Annex 10

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Particulate Matter and Health - from Air to Human Lungs

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Abstract

The aim of this project is to search for respiratory system particular aggressors to which workers are submitted in their labouring activity. The work plan under the current IAEA contract comprise a prospective study to identify particulate matter deposited in the human respiratory ducts and lung tissue and workers respiratory health status survey at a steel plant, *Siderurgia Nacional* (SN). So far, the selection of areas of interest at SN, workers exposed, airborne particulate monitoring sites according to the periodicity of labouring cycles, and the beginning of workers medical survey have been achieved and/or initiated. The SN selected area, where steel is processed and steel casting is achieved, involve approximately 80 workers, most of them working at that location for more than 15 years. Blood elemental content data determined by PIXE and INAA and a preliminary health status evaluation from 32 of the 80 workers included in this survey are presented and discussed.

1. Introduction

The awareness of environment degradation and of indoor pollution in a way that may influence health has increasingly been attested by several epidemiological and toxicological studies [1-4]. However, despite the considerable large amount of data on air constituents that has been gathered in the past decades, the verified levels of pollutants are not always compatible with the magnitude of respiratory complains. Actual concern on influence of particulate matter size in the respiratory health and on restricted milieus arose, among other reasons, from the awareness that dispersion and air recycling in confined places are less efficient than in natural conditions [4]. Therefore, the indoor exposure dose of an individual may be considerably higher and probably more hazardous than in a natural environment. Thus, scientific evaluation of the links between air pollution and respiratory symptoms seem to be incomplete.

Some metals have been pointed out to be toxic to cells and carcinogenic, as V, Cr, Co, Ni, Cd, and Pb. These elements are often associated with the particulate matter fraction of inhaled air constituents [3-6]. Also, asbestos and typical indoor pathogens as dust mites are known to increase respiratory responsiveness and prevalence of sensitisation. Individual exposure to such agents, usually related with professional activity, may lead to interstitial lung diseases and to the progression of lung fibrosis [2,7]. Lately, some organic constituents of the environmental tobacco smoke are being connected directly with cancer. Aside all knowledge acquired, how particulate matter interact, how and where it deposits in the human respiratory ducts are problems that have been only approached by theoretical models [8].

The working environment monitoring and workers respiratory health control is far from being routinely performed in many Portuguese industries. Even in those, where the generation of dust and gases is significant, as the case of the steel industry, Siderurgia Nacional (SN), data on air quality parameters are scarce. Also, the workers medical exams performed periodically by the company physicians do not account for respiratory health monitoring. Therefore, statistic data on the harmful effects of airborne particulate in human beings inferred through affections or complains of workers exposed have not been regularly produced.

In the particular case of Portugal, some epidemiological surveys at industrial areas have been carried out since 1972. From 1974 to 1980, 3359 steel industry workers and 400 cement industry workers have been studied aiming at the evaluation of their respiratory health. It was then observed a high incidence of upper respiratory system affections, although lung diseases had low occurrence [9,10]. Also, for cement industry workers the duration of exposure was found to be related with the lung capacity function [10].

On the other hand, an evaluation of airborne suspended matter at several working places of the steel plant SN took place in 1988. The objectives were then, to initiate a survey to establish human exposure, although the project was not accomplished. However, the airborne particulate matter elemental characterisation have been performed since 1993 (some projects under the IAEA co-operation programmes) both for rural and urban areas, that include the industrial belt where SN is located.

More recently, collaboration with medical teams, permitted to start a prospective study to identify particulate matter deposited at respiratory ducts and to draw up a reference data set for healthy individuals [11]. The increase of constitutive and exogenous elements data basis for the human respiratory system as well as a micro-distribution approach of elements in the tissues, are objectives to be achieved in a near future, in collaboration with the Portuguese TXRF group (INETI) and the Oxford

Nuclear Microprobe group (Oxford, England). The need to assess this information may be crucial to the understanding of sensitiveness and response of the respiratory system to a specific aggressor. Also, the recognition of specific particles of atmospheric origin in the human respiratory system may give information about its preferential size and elemental composition as well as the deposition local. Therefore, health effects of the inhaled particulate matter, in specific environments as working places may be foreseen. Moreover, incentives to the environmental health research will eventually permit the establishment of more adequate air quality standards for indoor and outdoor environments.

2. Methods

2.1. Worker's Medical Inspection

The lung function evaluation was performed with a Vitalograph Compact II spirometer equipment that enables the determination of flow volume curves. The basal values were registered before a non-specific bronchial provocation test was done, using metacholine, to determine an eventual bronchial hyper-reactivity.

2.2. Blood Samples Collection and Preparation Procedures

Blood for elemental analysis purposes was drawn to polycarbonate centrifuge tubes using standard methods [12] to avoid contamination. All containers were previously thoroughly cleaned with analytical grade nitric acid and serum separated from the packed blood cells (PBC) fraction by centrifuging with 10000 rpm during 30 min at 4°C. All samples were freeze-dried and stored at -30°C. The wet and dry weight were determined for both blood fractions. Serum and PBC sub-samples to be irradiated for Neutron Activation Analysis purposes were separated to special polyethylene containers.

The samples to be analysed by PIXE were then subjected to an acid digestion procedure with Suprapure® nitric acid and hydrogen peroxide, together with Y as an internal standard in closed Teflon™ vessels (Parr® Bombs for microwave digestion). A commercial microwave oven was employed for sample digestion purposes using 300W power during 3min. Targets for PIXE analysis were produced by pipetting 10 μl of the resulting solution on to a 1.5 μm thick polycarbonate film previously washed with suprapure® nitric acid to make it hydrophilic. At least three targets are analysed for each sample.

2.3. PIXE and INAA Techniques

For all blood samples collected elemental analysis were carried out by PIXE. Some of these samples were also analysed by INAA technique.

The PIXE experimental set-up is installed at the ITN 3 MV Van de Graaff accelerator. The samples were irradiated with a 2.4 MeV proton beam under vacuum keeping the current density at the target below 250 $nA.cm^{-2}$. X-rays were detected by a Link Si(Li) detector, positioned at an angle of 110° relative to the beam direction, in front of a chamber window of 6.3μm Mylar™. To absorb the low energy radiation originating from the matrix and minor elements of the sample, an absorber of 350 μm Mylar™ was used between the detector and the chamber. The PIXE spectra were analysed by means of a non-linear least-squares fitting program AXIL [13] and the concentration calculations were performed with the DATTPIXE program [14].

The INAA experiments were performed at the ITN Portuguese Research Reactor – RPI. The homogenised powdered serum and PBC (approximately 100 to 200 mg) were kept *in a small container* and irradiated. Short irradiation took place at the RPI pneumatic system with thermal neutron flux of 2.8×10^{12} $cm^{-2}s^{-1}$. Longer irradiation of 5h was carried out in the core grid with typical neutron flux 1.2×10^{13} $cm^{-2}s^{-1}$. The k_0*-method* [15] was applied to elemental quantification purposes; the 0.1% Au-Al wire was used as comparator. For the gamma measurement a hyperpure germanium detector (1.76 keV resolution at 1.33 MeV was used. The spectra were fitted with SEQAL computer code and elemental concentrations were calculated with SOLANG and SINGCOMP programs.

3. Results

The SN selected area, where steel is processed and steel casting is achieved, involve approximately 80 workers. The sector is a confined area so, the permanence of airborne particulate matter at that working area may be larger, despite the existence of ventilator devices. Consequently, workers may be more continuously exposed to dust as long as a time scale is concerned.

The blood elemental contents were assessed for 32 workers by PIXE and for 12 of these by INAA. The PIXE technique permitted the determination of 9 elements in blood, i.e., K, Ca, Fe, Cu, Zn, Se, Rb and Pb. The available INAA results are relative to 8 elements, i.e., Mg, Na, Al, Cl, K, Mn, Br and Mo. In Table 1 the elemental contents determined by PIXE are listed, and compared with a Portuguese data base obtained for an urban healthy population [16] that will be taken as control values. Reference data set was found to be comparable with literature reported values [12,16].

A significant concentration decrease was found for Zn and Se in serum and K, Fe and Se in PBC. On the other hand an increase of K concentration in serum is verified (that may derive from incomplete serum and PBC fractions separation) while Ca is enhanced in PBC fraction. The Se concentration values obtained are close to PIXE detection limit (approximately 0.4 µg/g). Se average values are based on 28 individuals for serum samples and only 10 individuals for PBC samples.

Table 1 - Mean elemental concentrations and associated standard deviations ($x \pm st$) in $\mu g.g^{-1}$ dry weight based on N individuals and wet-to-dry weight ratio (RF) for serum and packed blood cells. Reference values listed are relative to a healthy population not directly exposed [16].

	Serum (N=32) x ± st	Serum Reference Values (N=27) x ± st	PBC N=32 x ± st	PBC Reference Values (N=27) x ± st
K	4473 ± 2212 *	2100 ± 423	8740 ± 1460 *	10820 ± 750
Ca	665 ± 374 *	1149 ± 129	202 ± 65 *	76 ± 26
Mn				0.77 ± 0.21
Fe	58 ± 38	50 ± 23	2845 ± 138 *	3085 ± 126
Cu	11.7 ± 3.0	13.3 ± 4.7	4.07 ± 0.87 *	2.38 ± 0.36
Zn	13.7 ± 3.2 *	9.6 ± 2.0	37 ± 6	34 ± 5
Se	0.59 ± 0.18 *	1.72 ± 0.26	0.4 ± 0.3 (*)	0.66 ± 0.11
Rb	8.6 ± 6.4 *	3.1 ± 1.0	15.2 ± 4.0	17.7 ± 2.9
Pb			0.95 ± 0.65	1.02 ± 0.03
FR	10.7 ± 1.3	12.6 ± 1.1	3.4[a] ± 0.2	3.7 ± 0.4

* Significant difference for p<0.01 in a Student t-test [17]

(*) Significant difference for a 0.05<p<0.1 in a Student t-test [17]

[a] N=19

Therefore, uncertainties on the Se determination by PIXE are likely the responsible for the deviation observed. The mean K, Ca, Fe and Zn values differences encountered with respect to the urban population, although significant for a $p<0.01$ have large standard deviations that for most of the cases overlap with reference group average values. Therefore, more cases have probably to be analysed to decide about the significance of differences encountered.

Comparing INAA results with literature values or with the reference Portuguese group a steady decrease of K values is observed (Fig.1). Literature reported average value for serum K is 2000 $\mu g.g^{-1}$ dry weight while in PBC, K contents have minimum values of 10000 $\mu g.g^{-1}$ and does not exceed 14000 $\mu g.g^{-1}$ [12,16]. For the apparent systematic deviation of K content, determined by INAA, the influence of sample preparation can not be invoked.

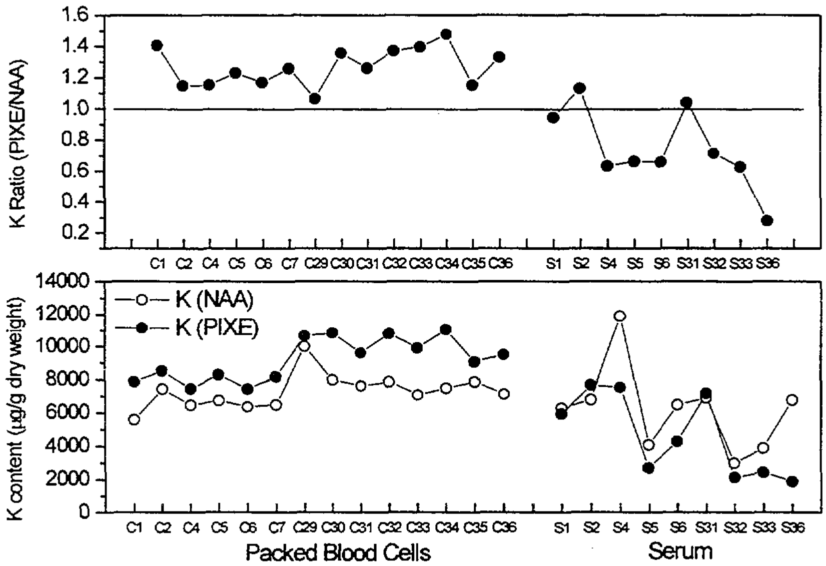

Fig. 1 - Comparison of K contents in packed blood cells and serum samples determined by PIXE and INAA.

Concerning the worker's health status evaluation, results are still not conclusive. As can be observed in Fig. 2, the number of years of exposure, smoking habits and respiratory symptoms are not clearly associated. So far, 18% of individuals observed have an apparent respiratory affection (lung function tests are not still completed evaluated neither thoracic radiographs). Also, if elemental contents are evaluated with respect to smoking habits, any significant differences are found between the three groups established: non-smoking, smoking and ex-smoking persons. The results obtained for serum and PBC are listed in Tables 2 and 3.

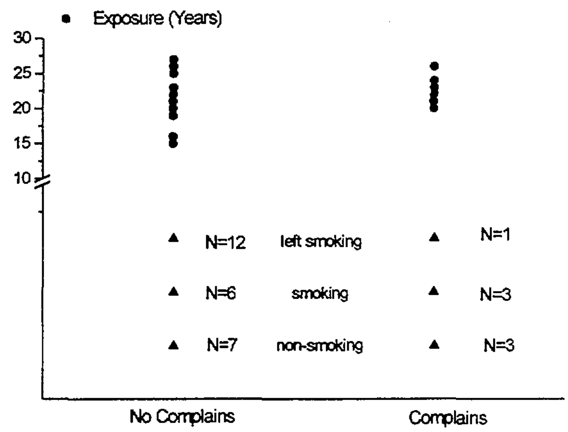

Fig.2 – Respiratory complains with respect to smoking habits and total number of years of exposure.

Table 2 - Elemental contents (x±st) in μg.g^{-1} dry weight in serum. The groups were established according to smoking habits (1 for non-smoking, 2 for smoking and 3 for ex-smoking). The elemental contents determined by INAA are indicated in table.

	Group 1 x ± st	N	Group 2 x ± st	N	Group 3 x ± st	N
Exposure (years)	21.6 ± 4.0	9	21.1 ± 3.5	10	21.4 ± 4.0	13
K	3300 ± 2041	9	4982 ± 2000	10	4892 ± 2332	13
Ca	809 ± 273	9	640 ± 500	10	586 ± 321	13
Fe	42 ± 20	9	70 ± 48	10	60 ± 38	13
Cu	13 ± 3	9	11.7 ± 2.6	10	12 ± 1	13
Zn	13 ± 2	9	14.3 ± 4.9	10	14 ± 2	13
Se	0.65 ± 0.18	9	0.47 ± 0.22	10	0.49 ± 0.26	13
Rb	6 ± 3	9	7 ± 5	10	11 ± 7	13
Ni	2.4	9	0.71 ± 0.12	10	0.76 ± 0.26	13
Sr	2.6	1	2.34 ± 0.96	3	4.4 ± 5.6	5
Na (INAA)	26500 ± 1480	5	28225 ± 1810	4	27175 ± 2990	4
Al (INAA)	576	1	±		±	
Cl (INAA)	27780 ± 3380	5	31250 ± 1723	4	30255 ± 3350	4
K (INAA)	5450 ± 1910	2	8530 ± 2920	3	4930 ± 1740	4
Br (INAA)	16 ± 2	3	27	1	24.5	1

Table 3 - Elemental contents (x±st) in µg.g^{-1} dry weight in packed blood cells. The groups established refer to smoking habits (1 for non-smoking, 2 for smoking and 3 for ex-smoking habits). The elemental contents determined by INAA are indicated in table.

	Group 1 x ± st	N	Group 2 x ± st	N	Group 3 x ± st	N
Exposure (years)	21.6 ± 4.0	9	21.1 ± 3.5	10	21.4 ± 4.0	13
K	9700 ± 1240	9	8380 ± 1320	10	8350 ± 1480	13
Ca	160 ± 62	9	220 ± 19	10	216 ± 80	13
Fe	2830 ± 130	9	2840 ± 100	10	2860 ± 170	13
Ni	1.7 ± 0.9	9	1.6 ± 1.1	10	1.9 ± 1.1	9
Cu	4.2 ± 0.7	9	4.3 ± 1.2	10	3.8 ± 0.7	13
Zn	39 ± 8	9	36 ± 6	10	36 ± 5	13
Se	<MDL		<MDL		0.24 ± 0.32	13
Rb	16 ± 5	9	15 ± 4	9	15 ± 4	13
Sr	2.5	1	< MDL		1.4	1
Pb	0.95 ± 0.79	6	0.87 ± 0.79	5	1.1 ± 0.3	4
Mg (INAA)	131 ± 51	3	159 ± 14	2	143 ± 53	2
Na (INAA)	2950 ± 490	5	2880 ± 620	4	2670 ± 707	5
Cl (INAA)	6860 ± 714	5	6850 ± 320	4	6500 ± 785	5
K (INAA)	7210 ± 570	5	7810 ± 1530	4	6980 ± 1010	5
Mn (INAA)	0.26 ± 0.01	2			0.83	1
Br (INAA)	5.12 ± 1.70	4	5.9 ± 0.8	3	5.4 ± 0.8	4
Mo (INAA)	0.50 ± 0.23	2	0.53	1	0.74	1

If cases and/or variables in serum are examined for their similarity using a statistical procedure called cluster analysis [17], the individuals that had respiratory complains, i.e. 3, 9, 17, 19, 20, 21 and 23, are aggregated in the same group, as can be observed in Fig. 3. The variables used in the statistical analysis were the elements determined by PIXE, as referred above, total number of working years at the SN steel casting sector (variable "Yexp"), smoking habits (variable "SmHa") and occurrence of complains (variable "Complain"). On the other hand, for PBC samples the separation of cases with positive symptoms is not evident. The major feature found for the PBC data set, is the separation of two large groups that identify individuals where Pb concentration is more elevated and individuals with lower Pb blood content.

The variables were also clustered to infer any common behaviour or identification of similar episodes, and the resulting dendrogram are displayed below the correspondent cases agglomeration in Fig. 3. It can then, be observed that for both serum and PBC data sets the occurrence of complains is consistently associated with the total working years.

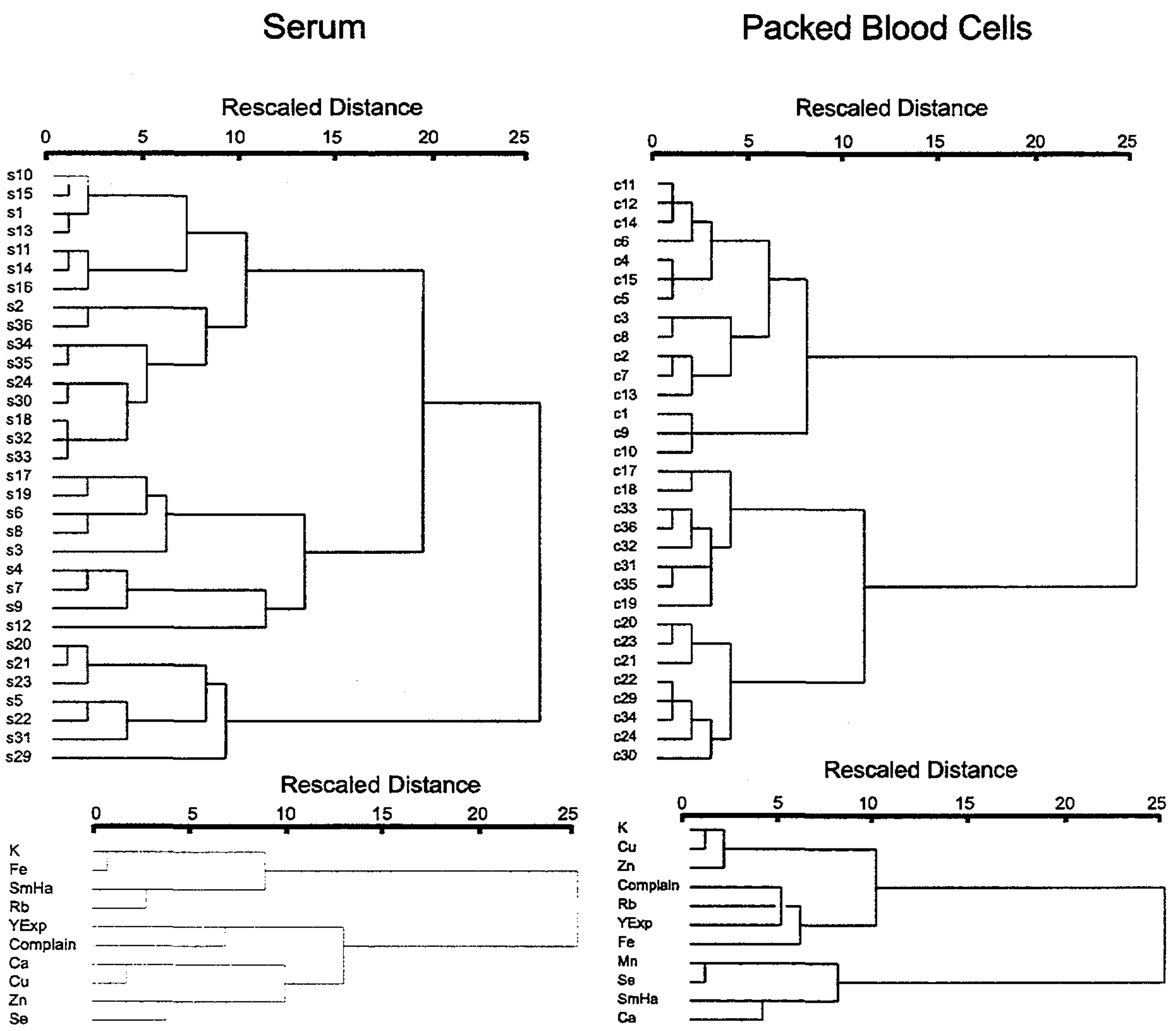

Fig. 3 – Dendrograms for Serum and PBC data sets. Both cases and variables are grouped. The Ward's method was used for the agglomeration procedure and the distance measurement obtained by the squared Euclidean distance method.

5. Plans for Future Work

The progression of the work plan, as presented in the research contract proposal submitted to the IAEA Co-ordinated Research Programme, will permit:

- the continuation of elemental analysis on samples already collected: blood and aerosol samples to check whether the elemental associations and alterations found in blood derive from exposure;
- to collect air particulate air samples repeatedly (not only the survey that is starting in October), that will allow to estimate indoor air quality and airborne particulate matter characterisation using both PIXE and INAA techniques;

- to continue medical examinations to workers exposed in order to infer and/or confirm any alteration that can be related to the working environment exposure;
- to gather data on respiratory system particulate matter deposition, through autopsy samples collected from healthy individuals and occasionally from individuals with respiratory affections due to professional activity (data is being collected since June 97 although elemental analysis had not yet been initiated). Samples will be analysed by PIXE and INAA whenever possible.
- Therefore, increasing data both from indoors airborne particulate and health status indicators (lung function response, blood exogenous elemental burden, etc.) will conduct to a better approach of risks to human respiratory health. Also, data gathered on a routine basis on air particulate matter elemental characterisation from an area comprising the Siderurgia Nacional and living area of individuals observed, will be useful to identify similar elemental combinations that may be found at the human respiratory system.

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