



**A newsletter of the IAEA's Laboratories, Seibersdorf**  
**Issue No. 15, July 2008**

ISSN 1608-4632

## X ray Fluorescence in the IAEA and its Member States

### ***In This Issue***

- Activities in the IAEA XRF Laboratory, 1
  - X ray phase-contrast tomography based on synchrotron radiation sources, 1
  - Workshops and Meetings, 3
    - Advanced School on Synchrotron and Free-Electron Laser Sources and their Multidisciplinary Applications, 3
    - European Conference on X ray Spectrometry, EXRS2008, 4
    - 9<sup>th</sup> International Art Conference, ART2008, 5
    - Technical Meeting on Nuclear Spectrometric Methods for in situ Characterization of Materials, 5
- X ray fluorescence in Member States, 7
  - Cameroon, 7
  - Poland, 9
  - Portugal, 12
  - Venezuela, 16
- Publications of potential interest to the XRF community, 19

## Activities in the IAEA XRF Laboratory

A few selected examples of recent activities and results in the field of XRF are presented.

### **X ray phase-contrast tomography based on synchrotron radiation sources**

The experiments were carried out at the ANKA synchrotron radiation facility, Institute for Synchrotron Radiation (ISS), Forschungszentrum Karlsruhe, Germany, in collaboration with the Entomology Unit (IAEA Laboratories), the Atominstitut (Vienna) and the ANKA synchrotron facility Tomo-Topo beamline team. The purpose was to perform X ray phase-contrast tomography of malaria transmitting mosquitoes (genus *Anopheles*) selected from irradiated and control groups as well as radiographic imaging of living and chemically fixed Tsetse flies (*Glossina pallidipes*). The X ray phase-contrast imaging technique allows radiographic-like imaging of samples characterized by very weak or no absorption contrast. This technique is of special interest for radiography of soft tissue, small animals, and insects where the available material is not sufficient to produce satisfactory absorption contrast. Another advantage of the technique over the X ray absorption radiography is minimization of the radiation dose to the sample [1]. The technique enables the investigation of living animals or the performance of tomographic imaging with longer exposition time with minimum radiation damage to the otherwise sensitive and delicate samples. Images of the malaria transmitting mosquitoes can provide information about morphological differences between the irradiated and control groups of specimens. It is of prime importance for a successful application of the sterile insect technique (SIT) that the irradiated (sterile) group of male mosquitoes is healthy and can compete with the local population of males during mating. In the case of Tsetse flies there is also an interest in *in vivo* investigation of the mating process as well as in learning more about the morphology of specific organs, such as salivary glands and reproductive organs.

Two measurement set ups were utilized. Set up #1 was equipped with a high magnification X ray microscope consisting of a single crystal scintillator, magnifying optics coupled to a high-resolution, cooled CCD camera. Image resolution for set up #1 was about 2.5 micrometers and the field of view about 1 mm x 1mm. Set up #2 consisted of a medium magnification X ray microscope with

High brilliance optics/single crystal scintillator coupled to an ultrafast, cooled CCD camera. The image resolution of the set up #2 was about 20 micrometers and the field of view about 10 mm x 10 mm.

Two groups of samples were measured: (1) irradiated and control group mosquitoes: fixed abdomens and heads; (2) live mating Tsetse flies and fixed Tsetse flies. The chemically fixed mosquito and Tsetse fly samples were measured in computed tomography (CT) mode in the set up #1. The *in vivo* mating of Tsetse flies was captured by using the set up #2. Figure 1 shows the picture of the Topo-Tomo beam line and the set up #2.

During the experiment around 230GB of data, in the form of X ray phase contrast enhanced images, were collected. These data are now being evaluated. Two examples of reconstructions of the collected data sets are given in Figs. 2 and 3. In Fig. 2 a volumetric reconstruction of a mosquito head is presented. This data set comes from CT measurement of a chemically fixed sample. The surface and internal morphological details can be inspected. However, the grey level detail allows for differentiation between the voids and the fixed tissue only. A dedicated computer code has been developed to extract the projected sample thickness from X ray phase contrast images. The code was based on a theoretical approach proposed in [2].

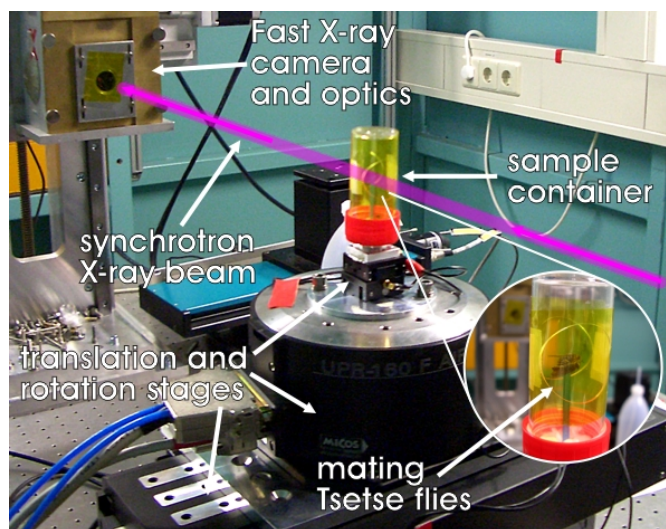


Fig. 1. Picture of the set up no. 2 at the Tomo-Topo beam line. The set up no. 2 comprised a fast X ray camera/optics capable of taking phase-contrast image sequences at 125 frames per second. A close-up view of the sample container with mating Tsetse flies is also shown.

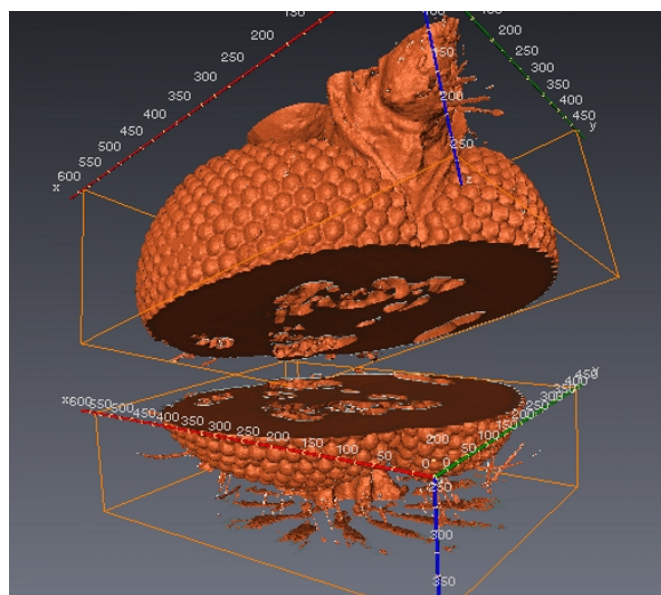
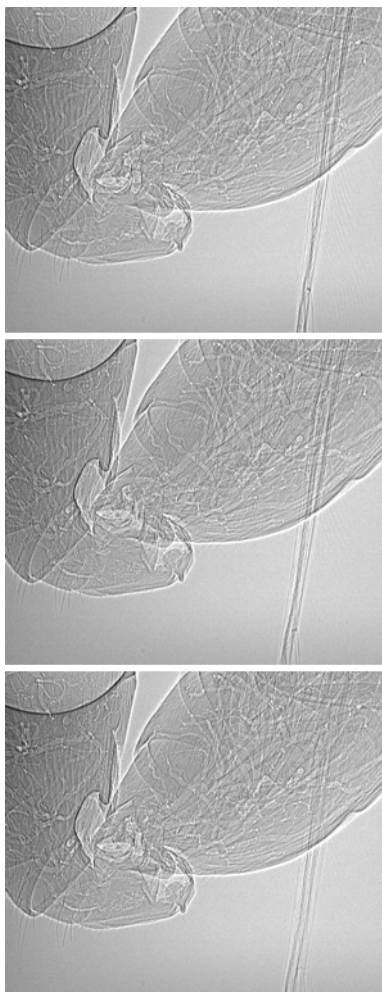


Fig. 2. Volumetric reconstruction of mosquito head (control population). A virtual cross-section through the sample shows the internal void regions. The CT scan consisted of 1000 projections; the distances in the image are given in micrometers.

The imaging of live species of Tsetse flies was performed inside specially designed plastic containers. In the container a single female species was attached with glue to a pole and the male was let free. The container was closed. The X ray beam passed through two windows (entrance and exit) made of 7.5 micrometers thick Kapton foil. During the mating a sequence of phase-contrast images was taken with a speed 125 frames per second and duration of a few minutes. A few frames from such a sequence are shown in Fig. 3.

## Conclusions

The experiments confirmed a possibility to perform imaging of the morphological details for live species of mosquito and flies as well as to study *in vivo* processes by using X ray phase contrast technique based on synchrotron radiation sources. Data for CT imaging of mosquito species require further examination by specialists from the Entomology Unit. There is also a need to modify or develop new sample fixing procedures which would improve X ray phase contrast for specific organs and structures inside the mosquito body.



*Fig. 3. From top to bottom: frames #1776, #1791, and #1801 form a sequence of 14687 images taken by a fast X ray camera/optics (set up #2) during mating of Tsetse flies.*

## References

- [1] J.J. Socha, M.W. Westneat, J.F. Harrison, J.S. Waters, W.K. Lee, Real-time phase-contrast X ray imaging: a new technique for the study of animal form and function, *BMC Biology* 5:6, (2007), available from: <http://www.biomedcentral.com/1741-7007/5/6>.
- [2] D. Paganin, S.C. Mayo, T.E. Gureyev, P.R. Miller, S.W. Wilkins, Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object, *Journal of Microscopy* 206, 33-40 (2002).

Further information on the development and applications of X ray phase contrast tomography is available from Dariusz Wegrzynek ([D.Wegrzynek@iaea.org](mailto:D.Wegrzynek@iaea.org)).

## Workshops and Meetings

### ***Advanced School on Synchrotron and Free-Electron-Laser (FEL) Sources and their Multidisciplinary Applications, Trieste, 7 – 25 April 2008***

The School was organised jointly by the Abdus Salam International Centre for Theoretical Physics (ICTP), Trieste, Italy; Elettra Synchrotron Light Laboratory, Trieste and IAEA (Directors: Ms. N. Binggeli, ICTP, Ms. M. Kiskinova, Elettra and Mr. A. Markowicz, IAEA). The Advanced School has been addressed to PhD students and young researchers interested in modern developments in physics and applications of synchrotron and FEL light sources. Emerging new light sources (FEL) provide IR, VUV and X rays with unprecedented coherence, brightness and pulse length. Recent progress in the development of spectroscopic, diffractive, microscopy/imaging and time-resolved methods was presented through lectures and practical demonstrations at the Elettra Synchrotron Light Laboratory (see photo).



*Visit to the Elettra Synchrotron Light Laboratory (credit: A. Markowicz)*

The programme of the School (available at [http://cdsagenda5.ictp.trieste.it/full\\_display.php?smr=0&ida=a07145](http://cdsagenda5.ictp.trieste.it/full_display.php?smr=0&ida=a07145))



included the topics related to generation and properties of SR and FEL radiation, photon optics and beam line instrumentation as well as SR-based experimental techniques and their applications in physics, chemistry, biology, environmental sciences, medicine, materials sciences, study of cultural heritage, nanotechnology and engineering. More than 85 participants and lecturers from Argentina, Austria, Bangladesh, Belgium, Brazil, Democratic Republic of the Congo, Ethiopia, France, Germany, India, Islamic Republic of Iran, Italy, Kenya, Mexico, Netherlands, Nigeria, China, Philippines, Romania, Russian Federation, South Africa, Sri Lanka, Switzerland, Thailand, Ukraine and USA attended the School. The following topics were of particular interest for the XRF community: X ray imaging techniques, European X-FEL (X ray FEL) project, applications of X ray microscopy and spectroscopy in environmental science, medicine and cultural heritage, development and applications of X ray microanalytical techniques based on X ray tubes and synchrotron radiation (SR) – summary of the results obtained by the XRF Group of the IAEA Laboratories at Seibersdorf (full presentations are available at

[http://cdsagenda5.ictp.trieste.it/full\\_display.php?smr=0&ida=a07145](http://cdsagenda5.ictp.trieste.it/full_display.php?smr=0&ida=a07145)).

### *Conclusions*

The Advanced School on Synchrotron and Free-Electron-Laser (FEL) Sources and their Multidisciplinary Applications was a very successful event. The professional level of presentations was very high and the lecturers presented up to date knowledge related to the applications of synchrotron and FEL sources in the physical and chemical sciences, medicine, biology, environmental science, study of cultural heritage and nanotechnology. The collaboration between the IAEA, ICTP and Elettra Synchrotron Light Laboratory in organization of the joint schools and workshops is extremely cost effective and results in events of a very high standard. Future events might be focused on specific applications in such areas as environment, study of cultural heritage, and medicine.

Further information on the Advanced School is available from Andrzej Markowicz ([A.Markowicz@iaea.org](mailto:A.Markowicz@iaea.org)).

## ***European Conference on X ray Spectrometry, EXRS2008, Cavtat, Dubrovnik, Croatia, 16-20 June 2008***

The Conference was sponsored by the Ministry of Science, Education and Sports, Croatia, the International Atomic Energy Agency, and the European X ray Spectrometry Association. 109 oral and 150 poster contributions were presented during the Conference. About 250 participants from 40 countries worldwide attended the event. The Conference was accompanied by an exhibition where a number of commercial companies presented their products related to X ray spectrometry. More information about the Conference can be found in the Conference web page: <http://exrs2008.irb.hr/>

The Conference sessions covered the following topics: applications of X ray spectrometry (XRS) in archaeometry, quantification methodology, synchrotron radiation-based XRS, particle induced X ray emission (PIXE) and electron induced XRS, X ray sources, optics and detectors, X ray imaging and tomography, applications in materials and nano-science, X ray absorption, applications in earth and environment sciences, wavelength dispersive XRS, applications in life sciences, interactions of X rays with matter, micro-beam techniques, and total reflection XRF and related techniques.

### *Conclusions*

A substantial number of contributions presented during the Conference were focused on biomedical applications, assessment of environmental pollution, in-situ analysis of cultural heritage objects, and integration of several X ray emission techniques into one instrument. There were also papers on data analysis and improvement in the accuracy of physical databases and so-called fundamental parameters. The Conference was a good opportunity for the IAEA representative (D.Wegrzynek) to discuss with the participants from developing countries issues related to the IAEA support in the field of XRS as well as to collect relevant feedback. The participants provided very positive opinions about the activities carried out in the Agency's Laboratories in Seibersdorf and Monaco with a special emphasis on the importance and usefulness of the fellowship training programmes, provision of guidelines and software related to utilization of nuclear analytical techniques, availability of well characterized reference materials and organization of cost-free proficiency test exercises.

Further information on the Conference is available from Dariusz Wegrzynek ([D.Wegrzynek@iaea.org](mailto:D.Wegrzynek@iaea.org)).

## ***9<sup>th</sup> International Art Conference, ART2008, Jerusalem, Israel, 25-29 May 2008***

The Conference attended by more than 250 scientists and curators from all over the world provided a forum to exchange information and discuss topics related to non-destructive analysis in support of investigation, diagnostics and conservation of cultural and environmental heritage. Five oral sessions were entirely devoted to X ray emission techniques. Other techniques included infrared, visible, and ultraviolet light spectroscopy, acoustic sensors, and applications of lasers in analysis and cleaning. Altogether 101 oral presentations and 75 posters were presented by the participants from 26 countries in Europe, North America, Latin America and Asia. X ray spectrometry techniques provide information about the chemical composition of an object exposed to electrons, ions or electromagnetic radiation. The useful features of XRS include non-destructiveness, multielemental capability, and high sensitivity for inorganic components. X ray fluorescence (XRF) based on laboratory sources or synchrotron radiation facilities appeared to be the analytical technique which is applied most frequently for the characterization of cultural heritage objects. Moreover, XRF technique can easily be adapted for *in situ* measurements. In this context a portable XRF spectrometer designed and constructed in the IAEA Seibersdorf Laboratories was presented including selected applications. The key features of the portable XRF system are the use of polycapillary optics to reduce the beam spot diameter below 200 micrometers,

and a vacuum chamber attachment to enable determination of low atomic number elements such as Mg, Al, Si, P, S, and Cl. The spectrometer was applied for chemical composition analysis of archaeological artefacts and works of arts from various collections of the Museum of Fine Arts, Vienna, Austria including such objects as ancient bronzes, coins, samples of pigments, and the famous goldsmith work "Saliera" by Benvenuto Cellini (1500-1571).

### ***Conclusions***

The participants agreed that curators, archaeologists and conservators should meet regularly with analytical scientists, physicists and chemists to learn from each other about the needs and opportunities, available tools and analytical performance in order to initiate cooperation between museums, conservation institutes and analytical laboratories/research institutes. Due to the unique features and capabilities, in particular non-destructive character of analysis, the nuclear analytical techniques are becoming widely accepted by the cultural heritage community. The IAEA has a special role in promotion, development and applications of these techniques in support of both developing and developed Member States.

Further information on the Conference is available from Dariusz Wegrzynek ([D.Wegrzynek@iaea.org](mailto:D.Wegrzynek@iaea.org)).

## ***Technical Meeting on Nuclear Spectrometric Methods for in situ Characterization of Materials, Vienna, Austria, 19 – 23 May 2008***

The Technical Meeting (TM) was organised by the IAEA Laboratories at Seibersdorf (A. Markowicz) under the IAEA project 1.4.3.4. Nuclear Spectrometry for Analytical Applications. Various nuclear spectrometry methods can be successfully applied in the field and in industrial environments for *in situ* analysis (*in situ* is a Latin phrase meaning *in the place* which might be used in many different contexts); this meeting covered the analysis of artefacts and materials that have not been moved from their original place of deposition/storage. A modern nuclear spectrometry portable analyser brings to the field site unsurpassed savings in time and labour as well as an excellent performance often matching that of the laboratory instrument. Major features of portable (or transportable/movable) analysers include simplicity, speed of operation and flexible requirements for sample preparation. Nuclear spectrometry methods applied for

*in situ* analysis can provide immediate analytical results (very important for interactive measurement programmes, e.g. assessing contaminated sites) in a truly non-destructive way (critical for study of museum samples, works of art and archaeological objects). A range of possible applications of *in situ* analysis is very wide and covers the following fields:

- Industrial applications including mineral exploration, mining, process control, alloy sorting, measurement of coating thickness, plastic scrap sorting, monitoring of corrosion processes, study of catalytic materials
- Environmental applications including soil screening for metals, indoor and outdoor air pollution monitoring, water monitoring, assessment of radiation exposure, decontamination assessment etc.

- Archaeological applications and study of cultural heritage objects including analysis and identification of artefacts in museums and at archaeological sites for authentication and provenance studies, analysis of artefacts for purposes of their preservation and restoration
- Extraterrestrial applications

Nuclear spectrometry techniques which are used for *in situ* characterization of materials include:

- X ray fluorescence (XRF)
- Gamma spectrometry (passive and active, e.g., following neutron activation)
- Mössbauer spectrometry
- Raman spectroscopy
- Alpha spectrometry

A major objective of the Technical Meeting was to review the current status, developments, and trends in portable nuclear spectrometry instrumentation and associated analytical methodologies for *in situ* characterisation of materials, and to produce a relevant report (proceedings).

The following specific topics were discussed:

- Nuclear spectrometry instrumentation for *in situ* measurements
- Analytical methodologies for *in situ* analysis
- Advantages and limitations of nuclear spectrometry methods for *in situ* measurements
- Selected *in situ* applications
- Further improvements in analytical performance of portable nuclear spectrometry instrumentation and methodologies for *in situ* applications
- Role of the IAEA in promotion and effective use of nuclear spectrometry instrumentation and associated analytical methodologies for *in situ* applications in the developing Member States.

The TM was attended by the participants from Australia, Cuba, Finland, Germany, Italy, Niger, South Africa and United Kingdom as well as by IAEA staff members from the Department of Nuclear Sciences and Applications and the Department of Safeguards (see photo).

The nuclear analytical techniques for *in situ* characterization of materials were presented and reviewed with emphasis on their advantages, limitations, typical applications, current developments and trends.



*Group photo of the participants of the Technical Meeting (credit: P. Potts).*

### Conclusions

*In situ* techniques have the capability of bringing substantial benefits to Member States in the following areas:

- Nuclear safeguards, safety and security
  - Control of nuclear materials
  - Particle characterisation
  - Mapping of environmental radioactivity for the estimation of doses
- Environmental applications
  - efficient water resource management (surface and ground water hydrology)
  - soil chemistry and preventing erosion
  - agricultural productivity (e.g. more efficient use of fertilisers)
  - air pollution monitoring
- Mineral exploration and mining
- Cultural heritage
  - characterisation,
  - restoration
  - conservation
- Extraterrestrial exploration (e.g. evidence for water-rock interactions on Mars)

In all these applications, *in situ* techniques offer advantages compared to conventional sampling and laboratory analysis including:

- higher speed
- lower cost per determination
- more effective and representative spatial and temporal coverage

- immediate access to data for decision making

The participants concluded that there is an outstanding opportunity for the IAEA to take an international leadership role in *in situ* nuclear measurements. This should involve further development of *in situ* nuclear measurement capabilities and methodologies as well as promotion of uptake of these techniques in various

fields including training and capacity building to encourage the migration of techniques from the laboratory to field applications in Member States.

Further information on the Technical Meeting and its outcomes is available from A. Markowicz ([A.Markowicz@iaea.org](mailto:A.Markowicz@iaea.org)).

## X ray fluorescence in Member States

During the last months we have received contributions from Cameroon, Poland, Portugal and Venezuela on the current XRF activities. Below there are communications based in the original submissions (with only minor editorial changes).

### Cameroon

#### ***Quantitative determination of trace elements in *gnetum africanum* and *gnetum buchholzianum* with energy-dispersive X ray fluorescence***

Department of Inorganic Chemistry, Faculty of Sciences, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon  
**Contributor:** J.F. SABOUANG and R.M.NEMBA (correspondence to R.M.NEMBA [memba@yahoo.fr](mailto:memba@yahoo.fr))

*Gnetum africanum* and *Gnetum buchholzianum* are two ligneous plants which produce edible leaves. Using energy-dispersive X ray fluorescence analysis, it was possible to determine the mean concentrations of several metals, like K, Ca, Mn, Fe, Ni, Cu, Zn, Rb and Sr, at ppm levels in the leaves of these plants. This paper aims at investigating the potential intake of mineral elements and the bio availability of the oligo-elements in *gnetaceae* plants. This information is of great importance for the detection of a potential metal contamination of the vegetables which may be collected in suburban forests.

#### **Introduction**

In many developing countries of Sub-Saharan Africa, products of the forest such as fruits and/or plants leaves are edible and contribute significantly to the nutrition of the population <sup>[1]</sup>. Therefore scientific studies of such plants are necessary to determine their nutritional importance and to insure their quality control. It is well known from the chemical literature that some elements found naturally in soils are essential for the growth of and nutrition of the plants are classified according to their needs in the following three categories: the primary elements are those which the plant need in large quantities such as carbon, hydrogen and nitrogen, the secondary ones include calcium, magnesium and potassium and the trace elements are those which the plants needs in small quantities (ppm or ppb) such as Co, Mo, Se and others. It is noticed that the trace

elements play a key role in some biological metabolisms <sup>[2]</sup> and their deficiencies or excesses in the human body can result in some physiological disorders. In fact some trace elements are toxic and harmful even at extremely low concentrations and conversely some micro-nutrients deficiencies in food remain a serious problem, notably in some developing countries <sup>[3]</sup>.

The objective of this work was to investigate the oligo-elements of mineral origin in *Gnetum africanum* and *Gnetum buchholzianum* which are two *gnetaceae* plants encountered in the forests of the coastal regions of Central and West Africa and which produce edible leaves which are collected by the population for nutritional needs and commercial transactions. A full description of these two species is given by Hutchinson et al. <sup>[4]</sup>. The investigations were performed by energy-dispersive X ray fluorescence (EDXRF). This technique has been used by many authors for trace elements analysis <sup>[5]</sup>.

In EDXRF, the determined elements range from Al to U. The principle of analysis usually consists of four stages: the excitation of the characteristic radiations of the specimen; the selection of the characteristic emission lines from specific elements; the detection and integration of the characteristic photons to obtain the intensity of the emission line and the conversion of these lines into elemental concentrations <sup>[6]</sup>. In this work, a comparative method was applied to determine the real elemental concentrations, i.e. a standard



reference material was used which has practically the same matrix as the analysed specimens.

## Experimental

The measurements were realized using a Tracor Spectrase 5000 EDXRF system. This system has three main units: the sample chamber, the X ray excitation and X ray detection sub-systems. The major functional components in the sample chamber are an automated sample changer with a ten-position tray, and an X ray filter wheel with five filters of different composition. The Spectrase 5000 uses a Rh-anode X ray tube with an operational range between 6 and 50 keV and a current up to 0.35 mA. Emergent X rays are detected at 90° relative to the incident X ray beam by a Si(Li) detector. The energy resolution is about 150 eV. The following excitation conditions were used: U=25 kV, I=0.25 mA operating in vacuum. The spectrum acquisition time was 5000 s for all samples. The dead time was around 50%.

The collection of leaves was done in Cameroon. The different species were identified according to their characteristics as described by Hutchinson et al. <sup>[4]</sup>. *Gnetum africanum* is characterized by an internode of male spike of the same diameter throughout its length, slender in dried (or old) specimens; staminal column exerted from the mouth of the envelope; leaves ovate-oblong or elliptic oblong, more rarely lanceolate, attenuate at the base, abruptly acuminate, 10-13 cm long, 3.5-5 cm broad. *Gnetum buchholzianum* is

characterized by: an internode of male spike in the fresh condition, much thicker at the base than higher up; staminal column hardly exerted from the mouth of the envelope, leaves ovate/elliptic or broadly elliptic, rounded or very slightly cuneate at the base, abruptly acuminate, 9-14 cm long, 4-7 cm broad.

The leaves were cut and dried at 105°C for 24 hours. These grass materials were ground and sieved to a mesh size of 2 mm to obtain a fine powder. The pellets were prepared with cellulose as a binder material. The mixture consisted of 80 wt % of grass powder and 20 wt % of cellulose. One gram of each mixture was pressed gradually by a hydraulic press from 5 to 15 tons. Finally we obtained pellets of 32 mm diameter. A mylar film was used as a support for pellets in the sample holder. Standard samples were prepared in the same way using standard reference material NIST 1571 (Orchard leaves). Three replicate were made for each sample measurement.

## Results and discussion

The accuracy of this analytical method was evaluated through the analysis of the standard material IAEA-V-10 (Hay Powder). In Table 1, the measured concentrations of different elements in Hay Powder are given together with the certified values. The results are based on triplicate measurements and had an error of the order of 10 % except for Br and Rb. In general the agreement between the measured and certified concentrations appears to be good.

Table 1. Measured concentrations of trace elements in standard reference material IAEA-V-10 (Hay Powder) together with the certified values.

Element	Measured concentration (ppm)		Certified value (ppm)	
	Mean value	Standard deviation	Mean value	Standard deviation
K	21800	400	21050	1450
Ca	23000	500	21600	600
Mn	49	1	48	4
Fe	190	10	184	6
Ni	4	1	4	1
Cu	10	1	9	1
Zn	23	2	24	1
Br	7	1	9	2
Rb	11	1	7.5	0.5
Sr	40	2	40	4

The elements detected in *Gnetum africanum* and *Gnetum buchholzianum* are K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr. Table 2 shows the mean concentrations of each element in both species. A great difference of elemental concentration is observed for Ca and Sr. Both these elements appear to be more available in *Gnetum*

*buchholzianum* than in *Gnetum africanum*. In both vegetables we have two groups of elements; the secondary and the trace elements. The secondary elements include K and Ca. The other elements are present at low concentrations ranging from 480 to 4 ppm.



Table 2. Average elemental concentrations obtained for samples of *Gnetum africanum* and *Gnetum buchholzianum*.

Element	Concentration in <i>Gnetum africanum</i> (ppm)		Concentration in <i>Gnetum buchholzianum</i> (ppm)	
	Mean value	Standard deviation	Mean value	Standard deviation
K	13500	600	12100	400
Ca	6700	200	17500	500
Mn	1250	50	1550	50
Fe	390	10	480	20
Ni	6	1	5	1
Cu	9	1	9	1
Zn	21	1	19	1
Cr	4	1	5	1
Rb	11	1	7.5	0.5
Sr	40	2	40	4

The essential macro minerals found are Ca and K. It is known that K is necessary for both muscular and nerve functions. Calcium is the main component of bones and teeth. It also acts in the muscular and nerve functions. The oligo-elements available at great concentration are Fe and Mn. Manganese is a cofactor of some enzymes and acts in the synthesis of glycoproteins. Iron is involved mainly as a component of haemoglobin, the molecule that carries oxygen in red blood cells. It also occurs in myoglobin (oxygen carrier in muscle cells). On the other hand, these elements can cause toxic effects due to an excess of consumption.

It appears that the relative percentages of Mn and Fe are practically the same for both species of *Gnetum*. Potassium is more available in *Gnetum africanum* than in *Gnetum buchholzianum*.

#### Acknowledgement

The EDXRF measurements were performed when one of us (JFS) was on a fellowship from the International Atomic Energy Agency in the Department of Chemistry at the University of Antwerp, Belgium. We

thank IAEA for the financial support and Prof. R. Van Grieken and his co-workers for their guidance during this work.

#### References

- [1] Andre RW, Roger S. Les plantes utiles du Gabon. (eds.). Lechvalier: Paris-VI<sup>e</sup>, 1961; 184.
- [2] Harper. Précis de Biochimie. De Boeck Université : Presses de l'Université Laval, 1995 ; 691-694.
- [3] Venkatesh GI, Padmanabhan PN. The science of the Total Environment. 2000; 249: 331-346.
- [4] Hutchinson J, Dalziel JM. Flora of West Tropical Africa. Millbank: London, 1954; 33.
- [5] Anjos MJ, Lopes RT, Jesus FO, Simabuco SM. X-Ray Spectrom. 2002; 31: 120-123.
- [6] Van Grieken R, Markowicz AA. Handbook of X-ray Spectrometry, Second Edition, Marcel Dekker Inc.: New York, 2002.

## Poland

### *Recent activities in X ray fluorescence laboratory of the University of Science and Technology AGH, Krakow, Poland*

AGH-University of Science and Technology, Faculty of Physics and Applied Computer Science Bldg. D-100, Room 19, al. Mickiewicz 20, Krakow, Poland

**Contributors:** M. Lankosz ([Lankosz@novell.ftj.agh.edu.pl](mailto:Lankosz@novell.ftj.agh.edu.pl)), L. Samek, J. Chwiej, M. Szczerbowska-Boruchowska

The X ray Fluorescence Laboratory is a part of Department of Applied Nuclear Physics at the Faculty of Physics and Applied Computer Science, University of Science and Technology AGH, Krakow, Poland.

Staff members include M. Lankosz – Head of the Department, L. Samek, B. Ostachowicz, M. Szczerbowska-Boruchowska, J. Chwiej, A. Ostrowski

and PhD students: S. Wójcik, M. Kastyak, and M. Czyżycki.

The XRF laboratory is equipped with the multifunctional energy-dispersive X ray fluorescence system (see Fig. 1) and microbeam X ray fluorescence confocal microscope (see Fig. 2). The multifunctional system consists of micro-beam X ray fluorescence spectrometer with capillary X ray optics, the broad X ray beam from Mo secondary target for XRF analysis of bulk samples and total reflection X ray technique. Chemical laboratory is equipped with pressure digestion system used for the sample preparation.



*Fig.1. Multifunctional X ray Fluorescence Spectrometer.*



*Fig. 2. Confocal X ray Fluorescence Spectroscopy.*

The group takes part in the investigations related to various fields including biomedical research, environment science and cultural heritage conservation.

The investigation of the biochemical mechanisms that may participate in the pathogenesis of brain gliomas is of great importance. The elemental and molecular investigation was performed with the use of the techniques based on synchrotron radiation i.e. synchrotron radiation X ray fluorescence (SRXRF), X ray absorption near edge structure (XANES) spectroscopy and Fourier transform infrared microspectroscopy (FTIR). The topographic and quantitative elemental analysis of thin tissue samples was performed using SRXRF technique. Two dimensional mapping of the different forms of sulfur and iron in the gliomas samples was performed with the use of XANES spectroscopy. The investigation of changes in main biological molecules (carbohydrates, lipids, proteins, nucleic acids) in case of brain gliomas was performed with the use of FTIR microspectroscopy.

Tissue samples were taken intraoperatively from patients with different grades of brain gliomas. The specimens were frozen and cut into sections of 20 or 10 micrometers thick in a cryo-microtome. The slices were fixed onto ultralene foil and freeze-dried.

Two-dimensional maps of distribution of selected element were performed. The preliminary investigation showed that for all cases the elements such as P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb were present in human brain glioma samples (see Fig. 3). The higher levels of S, K and Cl were found in glioma cells than in surrounding tissue.

It was noticed that cancer cells accumulate sulfur mainly as sulfide ( $S^{2-}$ ) form. Moreover, the results indicated higher accumulation of this form of sulfur in glioma of IV grade of malignancy in comparison with the samples of II grade neoplasm. The presence of sulfate ( $S^{+6}$ ) species was revealed in histological structures outside the cancer cells. The analysis of iron oxidation states showed that Fe occurs in the glioma tissue mainly in the oxidized form ( $Fe^{3+}$ ). Moreover, low intensity of pre-edge peak indicates that Fe occurs mainly in the complexes of octahedral geometry, in which the number of ligands bound to the transition metal ion is 6.

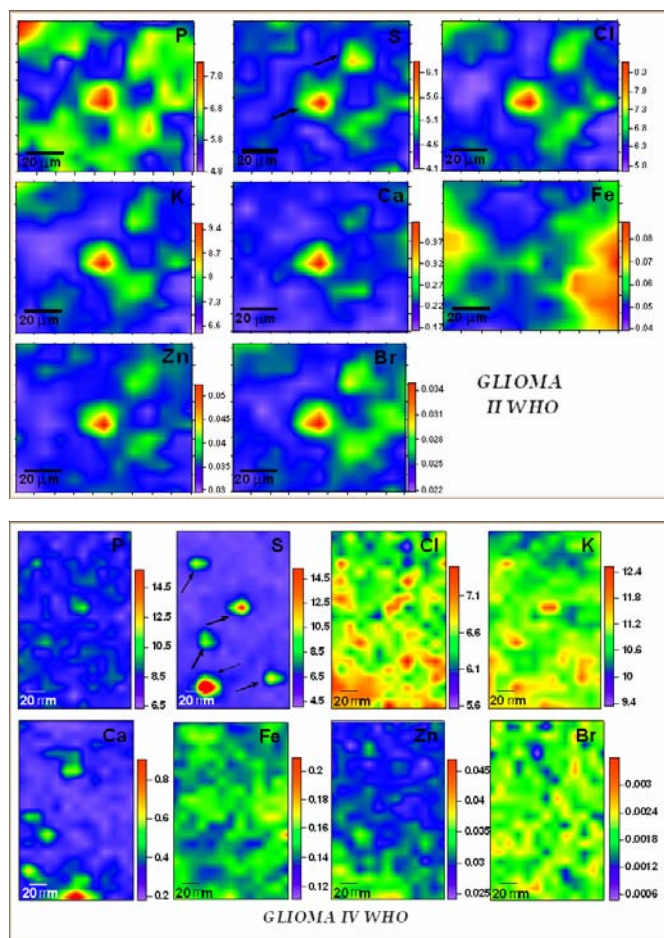


Fig. 3. Distribution of selected elements in human brain glioma tissue for II and IV grade malignancy. The arrows show cancer cells. Data presented in  $\mu\text{g}/\text{cm}^2$ .

The elemental analysis was coupled with determination of main biomolecules of the samples. The tissue areas were mapped to generate two-dimensional images of the molecules of interest. The major spectral differences between control and cancerous tissues were identified for the vibrational frequencies characteristic for proteins and lipids.

The etiology of epilepsy being the most common neurological disease in most of cases has been still unknown. Trace metals such as Ca, Fe, Cu or Zn participating in the processes like oxidative stress, excitotoxicity, mitochondrial dysfunction and protein aggregation are suspected to lead to the atrophy and death of nerve cells in case of serious neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, etc.). Because epileptic seizures induce neurodegenerative changes in selected areas of brain, it is expected that metals may play an important role in the pathogenesis and progress of epilepsy as well.

The X ray fluorescence microscopy was used for topographic and quantitative elemental analysis of the

areas of rat brain that are involved in epileptic activity. Two groups of animals were examined. First group included rats with pilocarpine induced epilepsy and the second naive control animals. For each sample two-dimensional analysis of elemental distribution was performed for the areas of hippocampus and brain cortex. For four regions of hippocampus formation (CA1, CA3 of Cornu Ammonis, DG-Dentate Gyrus and H-Hilus) and cortex the mean masses per unit area were evaluated. The areas taken into account in calculations were equal to  $300 \mu\text{m} \times 300 \mu\text{m}$ . The level of Ca was higher for CA1 and CA3 region of hippocampus and cortex in case of animals with pilocarpine induced epilepsy. The opposite relation was observed for Cu level in the area of DG and Zn in the areas of CA3 and DG. The statistical significance of the obtained results was confirmed using U (Mann-Whitney) test of median values. The investigations will be continued; mainly the elemental analysis will be done for animals with increased susceptibility to epileptic convulsions caused by brain injury. Additionally, influence of neuroprotective drugs on the composition of nervous tissue will be analyzed.

The X ray fluorescence technique was also applied in cultural heritage conservations and environment sciences. The analysis of aerosol samples (indoor, outdoor) collected on filters in the museums, historic churches were performed (see Fig. 4).

The aim of this research was selection and modification of conservation methods of art objects, proper heating of historic wooden churches as well as characterization of air flows, particulate deposition and strains in the wood. The laboratory is participating in a development of intelligent system for air pollution measurement including instrument for predicting air protection and influence of meteorological conditions on elemental content of particulate matter. Our effort is dealing with collection of air particulate matter, evaluation of concentration of particulate matter and concentration of elements in particulate matter by using EDXRF method. In the frame of these projects samples of particulate matter were collected in Krakow and Otwock, Poland. Particulate matter was separated according to size grain ( $0.4 \mu\text{m}$ - $2.0 \mu\text{m}$ ,  $2.0$ - $8.0 \mu\text{m}$  and above  $8 \mu\text{m}$ ). The mass of particulate matter was evaluated. Meteorological data were collected at the same place and stored in a computer.



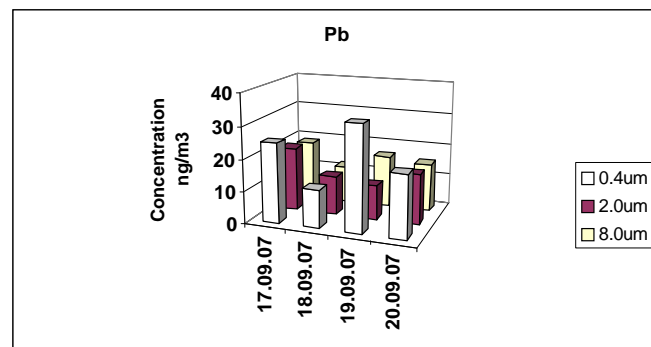
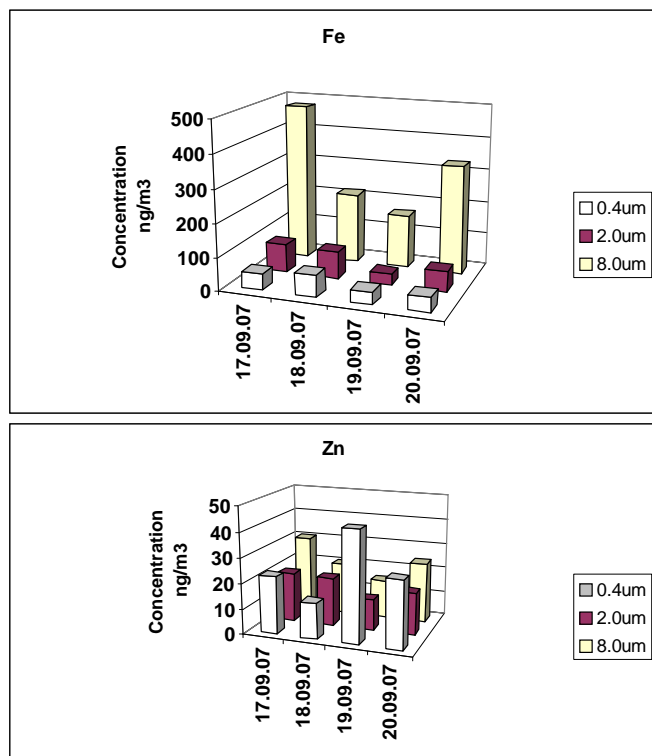


Fig. 4. Concentration of elements in the air particulate matter.

Another application was study of elemental content of archeological glasses by XRF method. The main research aims of this work were the following: (i) identification of the materials used to melt glass and determination of their origin using chemical analyses, (ii) reconstruction of the methods for modification of glass coloration by changing the conditions in the furnace and the time of melting, and (iii) determination of the chemical characteristics of the glass artifacts and identification of references to places where they had been produced.

## Portugal

### *Non-destructive elemental characterization in art and cultural heritage research*

*Centro de Física Atómica, Universidade de Lisboa, Faculdade de Ciências, Av. Prof. Gama Pinto 2, 1649-003 Lisboa, Portugal*

**Contributors:** S. Pessanha, M. Manso, A. Guilhere, M.L. Carvalho

(Corresponding author: Tel.: +351961051293; Fax: +351217954288, E-mail address: [luisa@cii.fc.ul.pt](mailto:luisa@cii.fc.ul.pt))

The Atomic Physics Group of the University of Lisbon (CFA)<sup>1</sup> has been giving evidence over the past 40 years of its suitability and proficiency in fundamental atomic physics and trace element determination regarding environmental contamination, toxicology and biophysics studies<sup>2-7</sup>.

The group also carried out a great investigative work in the field of Cultural Heritage as member of the PAPERTECH project (Innovative Materials and technologies for the Conservation of Paper of Historical Artistic and Archaeological Value)<sup>8-14</sup>.

Nowadays, and triggered by the acquisition of two portable EDXRF equipments, the Center is focusing its activities in Cultural Heritage studies, so that it is involved in two projects: “Morphological characterization of paper stains and treatment methodologies” financed by the Portuguese Foundation for Science and Technology (FCT), and a bilateral cooperation GRICES/CSIC with the Institute of Earth

Sciences Jaume Almera, in Barcelona entitled “Studies for Preventive Conservation of the Portuguese Patrimony by means of spectrometry techniques”, also financed by FCT.

Another project “Vibrational Spectroscopy and X ray Fluorescence on Art and Cultural Heritage” is starting under the programme Hubert Curien (PHC) between Portugal and France (CNRS), with Institut des Sciences Moleculaires, Université Bordeaux.

Some of the results obtained in the studies of Cultural Heritage are presented below.

### *In situ analysis by means of a portable EDXRF spectrometer*

The equipment used in the following studies consists on an X ray generator from Amptek, the ECLIPSE III with Ag anode (30 kV, 50 µA). The detector is an Amptek

XR-100CR of Si(Li) thermoelectrically cooled (Peltier effect), with a resolution of 190 eV at 6.39 keV.

The acquisitions were obtained through the Amptek software PMCA and the spectra analyses were performed by AXIL. The equipment is assembled on an

aluminium structure and mounted in a tripod that allows to cover a range of 1.5 m. Once assembled, two laser beams show where the sample should be positioned.

Table 1 – Pigments found in the Josefa de Óbidos easel paintings.

AGNUS DEI				SACRED FAMILY		
Colour	nº spots	Major elements	pigments	nº spots	Major elements	pigments
White	4	Pb	Lead white [2PbCO <sub>3</sub> .Pb(OH) <sub>2</sub> ]	4	Pb	Lead white
Yellow	2	Fe	yellow ochre (Fe <sub>2</sub> O <sub>3</sub> .nH <sub>2</sub> O)	6	Fe	yellow ochre
Orange	1	Hg, Pb, Fe	Cinnabar (HgS), Lead white and yellow ochre			
Pink	1	Hg, Pb	Cinnabar and Lead white			
Red	4	Hg, Pb	Cinnabar and Lead Red (Pb <sub>3</sub> O <sub>4</sub> )	4	Hg	Cinnabar
Blue	2	Cu	Azurite [2CuCO <sub>3</sub> .Cu(OH) <sub>2</sub> ] Malachite (CuCO <sub>3</sub> .Cu(OH) <sub>2</sub> )	6	Cu	Azurite
Green	6	Cu	or Verdigris [Cu CH <sub>3</sub> COO) <sub>2</sub> .2Cu(OH) <sub>2</sub> ] Umber (Fe, Mn oxide) or	1	Cu	Malachite or Verdigris
Brown	1	Fe	brown ochre (Fe <sub>2</sub> O <sub>3</sub> .MnO <sub>2</sub> .nH <sub>2</sub> O)	4	Fe, Mn	Umber or brown ochre
Flesh				3	Hg	Cinnabar

Analysis of two easel paintings of one of the most prominent Portuguese painters, Josefa de Óbidos (1630-1684), *Agnus Dei* and “Sagrada família” were performed *in situ* at the Évora Museum (Fig. 1) in order to identify the used pigments. Table 1 shows that the identified pigments were the same for both paintings. Doubts remain in the green and brown pigments that would only be determined using a molecular analysis technique

The study of one of the most important Portuguese masterpieces, the XV<sup>th</sup> century “São Vicente de Fora” polyptych was also performed *in situ* at the Museum of Fine Arts in Lisbon (Fig. 2). The analysis of the six panels attributed to Nuno Gonçalves showed pigments like cinnabar (HgS) and red ochre (Fe<sub>2</sub>O<sub>3</sub>) for the reds, lead white [2PbCO<sub>3</sub>.Pb(OH)<sub>2</sub>], lead-tin yellow (Pb<sub>2</sub>SnO<sub>4</sub>), and gold (Au) in the halos. The strong presence of Cu in the blue and green pigments revealed the use of azurite [2CuCO<sub>3</sub>.Cu(OH)<sub>2</sub>] for the blue. As for the greens the pigment can either be Malachite [CuCO<sub>3</sub>.Cu(OH)<sub>2</sub>] or Verdigris [Cu CH<sub>3</sub>COO)<sub>2</sub>.2Cu(OH)<sub>2</sub>].



Fig. 1. *In situ* analysis of two Josefa de Óbidos paintings a) “Agnus Dei” b) “Sagrada Família”.



Fig. 2. In situ analysis of the “São Vicente de Fora” polyptych a) “Frades” panel b) “Infante” panel.

A harpsichord, a clavichord and a pianoforte belonging to the XVIII<sup>th</sup> Portuguese School of keyboard making, collection of the Museum of Music in Lisbon, were also analyzed *in situ*. It is believed that these instruments were painted green as a strategy to give them a non-attractive appearance during the French invasions in the beginning of the XIX<sup>th</sup> century. The green ink, commonly called “merde d’oie” revealed to have mainly Cu mixed with Ba, probably barium white ( $\text{BaSO}_4$ ). Other colors and pigments were found in the decoration of the instruments like cinnabar (Fig. 3) and gold pigment.

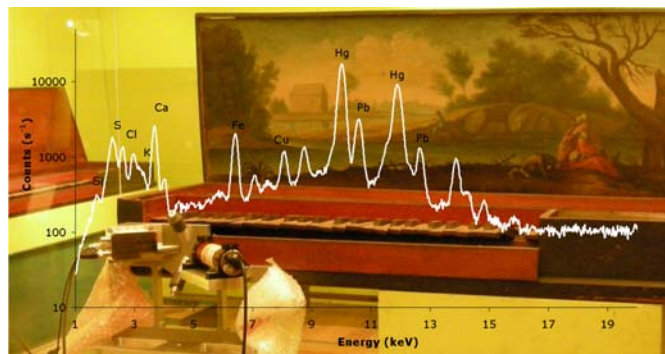


Fig. 3. Spectrum obtained in situ for the red pigment in the clavichord.

### Quantitative and micro-analysis of ancient papers

The Atomic Physics Group of the University of Lisbon also has a non portable EDXRF.

The spectrometer consists of an X ray tube equipped with a changeable secondary target, in Mo. The X ray tube, the secondary target and the sample are in a triaxial geometry.

The characteristic radiation emitted by the elements present in the sample is detected by a Si(Li) detector, with a 30 mm<sup>2</sup> active area and 8  $\mu\text{m}$  beryllium window. The energy resolution is 135 eV at 5.9 keV and the acquisition system is a Nucleus PCA card. Quantitative calculations are made through the fundamental parameters method. The absorption corrections are made using the information for coherent and incoherent scattering intensities. Experimental parameters are obtained through calibration, using standard reference materials.

Paper documents from XVIII<sup>th</sup> century were analyzed with this equipment in order to characterize them through their elemental composition. With this technique it was possible to distinguish the different papers specially the 1779 document that presented Co, Ni, As and Bi in high concentrations (table 2). It is worth mentioning that these elements have never been found together and none of them in so high concentrations.

Table 2 – Mean elemental concentration and standard deviation ( $\mu\text{g.g}^{-1}$ ) in XVIII<sup>th</sup> century paper documents.

Elements	Paper document		
	1779	1786	1788
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
K	2680 $\pm$ 200	890 $\pm$ 100	330 $\pm$ 30
Ca	820 $\pm$ 50	1500 $\pm$ 100	5500 $\pm$ 200
Fe	860 $\pm$ 50	250 $\pm$ 30	230 $\pm$ 30
Co	410 $\pm$ 30		
Ni	270 $\pm$ 20		
Cu	90 $\pm$ 10	30 $\pm$ 5	30 $\pm$ 5
Zn	90 $\pm$ 10	15 $\pm$ 3	50 $\pm$ 8
As	1650 $\pm$ 50		
Pb	100 $\pm$ 10	30 $\pm$ 5	
Bi	250 $\pm$ 20		

In order to perform the study of the inks present in the documents, micro-analysis was necessary. For this purpose a third EDXRF equipment was used (Fig. 4).

This portable  $\mu$ -EDXRF spectrometer consists in an OXFORD X ray generator (Model XTF 5011) with Mo anode (50kV, 1mA). The detector used is a VORTEX-EX SDD (Si) with a nominal area of 50mm<sup>2</sup> and a Be window thermoelectrically cooled. The X ray generator and detector are coupled to a vacuum chamber (0.5 mbar) in 45° geometry. This chamber was designed to allow change between a simple collimator and a polycapillary. The spectra acquisition is obtained by the PI-SpecA software and performed by the AXIL program.





Fig. 4. Micro-analysis performed for paper documents.

With this equipment it was possible to distinguish two different inks present in the 1779 Dirk & Cornelius Blaw document, the one in the text with Cu, Zn and S, and the one used in the text with that presented more Fe (Fig. 5).

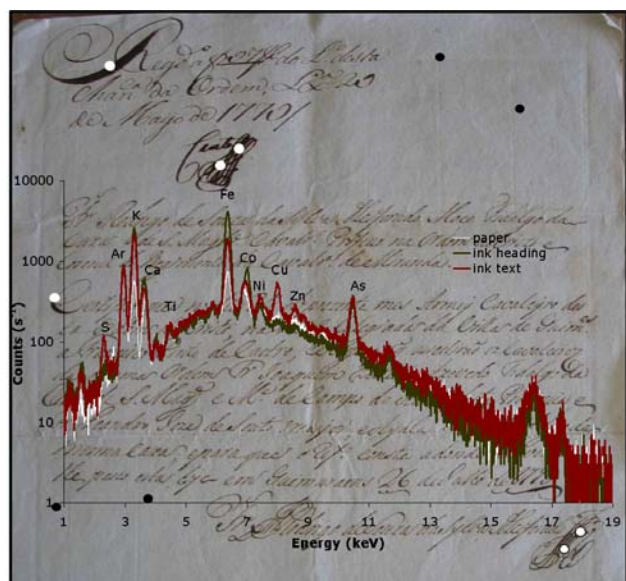


Fig. 5. Spectra obtained for the paper and inks of the 1779 document.

## References

- [1] M. L. Carvalho, F. Parente and Lúcia Salgueiro, "History of the Atomic Physics Group of the University of Lisbon", *X-ray Spectrometry*, 35, 271-275 (2006)
- [2] A.F. Marques, I. Queralt, M.L. Carvalho and M. Bordalo; "TXRF and EDXRF analysis of runoff water and vegetation from abandoned mining of Pb-Zn ores", *Spectr. Chim. Acta B, Atomic Spectroscopy* 12: 2191-2199 (2003).
- [3] P.J. Custódio, M.L. Carvalho and F.Nunes; "Direct analysis of human blood, mother and newborn baby, by energy dispersive X-ray fluorescence (EDXRF)", *Journal of Trace Elements in Medicine and Biology* 19: 151-158 (2005).
- [4] E. Marguí, I. Queralt, M.L. Carvalho and M. Hidalgo; "Determination of K, Ca, Mn, Fe, Cu, Sr, Pb and Zn in different vegetation specimens collected at an abandoned mining area by EDXRF: Comparison with microwave-assisted digestion plus ICP-OES", *Analytica Chimica Acta* 549: 197-204 (2005).
- [5] M. Manso and M.L. Carvalho; "Characterization of essential and toxic elements in cephalopod tissues by EDXRF and FAAS", *X-Ray Spectr.* 36: 413-418 (2007).
- [6] J.A.A. Brito, D.E.B. Fleming and D.R. Chettle; "A review of EDXRS in the study of human lead metabolism", *X-Ray Spectrom.* 37: 84-88 (2008)
- [7] M.L. Carvalho, T. Magalhães, M. Becker, and A. von Bohlen; Trace elements in human cancerous and healthy tissues: A comparative study by EDXRF, TXRF, synchrotron radiation and PIXE-a Review, *Spectrochim. Acta B* 62: 1004-1011 (2007)
- [8] M. Manso, S. Pessanha, and M.L. Carvalho; "Artificial aging processes in modern papers: X ray spectrometry studies", *Spectr. Chim. Acta B* 61: 922-928 (2006).
- [9] M. Manso and M.L. Carvalho; "Elemental characterization of a document paper by X ray fluorescence spectrometry", *J. Anal. At. Spectrom.* 22: 164-170 (2007).
- [10] M. Manso, M. Costa and M.L. Carvalho; From papyrus to paper: elemental characterization by X ray fluorescence spectrometry, *Nuclear Instruments and Methods in Physics Research A* 580: 732-734 (2007).
- [11] A. Guilherme, A. Cavaco, S. Pessanha, M. Costa and M.L. Carvalho; Comparison of a portable and a stationary EDXRF spectrometers in the study of ancient metallic artefacts DOI: 10.1002/xrs.1016
- [12] K. Castro, N. Proietti, E. Princi, S. Pessanha, M. L. Carvalho, S. Vicini, D. Capitani, J. M. Madariaga Multianalytical spectroscopic analysis of a coloured Dutch map from the XVIII century, doi:10.1016/j.aca.2008.06.019
- [13] M. Manso, M. Costa and M.L. Carvalho; Comparison of elemental content on modern and ancient paper by EDXRF, *Applied Physics A* 90: 43-48 (2008).
- [14] K. Castro, S. Pessanha, N. Proietti, E. Princi, D. Capitani, M. L. Carvalho, J. M. Madariaga, Non-invasive and non-destructive NMR, RAMAN and XRF analysis of a Blaeu's coloured map from the XVII century *Anal Bioanal Chem*, 391: 433-441 (2008).

## Venezuela

*Venezuela. Unidad De Análisis Instrumental, Decanato De Agronomía, Dpto. Química Y Suelos, Universidad Centroccidental Lisandro Alvarado, Tarabana, Cabudare, Edo. Lara, Venezuela*

**Contributor:** Profa. Lué-Merú Marcó Parra ([luemerumarco@yahoo.es](mailto:luemerumarco@yahoo.es) and [mparra@ucla.edu.ve](mailto:mparra@ucla.edu.ve))

The direct analysis of biological samples is the main research challenge of the scientific group at the Unidad de Análisis Instrumental of the Agronomy Faculty, Universidad Centro Occidental Lisandro Alvarado, (UCLA) Edo. Lara, Venezuela. The technique of total reflection X ray fluorescence (TXRF) plays an important role in the scientific activities of the laboratory. In this field, the research is devoted to the design, development, evaluation and application of methods for the analysis of biological and related samples, such as biological tissues, fluids, biota, soil and water samples. The studies involve the evaluation of feasibility for direct determination of analytes, the in situ (in quartz sample holder) preparation techniques, the in situ preconcentration and speciation among

others studies. The methods are developed for the application in clinical studies, agronomy, environmental monitoring, bioremediation, statistical processing of data and neural network applications. The research group works in joint projects with the laboratory of Nuclear Physics at Universidad Simón Bolívar, Caracas, Venezuela, Universidad Nacional Experimental Politécnica, Barquisimeto, Venezuela, The Comisión Nacional de Energía Atómica de Argentina, Universidad de Buenos Aires-Argentina, Universidad de Concepción-Chile, Hungarian Academy of Sciences-University Eotvoes Lorand and with the IAEA technical cooperation project RLA 010 in the frame of the ARCAL program.

### ***Direct analysis of biological samples by TXRF***

*(joint project with the Laboratorio de Física Nuclear, Universidad Simón Bolívar, Sartenejas, Baruta Venezuela)*

The biological organic samples are frequently analysed after digestion procedures. The direct determination of analytes requires shorter analysis time, low reactive consumption and simplifies the whole analysis process. On the other hand, the biological clinical samples have often minimal amounts and routine studies require the analysis of high number of samples. To overcome the difficulties associated to the analysis of organic samples, especially solids one, different procedures of sample preparation and calibration to approach the direct analysis were evaluated:

1. the slurry sampling;
2. Compton peak standardization;
3. in situ microwave digestion;
4. in situ chemical modification, and
5. direct analysis with element as internal standard.

The analysed samples were human brain tissue, serum blood, amniotic fluid, urine and the Bovine liver standard 1577a. The elements Fe, Cu, Zn, Se, Ca, K and Pt among others were determined. The accuracy was evaluated by comparison of the results to those obtained after the conventional digestion, with an element as internal standard and to the independent technique flame atomic absorption spectrometry (FAAS). No significant differences were found between the TXRF and FAAS technique results when the last technique was used for accuracy evaluation.

The precision of TXRF results depended mainly on the ratio of element level to detection limit and was less than 10% of relative standard deviation (RSD) for most of the quantified elements. The feasibility of the direct analysis using element as internal standard, Compton peak as internal standard, slurry preparation and also the alternative in situ microwave digestion and in situ chemical modification procedures for saline matrix elimination was demonstrated.

### **References:**

- [1] Determination of platinum levels in serum and urine samples from paediatric cancer patients by TXRF. Greaves E.D., Marcó L.M., Rojas A. Y Sajo L., X-Ray Spectrometry, vol. 29, (2000), 349-353.
- [2] Feasibility study on in situ microwave digestion prior to analysis of biological samples by Total Reflection X ray Fluorescence. L. M. Marcó P., T. Capote, E. A. Hernández and E.D. Greaves. Spectrochimica Acta B, vol 56, (2001), 2187-2193.
- [3] Determination of Zn/Cu ratio and oligoelements in serum samples by total reflection X ray fluorescence spectrometry for cancer diagnosis. L. M. Marcó P., E. Jiménez, E. A. Hernández C., A.

- Rojas, and E. D. Greaves, *Spectrochimica Acta B*, vol. 56, (2001), 2195-2201.
- [4] Evaluation of artificial neural networks as tools for cancer diagnosis based on the Fe, Cu, Zn and Se concentrations in blood serum samples determined by total reflection X ray fluorescence spectrometry. Edwin A. Hernández C. Y Lué Merú Marcó., *Spectrochimica Acta B* 58, (2003), 2205-2213.
- [5] Direct analysis of biological samples by TXRF, Edwin A. Hernández C. Y Lué Merú Marcó P., *Spectrochimica Acta B*, 59, (2004), 1077-1090.
- [6] Evaluation of chemometrics techniques and artificial neural networks for cancer screening using Cu, Fe, Se and Zn concentrations in blood serum. Edwin Hernández Caraballo, Franklin Rivas, Anna G. Pérez, Lué-Merú Marcó., *Analytica Chimica Acta*, 533, (2005), 161-168.

***Determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars using TXRF and ultrasound extraction procedure***

*(joint project with the Laboratorio de Física Nuclear, Universidad Simón Bolívar, Sartenejas, Baruta Venezuela)*

The chemical characterization of onion cultivar samples is an important tool for the enhancement of their productivity due to the fact that chemical composition is closely related to the quality of the products. A new sample preparation procedure for elemental characterization is proposed, involving the acid extraction of the analytes from crude samples by means of an ultrasonic bath, avoiding the required digestion of samples in vegetable tissue analysis. The technique of Total Reflection X ray fluorescence (TXRF) was successfully applied for the simultaneous determination of the elements Ca, K, Mn, Fe, Cu and Zn. The procedure was compared with the wet ashing and dry ashing procedures for all the elements using multivariate analysis and the Scheffé Test. The technique of Flame Atomic Absorption Spectrometry (FAAS) was employed for comparison purposes and accuracy evaluation of the proposed analytical method.

A good agreement between the two techniques was found when using the dry ashing and ultrasound leaching procedures. The levels of each element found for representative samples of two onion cultivars (Yellow Granex PRR 502 and 438 Granex) were also compared by the same method. Levels of K, Mn and Zn were significantly higher in the 438 Granex cultivar, while levels of Ca, Fe and Cu were significantly higher in the Yellow Granex PRR 502 cultivar.

**Reference**

- [1] Determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars using TXRF and three preparation procedures. Alvarez Jonathan, Marcó Lué Merú, Greaves E.D., Rivas Ricardo., *Spectrochimica Acta B* 58, (2003), 2183-2189.

***Development of off-line layer chromatographic and total reflection X ray fluorescence spectrometric methods for arsenic speciation***

*(joint project with the Hungarian Academy of Sciences, University Eotvoes Lorand, Budapest in the frame of the project FONACIT- Hungarian Academy of Sciences)*

Rapid and low cost off-line thin layer chromatography–total reflection X ray fluorescence spectrometry and overpressured thin layer chromatography–total reflection X ray fluorescence spectrometry methods have been developed for separation of 25 ng of each As(III), As(V), monomethyl arsenic acid and dimethylarsenic acid applying a PEI cellulose stationary phase on plastic sheets and a mixture of acetone/acetic acid/ water=2:1:1 (v/v/v) as eluent system. The type of eluent systems, the amounts (25–1000 ng) of As species applied to PEI cellulose plates, injection volume, development distance, and flow rate (in case of overpressured thin layer chromatography) were taken into consideration for the development of the chromatographic separation. Moreover, a micro-digestion method employing nitric acid for the As spots

containing PEI cellulose scratched from the developed plates divided into segments was developed for the subsequent total reflection X ray fluorescence spectrometry analysis. The method was applied for analysis of root extracts of cucumber plants grown in As(III) containing modified Hoagland nutrient solution. Both As(III) and As(V) were detected by applying the proposed thin layer chromatography/overpressured thin layer chromatography–total reflection X ray fluorescence spectrometry methods.

**Reference**

- [1] Development of off-line layer chromatographic and total reflection X ray fluorescence spectrometric methods for arsenic speciation,



Victor G. Mihucz, Ágnes M. Móricz, Krisztina Kröpfl, Szilvia Szikora, Enikő Tatár, Lué Merú

Marcó Parra, Gyula Záray. *Spectrochimica Acta. Part B. Volumen 61, (2006), 1124-1128.*

***Evaluation of vermicompost as bioadsorbent substrate of Pb, Ni, V and Cr for waste waters remediation using total reflection X ray fluorescence***

*(joint project with the Laboratorio de Física Nuclear, Universidad Simón Bolívar, Sartenejas, Baruta Venezuela, the Universidad Nacional Experimental Politécnica-Barquisimeto, The Comisión Nacional de Energía Atómica of Argentina and the Universidad de Buenos Aires)*

The use of vermicompost as adsorbent substrate for removing Pb, Ni, V and Cr from waste waters is proposed. In this work, after a preliminary physical and chemical characterization of the vermicompost, the optimal parameters for the heavy metals adsorption were obtained. A synthetic multielemental solution of Pb, Cr and Ni and a solution of  $\text{NH}_4\text{VO}_3$  for vanadium were evaluated. The optimized parameters were pH, vermicompost mass to volume ratio, and agitation time and particle size of the adsorbent. A batch system was employed for the assays. The elements were determined in the supernatant solution after filtration of the substrate. An optimum pH of 4.5 was found for ions removal. The agitation time slightly influences the adsorption of Pb and Cr, but it has a high influence on the Ni and V adsorption. The highest adsorption and

removal of the metals was observed for a vermicompost mass of 2 grams per 500 ml using a particle size between 75 to 841  $\mu\text{m}$  for Pb, Cr and Ni, and 841 to 1192  $\mu\text{m}$  for V. The mean removal percentage for each element is around 95% for Pb, Ni and Cr in the multielemental synthetic sample, demonstrating a high removal capacity of the substrate. For V it was found a removal efficiency of 50%.

**Reference**

Evaluation of vermicompost as bioadsorbent substrate of Pb, Ni, V and Cr for waste waters remediation using Total Reflection X ray Fluorescence. Cintia Urdaneta, Lué Marcó Saida Matute, Angel Garaboto y Cristina Vazquez. Accepted in *Spectrochimica Acta B*, June 2008.

***Determination by TXRF of total As in onion plants growing in contaminated substrates***

*(in process of publication). Lué Marcó, Saida Matute and Jesús Arroyo, Universidad Centro Occidental Lisandro Alvarado.*

The aim of this work is the evaluation of the effect of inorganic Arsenic on onion plants and the determination of the concentration of the element by TXRF. The technique was compared to the HG-AAS method.

Two treatments with 15 replicates were applied to the onion plants: As (III) contaminated, and control. After a careful selection of the onion plantlets (hybrid seminis 438), they were transplanted to dark polyethylene pots filled with prepared sand (sand was milled, washed with diluted HCl, distilled water and dried). The contaminant was added to the plants to an amount of  $0.5 \mu\text{g g}^{-1}$  in sand, in a single time three weeks after the transplant. Then the non contaminated Hoogland solution was added till the end of the experiments for both groups (55 days). The green leaves, bulbs and roots together with the steams were separated. Then the different parts were dried at  $60^\circ\text{C}$  and wet ash digested for total arsenic determination by TXRF and HG-AAS. For TXRF analysis aqueous, multielement (K, Ca, V, Mn, Cu, As, Se and Sr) standards were prepared with the element Co selected as internal standard. Five

microliters of standards or samples followed by 5 microliters of a  $10 \text{ mgL}^{-1}$  Co solution were deposited onto the reflector and vacuum dried. For quantification, the normalized sensitivities obtained with the standards were fitted by using a continuous curve. The TXRF analysis was carried out in a Canberra XRF Spectrometer with a modified TXRF module designed at the Atominstitut der Österreichischen Universitäten, Vienna. The excitation was performed with the  $\text{K}_\alpha$  (17.4 keV) line of a Molybdenum anode X ray tube, operated at 40 kV and 20 mA. A carbon-molybdenum multilayer crystal was used for monochromatization of the incident beam and a Si(Li) detector ( $R_{\text{Mn},5.8 \text{ keV}} = 180 \text{ eV}$ ) was used for the detection. The spectra were collected during 200 s of live time. The spectral data analysis was conducted with the QXAS package supplied by the IAEA. The total arsenic was determined in the tissue samples by HG-FAAS, in a PERKIN ELMER® Spectrometer 3110, with Hydride generation module. The analysis was carried out according to the method 3114C of the Standard Methods for Water and Wastewater Examination.

The total As concentration was above detection limits of both techniques used in this work (TXRF and HG-AAS) in the control group, for all the parts of the onion plants, green leaves, bulbs and roots together with the steam. A quantification limit of  $0.67 \mu\text{g g}^{-1}$  and  $0.15 \mu\text{g g}^{-1}$  (referred to 1 g of digested sample) was found for TXRF and HG-AAS respectively. In the experimental group the mean values were  $3.91 \mu\text{g g}^{-1}$  in the green leaves,  $11.6 \mu\text{g g}^{-1}$  for the bulb and  $37.48 \mu\text{g g}^{-1}$  for the root and steam. When the concentrations in the

different parts are compared at the 95% confidence level,  $n=15$ , significant differences were found. The As(III) at the level of  $0.5 \mu\text{g g}^{-1}$  in the substrate does not affect the plant development regarding to the bulb diameter and leaf size, Phosphorous concentration in the pholiar tissue, % of humidity. Significant differences were found regarding the root length, which was significantly higher in the contaminated group compared to the control group. HG FAAS and TXRF results were in agreement at the 95% confidence level.

### *On going research:*

1. In situ preconcentration and speciation of Arsenic III and Arsenic V in water samples using copper covering of the quartz reflector or adsorption with alumina. By Hayden Barros and Eduardo Greaves (Laboratorio de Fisica Nuclear, Univ. Simón Bolívar); Leonardo bennun (Universidad de Concepción) and Lué—Merú Marcó Parra (Unidad de Analisis Instrumental, Univ. Centroccidental Lisandro Alvarado).
2. Speciation of Arsenic III and Arsenic v by TXRF after in situ hydride generation on the quartz sample holder. By Merú Marcó Parra ((Unidad de Analisis Instrumental, Univ. Centroccidental Lisandro Alvarado) and Jorge Amaya (Univ. Nacional Experimental Politécnica, UNEXPO, Barquisimeto).

## **Publications of potential interest to the XRF community**

Applied Chemometrics for Scientists, Richard G. Brereton, John Wiley and Sons, New York, 2007

G. Buzanich, P. Wobrauschek, C. Streli, A. Markowicz, D. Wegrzynek, E.Chinea-Cano, S.Bamford, A portable micro-x ray fluorescence spectrometer with polycapillary optics and vacuum chamber for archaeometric and other applications, Spectrochimica Acta Part B 62, 1252-1256, 2007

E. Margui, M. Hidalgo and I. Queralt, XRF spectrometry for trace element analysis of vegetation samples. SpectroscopyEurope 19 (3), 13-17, 2007.

N. Zoeger, C. Streli, P. Wobrauschek, C. Jokubonis, G. Pepponi, P. Roschger, J. Hofstaetter, A. Berzlanovich, D. Wegrzynek, E. Chinea-Cano, A. Markowicz, R. Simon and G. Falenberg, Determination of the elemental distribution in human joint bones by SR micro XRF, X-Ray Spectrom. 37, 3-11, 2008.

Elisabeth Prichard, Vicki Barwick, Quality assurance in analytical chemistry, John Wiley & Sons, 2007, ISBN: 978-0-470-012031-1.

Werner Funk, Vera Dammann, Gerhild Donnevert, Quality assurance in analytical chemistry: applications in environmental, food, and materials analysis, biotechnology, and medical engineering, 2nd ed., Wiley-VCH, 2006, ISBN: 978-3-527-31114-9.



**IAEA**

International Atomic Energy Agency

## XRF Newsletter No. 15

July 2008

The XRF Newsletter is prepared twice per year by the IAEA Laboratories in Seibersdorf. Correspondence and materials to be considered for publishing should be sent to:

Dr. A. Markowicz  
IAEA Laboratories  
2444 Seibersdorf, Austria

Fax: (+43 1) 2600-28222 or (+43 1) 26007  
E-mail: A.Markowicz@iaea.org

International Atomic Energy Agency  
Wagramer Strasse 5, P.O. Box 100,  
1400 Wien, Austria

Printed by the IAEA in Austria,  
September 2008