Health and Environmental Studies Using Ion Beams

The group main activities are centred in applications of nuclear and nuclear-related techniques to different spheres of science, such as, Biology and Biomedicine, Eco-toxicology and Environment.

During the past five years, collaborations have been strengthened between other groups from ITN and University groups, where the share of different analytical facilities and expertise is the outstanding feature.

The work developed allowed the elaboration of proposals that partially become supported by international and national organisms for R&D financing.

The work has been centred in the study of trace element physiology, and on the response of living organisms to the presence of xenobiotic agents in the ecosystem. The running projects, so far, combine trace element, biochemical and physiological indicators that enable to infer the status of populations surveyed, with the purpose of interpreting pathological or toxicological mechanisms. However, for many toxicological relevant indicators, the analytical capabilities of ITN are already inadequate. The levels of chemical elements or are too low, or there are interference of other matrix compounds for the available techniques (X-ray and Gamma Spectrometry), or the matrix is itself inadequate (liquid form). Such is the case for As, Ni and Pb, in many biological samples.

In co-operation with Universities, training has been a major activity within the group that is expected to continue. During 1999, four students achieved their graduation and two doctorate students, from Biochemistry and Biology are making use of the existent facilities for trace element research at the van de Graaff accelerator.

On a short-term perspective, the use of the new Microprobe facility at the van de Graaff accelerator for Biomedical applications is a major objective to be accomplished by the group.

Relative to a mid-term standpoint, to expand the expertise and analytical capabilities achieved, should be a prerequisite in order to encourage the synergy achieved by the different gathered teams. These purposes are demonstrated in the projects presented by different ITN teams for a ***Multidisciplinary Laboratory*** and for the acquisition of an ***Inductive Coupled Plasma Mass Spectrometry instrument***.

However, to pursue these actions and to achieve the objectives there is a demand for research personnel in the group. The perspectives pointed out and the foreseen work, are strongly dependent on adequate human resources in order to assure the collaborations established and the multidisciplinary character of the work developed.

Eco-toxicological Studies: Biochemical Parameters as Indicators for Metal Toxicity*

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Objectives

These studies aim at the study of biochemical parameters, such as, anti-oxidant enzyme expression associated to oxidative stress pathways, as biomarkers for toxicity of environmentally relevant elements. In addition, the environmental susceptibility of certain species was assessed to evaluate their importance as bioindicators for metal pollution. The study focuses a Cu mining polluted area, Neves Corvo, at the Guadiana River basin (Portugal). Data is referred to populations from a remote ecosystem at São Mamede Natural Reservation.

Results

Anti-oxidant enzyme expression and elemental concentrations were determined at excretion and/or accumulating organs and body compartments for fish, amphibians and riverside micro-mammals.

Biological susceptibility was assessed for several species of micro-mammals, freshwater fish and amphibians. The intra_ and inter_species variability was studied relative to the different parameters determined. The most susceptible were subjected to laboratory tests, for acute and chronic toxicity response assess and detoxification rate evaluation. The biochemical parameters studied were the Superoxide Dismutase activity (SOD), and Glutathione S-transferases (GST). The Mn, Fe, Cu, Zn, and Se concentrations were determined for in liver of all species studied, in the amphibian skin and in fish gills. The influence of age, sex and sexual activity are confounders for both antioxidant enzyme activity and trace element level, especially for mammals species. Results also show a significant increase for GST activity in freshwater *Rutilus alburnoides* for polluted environments that correlates with elevated liver Cu and Se levels, as can be observed in Fig.1. Cu intoxication induced in laboratory indicates a correlation between Cu availability in water and GST expression.

Furthermore, very high levels of Cu were determined for fish gills and amphibian skin, suggesting an important role of these organs as a first line of defence against metal environmental pollution.

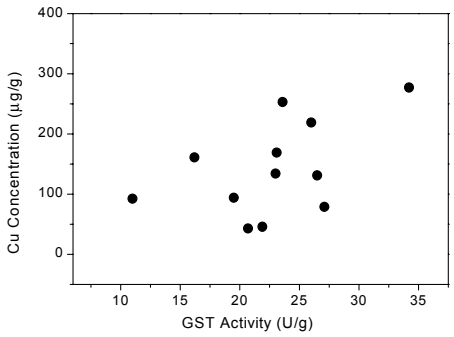

Fig. 1 - Variation of GST activity with liver Cu concentrations for pools of fish *Rutilus alburnoides* (Summer 1998, Neves Corvo area).

For mammal species liver metal accumulation and enzyme activity variations are not as striking as in fish, although an increase of Se levels, and GST activity in exposed individuals is observed. In addition, detoxification studies show a decrease on the trace elemental levels and GST expression, for individuals captured at the polluted area and maintained at laboratory for one month.

Results obtained for SOD activity, for the different species studied relative to the control area are not as significant as GST response, in consequence of intra_ and inter_species high variability.

The enhanced levels of Se in liver of exposed mammals and fish may express a protection mechanism to metal oxidative effect. Both GSH activity and Se alterations may constitute indicators for Cu toxicity.

Further work

The project will be closed in the first trimester of 2000. It is expected however that these studies will proceed supported by other existent projects, new proposals to submit to the FCT (Fundação para a Ciência e Tecnologia) and will be directed towards training activities (graduation and post-graduation levels).

Apart from the response of different body compartments, as amphibian skin and fish gills to environmental pollutants, the biological response of anti-oxidant processes to metals, still need to be further investigated. As an example, the identification of products of anti-oxidant enzymatic reactions may give valuable information on the oxidative stress processes and may constitute as well, biomarkers for environmental xenobiotic agents.

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Metal Exposure and Health Effects*

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Objectives

Assessing the respiratory health status and specific elemental concentrations in blood of steel processing workers (Siderurgia Nacional, EP) and aiming at the evaluation of the incidence rate of pulmonary affections due to metal exposure during labour activities are the major objectives of this study.

Results

The work comprehends aerosol elemental characterisation at the workplace, clinical examination, respiratory function evaluation, and the determination of trace element concentrations in blood, such as, essential elements, Cu, Zn, and Se, and toxic elements, Sb, Hg, and Pb.

So far, both the work atmosphere and a population exposed for more than 15 years were characterised.

Relative to the respirable aerosol fraction (by convention particles below 2.5μm aerodynamic diameter) the major source of pollution is originated by scrap material used in steel processing. This source contributes for the majority of Cu, Zn, As, Sb, and Cd and a significant part of the Cr and Pb present in the fine particulate fraction.

Concerning blood, high levels of Cu, Zn, Hg and Pb were found for the exposed workers with respect to a reference group of healthy non-exposed individuals (N=30). Cr, As and Cd concentrations in blood could not be conclusive, due to their extremely low levels and large variability observed as a result of their short-life period in blood.

Correlation between enhanced Cu, Zn, and Hg and respiratory affections were found. In addition, the increased Cu and Zn and decreased Se blood levels correlate with exposure time. The estimation of the weight of confounders in the associations verified is so far relative to smoking habits. Part of the Pb and Sb variability in blood seems to be dependent on smoking habits.

Experimental tests with laboratory animals exposed to the working environment revealed a significant accumulation of Ca, Cr, Mn, Fe, Zn, and Pb at both trachea and lung tissue. The difference of concentrations between exposed and control groups show that Mn, Fe and Zn present higher deposition rates in lung tissue while Cr and Pb are similarly distributed for both airway levels studied (trachea and lung).

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Further work

Personal monitoring will be carried out to assess the health effects of exposure and to determine biological values of exposure. Selected workers will be surveyed for working shift periods. Respirable aerosols will be collected by personal samplers and elemental content of blood evaluated in the beginning and in the end of shift. If Hg and Pb can constitute biomarkers for exposure in blood, Cr, As and Cd exposure should be assessed in urine, that requires specific collection protocols and elemental analytical methodologies. Therefore, it will be necessary to establish the appropriate methodologies and to perform the necessary tests in order to expand the survey, to this biological matrix and to toxicologically relevant elements.

Also, the studies focusing the localisation and characterisation of aerosol particles at different levels of the human respiratory tree are foreseen to be revived. The NMP technique installed at the Van de Graaff accelerator will be used and the utilisation of Synchrotron Radiation Microprobe (Grenoble) will be considered to assess specific elemental distributions.

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Microprobe Synchrotron Radiation Applied to the Analysis of Human Teeth*

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Objectives

The purpose of this work is to study trace elemental distribution across the entire thickness of teeth and conclude whether tooth elemental content reflects environmental influence, dietary habits and, the role played by some trace elements in dental and human health.

Results

Microprobe analysis using a spatial resolution of 100 μm had revealed to be very useful to assess elemental distributions in teeth, from the root to the enamel.

Concerning the role played by trace elements in dental health, the existent information is scarce and contradictory in some cases. Also, the characterisation of teeth composition according to living habits or occupation is not well established. These aspects are not only relevant for the study and comprehension of phisiological processes of contemporary populations but for archaeological studies.

The work carried out permitted to establish concentration ranges for Mn, Fe, Cu, Zn, Br, Sr and Pb in the different teeth regions in different subjects. So far, the drift of amalgam materials in teeth matrix and the study of several groups of subjects with different labour activities and living habits have been carried out. Despite the few cases analysed so far for each category, differences in the elemental distributions along teeth can be found according to living habits or environmental contamination.

Micro-analytical techniques can give a significant contribute to the study of elemental distribution in these samples. The microprobe synchrotron radiation X-ray fluorescence (SRXRF) technique, due to the brilliance and brightness characteristics of the radiation source, permit a high efficiency for trace element determination, and short time of analysis requirement. In addition, the wide range of spatial resolutions permitted, non-destructive characteristics, and the multielemental capabilities, make of SRXRF a highly attractive technique.

References

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Further work

More data from the different population groups will need to be analysed and the participation in archaeological studies will be reinforced.

It is foreseen to initiate a comparative study of teeth elemental distribution using nuclear microprobe (NMP) installed at the Van de Graaff accelerator of ITN, and synchrotron radiation microprobe (LURE). Data will be compared for quantitative determination accuracy, detection limits, and spatial resolution.

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