tr>
<td>photometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetic analysis</td>
<td>mg-ng</td>
<td>Highly selective, Sensitive, Require small sample.</td>
<td></td>
</tr>
<tr>
<td>(of reaction in which metal ion acts as a catalyst)</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>
Table 10
Standard conditions and related details for various metal determination on AAS
(Perkin-Elmer instrument setting)

<table>
<thead>
<tr>
<th>Element</th>
<th>Wave length setting</th>
<th>Slit</th>
<th>Flame type</th>
<th>Sensitivity linearity for 1% absorption range</th>
<th>(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.7</td>
<td>Air-acetylene</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Mn</td>
<td>279</td>
<td>0.2</td>
<td>do-</td>
<td>0.055</td>
<td>3</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.2</td>
<td>do-</td>
<td>0.12</td>
<td>5</td>
</tr>
<tr>
<td>Ni</td>
<td>232</td>
<td>0.2</td>
<td>do-</td>
<td>0.15</td>
<td>5</td>
</tr>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>0.7</td>
<td>do-</td>
<td>0.09</td>
<td>5</td>
</tr>
<tr>
<td>Zn</td>
<td>214</td>
<td>0.7</td>
<td>do-</td>
<td>0.018</td>
<td>1</td>
</tr>
<tr>
<td>Cd</td>
<td>229</td>
<td>0.7</td>
<td>do-</td>
<td>0.025</td>
<td>2</td>
</tr>
<tr>
<td>Pb</td>
<td>283</td>
<td>0.2</td>
<td>do-</td>
<td>0.50</td>
<td>20</td>
</tr>
</tbody>
</table>
Table II

Types and number of samples included in the present studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Environmental monitoring</th>
<th>Other complexes</th>
<th>Biological monitoring</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air</td>
<td>Water</td>
<td>Food</td>
<td>Blood</td>
</tr>
<tr>
<td>A. Background exposure to trace heavy metals.</td>
<td>19</td>
<td>7*</td>
<td>Raw food Cigars</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>Water</td>
<td>Food</td>
<td>Blood</td>
</tr>
<tr>
<td>B. Occupational exposure in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Dry Cell Mfg.</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii) Type Foundry</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii) Welding Lines</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iv) Ferrous/non-Ferrous Foundry</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v) Ceramic glaze</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total samples</td>
<td>139</td>
<td>7</td>
<td>43</td>
<td>21</td>
</tr>
</tbody>
</table>

*Samples taken in duplicate.
Air sampling Instruments
Figure 4: Nature of the peaks obtained for metals concentrations by AAS

- MANGANESE

- CADMIUM
SECTION TWO

EXPERIMENTAL

PART I: BACKGROUND EXPOSURE
PART II: OCCUPATIONAL EXPOSURE