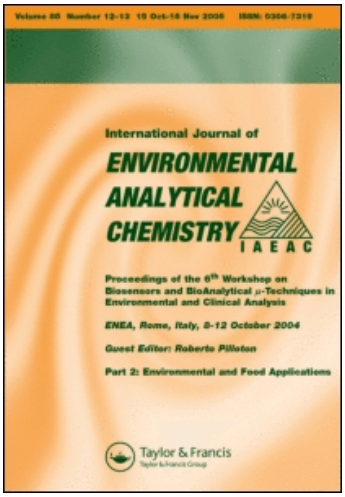

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K. K. Dwivedi[a]; M. S. Prasad[a]; G. N. Rao[a]; R. K. S. Dogra[b]; R. K. Upreti[b]; Ravi Shanker[b]; C. R. Krishna Murti[b]; S. S. Kapoor[c]; Madan Lal[c]; K. V. Viswanathan[c]
[a] Department of Physics, Indian Institute of Technology, Kanpur, India [b] Industrial Toxicology Research Centre, Lucknow, India [c] Nuclear Physics Division, Bhabha Atomic Research Centre, Bombay, India

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Trace Elemental Analysis of Extracted Dust from Lungs and Lymph Nodes of Domestic Animals Using X-ray Fluorescence Technique

K. K. DWIVEDI, M. S. PRASAD and G. N. RAO

Department of Physics, Indian Institute of Technology, Kanpur 208016, India

R. K. S. DOGRA, R. K. UPRETI, RAVI SHANKER and C. R. KRISHNA MURTI

Industrial Toxicology Research Centre, Lucknow 226001, India

S. S. KAPOOR, MADAN LAL and K. V. VISWANATHAN

Nuclear Physics Division, Bhabha Atomic Research Centre, Bombay 400 085, India

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Samples of dust extracted from the lungs and lymph nodes of certain domestic animals from West Bengal, Orissa, Bihar provinces of India were quantitatively analysed using photon excited energy dispersive X-ray fluorescence technique. Thin specimens were prepared for analysis to minimize matrix enhancement and absorption effects. Amongst the various elements analysed, Hg, Fe, Cu, Zn and Pb were found to be present in appreciable amounts. An important finding was the presence of a very high concentration of Hg in extracted dust samples from West Bengal. The significance of the various results obtained in this investigation is discussed.

KEY WORDS: Lungs and lymph nodes, mercury toxicity, siliceous particulate matter, Si(Li) X-ray detector, trace elemental analysis and X-ray fluorescence technique.

INTRODUCTION

Trace elemental analysis of environmental and biological samples by

photon excited X-ray Fluorescence (XRF) Technique has made major advances in the past few years.[1-7] This technique provides a rapid, non-destructive multielemental analysis of samples with concentration levels as low as a few micrograms per gram (μg/g) or parts per million (ppm). A unique feature of energy dispersive X-ray fluorescence analysis is that it permits the simultaneous determination of all the elements ($Z > 11$) in a single run. Further, X-ray fluorescence technique appears most attractive for fairly accurate detection of trace elements even in the presence of large amounts of other elements.

With the increasing industrial activities of man, an enormous number of pollutants of diverse complexity have been entering our environment and their effects are seemingly increasing. In recent years attention has been paid to animals as indicators of environmental hazards since they share man's environment. In one of the joint FAO/WHO meetings it was recommended that the food animals should be subjected routinely to post-mortem examination not merely from the point of view of livestock disease surveillance but also the environmental monitoring through them.[8] Accordingly a few preliminary surveys were attempted[9] in the industrial and mining belts of Eastern part of our country and large number of food animals examined at post-mortem. Amongst the various atmospheric pollutants, increasing awareness has been aroused during the past decade regarding the possible chronic deleterious effects of trace element burdens. Role of trace elements in human health and disease has been ably presented by Prasad,[10] in which Darby commented "... the ever expanding appreciation of the toxicity of excessive quantities of each element and the complexities of biologic and chemical interactions make the study of trace elements a most demanding field". Both for diagnostic and monitoring purposes the trace elemental analysis performed in the extracted dust samples of tissues by XRF technique in the present study provides an opportunity to determine the usefulness of such procedures.

EXPERIMENTAL

Sampling and extraction of dust from tissues

Various slaughter houses located in different districts of Bihar, Orissa and West Bengal provinces (Figure 1) were visited from time to time for collection of lungs and regional lymph nodes from bovine, ovine and caprine species brought for meat purposes and which represented the industrial and mining complexes. Those lungs and lymph nodes which exhibited any gross abnormality at post-mortem examination were collected and brought to laboratory after proper inflation with 10% formol

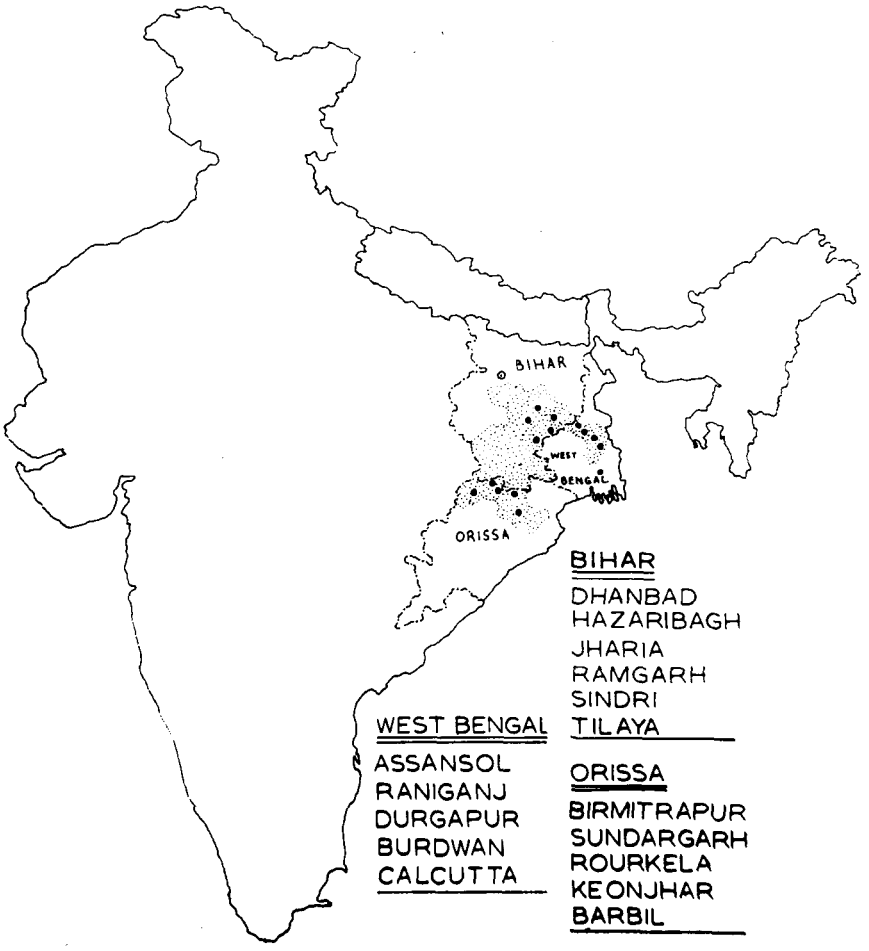

FIGURE 1 Map showing mining and industrial regions in Bihar, West Bengal and Orissa.

saline. Part of the tissue was processed and blocks embedded in paraffin. Representative 5 μm thick sections were cut, stained with hematoxylin and eosin for routine examination,[11] and also under polarising microscope to locate the dust deposits.[12] Suitable fields were selected and photomicrographed. The remaining formalin fixed tissues were used for the extraction of dust. The method of Guest[13] was followed and finally the extracted dust was dried in an oven at 110°C. In all, seven samples of lungs and one lymph node sample were prepared for the dust content and subsequently used for trace elemental analysis.

Preparation of thin samples and standards

Each sample consisting of very fine dust particles (<5 μm) was weighed (~ 20 mg) and transferred to a 100 ml beaker. About 50 ml of distilled water with 1–2 drops of Digol (diethylene glycol) was slowly added to the sample and the beaker was kept in an ultrasonic vibrator for 15 minutes to make a uniform suspension. This was filtered through preweighed Selectron filter (Type BA 85, Schleicher and Schüll, West Germany) mounted on a microfiltering unit with deposition area of 2.05 cm^2 to produce a uniform sample of thickness approximately 10 mg/cm^2. Each sample thus prepared was placed between two perspex rings to avoid curling while drying under an IR lamp. Dried samples were weighed accurately to determine the actual amount of dust deposited on the filter. All the prepared samples were stored in air-tight plastic boxes for their analysis.

High purity (99.99%) oxides of copper (CuO), lanthanum (La_2O_3) and lead (PbO_2) were used to prepare thin standard samples for calibration purposes. At room temperature, these oxides are insoluble in water, therefore, thin standard samples in the thickness range of 0.07–3.04 mg/cm^2 were prepared using the same method as described above.

XRF spectrometer

The experimental arrangement of the X-ray fluorescence spectrometer used for the present measurement is shown in Figure 2. An annular 100 mCi source of ^{241}Am with stainless steel window to absorb all lower energy

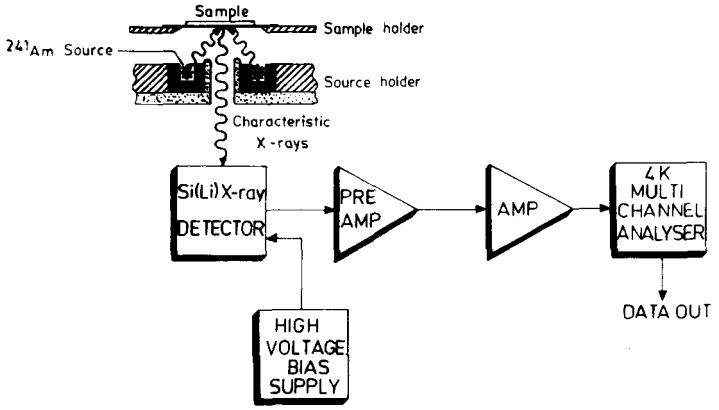

FIGURE 2 Typical block diagram of the experimental set-up for the X-ray fluorescence analysis.

gamma-ray lines except 59.5 keV gamma, was used for the excitation of the samples. This source was placed in a brass source holder having lead and aluminium linings to shield the detector from the source. The source-assembly was mounted on top of the vertical head of a Si(Li) X-ray detector having 30 mm^2 area and 3 mm sensitive depth and the X-rays were detected through a 5 mil Be window. A drain feedback preamplifier was used to amplify the detector signals which were further amplified before being analysed by a 4K pulse height analyser. The energy resolution of the Si(Li) detector X-ray spectrometer was about 235 eV for 5.94 keV Mn K X-rays.

Energy calibration of the spectrometer was done before and after the sample runs using a number of pure metals and standard sources of ^{55}Fe and ^{241}Am. In a few cases internal calibration was also done to identify certain X-ray peaks. Samples prepared on selectron filters were placed (facing up) on sample holder and were exposed to exciting radiations and their X-ray fluorescence spectra were recorded. The distance between the sample and the detector was optimised ($\sim$4 cm) to give maximum count rate for fluorescent radiation. The geometry of the source, sample and the detector was kept unchanged throughout this analysis. Using a blank selectron filter, the background spectrum was also recorded for almost the same period of time. Thin standard samples were also exposed to the exciting radiation and the fluorescent radiation spectra were recorded for calibrating the peak counts to the amount of element present, as explained later.

XRF analysis

The identification of the peaks corresponding to different elements in the observed spectrum was done with the help of the energy calibration curve. Integrated peak counts in the background spectrum were normalized with respect to the incoherent (Compton) scattered peaks of background plus sample spectra and then subtracted from the corresponding peak intensities of the sample spectrum. Silver peaks appearing in XRF spectra of the samples were found to be present due to background only. In the present analysis we have considered either Kα or Lα characteristic X-rays of elements detected in these samples for quantitative estimation. In case of certain overlapping peaks, mathematical unfolding and peak smoothening were done using data for relative peak intensities.[14] Thus, the intensity I_j of the characteristic X-ray of jth element present in the sample was determined.

This value of I_j is related to the concentration M_j of the jth element as

given in the following equation:

$$I_j = I_0 G K_j M_j C_j \tag{1}$$

where

I_0 is the intensity of the primary excitation radiation(s),
G is the geometrical factor,
K_j is the relative ability to excite and detect the X-ray line of interest,
C_j is the self absorption term and is due to absorption of exciting and fluorescent radiation in the sample itself.

For a particular X-ray fluorescence system the value of K_j remains constant for any element j, and could be expressed as

$$K_j = \tau\left(1 - \frac{1}{J_{K,L}}\right)\omega_{K,L} T \varepsilon f \tag{2}$$

where

τ is the total photoelectric cross-section for the exciting radiation,
$J_{K,L}$ is the jump ratio which is the same as the ratio between the photoelectric mass absorption coefficients at the top and bottom of the K or L edge energy, and the term $(1-1/J_{K,L})$ is the fraction of total photoelectric events which occur in K or L shell,
$\omega_{K,L}$ is the fluorescent yield of K or L shell,
T is the transmission of fluorescent radiation through the air column, beryllium window of the detector and the specimen backing material,
ε is the detector efficiency, and
f is the fractional intensity of the X-ray lines under study.

In Eq. (2) values of $\omega_{K,L}$, τ, $J_{K,L}$ and f were obtained from the literature[14-16] and values of T and ε were calculated for the detector system. Thus, the values of K_j were obtained for all the elements detected in the samples.

The self absorption term C_j for the near normal incidence geometry can be written as

$$C_j = \frac{1-A}{\ln A} \tag{3}$$

and

$$A = e^{-(\mu_e + \mu_f)X_s} \tag{4}$$

where, μ_e and μ_f are the mass absorption coefficients of the specimen for the exciting and fluorescent radiation and X_s is the specimen thickness. Values of self-absorption term C_j were experimentally determined for each element for individual samples.

Thin standards of copper, lead and lanthanum with precisely known mass M_j were exposed to the exciting source and their fluorescent X-ray intensities (I_j) were obtained. Knowing the values of K_j for these elements, the term I_oG was evaluated using Eq. (1). The self-absorption term C_j was negligible for these very thin standards.

Hence in Eq. (1) substituting the values of I_oG and K_j, C_j and I_j for a jth element, M_j can be determined. If K_j is expressed in $cm^2/\mu g$, one gets M_j in units of $\mu g/cm^2$. Dividing this by the sample thickness (g/cm^2), the concentration could be obtained in $\mu g/g$ or parts per million (ppm).

The quantitative results as obtained in the present experiment are listed in Table I. The errors quoted within parentheses are percentage statistical uncertainties. The actual errors could be more by about 15% because of the systematic errors introduced by non-uniformity of the samples and standards, detector efficiency, self absorption in sample and backing materials. Matrix enhancement errors have been neglected because the samples were thin.

RESULTS

Histopathological findings

Lungs: Focal deposits of particulate dusts were encountered in the alveolar parenchyma especially around the fixed structures like bronchi, bronchioles and blood vessels. In majority of sections the dust lesions were comprised to aggregates of dust-laden macrophages infiltrated with epithelioid cells, lymphocytes and a few fibroblasts. The macrophages had phagocytosed the blackish siliceous particulate matter and gave intense birefringence under polarised light, as shown in Figures 3 and 4. Very little fibrotic reaction was evident in some of the sections of lung attributable to cytotoxic nature of the inhaled dust.

Lymph nodes: Unlike lungs the deposition of inhaled dust in lymph nodes was more and dust lodged in the paracortical areas and modullary cords. The density of inhaled particles was fairly high in the large animals. There was no significant fibrotic reaction in such area in spite of large deposits of dust. Figure 5 shows a microphotograph of particulate deposits in tracheobronchial lymph nodes of a goat.

TABLE I

Trace elemental concentration (in ppm) of various extracted dust samples

	West Bengal					Orissa	
Elements	ED-1	ED-3	ED-6	ED-7	ED-8	ED-2	ED-5
K	—	—	—	—	—	—	—
Ca	—	—	2800(20.7)	—	—	—	—
Cr	1000(14.7)	—	—	—	—	—	900(13.4)
Mn	—	1000(17.8)	—	—	—	—	700(14.1)
Fe	19600(0.6)	26400(1.8)	8300(1.3)	32800(0.5)	10300(1.2)	15500(0.7)	5600(1.5)
Ni	(bkgd)	290(10.0)	(bkgd)	25(12.9)	(bkgd)	(bkgd)	(bkgd)
Cu	100(7.3)	9000(0.9)	4500(1.0)	4500(1.3)	2300(1.9)	570(4.0)	1000(2.6)
Zn	10600(0.5)	20500(0.4)	7300(0.7)	13300(0.5)	4500(1.0)	10200(0.5)	4400(0.8)
Br	250(3.4)	—	—	—	—	—	—
Sr	60(8.8)	—	—	—	110(4.9)	80(7.0)	—
Zr	20(16.3)	60(7.9)	20(15.5)	80(5.0)	30(12.0)	45(8.7)	35(9.5)
Mo	—	60(8.0)	70(10.0)	55(5.0)	30(12.0)	—	—
In	7(4.3)	4(5.6)	60(9.8)	(bkgd)	35(10.0)	(bkgd)	60(8.0)
Sn	760(0.5)	(bkgd)	120(1.2)	30(1.6)	75(1.2)	670(0.5)	500(0.6)
I	—	100(2.8)	—	75(3.4)	—	—	—
Ba	790(0.7)	800(1.0)	200(2.0)	350(1.2)	280(1.5)	1660(0.4)	120(2.6)
Ce	15(19.6)	—	—	—	—	—	—
Hg	490(4.5)	124800(0.1)	9100(0.5)	122000(0.1)	7000(0.6)	1300(1.9)	4500(0.7)
Pb	220(4.8)	930(3.2)	740(2.4)	4300(1.0)	700(2.4)	1600(1.5)	230(4.3)
Bi	—	—	—	—	—	1700(1.4)	—

(bkgd): Presence due to background only.

ED-1, ED-3 and ED-7: Dust extracted from lungs of bovine of different places in West Bengal.

ED-6: Dust extracted from lungs of caprine of West Bengal.

ED-8: Dust extracted from lymph nodes of bovine of West Bengal.

ED-2 and ED-5: Dust extracted from lungs of caprine of Orissa.

ED-4: Dust extracted from lungs of caprine of Bihar.

Values within parentheses represent percentage statistical uncertainties.

FIGURE 3 Lung of buffalo, perivascular and parabronchiolar deposits of siliceous coal dust. Hematoxylin and eosin, X 296.

FIGURE 4 Lung of buffalo, perivascular and parabronchiolar deposits of siliceous coal dust. Hematoxylin and eosin, X 296, under polarized light.

FIGURE 5 Tracheobronchial lymph node of goat, particulate deposits in paracortical region and medullary cords. Hematoxylin and eosin, X 95.

Analytical findings

Typical observed X-ray fluorescence spectra of representative samples from various regions are shown in Figures 6–9. Table I summarizes the analytical findings of the present investigation. The description of the samples analysed is given at the bottom of the table. It can be seen that the present investigation has revealed the presence of a number of elements in various concentrations in the dust samples studied. It has been noted that West Bengal samples are in general richer in trace elements as

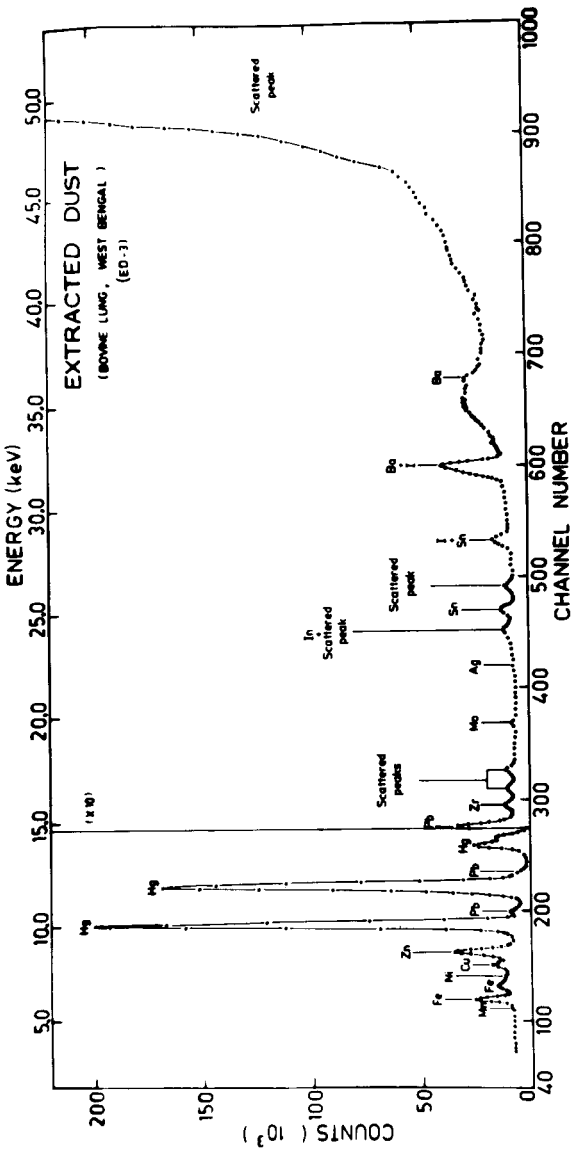

FIGURE 6 X-ray fluorescence spectrum of extracted dust from bovine lungs of West Bengal regions.

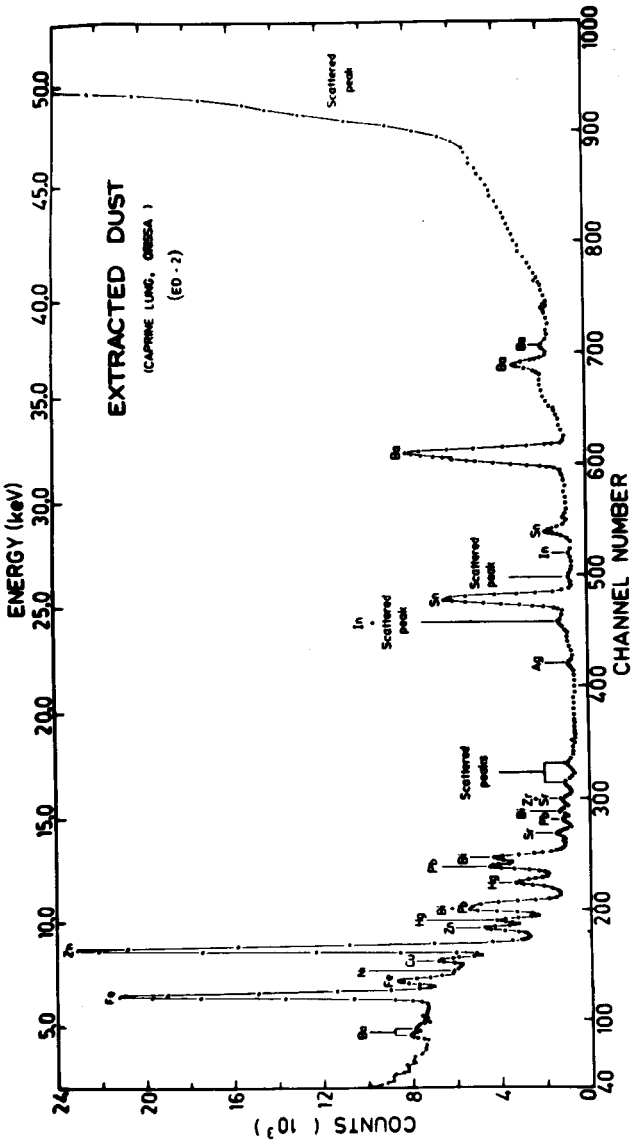

FIGURE 7 X-ray fluorescence spectrum of extracted dust from caprine lungs of Orissa regions.

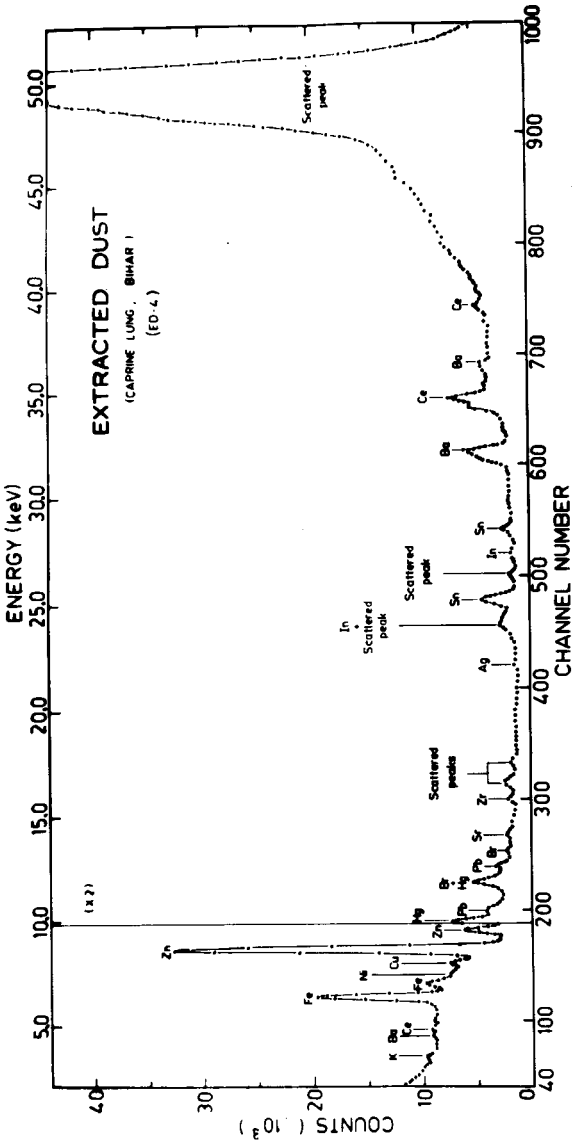

FIGURE 8 X-ray fluorescence spectrum of extracted dust from caprine lungs of Bihar regions.

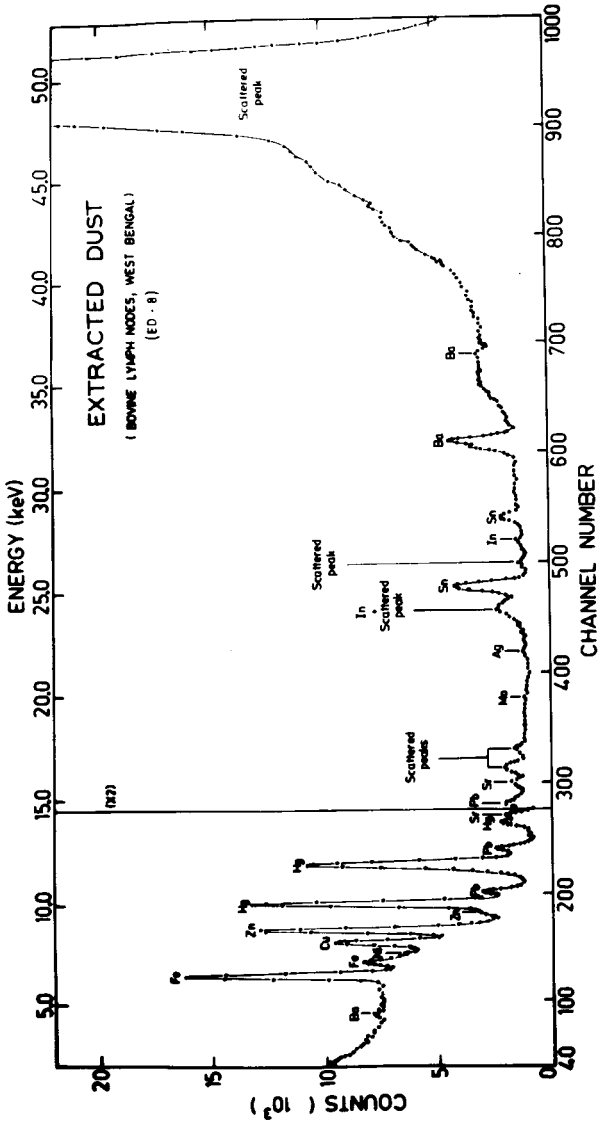

FIGURE 9 X-ray fluorescence spectrum of extracted dust from bovine lymph nodes of West Bengal regions.

compared to those of Bihar and Orissa. Mercury has been found in unusually large concentrations in West Bengal samples. Besides mercury, Fe, Cu, Zn and Pb are found to be present in appreciable amounts in almost all samples representing the three provinces.

DISCUSSION

While describing the recondite toxicity of trace elements in the biologic system, Schroeder[17] was of the opinion that elements like Al, Be, Cr, Fe, Sn, Sr, Ti and V accumulate in human lung with age and the elements which are absorbed from human lung into other tissues are Ba, Be, Cd, Cr, Mn, Pb, Sn and Sr. He further stated that under conditions of heavy exposure from mine dusts or badly polluted air of the industrialized area, metals which are absorbed from the lung could accumulate in the body and some of them cannot be eliminated easily. Harington[18] suggested the possibility that the metal constituents present in traces in different varieties of asbestos may play a role in asbestos carcinogenesis. Several metal complexes especially those containing iron have been shown[19] to induce cancer and several metals including Cr, Ni, Pb etc are carcinogenic in their own right.

In the present investigations, the finding that Cu, Zn and Pb are present in almost all dust samples, is of significance. The possibility of interaction of these elements with Zr, Sn, Ba and Mo etc. both at enzymatic and molecular level in the system, could not be excluded from the possible adverse effects at later periods.

The relatively high concentration of Hg in samples from West Bengal seems quite amazing. Monkman *et al.*[20] stated that traces of mercury in biologic and other natural materials including air are useful guides for locating sources of contamination and surveying health hazards. Recently mercury concentrations in different types of coal were reviewed,[21] estimated[22] and analysed,[23,24] and the content in coal samples ranged between 0.07 and 33 ppm in U.S.A. Also the presence of mercury in coal and a pitch coke plant in Germany was reported but the concentrations were significantly less than the MAK value for mercury.[25] Recently Falchuk *et al.*[26] emphasized that increasing quantities of mercury are both accumulating in the environment and entering the food chain of a variety of animals. The significance of this accumulation must be assessed especially from the functional consequences of its presence in the tissues and organs.

On the basis of the present exploratory study in which high concentration of mercury was detected in the lungs and lymph nodes of food animals especially derived from the West Bengal province it could

tentatively be predicted that similar exposure of mercury may also occur in the human population residing in such industrial and mining complexes. However, before it is accepted as a potential health risk, more systematic surveys are required to be carried out to confirm the present results of relatively high concentration of mercury in the environment and biologic system of some of the geographical areas.

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