# Determination of Trace Elements in Human Lung Samples

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## ABSTRACT

Lung samples from smokers, nonsmokers, and one stillborn were analyzed by an instrumental neutron activation analysis (INAA) method. Pulmonary tissue and hilum lymph node samples were obtained separately from autopsies, and then submitted to cryogenic homogenization, lyophilization, and sterilization. Short and long irradiations were performed in an IEA-R1 nuclear reactor, and γ-ray activities were measured using a Ge(Li) or hyperpure Ge detector. Precision of results was evaluated by analyzing one lung tissue in replicates and their accuracy by analyzing reference materials. Comparisons were carried out between results obtained in pulmonary tissues and lymph nodes, as well as those obtained in samples from different groups of individuals.

**Index Entries:** Instrumental neutron activation analysis; lungs; lymph nodes; pulmonary tissues; smokers; nonsmokers; stillborn.

## INTRODUCTION

Determination of trace elements in lung samples has been of great interest, since the inhalation of toxic atmospheric contaminants presents risk not only to the respiratory tract, but also to other organs of the body. Since the air and blood are in contact in the lungs, the inhaled toxic substances are transferred into the bloodstream, and dispersed in the body by both lymphatic and circulatory systems or else by mucocilliar depurations and swallowing.

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Epidemiological studies *(1–3)* and the reviews of several papers *(4,5)* have also shown that various inhaled particles or gases are capable of producing harmful health effects. Consequently, lungs are considered to be a critical organ.

In this context, the determination of normal levels of elements in pulmonary tissues of a selected population group has been very useful for a comparative study with results obtained for individuals exposed to pollutants or in the study of pulmonary diseases related to trace elements. In addition to direct determination of trace elements in the lung tissue, bronchoalveolar lavage has been suggested to be an indicator of lung disorders of occupational origin *(6)*. Analysis of the bronchoalveolar lavage is advantageous from the diagnostic point of view, since the collection of lung biopsies is traumatic and can present risk of complications to the patient. In the present work, INAA was used to determine the trace elements in pulmonary tissues and lymph nodes from smokers, nonsmokers, and from one stillborn.

## EXPERIMENTAL

### *Lung Sample Collection and Treatment*

Lung samples were obtained on autopsies performed at the Institute of Forensic Medicine of the São Paulo University. They were collected from seven smokers with age varying from 34 to 69 yrs and from six nonsmokers aged from 43 to 74 yr. As an internal control, a sample was collected from one unborn child of about 9 mo of age deceased by intrauterine events. The *causa mortis* of these donors were not chronic pulmonary diseases. The lung samples were collected within 6–12 h postmortem. Information concerning the residence, profession, smoking habits, and length of work of each individual was provided by their closest relatives.

About 10–30 g of pulmonary tissue macroscopically normal from the right inferior lobe and samples of lymph nodes from pulmonary hilum were wrapped in clean polyethylene foil, stored in liquid nitrogen, and sent to the laboratory, where these samples were stored in a freezer at a temperature below –10°C until cryogenic homogenization was carried out as follows.

The pulmonary tissue and lymph node samples were separately placed in a Teflon™ vessel immersed in liquid nitrogen. By using a Teflon™ pestle, the frozen solid material was fractured, ground, and then lyophilized. During freeze-drying, a mean weight loss of 82% was found. The resulting fine powder of each sample was sterilized by irradiation in a $^{60}Co$ source and placed in a polyethylene container that was stored in a dessicator.

To avoid contamination of the samples, a series of precautions were taken. A titanium knife was used for cutting the samples. A polyethylene spatula was used for their transfer. Handling of the samples was performed inside a glove box or a class 100 laminar flow hood.

### *Preparation of Standards*

Multielement and single-element standard solutions of appropriate concentrations were pipeted onto a small sheet of Whatman No. 42 filter paper using an Eppendorf pipet. After drying at room temperature, these sheets were placed into polyethylene bags and irradiated together with the samples.

### *Instrumental Neutron Activation Analysis*

Irradiations were performed at the IEA-R1 research nuclear reactor at the IPEN-CNEN/SP. Aliquots of 50–100 mg of pulmonary tissues or lymph nodes were weighed and heat-sealed in clean polyethylene bags. Two irradiations were carried out for each sample: Short irradiations (30 min) using pneumatic system facility, in a thermal neutron flux of $3.7 \times 10^{11}$ $n/cm^2/s$ to determine Cl, K, Mn, and Na, and long irradiations (16 h) in a thermal neutron flux of about $10^{13}$ $n/cm^2/s$ to determine Br, Cr, Cs, Fe, Hf, La, Rb, Sb, Sc, Se, Th, and Zn.

The irradiated samples and standards were fixed in stainless-steel planchets and counted using hyperpure Ge or Ge(Li) detector coupled to an EG & G Ortec 4096 channel pulse height analyzer connected to a Monydata PC 200 Plus microcomputer. The detectors used had a resolution (FWHM) better than 1.35 keV for the 122-keV γ-ray of $^{57}Co$ and 2.4 keV for the 1332-keV γ-ray of $^{60}Co$. The spectral data were processed using VISPECT software. Element concentrations were calculated by the comparative method.

### *Analysis of Reference Materials*

Four certified reference materials (CRMs), bovine liver 1577a from NIST USA, Bowen's kale from IUPAC, vehicle exhaust particulates No. 8 from NIES, Japan, and animal muscle H-4 from IAEA Austria, were analyzed for the evaluation of the accuracy of the method. The moisture content in reference materials was ascertained by drying in accordance with the producer's recommendations. The following values (in percent) of the weight loss were found and used for correcting the final results: 5.48 for bovine liver, 6.95 for H-4 animal muscle, 1.35 for vehicle exhaust particulate, and 12.77 for Bowen's kale.

## RESULTS AND DISCUSSION

Table 1 shows that our results obtained for the CRMs agree well with the respective certified or information values. The relative errors for most of the elements determined were found to be <10%. Precision of the results was also satisfactory for most elements. The relative standard deviations varied from 0.4 to 11%. Less precise results were obtained for Br and Fe in vehicle exhaust particulate reference material and for some elements present at the concentrations lower than 1 μg/g.

Table 1
Results of Control Analyses of Certified Reference Materials (µg/g Unless Otherwise Indicated)

| Element | Bovine Liver 1577a | | Bowen's Kale | | Vehicle Exhaust Partic. | | H-4 Animal Muscle | |
|---|---|---|---|---|---|---|---|---|
| | This Work | Literature Values (7) | This Work | Literature Values (7) | This Work | Literature Values (7) | This Work | Literature Values (8) |
| Br | 9.2+-0.6 (4)* | (9)** | 26.7+-1.7 (6) | 24.9+-2.5 | 67 +-12 (5) | (56) | 4.5+-0.4 (4) | 4.1+-0.6 |
| Cl | 2769+-141 (6) | 2800+-98 | 4443+-453 (8) | 3560+-427 | | | 2019+-55 (4) | 1890+-83 |
| Cr | 0.84+-0.19 (5) | | 0.89+-0.14 (4) | (0.369) | 23.5+-1.3 (4) | 24.5+-1.5 | | (0.010) |
| Cs, µg/kg | 18.56+-0.08 (2) | | 95+-10 (8) | 76.3+-5.9 | 246+-7 (4) | (240) | 135+-13 (5) | 120+-10 |
| Fe | 194+-12 (5) | 194+-19 | 120+-12 (9) | 119.3+-14.3 | 4510+-687 (4) | | 51.7+-4.6 (6) | 49+-3 |
| Hf, µg/Kg | | | | | 194+-4 (4) | | | |
| K, % | 0.992+-0.063 (8) | 0.996+-0.007 | 2.64+-0.16 (7) | 2.436+-0.146 | | 0.115+-0.008 | 1.56+-0.13 (3) | 1.58+-0.36 |
| La, µg/kg | | | 87+-20 (8) | 86.4+-13.0 | 1190+-50 (5) | (1200) | | |
| Mn | 10.7+-0.8 (9) | 9.9+-0.8 | 14.3+-3.2 (7) | 14.82** | | | 0.70+-0.30 (4) | 0.52+-0.04 |
| Na | 2447+-154 (9) | 2430+-129 | 2583+-258 (8) | 2366+-284 | | | 2073+-87 (4) | 2060+-126 |
| Rb | 12.1+-0.4 (5) | 12.5+-0.1 | 54+-6 (9) | 53.4+-5.3 | 4.0+-0.2 (5) | (4.6) | 19.5+-1.6 (6) | 19.0+-1.5 |
| Sb, µg/kg | | | 70+-18 (4) | 68.5** | 5990+-360 (4) | 6000+-40 | | |
| Sc, µg/kg | | | 15.1+-1.0 (9) | 9.48** | 564 +-21 (4) | (550) | | |
| Se | 0.72+-0.07 (5) | 0.71+-0.07 | | | 1.45+-0.04 (5) | (1.3) | 0.32+-0.05 (4) | 0.33+-0.03 |
| Th, µg/kg | | | 12.2+-2.2 (5) | 10.4** | 344+-9 (4) | (350) | | |
| Zn | 127+-4 (5) | 123+-8 | 33.7+-2.3 (9) | 32.26+-2.74 | 879+-39 (5) | 1040+-50 | 90+-6 (6) | 86+-3 |

*Number of determinations.
**Information values.

Table 2
Precision of Analysis of One Sample of Pulmonary Tissue in
(μg/g, dry wt, Unless Otherwise Indicated)

| Element | XM +- S (N) | SR, % |
|---|---|---|
| Br | 16.8 +- 1.5 (4) | 8.9 |
| Cl, % | 1.73 +- 0.18 (5) | 10.4 |
| Cs | 0.96 +- 0.01 (4) | 1.0 |
| Fe | 2423 +- 73 (4) | 3.0 |
| K, % | 0.82 +- 0.05 (6) | 6.0 |
| Mn | 1.46 +- 0.15 (3) | 10.2 |
| Na, % | 1.42 +- 0.14 (5) | 9.8 |
| Rb | 32.8 +- 0.7 (4) | 2.1 |
| Sb | 0.30 +- 0.02 (4) | 6.7 |
| Sc ug/kg | 1.39 +- 0.07 (4) | 5.0 |
| Se | 0.66 +- 0.03 (4) | 4.5 |
| Zn | 69 +- 2 (4) | 2.9 |

$X_M \pm S$—arithmetic mean and standard deviation.
$S_R$—relative standard deviation.
*n*–number determinations

The precision of the results was also evaluated by analyzing one sample of pulmonary tissue in replicate as shown in Table 2. Element concentrations found in the pulmonary tissues from smokers, nonsmokers, and stillborn are presented in Table 3 together with the data published by Vanoeteren et al. *(5)* for comparison. It can be seen from this table that the elements determined exhibit considerable intersubject variability. However the results for nonsmokers are within the range reported in the literature, except for the elements Cl and Hf.

Table 4 shows the results obtained for lymph node samples from nonsmokers, smokers, and stillborn. No literature data for this tissue were found for comparison.

The concentration levels of the trace elements in pulmonary tissues from smokers and nonsmokers are, in general, of the same order of magnitude. One exception was Sb, for which a higher value was found for smokers. The results for lymph nodes (Table 4) indicate the same pattern as observed for the lung samples. This finding indicates that hilum

Table 3
Elemental Concentrations of Pulmonary Tissue of Nonsmokers (NS), Smokers (S), and Stillborn (SB) (dry wt)

| Element | Smoking Habit | $X_M$ +- s | $X_G$ x: $S_G$ | Median | Range | Vanoeteren et al[5] Range |
|---|---|---|---|---|---|---|
| Br (ug/g) | NS | 29 +- 11 | 27 x: 1.46 | 28 | 17—46 | 2—120 |
| | S | 29 +- 8 | 28 x: 1.30 | 27 | 19—40 | |
| | SB | 22.4 +- 0.4** | | | | |
| Cl (%) | NS | 1.4 +- 0.4 | 1.3 x: 1.33 | 1.2 | 1.0—1.9 | 0.5—1.1 |
| | S | 1.1 +- 0.2 | 1.1 x: 1.22 | 1.0 | 0.9—1.6 | |
| | SB | 1.46 +- 0.04 | | | | |
| Cr (ug/g) | NS | 1.6 +- 1.0 | 1.4 x: 1.76 | 1.3 | 0.6—3.4 | 0.01—2.5 |
| | S | 1.4 +- 0.7 | 1.3 x: 1.72 | 1.2 | 0.5—2.5 | |
| | SB | 0.6 +- 0.1 | | | | |
| Cs (ug/g) | NS | 0.29 +- 0.07 | 0.28 x: 1.33 | 0.31 | 0.16—0.35 | 0.015—1.5 |
| | S | 0.18 +- 0.05 | 0.18 x: 1.29 | 0.18 | 0.12—0.24 | |
| | SB | 0.15 +- 0.01 | | | | |
| Fe (%) | NS | 0.22 +- 0.09 | 0.20 x: 1.64 | 0.23 | 0.08—0.32 | 0.02—0.25 |
| | S | 0.17 +- 0.08 | 0.16 x: 1.50 | 0.14 | 0.10—0.32 | |
| | SB | 0.106 +- 0.001 | | | | |
| Hf (ug/kg) | NS | 22 +- 8 | 21 x: 1.41 | 20 | 15—38 | (0.01) |
| | S * | 24 +- 17 | 20 x: 1.87 | 17 | 12—49 | |
| K (%) | NS | 0.9 +- 0.1 | 0.9 x: 1.16 | 0.9 | 0.8—1.2 | 0.25—1.0 |
| | S | 1.0 +- 0.2 | 0.9 x: 1.24 | 0.9 | 0.7—1.3 | |
| | SB | 0.86 +- 0.12 | | | | |
| La (ug/g) | NS | 0.3 +- 0.1 | 0.3 x: 1.48 | 0.2 | 0.2—0.5 | 0.005—1 |
| | S | 0.28 +- 0.09 | 0.26 x: 1.48 | 0.29 | 0.13—0.38 | |
| Mn (ug/g) | NS | 1.3 +- 0.3 | 1.2 x: 1.24 | 1.2 | 1.0—1.6 | 0.05—15 |
| | S | 1.3 +- 0.4 | 1.2 x: 1.44 | 1.2 | 0.7—1.8 | |
| | SB | 1.2 +- 0.5 | | | | |
| Na (%) | NS | 6.0 +- 0.4 | 1.0 x: 1.39 | 1.0 | 0.6—1.6 | 0.5—1.5 |
| | S | 7.0 +- 0.3 | 1.0 x: 1.31 | 1.0 | 0.6—1.5 | |
| | SB | 1.19 +- 0.01 | | | | |
| Rb (ug/g) | NS | 32 +- 6 | 31 x: 1.19 | 31 | 26—39 | 2.5—50 |
| | S | 27 +- 7 | 27 x: 1.30 | 23 | 19—37 | |
| | SB | 33.9 +- 0.5 | | | | |
| Sb (ug/g) | NS | 0.29 +- 0.07 | 0.28 x: 1.29 | 0.31 | 0.20—0.36 | (0.01—0.5) |
| | S | 0.7 +- 0.5 | 0.5 x: 2.52 | 0.9 | 0.14—1.2 | |
| | SB | 0.04 +- 0.01 | | | | |
| Sc (ug/g) | NS | 0.03 +- 0.03 | 0.025 x: 1.97 | 0.02 | 0.01—0.08 | 0.0005—0.035 |
| | S | 0.02 +- 0.02 | 0.013 x: 2.71 | 0.02 | 0.003—0.05 | |
| | SB | 0.002 +-0.0007 | | | | |
| Se (ug/g) | NS | 0.6 +- 0.1 | 0.6 x: 1.22 | 0.6 | 0.5—0.9 | 0.25—25 |
| | S | 0.6 +- 0.1 | 0.6 x: 1.22 | 0.6 | 0.5—0.8 | |
| | SB | 0.6 +- 0.1 | | | | |
| Th (ug/g) | NS | 0.03 +- 0.01 | 0.03 x: 1.41 | 0.03 | 0.02—0.05 | (0.005—0.1) |
| | S | 0.04 +- 0.03 | 0.03 x: 2.29 | 0.02 | 0.01—0.10 | |
| Zn (ug/g) | NS | 73 +- 21 | 71 x: 1.28 | 66 | 58—113 | 5—150 |
| | S | 57 +- 4 | 57 x: 1.08 | 58 | 52—65 | |
| | SB | 67.7 +- 0.8 | | | | |

Literature values given on wet wt basis were converted to dry wt basis using a factor of 0.2 according to Ref (5)

Values in parenthesis are speculative data.

*Results for four samples.

**Statistical counting errors.

$X_M \pm S$—Arithmetic means and their standard deviations.

$X_G$ X:$S_G$—Geometric means and their standard deviations.

lymph nodes may be used as an indicator of elemental profile in the lungs because of their physiological function of draining and clearance of exogenous materials. Especially for epidemiological purposes, it is important to choose a determined sampling point because of the possible differences in distribution of elements in lungs.

Elements Hf, La, Sb (in lymph node), and Th were not detected in the sample from stillborn. The levels of Cr, Cs, Sb, and Sc were significantly lower in stillborn than those for adults. This comparison indicates

Table 4
Elemental Concentrations of Lymph Nodes for Nonsmokers (NS), Smokers (S), and Stillborn (SB) (dry wt)

| Element | Smoking Habit | $x_M$ +- s | $x_G$ x: $s_G$ | Median | Range |
|---|---|---|---|---|---|
| Br (µg/g) | NS | 17 +- 6 | 16 x: 1.37 | 14 | 12—25 |
| | S | 11 +- 7 | 9 x: 1.73 | 8 | 5—26 |
| | SB | 13.8 +- 0.5 | | | |
| Cl (%) | NS | 0.89 +- 0.11 | 0.88 x: 1.14 | 0.88 | 0.7—1.0 |
| | S | 0.65 +- 0.21 | 0.61 x: 1.51 | 0.67 | 0.26—0.88 |
| | SB | 1.03 +- 0.05 | | | |
| Cr (µg/g) | NS | 5.9 +- 1.5 | 5.8 x: 1.28 | 5.6 | 4.2—8.2 |
| | S | 8.8 +- 11.0 | 5.3 x: 2.82 | 6.6 | 1.7—33.2 |
| | SB | 0.62 +- 0.08 | | | |
| Cs (µg/g) | NS | 0.49 +- 0.25 | 0.44 x: 1.72 | 0.40 | 0.20—0.82 |
| | S | 0.21 +- 0.08 | 0.19 x: 1.60 | 0.21 | 0.07—0.29 |
| | SB | 0.10 +- 0.01 | | | |
| Fe (%) | NS | 0.23 +- 0.15 | 0.19 x: 2.05 | 0.21 | 0.06—0.47 |
| | S | 0.15 +- 0.08 | 0.13 x: 1.66 | 0.13 | 0.06—0.30 |
| | SB | 0.073 +- 0.001 | | | |
| Hf (µg/g) | NS | 0.63 +- 0.86 | 0.35 x: 3.15 | 0.33 | 0.1—2.36 |
| | S | 0.13 +- 0.15 | 0.07 x: 3.51 | 0.06 | 0.01—0.45 |
| K (%) | NS | 0.70 +- 0.17 | 0.68 x: 1.33 | 0.77 | 0.42—0.89 |
| | S | 0.59 +- 0.18 | 0.56 x: 1.39 | 0.56 | 0.33—0.84 |
| | SB | 0.65 +- 0.04 | | | |
| La (µg/g) | NS | 1.5 +- 0.5 | 1.4 x: 1.56 | 1.5 | 0.6—2.2 |
| | S | 1.1 +- 0.8 | 0.8 x: 2.29 | 0.9 | 0.4—2.2 |
| Mn (µg/g) | NS | 7.1 +- 3.4 | 6.4 x: 1.68 | 7.1 | 3.4—10.6 |
| | S | 5.0 +- 3.5 | 3.8 x: 2.39 | 5.0 | 0.9—11.7 |
| | SB | 0.15 +- 0.05 | | | |
| Na (%) | NS | 0.9 +- 0.1 | 0.9 x: 1.12 | 0.9 | 0.8—1.0 |
| | S | 0.7 +- 0.2 | 0.7 x: 1.41 | 0.7 | 0.36—0.93 |
| | SB | 0.967 +- 0.006 | | | |
| Rb (µg/g) | NS | 23 +- 7 | 22 x: 1.37 | 22 | 14—34 |
| | S | 17 +- 7 | 16 x: 1.59 | 19 | 8—27 |
| | SB | 22.4 +- 0.4 | | | |
| Sb (µg/g) | NS | 1.7 +- 1.1 | 1.3 x: 2.37 | 1.7 | 0.3—3.2 |
| | S | 5.7 +- 8.5 | 2.6 x: 3.68 | 2.2 | 0.5—24.1 |
| Sc (µg/g) | NS | 0.43 +- 0.25 | 0.36 x: 1.98 | 0.45 | 0.14 - 0.66 |
| | S | 0.17 +- 0.16 | 0.09 x: 4.09 | 0.13 | 0.01 - 0.42 |
| | SB | 0.0042+-0.0006 | | | |
| Se (µg/g) | NS | 0.94 +- 0.23 | 0.92 x: 1.31 | 1.0 | 0.6—1.2 |
| | S | 0.78 +- 0.29 | 0.74 x: 1.44 | 0.7 | 0.5—1.3 |
| | SB | 0.49 +- 0.08 | | | |
| Th (µg/g) | NS | 0.35 +- 0.14 | 0.33 x: 1.56 | 0.37 | 0.18—0.50 |
| | S | 0.20 +- 0.17 | 0.13 x: 3.02 | 0.12 | 0. 3—0.45 |
| Zn (µg/g) | NS | 67 +- 7 | 67 x: 1.10 | 67 | 57—77 |
| | S | 68 +- 50 | 58 x: 1.73 | 59 | 36—178 |
| | SB | 68.3 +- 0.4 | | | |

that several elements accumulate in the lungs from air pollution and/or from tobacco smoke.

## ACKNOWLEDGMENTS

The authors are grateful to CNPq and FAPESP from Brazil for financial support.

## REFERENCES


1. N. Neuberger and M. Kundi, *Arch. Geschwulstforch* **61,** 47–53 (1991).
2. J. T. Hodgson and R. D. Jones, *Br. J. Ind. Med.* **47,** 665–676 (1990).
3. J. C. Mc Donald, B. W. Case, P. E. Enterline, V. Henderson, A. D. Mc Donald, M. Plourde, and P. Sebastien, *Ann. Occup. Hyg.* **34(5),** 427–441 (1990).
4. M. Chiba and R. Masironi, Toxic and trace elements in tobacco, World Health Organization/TOH/CLH/91.1, 1991.
5. C. Vanoeteren, R. Cornelis, and E. Sabbioni, Critical evaluation of normal levels of major and trace elements in human lung tissue, EUR 10440 EN, March 1986.
6. E. Sabbioni, R. Pietra, F. Mousty, F. Colombo, G. Rizzato, and G. Scansetti, *J. Radioanal. Nucl. Chem., Articles* **110(2),** 595–601 (1987).
7. E. Cortes Toro, R. M. Parr, and S. A. Clements, Biological and environmental reference materials for trace elements and organic microcontaminants. IAEA/RL/128 (Rev.1) Vienna, IAEA, 1990.
8. Y. Muramatsu and R. M. Parr, Survey of currently available reference materials for use in connection with the determination of trace elements in biological and environmental materials, IAEA/RL/128 Vienna, IAEA, Dec. 1985.