Activities of the Department of Atomic Physics and Nanophysics of Institute of Physics within the project ENFORCE TXRF (COST ACTION CA18130)

ENFORCE TXRF - European Network for Chemical Elemental Analysis by **Total Reflection X-Ray Fluorescence (TXRF)**

A. Kubala-Kukuś, D. Banaś, I. Stabrawa, K. Szary, P. Jagodziński, M. Pajek, G. Wesołowski, R. Stachura, A. Foks, Ł. Jabłoński, D. Sobota

In collaboration with Holy Cross Cancer Center, Kielce, Poland

TOPICS

- ENFORCE TXRF (COST ACTION CA18130) - European Network for Chemical Elemental Analysis by Total Reflection X-Ray Fluorescence: the main aims and objectives of the Action
- Total-Reflection X-ray Fluorescence Analysis (TXRF)
- TXRF conference
- ENFORCE TXRF – structure, activities, support
- TXRF in Institute of Physics at Jan Kochanowski University
- Activities within the project ENFORCE TXRF
- Joint experimentation within the COST - VMG: TXRF CENSORED DATA
- Joint experimentation within the COST - VMG: WATER INTERLAB TEST
- Joint experimentation within the COST - VMG: HAIR - PROFICIENCY TEST

Cost Actions

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.
www.cost.eu

ENFORCE TXRF (COST ACTION CA18130)

ENFORCE TXRF - European Network for Chemical Elemental Analysis by Total Reflection X-Ray Fluorescence

The Challenge

The main aims and objectives of the Action is to coordinate the efforts made at the national and transnational level to establish total reflection X-ray fluorescence (TXRF) as a reference technique for reliable elemental analysis of solid and liquid matrices, for the purposes of both fast screening and accurate quantitative determination. These objectives will be achieved via the development of a strong TXRF network, building capacity by training, connecting and involving stakeholders.

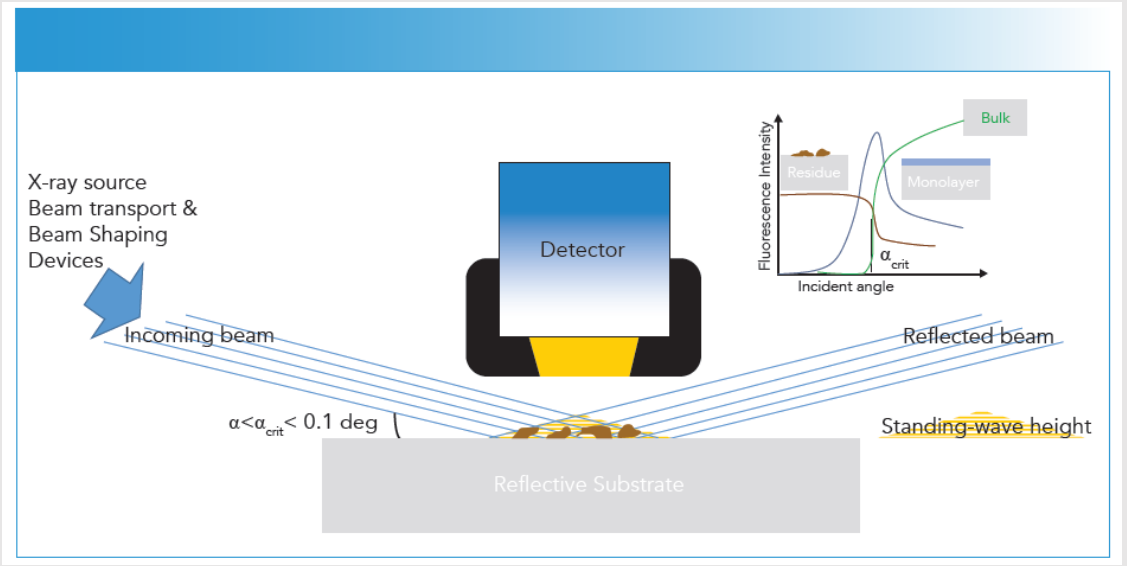
https://enforcetxrf.eu/

Duration: 2019 March 13th → 2023 September 12th

Chair **Laura Borgese** (IT)
Vice chair **Jasna Jablan** (HR)

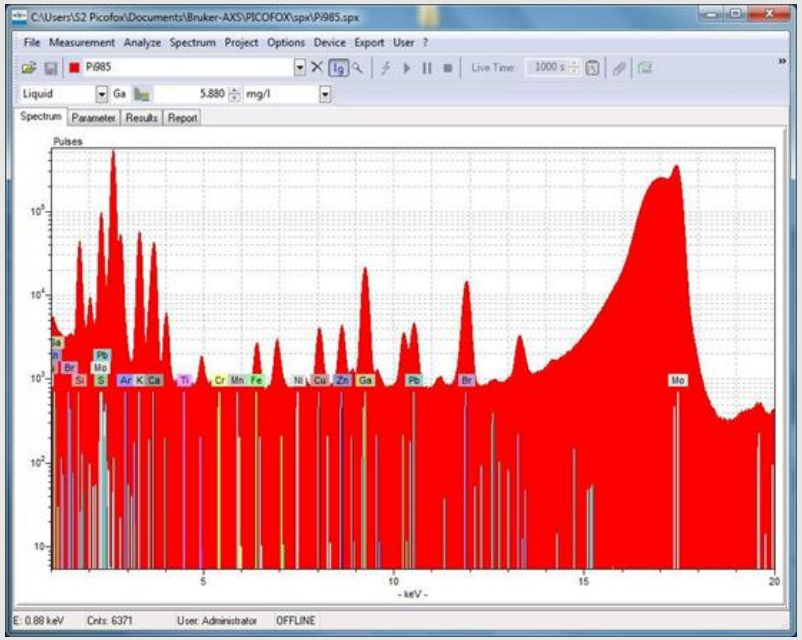
Total-Reflection X-ray Fluorescence Analysis (TXRF)

In the TXRF technique, the primary X-ray beam is directed on the studied sample (or sample carrier on which the sample is deposited) at an angle smaller than the critical angle, strictly defined for a given X-ray energy and type of sample/sample carrier material. In the TXRF technique, the registration angle is 90°.



D. Eichert, The fundametals of Total Reflection X-ray Fluorescence, Spectroscopy Spectroscopy-08-01-2020 35 (2020) 20-24.

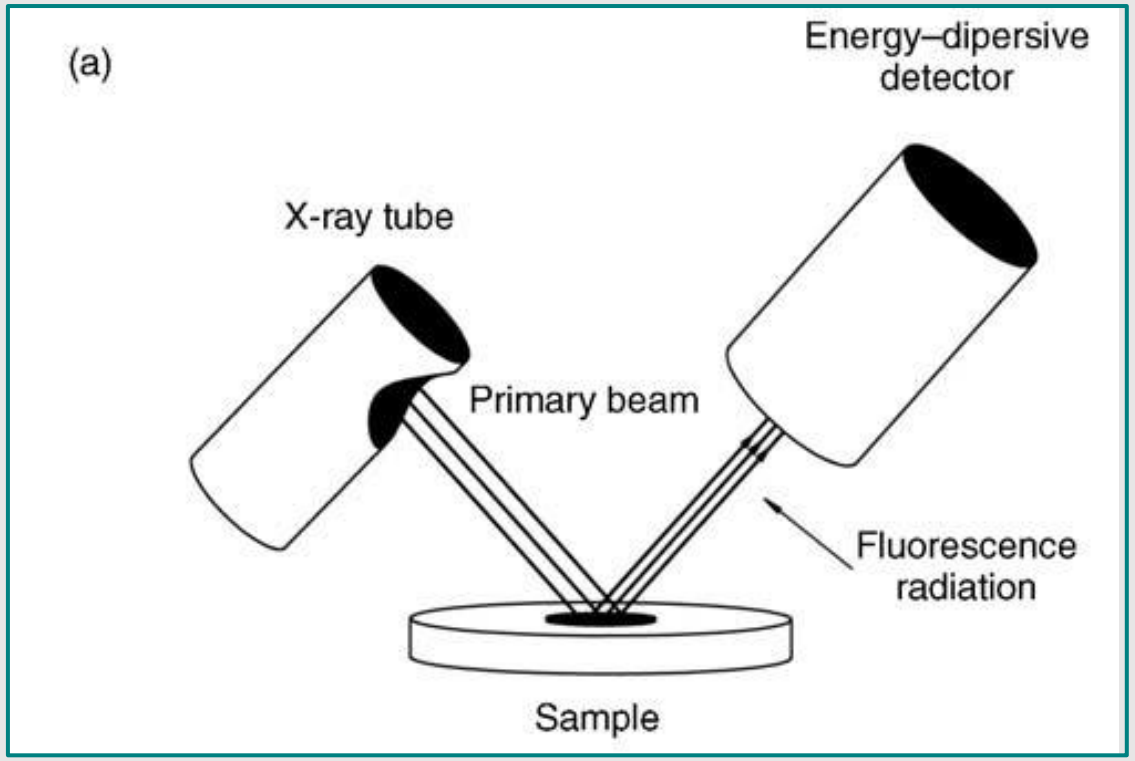
The main issue of the studied atomic processes is the registration of excited in photoelectric effect fluorescence radiation and primary radiation scattered elastically and inelastically (Rayleigh and Compton effects), determination of its intensity, which gives information, also quantitative, about the elemental composition of the sample and detection limit level. An important aspect is the selection of experimental conditions to maximize the emission probability of characteristic radiation and minimize the inelastic scattering of X-rays in the studied material.



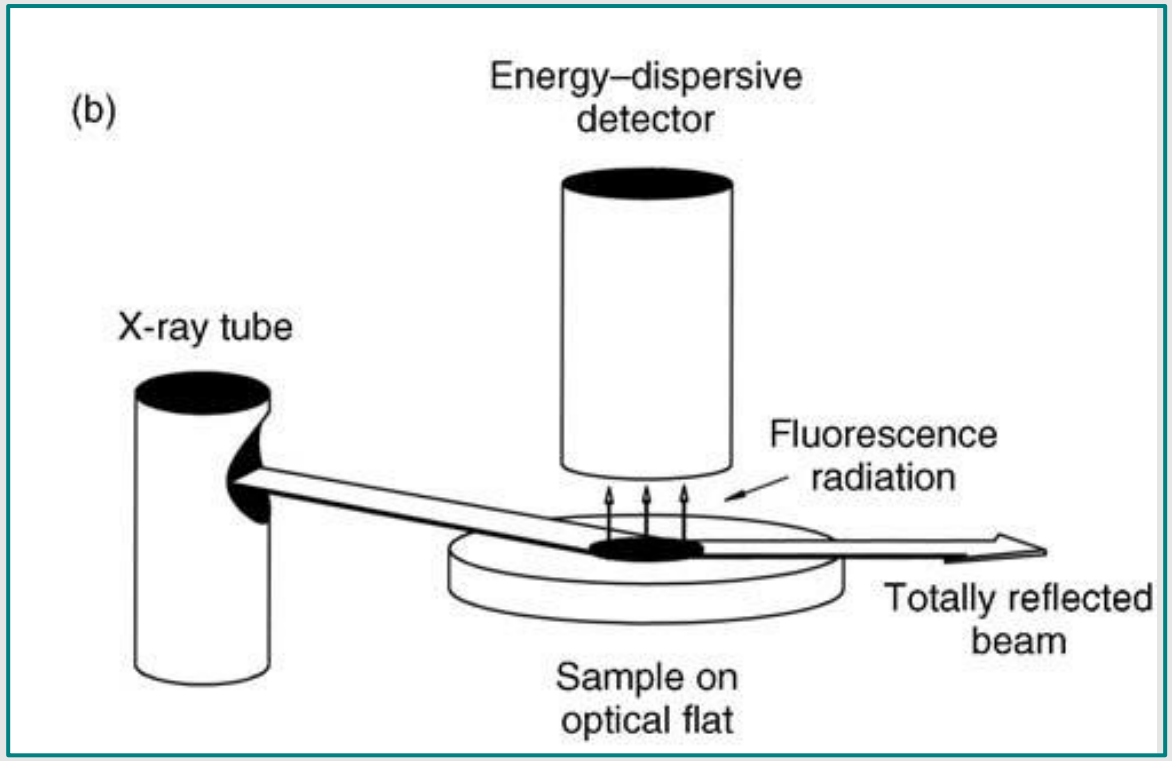
Total-Reflection X-ray Fluorescence Analysis (TXRF)

Instrumental arrangement for (a) conventional XRF and (b) TXRF. Comparison shows a difference in the geometric grouping of excitation and detection units.

XRF



TXRF



R. Klockenkämper, A. von Bohlen, Total Reflection X-Ray Fluorescence Analysis and Related Methods, Wiley: New Jersey, 2015

Total-Reflection X-ray Fluorescence Analysis (TXRF)

Historical synopsis

- In the year 1923 Arthur H. Compton described for the first time total reflection of X-rays under grazing incidence of the primary beam (*A. H. Compton, The total reflection of X-rays, Philosophical Magazine 45 (270) (1923) 1121-1131*).
- Nearly fifty years later, in 1971, Yomeda and Horiuchi discovered the possibility to use the phenomenon of external total reflection of X-rays at a flat reflector surface for spectra analyses (*Y. Yoneda, T. Houriuchi, Optical flats for the use in X-ray spectrochimical microanalysis, Review of Scientific Instruments 42 (1971) 1069-1070*).
- The promising idea was abandoned and was re-evaluated in the late 1970th and early 1980th by Aiginger and Wobrauschek in Austria and by Knoth and Schwenke in Germany.
- Few papers were published until 1986. They deal with theoretical estimation (*P. Wobraushek, H. Aiginger, Total-reflection X-ray fluorescence spectrometric determination of nanogram amounts, Analytical Chemistry 47 (1975) 852-855*) and the first spectrometer prototype and its features (*J. Knoth, H. Schwenke, Trace element enrichment on a quartz glass Surface used as a sample suport of an X-ray Spectrometer for the subnanogram range, Fresenius' Zeitschrift für Analytische Chemie 294 (1979) 273-274*).
- A first commercially available device was presented by Seifert & Co., Ahrensburg, Germany in 1980 and nearly at the same time a simple module built by Wobraushek and distributed by the IAEA (International Atomic Energy Agency) Vienna, Austria were available. Nearly 50 modules, as non-profit systems for academic use only, has been installed in developing countries around the whole world under the auspices of the IAEA. In the Institute of Physics, we started our work with the TXRF based on Wobraushek's module.

Critical angle in dependence on the primary X-ray beam energy and material properties

X-rays exhibit total reflection for a particular angle of incidence when passing from a material with a higher refractive index to a material with lower refractive index. This is fulfilled for X-rays when they pass from e.g. vacuum (or air) to any other material.

The phenomena of reflection and refraction of the X-ray beam at the border of the medium are described by three basic quantities characterizing these processes, namely: *critical angle* θ_c, *reflection coefficient R* and *penetration depth z* of X-ray. For angles $\theta < \theta_c$ the reflection coefficient is practically the same for all materials, reaching the value of R ≈ 1 (100% of reflection). The penetration depth of X-rays into the medium under conditions of total reflection of X-rays reaches a constant value, on the order of a few nanometers.

Values of critical angles for various materials at photon energy 17.47 keV (Mo-Kα line)

Medium:	θ_c [°]
Quartz glass	0.104
Aluminum	0.108
Silicon	0.102
Nickel	0.196
Copper	0.192

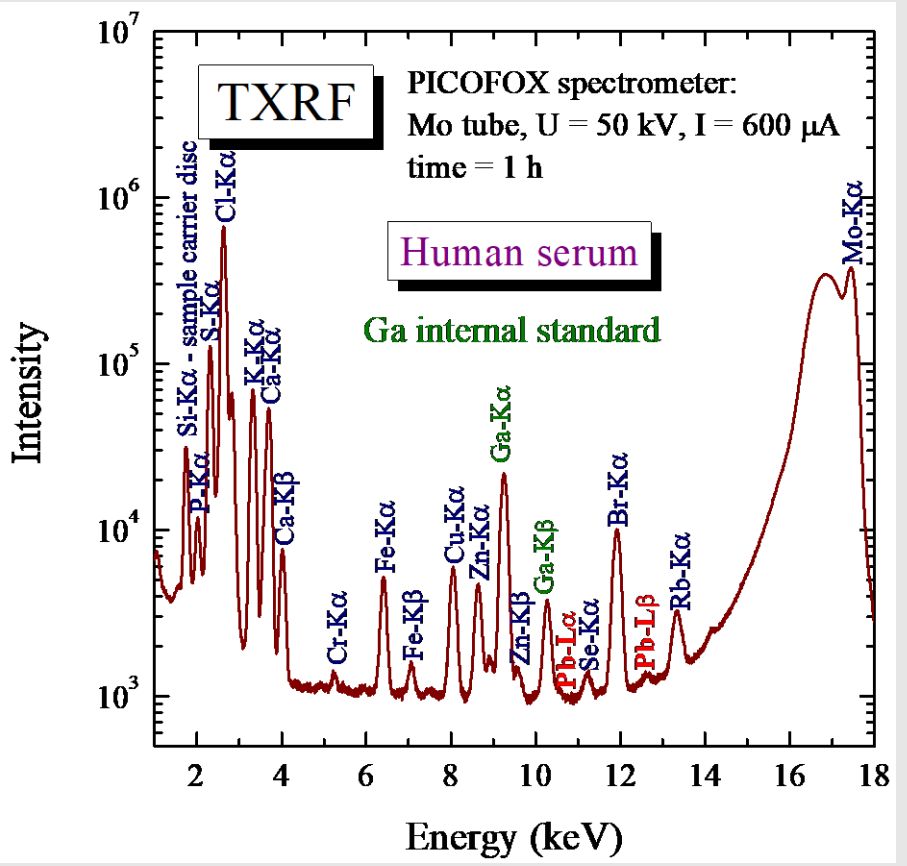
$$\theta_c = C\sqrt{\frac{Z\rho}{A}}\frac{1}{E}$$

where $C = \sqrt{\frac{N_a r_e h^2 c^2}{\pi}} = 1.65$ is constant value; density ρ in g/cm^3, photon energy E in keV, θ_c in degrees.

Total reflection of X-rays occurs at very small glancing angle.

Total-Reflection X-ray Fluorescence Analysis (TXRF)

- The method is applicable to a great variety of sample materials, a wide scope of applications, with simultaneous analysis of many elements.
- Usually a minute specimen, i.e., a single particle, some grains of a fine powder, a thin layer or some small droplets, has to be deposited in the center of a flat and clean carrier.
- In general, all the TXRF users can be divided into three main groups: (i) users from universities and scientific institutes; (ii) users at synchrotron beam-lines, and (iii) users in industry (for example semiconductor industry).
- Worldwide distribution of TXRF instrumentation (*R. Klockenkämper, A. von Bohlen, Spectrochimica Acta B 99 (2014) 133-137*): TXRF instrumentation is used in 57 countries on six continents (43% are localized in Europe), mainly in scientific institutes.
- Different fields of applications with:
  - environmental problems (different kinds of water, aerosols, biomonitors, nutrients),
  - industrial applications (ultrapure water, purified sewage, fuels, high tech materials),
  - chemical aspects (high-purity fluids and solids, ceramics, polymers),
  - biological background (biomolecules, cells, plants, beverage, food),
  - medical origin (urine, blood, serum, tissue, hair).



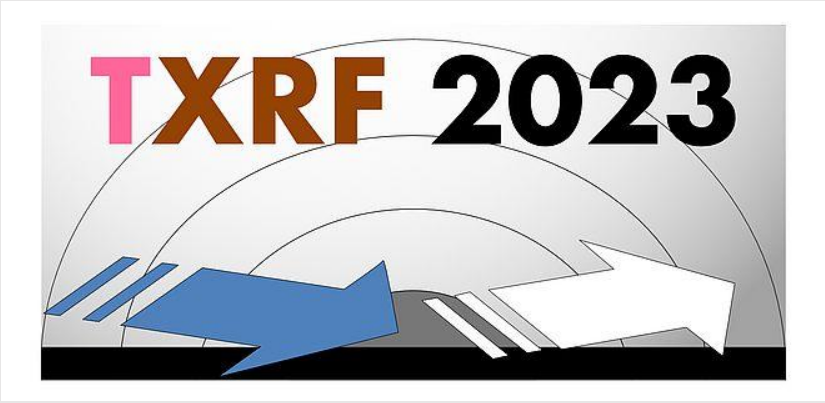
TXRF conference

History of the Conference

After long years of experimental work on instrumental development, the first International Conference on Total Reflection X-ray fluorescence Analysis and Related Methods (also known as TXRF conference) was held in 1986, in Geesthacht (near Hamburg), Germany. Subsequently, seventeen more conferences have been organized in different countries on a biannual basis.

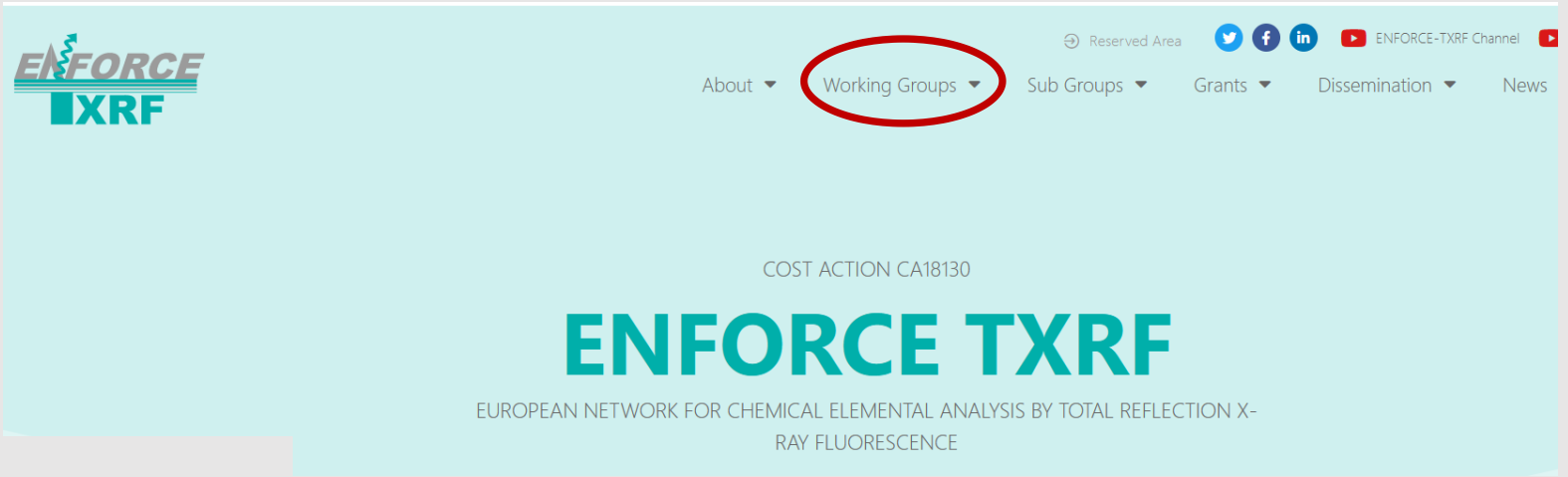
Scope of the Conference

The biannual TXRF conference is a forum for experts and users of TXRF and related techniques. The scope is to jointly present and discuss recent advances, latest research, and new perspectives in different fields of TXRF from the fundamentals to the applications.



TXRF 2023, 19th International Conference on Total Reflection X-ray Fluorescence Analysis and Related Methods took place from 5 to 8 September 2023 at Clausthal University of Technology, Germany

ENFORCE TXRF – structure, activities, support



Working groups

WG1: Instrumentation, modelling, data evaluation and software

WG2: Metrology and standardization

WG3: Sample preparation and analytical procedures

WG4: Performance assessment and data analysis

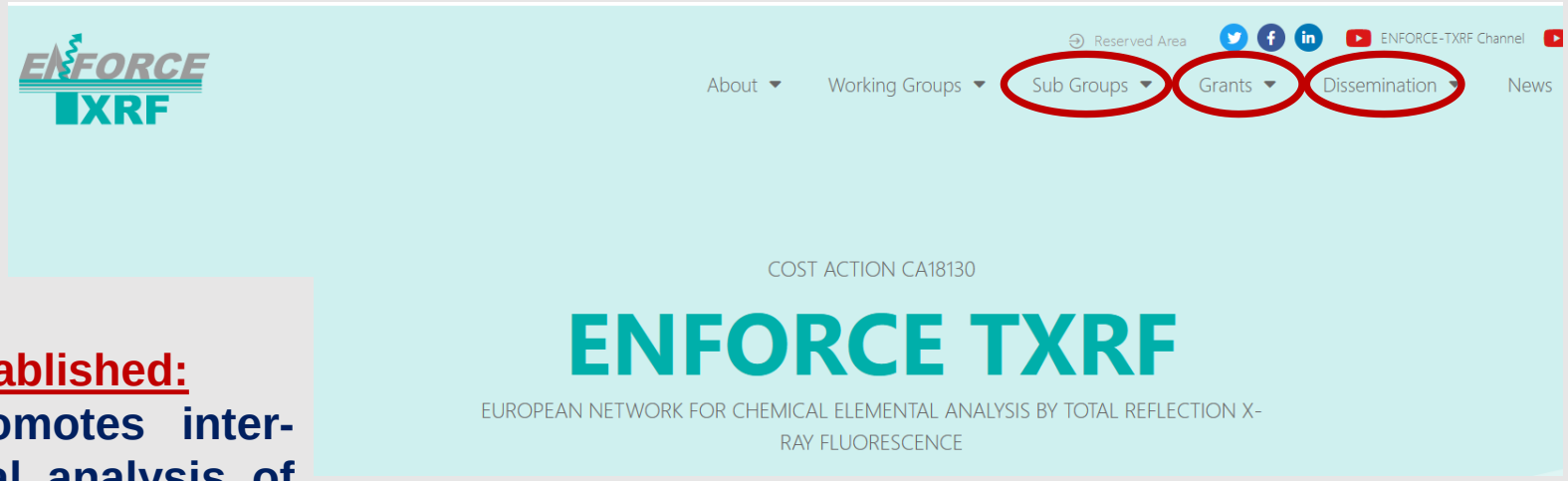
WG1 objectives:
- to enable equal access to research infrastructures, instrumentation and facilities,
- to understand the most relevant issues about measurements, software and data analysis affecting the quality of results.

WG2 objective:
- to help in establishing TXRF as a reference/certified technique for reliable elemental analysis, both for screening and accurate quantitative determination.

WG3 objectives:
- to contribute to the improvement of existing methodologies and development of new sample preparation strategies and analytical procedures,
- to promote TXRF application for screening, quality control and monitoring purposes in many different fields.

WG4 objectives:
- to assess the performance of TXRF methods and compare with complementary analytical techniques,
- data analysis performance assessment of different instrumental configurations and comparison with other analytical techniques used for elemental analysis, such as AAS and ICP spectroscopy, highlighting advantages, disadvantages and possibilities of use in different application fields.

ENFORCE TXRF – structure, activities, support



COST ACTION CA18130
Thematic Sub Groups were also established:

- the METROFOOD subgroup promotes inter-laboratory activities for elemental analysis of food candidate Reference Materials (RMs),
- the BIOMEDIAG subgroup brings together scientists interested in using the TXRF technique for advanced elemental analysis of biological and medical samples (leaders: Dariusz Banaś & Aldona Kubala-Kukuś).

COST ACTION CA18130
Grants:

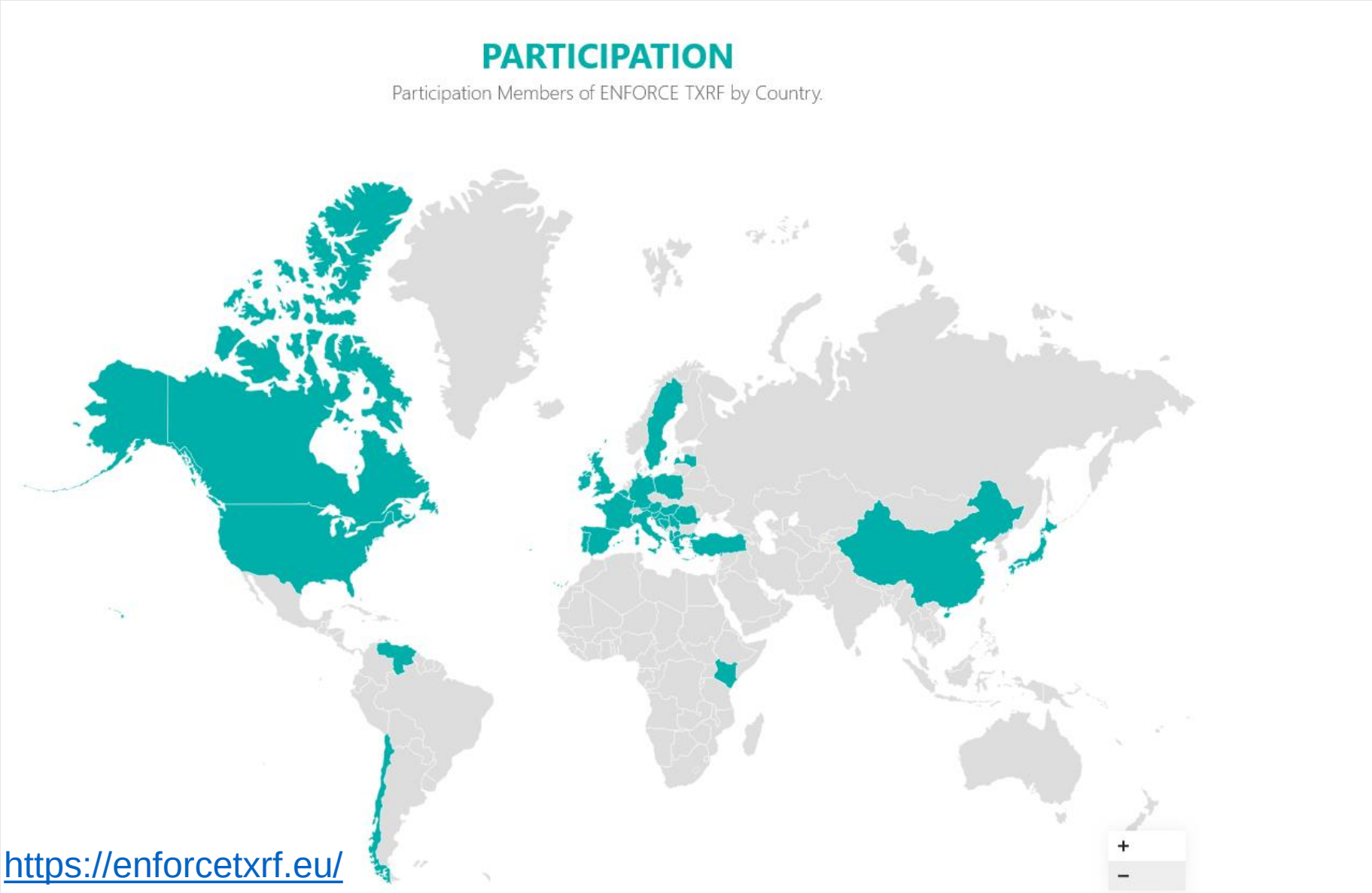
The ENFORCE TXRF Cost Action CA18130 funds contributions in the form of grants for:

- Short Term Scientific Missions (STSM),
- Inclusiveness Target Countries Conference Grants,
- Virtual Networking.

COST ACTION CA18130
Disseminations:

- events (management committee meetings, core group meetings, workgroup meetings, transversal workgroup meetings, workshops, training school),
- conferences and publications,
- TXRF Journal Club (a monthly 45 minute *remote* academic journal club lecture and discussion on research in TXRF),
- training materials.

Participants



https://enforcetxrf.eu/

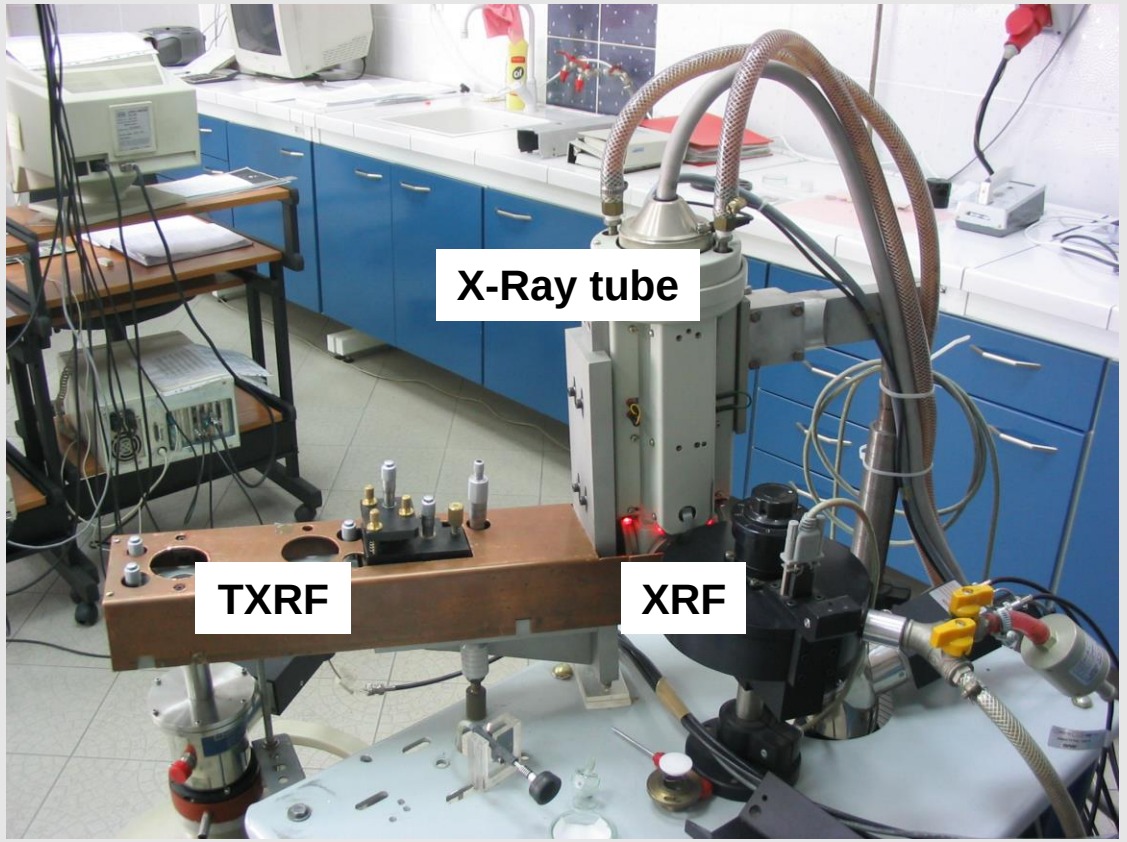
Country list

- Albania
- Argentina
- Austria
- Bosnia and Herzegovina
- Belgium
- Canada
- Egypt
- Switzerland
- Chile
- China
- Croatia
- Deutschland
- Greece
- France
- Hungary
- Ireland
- Italy
- Japan
- Kenya
- Lativia
- Montenegro
- North Macedonia
- **Poland**
- Portugal
- Romania
- Serbia
- Spain
- Sweden
- Slovenia
- Turkey
- United Kingdom
- United States
- Venezuela

TXRF in Institute of Physics at Jan Kochanowski University

We have been working with TXRF for about 30 years. This technique we use mainly in the medical applications but also in biological and environmental studies. We collaborate with Holycross Cancer Center in Kielce and routinely perform analysis of copper and selenium concentration in human serum of patients of the Center.

Wobraushek's TXRF module

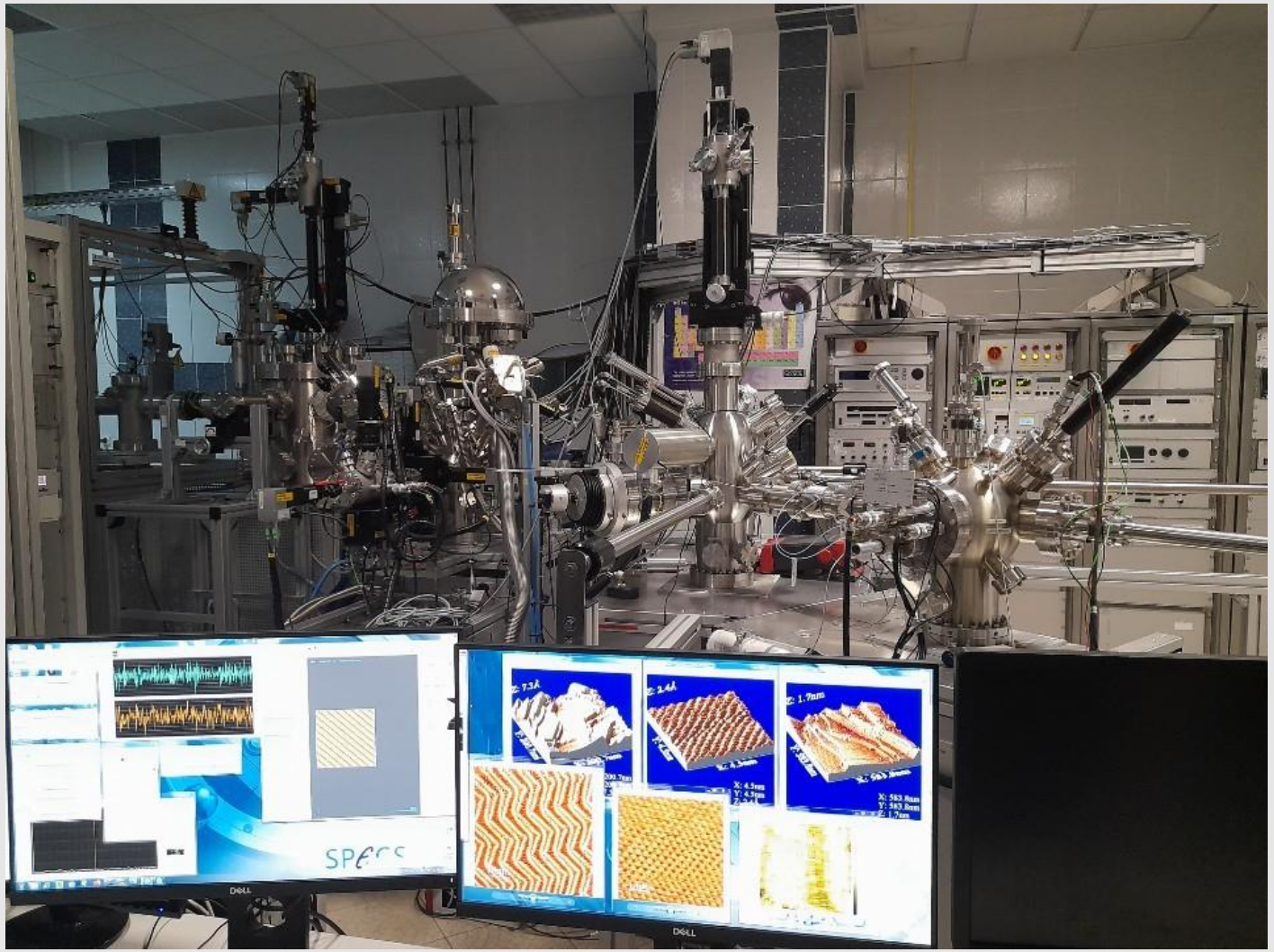


J. Braziewicz, E. Braziewicz, S. Chojnacki, M. Pajek, J. Semaniak, *Analiza rentgenowska próbek środowiskowych* (*X-ray analysis of environmetal samples*). Monitoring Środowiska Regionu Świętokrzyskiego, 1994, nr 2, s. 39-46, Kieleckie Towarzystwo Naukowe, Kielce.

TXRF in Institute of Physics at Jan Kochanowski University

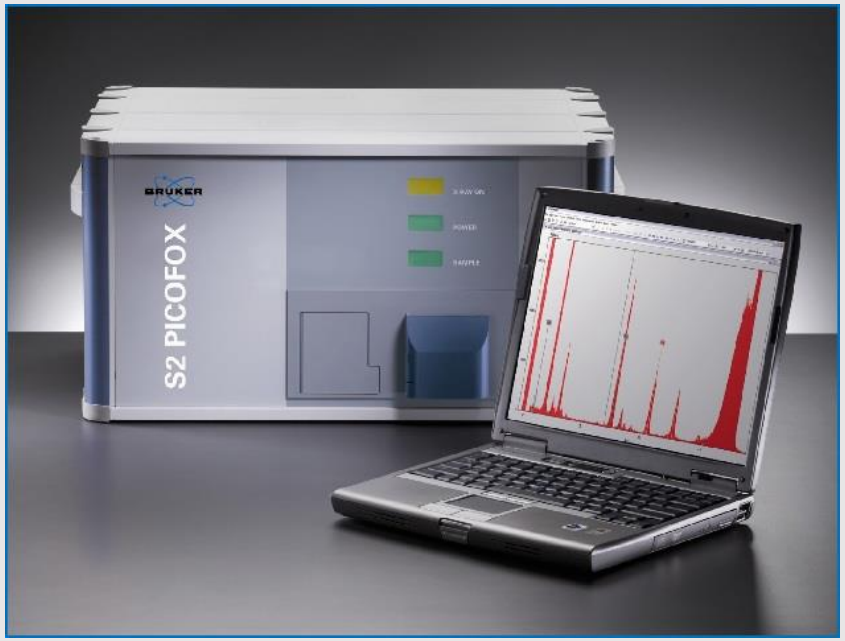
view in 2004 (from my PhD thesis) and …

present view (2024)



TXRF spectrometers

TXRF spectrometer in Institute of Physics



Mo-anode 30 W X-ray tube
Range of analyzed elements: from Mg to U

Note:
The new TXRF spectrometer will be purchased at the beginning of next year (financed by the Minister of Science under the “Regional Excellence Initiative” program (project no.: RID/SP/0015/2024/01)).

Accreditation of Polish Centre for Accreditation (ISO 17025)

We have also accreditation of Polish Centre for Accreditation (ISO 17025) for analysis of water and purified sewage using the procedure developed by us for TXRF technique.

CBiA Centrum Badań i Analiz

Uniwersytet Jana Kochanowskiego w Kielcach

Main Page | Structure | Offer | Quality politics | Accreditation | Contact

MAIN PAGE

Description from 02.04.2023

Basic information

The Center for Research and Analysis (CBiA) is an independent unit operating within the structure of the Faculty of Natural Sciences of the Jan Kochanowski University in Kielce (UJK). The center was created by the decision of the Rector and was established as a result of the transformation of the Laboratory of X-ray Methods. Currently, the CBiA consists of 3 laboratories: the Laboratory of X-ray Methods (PMR), the Laboratory of Environmental Studies (PBŚ) and Laboratory of Thermal and Chromatographic Analysis (PATCh). PMR was separated from the X-ray Spectrometry Laboratory of the Institute of Physics of the Jan Kochanowski University, PBŚ from the Environmental Research Laboratory (LBŚ), previously operating in the Department of Environmental Protection, while PATCh was separated from the Environmental Research Laboratory and the Group of Geomorphological-Hydrological Laboratories. The laboratories were established under the project "Development of the research base of specialized laboratories of public universities in the Świętokrzyskie region" (Operational Program Innovative Economy, Priority 2. R&D infrastructure, Measure 2.2. Support for development of research infrastructure of scientific entities).

The center is a research and service unit whose purpose is:

- conducting scientific research,
- the realization of of works commissioned by other entities, including business entities.

The organization of the Center is defined by the requirements of the Integrated Quality Management System, the principles of which are regulated by the PN-EN ISO/IEC 17025:2018-2 standard "General requirements for the competence of testing and calibration laboratories".

News

Accredited measurements of smoke optical density

August 22, 2023

PCA granted CBiA accreditation for measuring optical density of smoke from plastic and rubber products as well as construction products, materials and objects using the single-chamber test. The measurements are carried out in the Thermal and Chromatographic Research Laboratory of CBiA.

Positive PCA assessment

November 3, 2022

Based on the results of the assessment carried out on July 5, 2022, PCA decided to maintain the CBiA accreditation No. AB 1622 in the current scope of accreditation.

Accredited measurements PM2.5 and PM10 particulate matter

September 1, 2021

POLSKIE CENTRUM AKREDYTACJI
POLISH CENTRE FOR ACCREDITATION

PCA

Sygnatariusz EA MLA
EA MLA Signatory

CERTYFIKAT AKREDYTACJI
LABORATORIUM BADAWCZEGO
ACCREDITATION CERTIFICATE OF TESTING LABORATORY
Nr AB 1622

Potwierdza się, że: / This is to confirm that:

UNIWERSYTET JANA KOCHANOWSKIEGO W KIELCACH
ul. Żeromskiego 5, 25-369 Kielce
CENTRUM BADAŃ I ANALIZ
WYDZIAŁ NAUK ŚCISŁYCH I PRZYRODNICZYCH
UNIWERSYTET JANA KOCHANOWSKIEGO W KIELCACH
ul. Uniwersytecka 7, 25-406 Kielce

spełnia wymagania normy PN-EN ISO/IEC 17025:2018-02
meets requirements of the PN-EN ISO/IEC 17025:2018-02 standard

Akredytowana działalność jest określona w Zakresie Akredytacji Nr AB 1622
Accredited activity is defined in the Scope of Accreditation No AB 1622

Akredytacja pozostaje w mocy pod warunkiem przestrzegania wymagań jednostki akredytującej określonych w kontrakcie Nr AB 1622
This accreditation remains in force provided the Laboratory observes the requirements of Accreditation Body defined in the Contract No AB 1622

Akredytacji udzielono dnia 28.09.2016 r.
Accreditation was granted on 28.09.2016

DYREKTOR
POLSKIEGO CENTRUM AKREDYTACJI
LUCYNA OLBORSKA

Warszawa, dnia 7 września 2020 r.

Benefits of activities within the project ENFORCE TXRF

Collaboration (projects, conference presentations, articles)

- Institute of Physics, Jan Kochanowski University, Kielce, Poland
- Holy Cross Cancer Center, Kielce, Poland
- Institute of Health Sciences, Jan Kochanowski University, Kielce, Poland
- Institute of Medical Sciences, Jan Kochanowski University, Kielce, Poland
- Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Kraków, Poland
- Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland
- Department of Chemistry, University of Girona, Girona, Spain
- Interdepartmental Research Service (SIdI), Autonomous University of Madrid, Madrid, Spain
- Faculty of Pharmacy and Biochemistry, Department of Analytical Chemistry, University of Zagreb, Zagreb, Croatia
- Department of Physics, NOVA School of Science and Technology, Caparica, Portugal
- Bruker Nano GmbH, Berlin, Germany
- Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy
- Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade
- Elettra Sincrotrone Trieste, Trieste, Italy
- Toronto Metropolitan University, Toronto, Canada

Virtual Mobility Grants (VIRTUAL NETWORKING)

- VIRTUAL MOBILITY (VM) grant title: WATER INTERLAB TEST (Grantee name: Aldona Kubala-Kukuś)
- VM grant title: TXRF CENSORED DATA (Grantee name: Aldona Kubala-Kukuś)
- VM grant title: HAIR - PROFICIENCY TEST (Grantee name: Dariusz Banaś)

ITC (Inclusiveness Target Country) Conference Grant

- Conference Title: TXRF 2023, 19th International Conference on Total Reflection X-ray Fluorescence Analysis and Related Methods, Germany (Grantee name: Grzegorz Wesołowski)

BIOMEDIAG Subgroup Workshop

https://lmr.ujk.edu.pl/biomediag/

Welcome Agenda Registration Abstracts Contact

Welcome

Workshop date: 21-23 of April 2021

Workshop organizer: Institute of Physics at Jan Kochanowski University (UJK) and Holy Cross Cancer Center (Kielce, Poland); Dariusz Banaś and Aldona Kubala-Kukuś, leaders of the BIOMEDIAG subgroup of ENFORCE-TXRF COST CA18130 action.

cost
European Cooperation in Science and Technology

BIOMEDIAG Subgroup Workshop

General aims: The BIOMEDIAG SG aims to foster the knowledge exchange and the development of a joint research agenda about using TXRF in the new and emerging applications for advanced elemental analysis of biological and medical samples, acting transversely to all the Action working groups (WG1, WG2, WG3 and WG4) coherently with their specific objectives.
This workshop acts as a stakeholders platform bringing together scientists with interest in biological and medical applications, promoting the TXRF technique for the analysis of biological (plants and animals) and medical (human) samples.

Topics:

- Typical biological and medical samples analyzed by TXRF: aim of the studies, technical details of the measurements.
- Application of TXRF in laboratory diagnostics of human biological material samples.
- Standards/reference materials (biological and medical)
- Available in the literature and new procedures for the preparation of selected biological and medical samples for the TXRF analysis.
- TXRF instrumentation and laboratory equipment useful in the context of biomedical analysis.
- Presentation of other independent techniques used for elemental analysis of the biological and medical samples and comparisons of their features with the TXRF method.
- Further research direction (e.g. Development and verification of easy to implementation procedures allowing routine measurements with low detection limits for metals, metalloids and non-metals).
- Promotion of the ISO/IEC 17025:2017 standard “General requirements for the competence of testing and calibration laboratories”.

BIOMEDIAG Subgroup Workshop

- 70 participants
- Countries: India, Argentina, Austria, Canada, Croatia, France, Germany, Greece, Hungary, Indonesia, Italy, Japan, Latvia, Mexico, Morocco, Poland, Portugal, Spain, Switzerland, Turkey, UK, USA
- Oral and poster sessions

Articles published

Spectrochimica Acta Part B: Atomic Spectroscopy
Volume 198, December 2022, 106548

Evaluation of different analytical approaches using total reflection X-ray fluorescence systems for multielemental analysis of human tissues with different adipose content

Patrícia M. Carvalho [a], Eva Marguí [b], Aldona Kubala-Kukuś [c d], Dariusz Banaś [c d], Jorge Machado [a], Diogo Casal [e], Diogo Pais [e], José Paulo Santos [a], Sofia Pessanha [a]

Article | Open access | Published: 27 October 2023

Ketogenic diet influence on the elemental homeostasis of internal organs is gender dependent

Kamil Kawon, Marzena Rugiel, Zuzanna Setkowicz, Katarzyna Matusiak, Aldona Kubala-Kukus, Ilona Stabrawa, Karol Szary, Zuzanna Rauk & Joanna Chwiej

Scientific Reports **13**, Article number: 18448 (2023) | Cite this article

Spectrochimica Acta Part B: Atomic Spectroscopy
Volume 205, July 2023, 106695

The first total reflection X-ray fluorescence round-robin test of rat tissue samples: Preliminary results

Karolina Olbrich [a], Aldona Kubala-Kukus [b c], Eva Marguí [d], Ramón Fernández-Ruiz [e], Katarzyna Matusiak [a], Jolanta Wudarczyk-Mocko [c], Pawel Wrobel [a], Zuzanna Setkowicz [f], Joanna Chwiej [a]

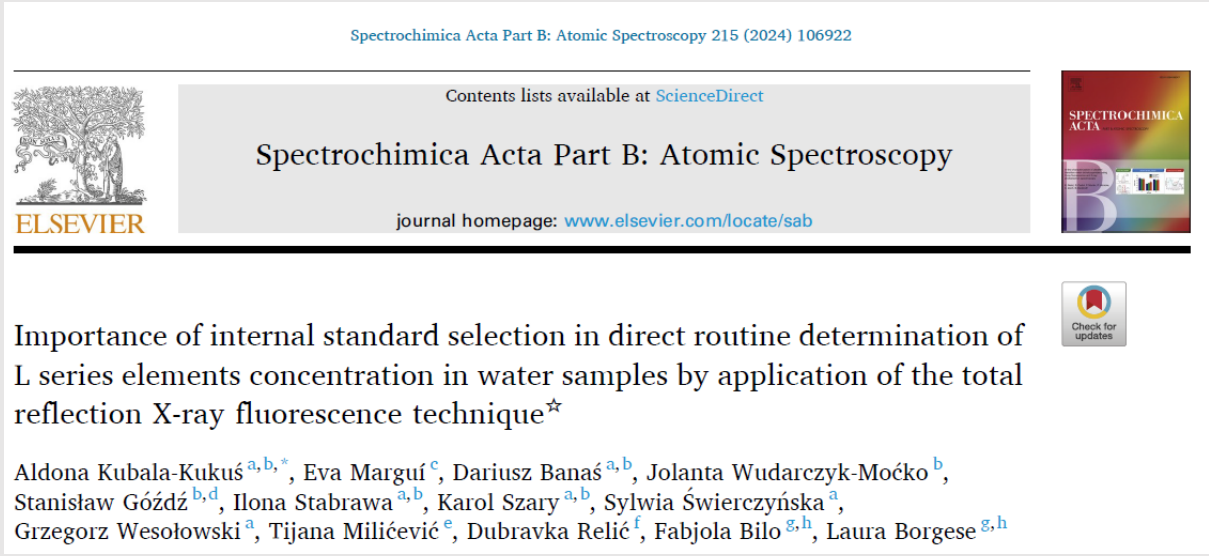
Spectrochimica Acta Part B: Atomic Spectroscopy
Volume 213, March 2024, 106865

Statistical analysis of analytical results near detection limits with illustrations using elemental determinations in medical and biological samples by total reflection X-ray fluorescence ☆

Aldona Kubala-Kukuś [a b], Dariusz Banaś [a b], Marek Pajek [a], Janusz Braziewicz [a b], Stanisław Góźdź [b c], Joanna Chwiej [d], Stanisław Głuszek [e], Paweł Jagodziński [a], Eva Marguí [f], Monika Pierzak-Stępień [c], Agata Pniak [a], Zuzanna Setkowicz [g], Ilona Stabrawa [a b], Regina Stachura [a], Karol Szary [a b], Grzegorz Wesołowski [a], Anna Wojsa [h], Jolanta Wudarczyk-Moćko [b]

Article published

A. Kubala-Kukuś, E. Marguí, D. Banaś, J. Wudarczyk-Moćko, S. Góźdź, I. Stabrawa, K. Szary, S. Świerczyńska, G. Wesołowski, T. Milićević, D. Relić, F. Bilo, L. Borgese
Importance of internal standard selection in direct routine determination of L series elements concentration in water samples by application of the Total Reflection X-ray Fluorescence technique
Spectrochimica Acta B 215 (2024) 106922



Articles in review

D. Banaś, A. Kubala-Kukuś, I. Stabrawa, K. Szary, J. Wudarczyk-Moćko, P. Jagodziński, G. Wesołowski, S. Góźdź, J. Chwiej, P. Wróbel, E. Marguí Grabulosa, J. Jablan, S. Pessanha, H. Stosnach, L. Borgese
Determination of minor and trace elements in human hair - an interlaboratory comparison
Spectrochimica Acta B (2024)

E. Marguí, D. Eichert, J. Jablan, F. Bilo, L.E. Depero, A. Pejović-Milić, A. Gross, H. Stosnach, A. Kubala-Kukuś, D. Banaś, L. Borgese
An overview of the applications of total reflection X-ray fluorescence spectrometry in food, cosmetics, and pharmaceutical research
Journal of Analytical Atomic Spectrometry (2024)

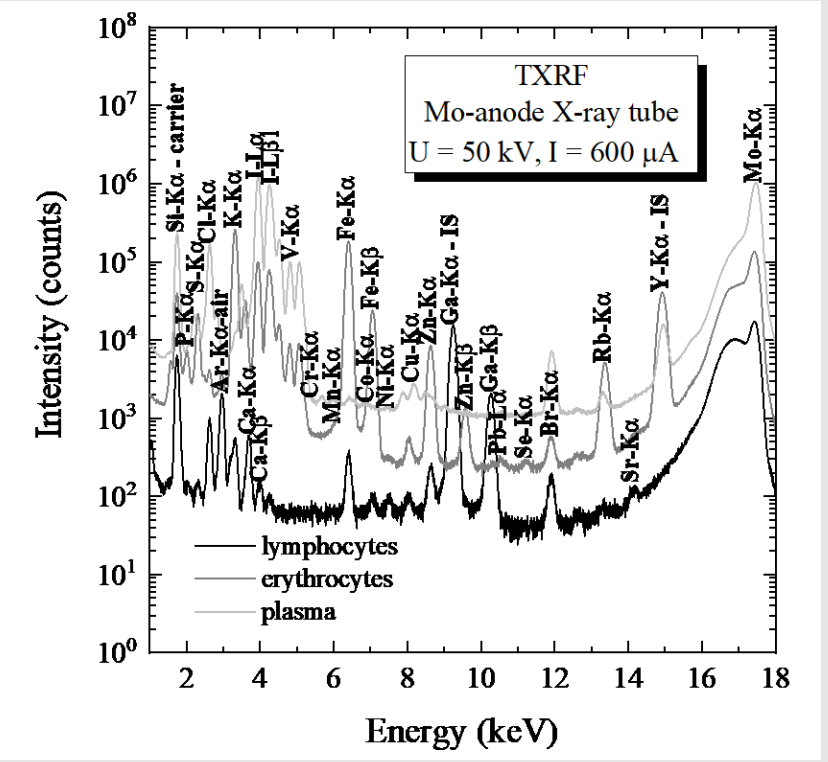
Virtual Mobility Grant: TXRF CENSORED DATA

Statistical analysis of analytical results near detection limits with illustrations using elemental determinations in medical and biological samples by Total Reflection X-ray Fluorescence

- The discussed studies concentrate on statistical analysis of the measurement results close to detection limit on the examples of the element concentrations analysis in medical and biological samples.
- In the presented examples, different experimental results are discussed, both almost complete with small fraction of data below the detection limit as well as with the main contribution of such data.
- Examples focus on different element concentration distributions obtained in analysis of various biomedical samples.

Criterion of a statistically significant number of counts N_{xDL} of X-ray lines, for which the element concentration is precisely determined, is assumed from the 3σ rule according to: $\boldsymbol{N_{xDL} > 3 \cdot \sqrt{N_{xBkg}}}$, where N_{xBkg} is the number of counts for background of analyte fluorescence line.

This condition determines value limit for the number of counts:
$N_{xDL} = 3 \cdot \sqrt{N_{xBkg}}$.
Value limit of counts can be next converted to the detection limit C_{xDL}:
$\boldsymbol{C_{xDL} = 3 \cdot \sqrt{N_{xBkg}} \cdot \frac{C_x}{N_x}}$.



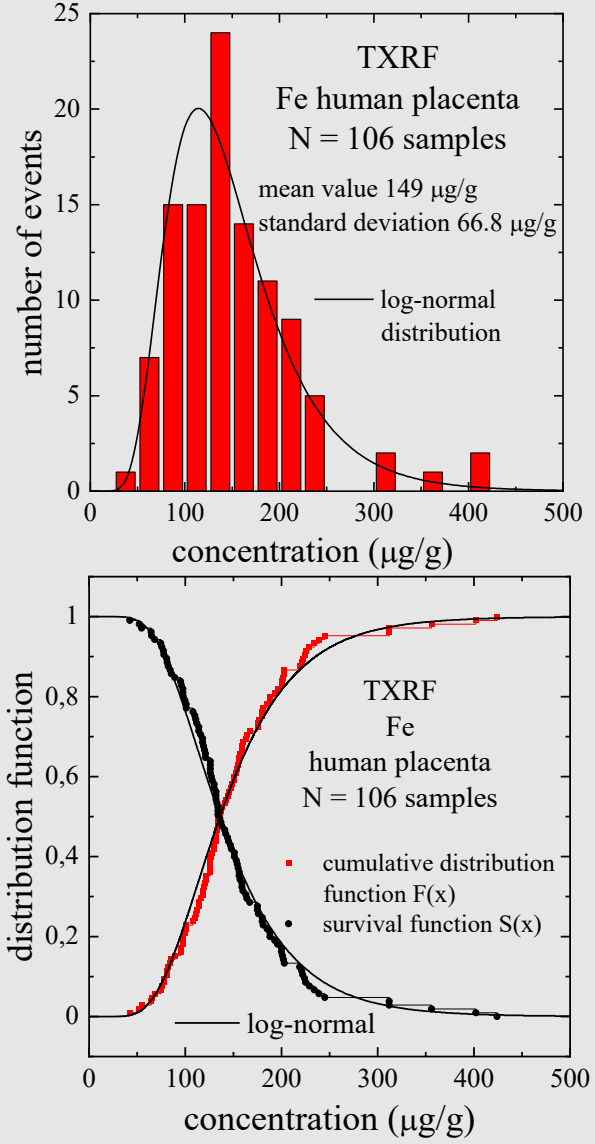
Examples of the X-ray spectra emitted by the lymphocytes, erythrocytes and plasma samples, registered using TXRF technique (IS – internal standard).

$\frac{1}{N_{IS}} \cdot \frac{B_{IS}}{B_x} \cdot C_{IS} = \frac{C_x}{N_x}$

C_{IS} is the concentration of an internal standard, N_x and N_{IS} are the net intensities of the analyte fluorescence line and internal standard, respectively. The quantities B_x and B_{IS} are the absolute sensitivities for the analyte and internal standard.

Virtual Mobility Grant: TXRF CENSORED DATA

- Analyzing set of samples in context of elemental composition, especially for trace element concentration, the measurement results for given element are obtained as set of values C_x or C_{xDL}.
- Any measurements in which the values are limited by the detection limit of the analytical technique (not only for X-ray spectroscopic analysis of element concentration), which may depend on many factors such as the apparatus used, the analyte to be determined, the presence of other analytes, the measuring conditions, represent ***random-left censored data***.
- In statistical software, the problem of the censored data is treated in ***Survival analysis procedure*** intended for consideration of time variable for which right-censored observations can occur. Statistical procedures dedicated to the random-right censored data can be, however, adopted in simple way to analyze measurement results close to the detection limit being example of left-censored data.
- A practical aspect of the discussed studies is the presentation of statistical procedures dedicated to censored data analysis in the selected statistical software (STATISTICA, OriginPro).
- These procedures allow inclusion of data below the detection limit in statistical analysis, in which the cumulative distribution function of element concentration is estimated, giving information about concentration quantiles such as for instance median, the lower (first) and the upper (third) quartiles.

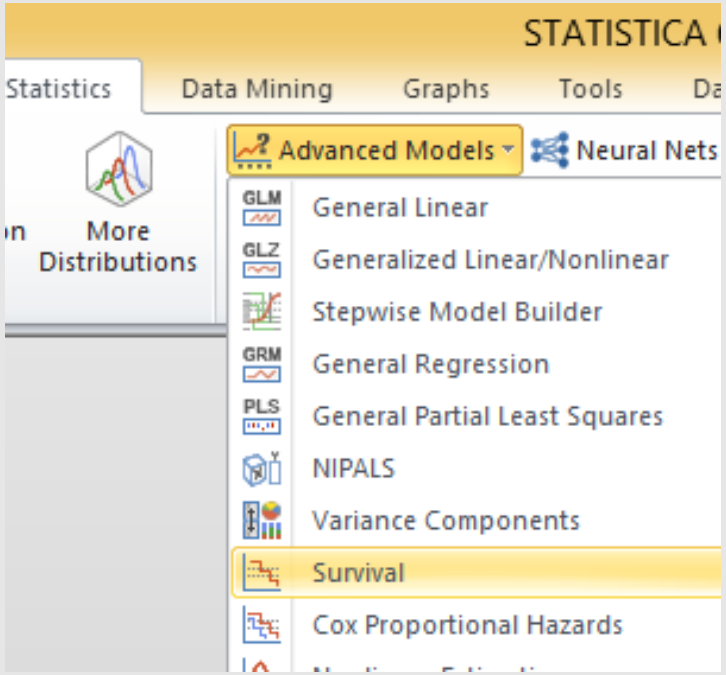


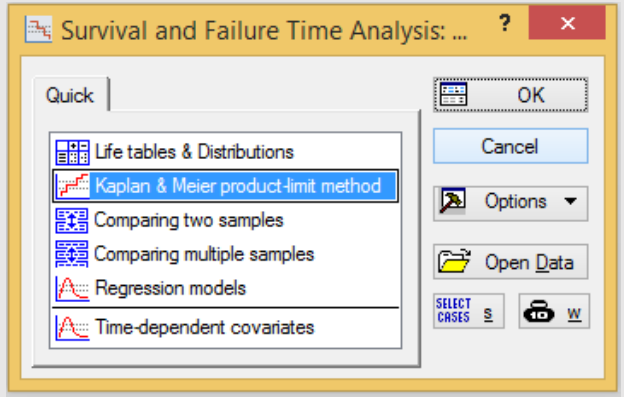
Graphical data presentation on example of Fe concentrations in N = 106 samples of human placenta: histogram of number of events (top panel) and plots for cumulative distribution function *F(x)* and survival function *S(x)* (bottom panel).

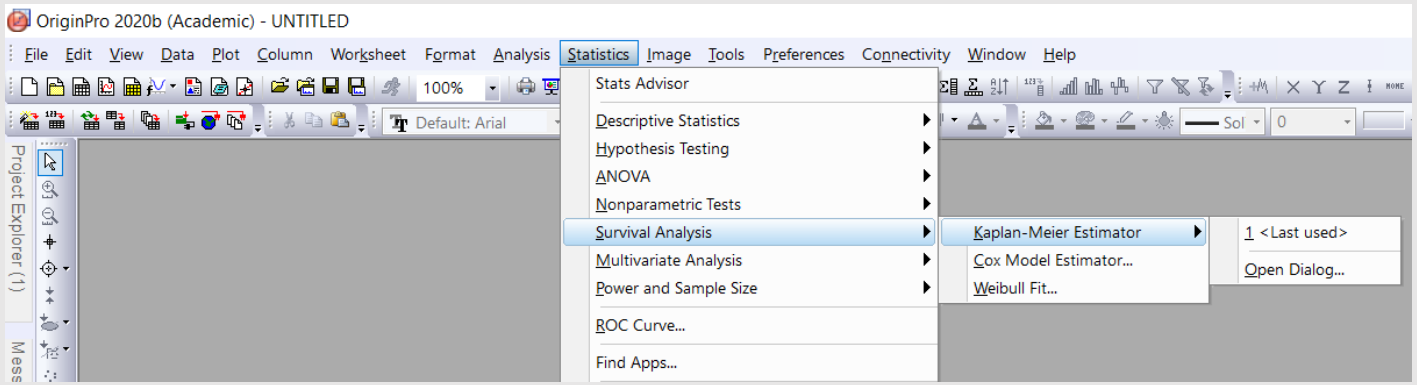
Virtual Mobility Grant: TXRF CENSORED DATA

PROCEDURE:

- calculating the survival function and estimating the value of quantiles;
- the survival analysis is discussed based on STATISTICA and OriginPro 2020;
- in STATISTICA the dialogue window is called *Survival and Failure Time Analysis* and offers several statistical procedures for typical survival and failure time analysis, including determination of the survival function and percentiles (*Kaplan & Meier product limit method*) as well as sample populations comparison (*Comparing two samples* or *Comparing multiple samples*);
- in OriginPro 2020 survival time analysis is placed in *Survival Analysis*. In order to estimate the survival function, the *Kaplan-Meier Estimator* option has to be chosen.







Note: the survival analysis procedure available in discussed software is dedicated to right censored data.

So, left-censored data need transformation (calculation of the reciprocal of element content).

Virtual Mobility Grant: TXRF CENSORED DATA

- **The analysis of measurement results limited by the detection limit based on survival analysis procedure was presented on the examples of the TXRF analysis of element concentrations in biological (rat organs, plant reference materials) and medical (human blood components: serum, erythrocytes, lymphocytes, and plasma) samples.**
- **These studies had various motivation, e.g. the detection of elemental abnormalities of various organs of rats resulting from brain tumor development, the determination of reference values in human blood components: erythrocytes, lymphocytes and plasma, the analysis of changes in element levels in the serum of patients with parenteral nutrition and Rb concentrations in plant reference materials.**
- **These studies were performed for different number of samples. In the research, many elements were determined, in a very wide range of concentration levels.**

Collaboration:
Institute of Physics, Jan Kochanowski University, Kielce, Poland
Holy Cross Cancer Center, Kielce, Poland
Institute of Health Sciences, Jan Kochanowski University, Kielce, Poland
Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Kraków, Poland
Institute of Medical Sciences, Jan Kochanowski University, Kielce, Poland
Department of Chemistry, Faculty of Sciences, University of Girona, Girona, Spain
Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland
Regional Hospital, Kielce, Poland

Virtual Mobility Grant: TXRF CENSORED DATA

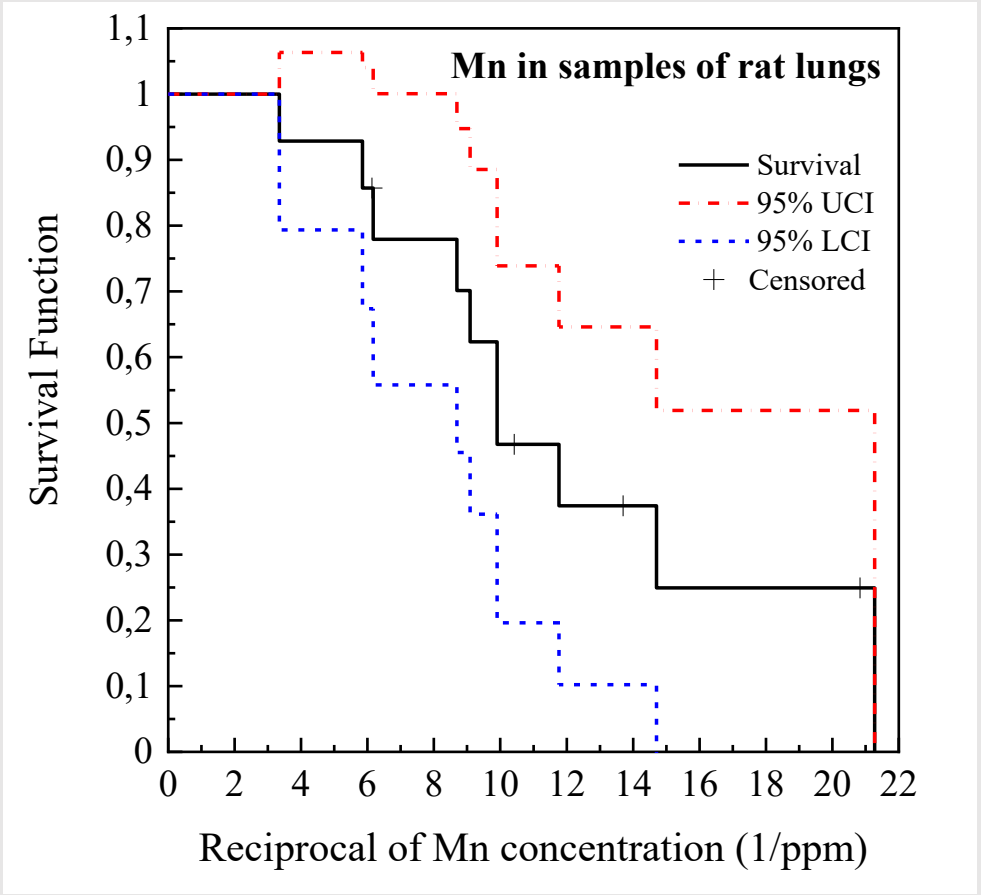
Element concentration in the organs of rats

- The experimental results concern the TXRF analysis of trace element contents in organs taken from male Wistar rats subjected to the intracranial implantation of various human cells of glioblastoma multiforme.
- The analysis was performed for the following rat organs: heart, lung, kidney, and spleen.
- For each organ, 14 samples were analyzed. The studies focus on concentrations of Mn, Br, Rb, Sr and Pb in the rat organ samples.

Censoring level (%) = (m/N)×100%					
Organ/Element	**Mn**	**Br**	**Rb**	**Sr**	**Pb**
All organs (N = 56)	7.14 (m = 4)	0 (m = 0)	0 (m = 0)	75.0 (m = 42)	71.4 (m = 40)
Heart (N = 14)	0 (m = 0)	0 (m = 0)	0 (m = 0)	85.7 (m = 12)	100 (m = 14)
Kidney (N = 14)	0 (m = 0)	0 (m = 0)	0 (m = 0)	64.3 (m = 9)	100 (m = 14)
Lung (N = 14)	28.6 (m = 4)	0 (m = 0)	0 (m = 0)	78.6 (m = 11)	78.6 (m = 11)
Spleen (N = 14)	0 (m = 0)	0 (m = 0)	0 (m = 0)	71.4 (m = 10)	7.14 (m = 1)

Censoring level (in percentage) for rat organ samples. Notation: N – number of samples, m – number of censored samples for which $C < C_{DL}$.

Multi-group comparisons of the censored data for different animal organs were also performed.

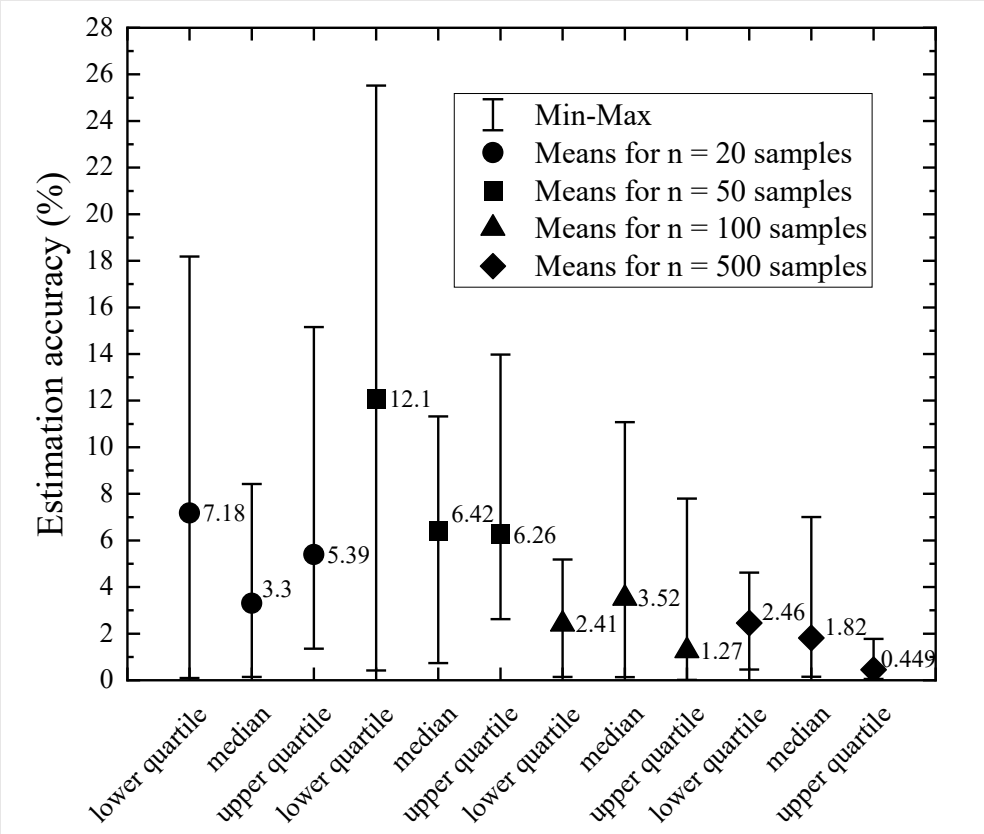


Reciprocal of Mn concentration $(\mu g/g)^{-1}$		Mn concentration (μg/g)	
Percentile	**Values**	**Values**	**Percentile**
Lower quartile	7.12	0.140	**Upper quartile**
Median	9.90	0.101	**Median**
Upper quartile	14.7	0.068	**Lower quartile**

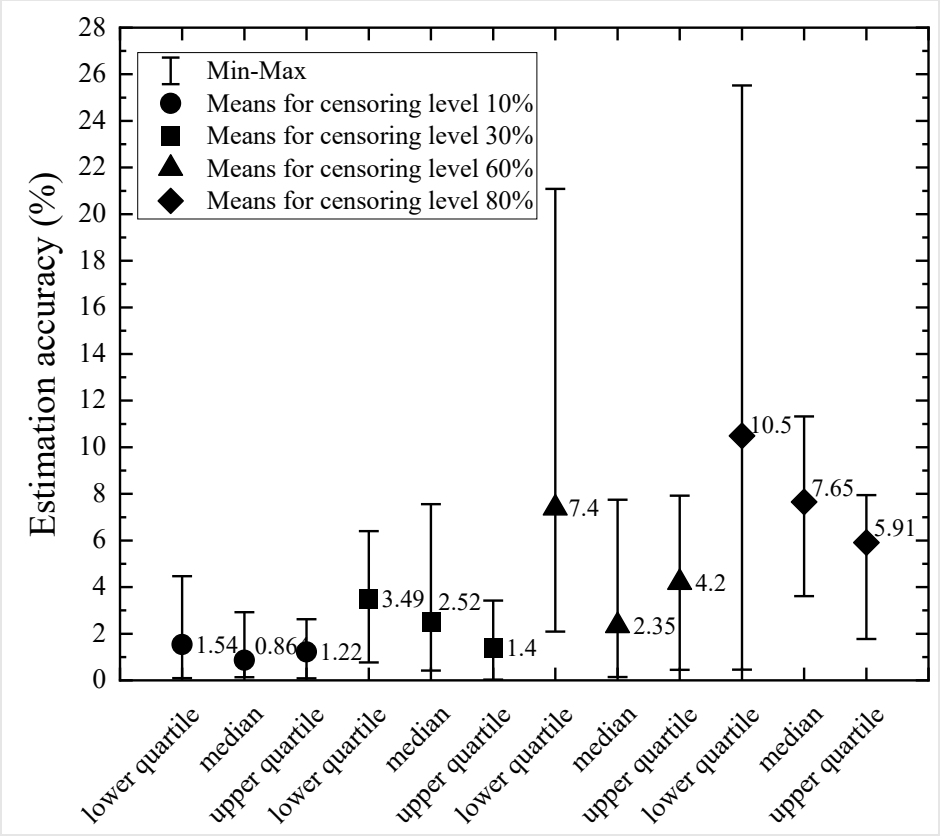
Virtual Mobility Grant: TXRF CENSORED DATA

Accuracy of survival analysis procedure

Simulations for N = 20, 50, 100, 500 number of samples and for 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% censoring levels.



Accuracy (in %) of quantile estimation from the survival function using *Survival analysis* procedure calculated for the simulated sets of the censored data for a various number of samples (N = 20, 50, 100, 500 samples).



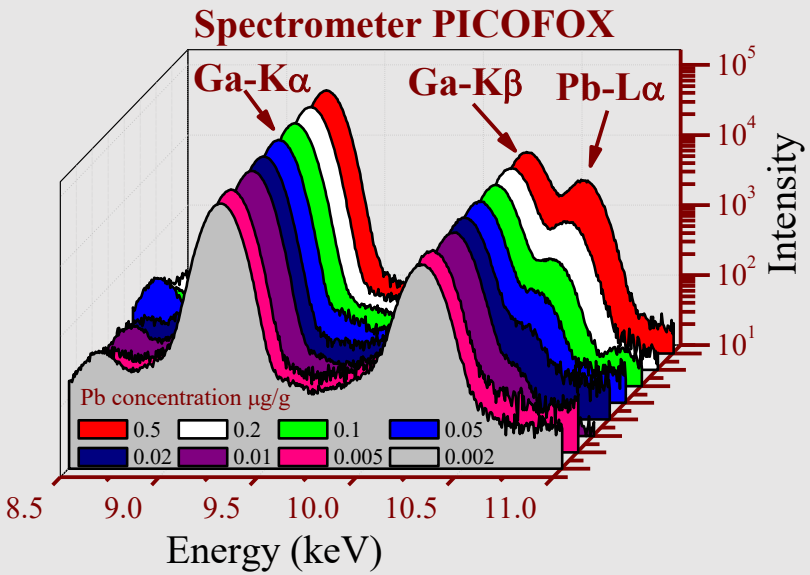
Accuracy (in %) of quantile estimation from the survival function using *Survival analysis* procedure calculated for the simulated sets of the censored data for various number of censoring levels (10%, 30%, 60%, 80%).

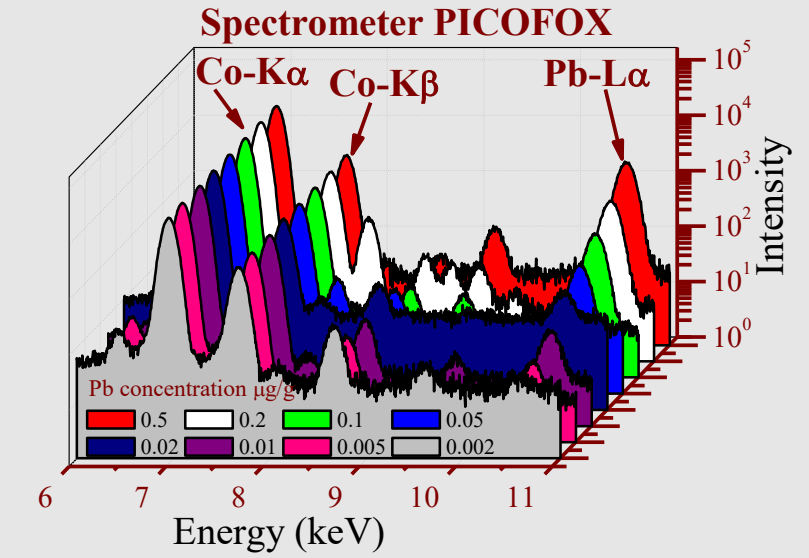
In general, the accuracy is at the level from about 0.5% to 12%, depending on the number of samples and the censoring level. It was shown that even for a relatively small number of measurement results (N = 20 samples) and for a relatively high censoring level (80%) accuracy is at a satisfactory level of 10%.

Virtual Mobility Grant: WATER INTERLAB TEST

Importance of internal standard selection in direct routine determination of L series elements concentration in water samples by application of the Total Reflection X-ray Fluorescence technique

- The aim of the research is to select best internal standard for use in the direct routine analysis of L series elements concentration in water samples by applying the Total Reflection X-ray Fluorescence (TXRF) technique.
- In the study, aqueous reference materials were used to determine detection limits of the following elements: palladium (Pd), silver (Ag), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), bar (Ba), neodymium (Nd), gadolinium (Gd), erbium (Er), tantalum (Ta), tungsten (W), thallium (Tl), lead (Pb), bismuth (Bi). The elements were in the form of: monoelemental, two-elemental (Ba and Ti, W and Cu, Pb and As), and multielemental solutions with different concentrations of elements.
- The detection limits of chemical elements were analyzed using various internal standards (Co, Ga, Nd, and Sr), and the TXRF spectrometers with the Mo-anode and W-anode X-ray tubes.
- For the analyzed L series elements, the detection limits, normalized to the same measurement time (t = 1000 s), were calculated. Finally, the internal standards were proposed to analyze the studied elements for two different X-ray sources (the Mo-anode and W-anode X-ray tubes).
- Based on the measurements, the relative sensitivities were calculated for each of the applied TXRF spectrometers.

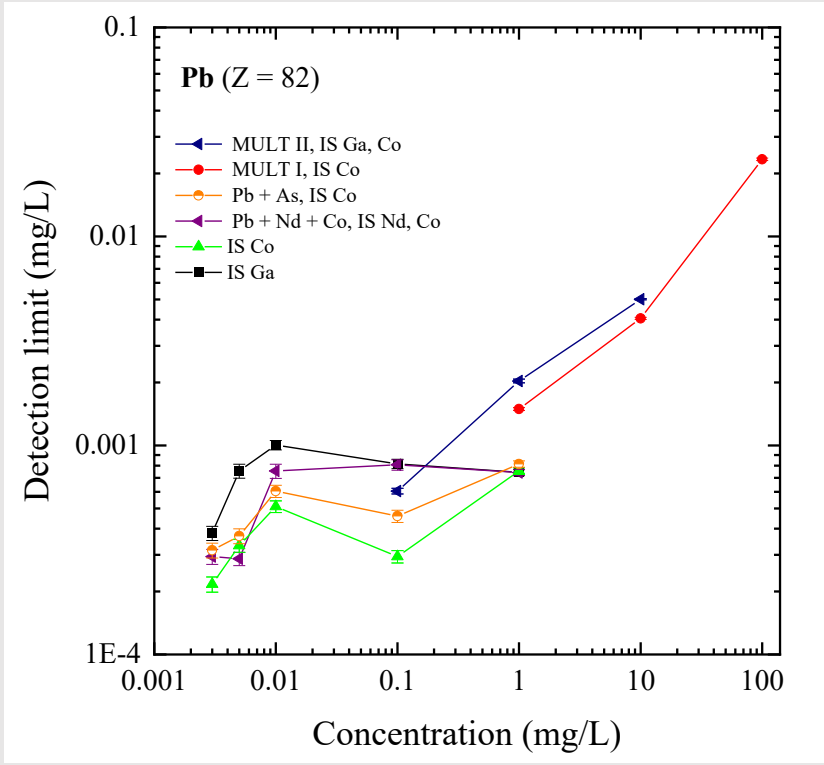




Virtual Mobility Grant: WATER INTERLAB TEST

Set of elements

No.	Element	Atomic number	Analyzed line Mo-anode X-ray tube ($L\alpha_1$ or $K\alpha_1$ line energy)	Analyzed line W-anode X-ray tube ($L\alpha_1$ or $K\alpha_1$ line energy)
1.	Pd	46	Lα (2.839 keV)	Kα (21.177 keV)
2.	Ag	47	Lα (2.984 keV)	Kα (22.163 keV)
3.	Cd	48	Lα (3.134 keV)	Kα (23.174 keV)
4.	In	49	Lα (3.287 keV)	Kα (24.210 keV)
5.	Sn	50	-	Kα (25.271 keV)
6.	Sb	51	-	Kα (26.359 keV)
7.	Ba	56	Lα (4.466 keV)	Lα (4.466 keV)
8.	Nd	60	Lα (5.230 keV)	Lα (5.230 keV)
9.	Gd	64	Lα (6.057 keV)	Lα (6.057 keV)
10.	Er	68	Lα (6.949 keV)	Lα (6.949 keV)
11.	Ta	73	Lα (8.146 keV)	Lα (8.146 keV)
12.	W	74	Lα (8.398 keV)	Lα (8.398 keV)
13.	Hg	80	Lα (9.989 keV)	Lα (9.989 keV)
14.	Tl	81	Lα (10.269 keV)	Lα (10.269 keV)
15.	Pb	82	Lα (10.552 keV)	Lα (10.552 keV)
16.	Bi	83	Lα (10.839 keV)	Lα (10.839 keV)
17.	Ti	22	Kα (4.511 keV)	Kα (4.511 keV)
18.	Cu	29	Kα (8.048 keV)	Kα (8.048 keV)
19.	As	33	Kα (10.544 keV)	Kα (10.544 keV)
20.	Co	27	Kα (6.930 keV)	Kα (6.930 keV)
21.	Ga	31	Kα (9.252 keV)	Kα (9.252 keV)
22.	Sr	38	Kα (14.165 keV)	Kα (14.165 keV)



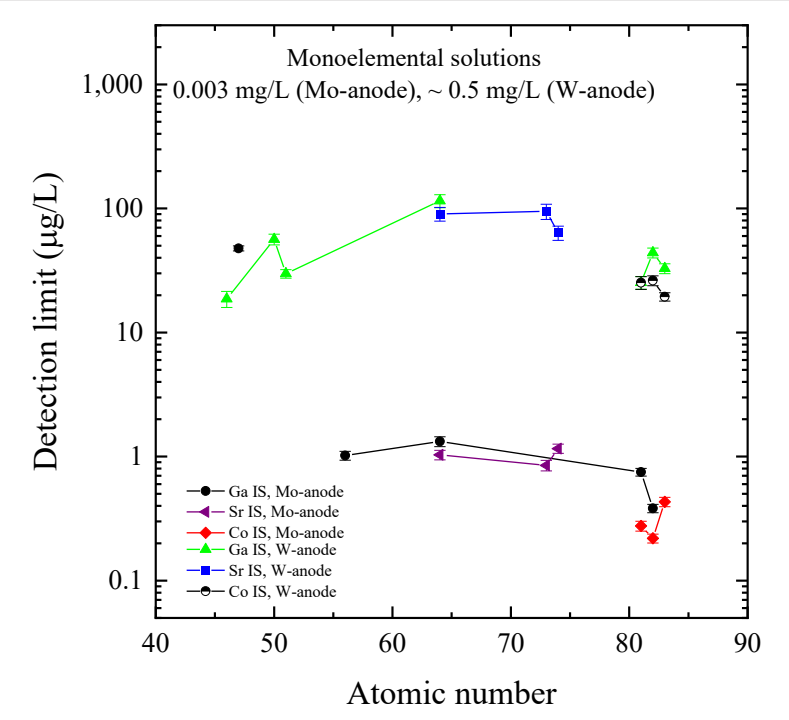
Dependence of the Pb detection limit on elemental concentration for various solutions (MULT I, MULT II, monoelemental, two-element) for the Mo-TXRF spectrometer.

Detection limits achieved:

- The detection limits values increase alongside an increase in the element content in the analyzed sample and are various for different internal standards. For the Mo-anode X-ray tube, detection limits achieve values from about 0.2 μg/L for the Pb solution (with Co applied as the internal standard) to 56 μg/L for the Pd solution (Ga internal standard). In most cases, however, it is at the level of several μg/L.
- For the W-anode X-ray tube, the detection limits achieve the values from about 10 μg/L for the Pd solution (with Ga used as the internal standard) to 543 μg/L for the Ba solution (Ga internal standard) but in n most cases it is at the level of several tens of μg/L.

Virtual Mobility Grant: WATER INTERLAB TEST

Dependence of the detection limit on the element atomic number for monoelemental solutions with concentration of 0.003 mg/L (for Mo-anode X-ray tube based TXRF spectrometer) and ~ 0.5 mg/L (W-anode X-ray tube) for Ga, Sr, and Co.



Internal standards proposed for analyzing the listed elements for two different X-ray sources (the Mo-anode and W-anode X-ray tubes).

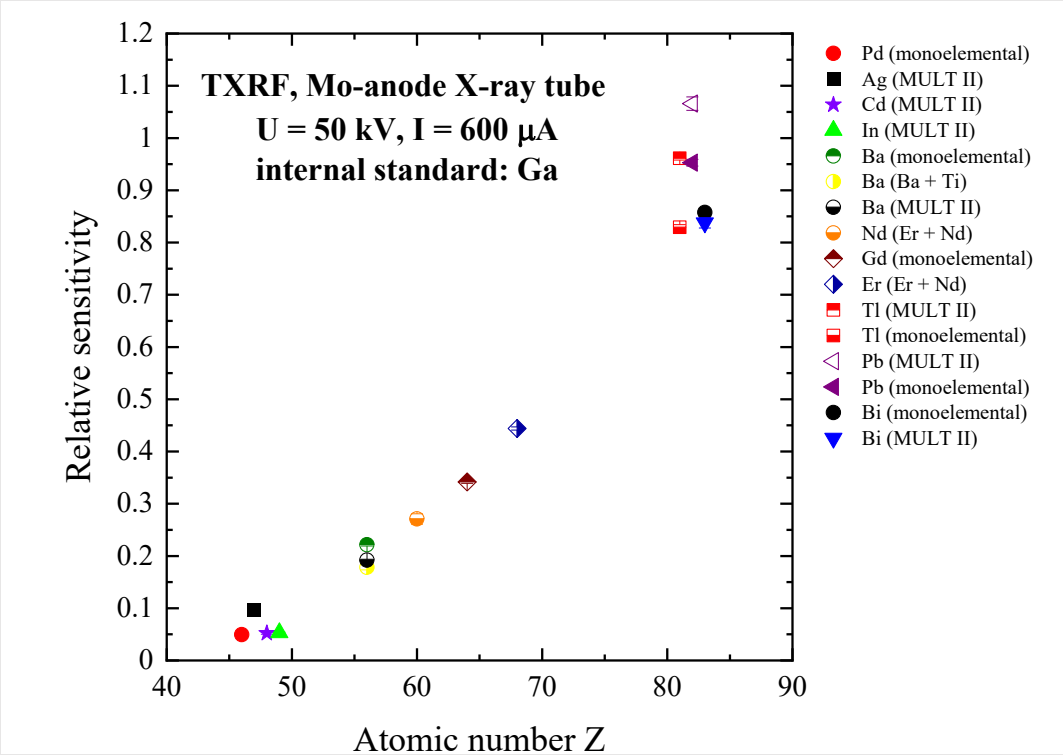
Element	Atomic number	Internal standard (Mo-anode X-ray tube)	Internal standard (W-anode X-ray tube)
Pd	46	Ga	Ga
Sn	50	Ga	Ga
Sb	51	Ga	Ga
Ba	56	Ga	Ga
Gd	64	Sr	Sr
Ta	73	Sr	Sr
W	74	Sr	Sr
Tl	81	Co	Co
Pb	82	Co	Co
Bi	83	Co	Co

- The values of DL are in the range from about 0.3 µg/L to about 100 µg/L.
- For Pd the detection limit for the W-anode X-ray tube is lower than for the Mo-anode X-ray tube. The reason is that these elements in the case of the W-anode are detected based on the K series lines.
- For the rest of the elements, lower values of the detection limits are achieved for Mo-anode X-ray tube. Additionally, the detection limit depends on the internal standard applied. The best detection limits are achieved for Co used as an internal standard.

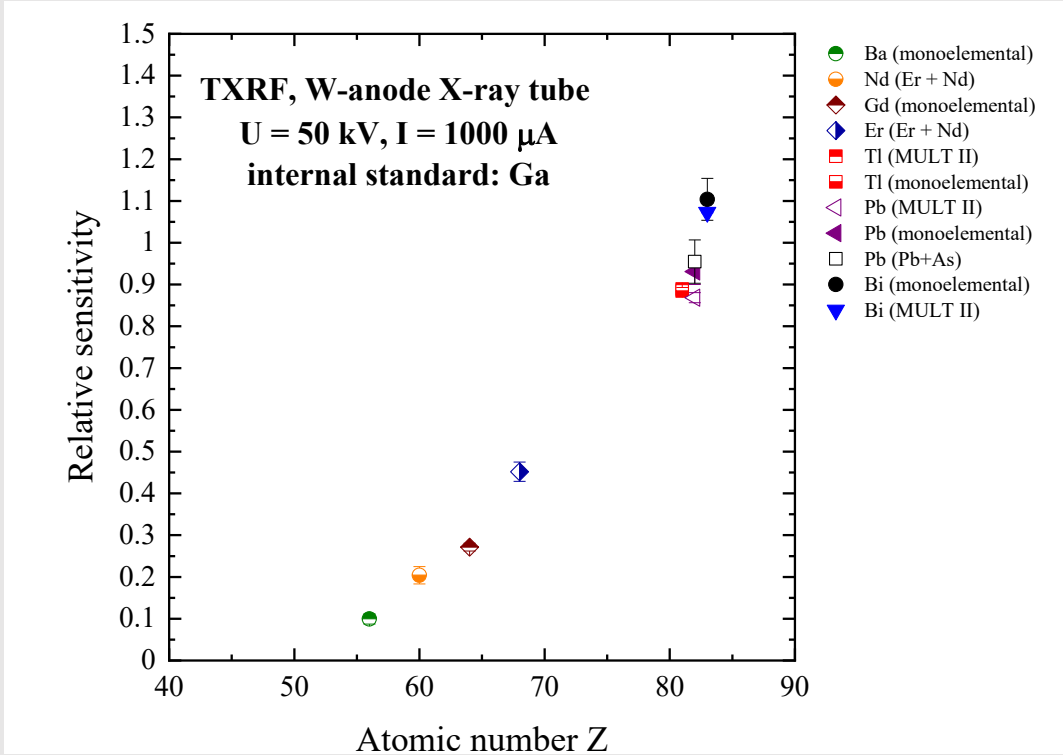
Virtual Mobility Grant: WATER INTERLAB TEST

Dependence of relative sensitivity of elements as a function of the atomic number Z of the element (using Ga as an internal standard).

Mo-anode X-ray tube spectrometer



W-anode X-ray tube spectrometer



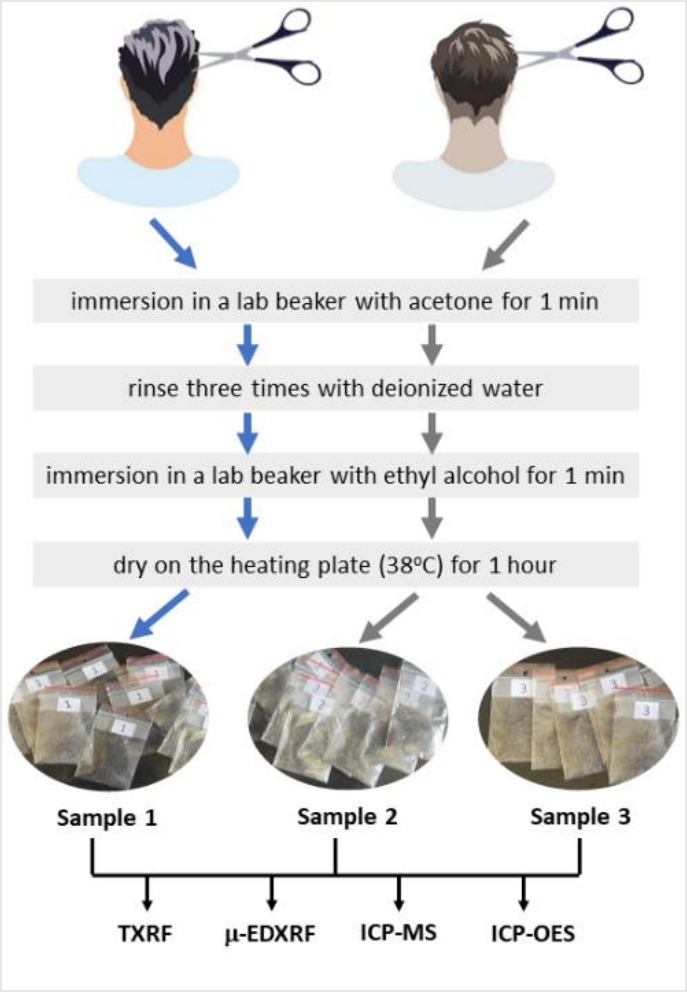
- The shape of the calibration curve reflects the dependence of the fluorescence yield on the atomic number Z of an element.
- In the case of Pb and Bi (for both X-ray tubes) as well as Ba and Tl (Mo-anode X-ray tube), the dependence of the relative sensitivity on the type of reference solution (single-element or MULT II) is visible.
- The observed dependence of the relative sensitivity on the type of the analyzed solution is associated with matrix effects, i.e., with the absorption of the fluorescent radiation of elements and the enhancement effect. The most significant difference between relative sensitivity is for Tl (about 16%), and, next, for Pb (12%), for the Mo-anode X-ray tube.
- The consequence of discrepancies in relative sensitivity is the uncertainty of element content determination.

Virtual Mobility Grant: HAIR - PROFICIENCY TEST

Determination of minor and trace elements in human hair - an interlaboratory comparison

- The aim of the research was an interlaboratory comparison for the analysis of elemental concentration in human hair samples as an example of a human biological material.
- The analysis concerned the determination of the content of the following elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Mo, Pb and Hg.
- Six laboratories from five countries (Poland, Germany, Spain, Portugal, and Croatia) participated in the interlaboratory comparison and performed the analyses using the following methods: TXRF (three laboratories), μ-EDXRF (one laboratory), ICP-OES (one laboratory), and ICP-MS (two laboratories).

Laboratory number	Method	Instruments	Preparation procedure	
			Amount of sample	Sample treatment
Lab 1	TXRF	S2 Picofox	0.025 g	digestion in acid
Lab 2	TXRF	Rigaku Nanohunter II	0.025 g	digestion in acid
Lab 3	TXRF	S4 T-STAR	0.025 g	digestion in acid
Lab 4	μ-EDXRF	M4 TORNADO	~ 3 g	grinding and pressing
Lab 5	ICP-MS ICP-OES	Agilent 7500c Agilent 5100	0.15 g	digestion in HNO_3 and H_2O_2 (7:1) and next dilution in ultra-pure water
Lab 6	ICP-MS	Agilent 7500cx	0.1 g	digestion in in HNO_3 and H_2O_2 (1:1) and next dilution in ultra-pure water



The idea of a double analysis of the same hair sample was to check the homogeneity of the material prepared for the interlaboratory comparison and to determine the repeatability of the results of the individual laboratories.

Virtual Mobility Grant: HAIR - PROFICIENCY TEST

Determination of minor and trace elements in human hair: TXRF analysis

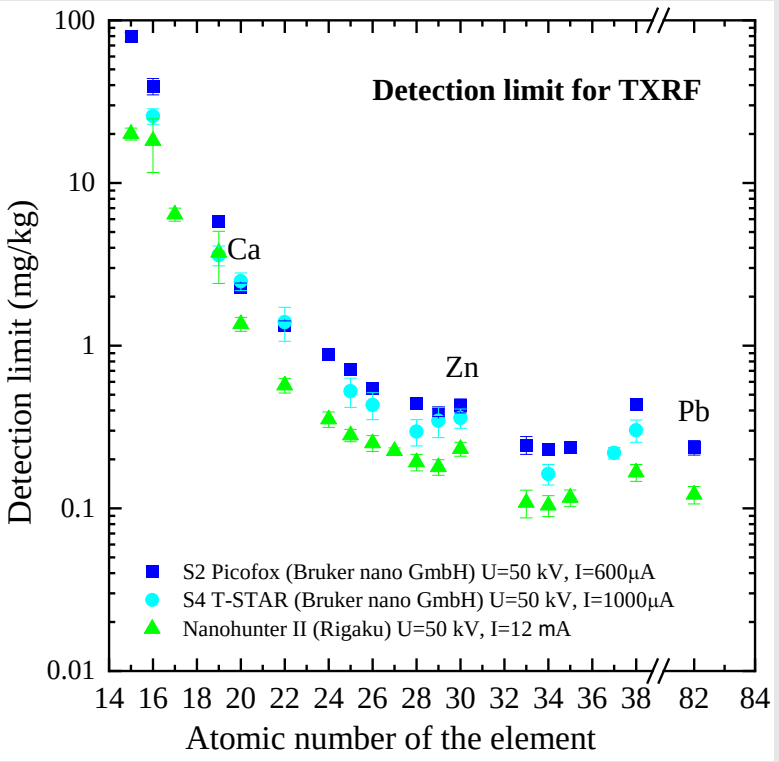
In the TXRF analysis, three instruments were used:

- S2 Picofox (Bruker Nano GmbH, Mo-anode X-ray tube of 30 W power, operated at 50 kV and 600 μA),
- S4 T-STAR (Bruker Nano GmbH, W-anode X-ray tube of 50 W, operated at 50 kV and 1000 μA),
- Nanohunter II (Rigaku, Mo-anode X-ray tube of 600 W, operated at 50 kV and 12 mA).

Quantification and quality control

IAEA-086 human hair								
Element	**Certified values (mg/kg)**	**95% Confidence Interval (mg/kg)**	TXRF Lab 1 (mg/kg)	Accuracy (%)	TXRF Lab 2 (mg/kg)	Accuracy (%)	TXRF Lab 3 (mg/kg)	Accuracy (%)
Ca	**1120**	**1000-1240**	839	25	933	17	1307	17
Mn	**9.6**	**8.9-10.3**	9.00	6.3	9.2	4.2	18.5	93
Fe	**123**	**110-136**	111	9.4	75	39	109	12
Cu	**17.6**	**16.6-18.8**	17.7	0.3	13.9	21	17.8	0.9
Zn	**167**	**159-174**	160	4.2	145	13	127	24
Se	**1.00**	**0.80-1.20**	0.63	37	1.32	32	0.85	15
Hg	**0.258**	**0.236-0.279**	-	-	-	-	0.212	18

The concentrations obtained for Ca, Mn, Fe, Cu, Zn, Se and Hg in the certified reference material IAEA-086 (human hair) using the TXRF method. The results are expressed as mean concentration values of three replicates (in mg/kg). Based on the certified values, the accuracies of the TXRF measurements are calculated.



Dependence of detection limits on the atomic number of an element in human hair samples obtained by 3 laboratories which participated in the interlaboratory comparison and used the TXRF technique for the sample analysis.

Virtual Mobility Grant: HAIR - PROFICIENCY TEST

Determination of minor and trace elements in human hair: statistical analysis

- The statistical analysis of the results focused on determining the consensus value of the element content, which is the average of the results after rejecting outliers and determining the standard deviation of the results.
- Information about these parameters makes it possible to calculate, for each laboratory participating in the interlaboratory comparison and for each element being determined, a z-score, which is a value informing about the compliance of the result obtained by the laboratory with the average value.
- Additionally, the ratio of the standard deviation to the consensus value, known as the variation coefficient, gives information about the dispersion of the results.
- In the first approach, only the measurement results obtained by laboratories employing the TXRF technique were subjected to statistical analysis (the same sample preparation and quantification procedures).
- In the next step, the results obtained using other measurement techniques (μ-EDXRF, ICP-OES, ICP-MS) were also included.

Example

Results of the statistical analysis for concentrations of: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, Mo, and Pb obtained with TXRF technique in the hair sample No. 1.

Element	Number of reported values	Outliers - (mg/kg)	Number of reported values without outliers	Minimum (mg/kg)	Maximum (mg/kg)	x_c (mg/kg)	σ_c (mg/kg)	Coefficient of variation $(\sigma_c/x_c) \cdot 100\%$
P	6	-	6	119	289	**205**	**71**	**35**
S	9	-	9	18800	32700	**25800**	**5100**	**20**
Cl	3	-	3	47.3	70.5	**61.3**	**12.3**	**20**
K	9	-	9	18.4	250	**88.8**	**79.1**	**89**
Ca	9	-	9	574	834	**683**	**92**	**13**
Ti	9	-	9	1.28	3.91	**2.82**	**1.09**	**39**
Cr	6	-	6	2.15	3.12	**2.69**	**0.43**	**16**
Mn	7	-	7	0.157	1.15	**0.591**	**0.418**	**71**
Fe	9	-	9	8.53	29.6	**20.5**	**8.8**	**43**
Ni	9	-	9	2.22	10.9	**6.87**	**3.87**	**56**
Cu	9	-	9	8.83	23.0	**13.4**	**5.0**	**37**
Zn	9	-	9	143	209	**178**	**27**	**15**
Se	9	0.143	8	0.358	0.475	**0.417**	**0.042**	**10**
Br	6	-	6	0.624	1.07	**0.792**	**0.179**	**23**
Rb	3	-	3	0.204	0.357	**0.294**	**0.080**	**27**
Sr	9	-	9	2.15	3.03	**2.72**	**0.35**	**13**
Mo	3	-	3	3.38	6.53	**4.81**	**1.60**	**33**
Pb	5	-	5	0.215	0.344	**0.292**	**0.048**	**16**

$$z = \frac{x_i - x_c}{\sigma_c}$$

Z-scores

Virtual Mobility Grant: HAIR - PROFICIENCY TEST

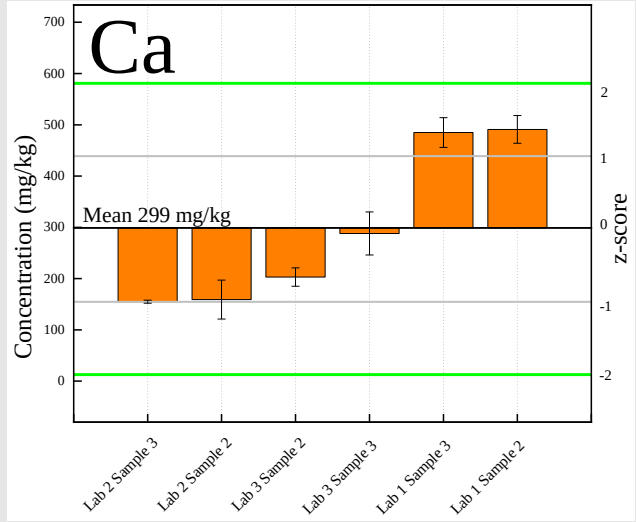
Determination of minor and trace elements in human hair: z-scores

Based on the values of z, the following result evaluation criteria were set:
|z| ≤ 2 refers to a satisfactory result,
2 < |z| < 3 corresponds to the questionable result,
|z| ≥ 3 marks the unsatisfactory result.

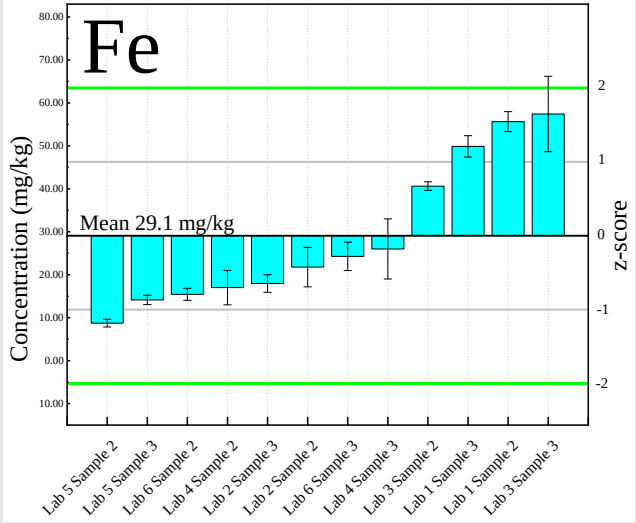
Conclusions:

- The statistical analysis included typical procedures, used in the evaluation of the results of interlaboratory comparisons, i.e. rejection of outlier values, determination of the consensus value, standard deviation, coefficient of variation and z-score parameter for the content of each determined element. It was noted that outliers occurred rarely and randomly, regardless of the measurement technique and laboratory.
- For TXRF, the average value of the coefficient of variation was approximately on the level 30-40%. The smallest dispersion of results (coefficient of variation below 30%) is observed for Se, Ca, Sr, Zn, Cr, Pb, S, Cl, Br, Rb. These elements include both light and heavy elements, with low and high concentrations in hair samples. The detection limit for TXRF was in the range from about 0.1 mg/kg to 100 mg/kg, while the precisions were on the level up to 20%, depending on the element and its concentration in human hair sample.
- The average value of the coefficient of variation for results analyzed by all techniques is approximately 55-65% and is higher than for TXRF results analyzed separately. The smallest dispersion of results is observed for: Se, Ca, Sr, S, and P.
- The calculated consensus values and standard deviations allowed for the calculation of the z-score parameter values, which took the values |z-score| ≤ 2 (satisfactory results).
- The result of the interlaboratory comparisons is the determination of the potential of the applied analytical techniques, mainly TXRF, as the methods allowing their use for routine analysis of the elemental composition of human hair samples used in human biomonitoring.

z-score analysis obtained using TXRF technique for Ca



z-score analysis obtained using all techniques (TXRF, μ-EDXRF, ICP-OES, ICP-MS) for Fe

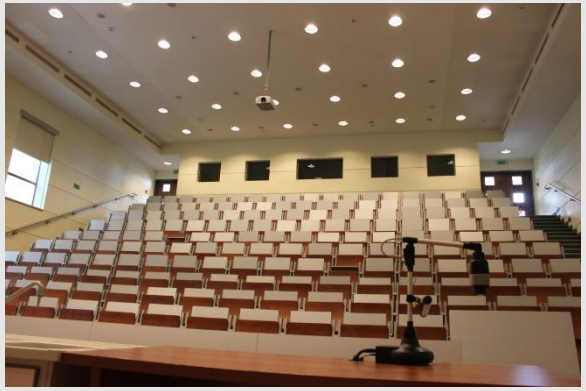


CONCLUSIONS

- ENFORCE TXRF Action coordinated the efforts made at the national and transnational level to establish total reflection X-ray fluorescence (TXRF) as a reference technique for reliable elemental analysis of solid and liquid matrices. The Action's objectives were achieved via the development of a strong TXRF network and joint research and development activities.
- Great interest in the project was related to the popularity of elemental analysis in many different fields of science and industry.
- The ENFORCE TXRF project fit perfectly into the scope of scientific activities conducted at the Institute of Physics.
- Activities of the Department of Atomic Physics and Nanophysics of Institute of Physics within the project ENFORCE TXRF focused mainly on research in area of samples of human biological material.
- Our participation in the project resulted in the implementation of many scientific projects conducted in international cooperation and publication of scientific articles.
- **Near future TXRF activities**:
  - finalization of the projects, which started within ENFORCE TXRF,
  - realization (in 2024-2025) two projects within Polish Metrology II program (1. MultiBioCRM: Multifunctional plant CRM with certified contents of metals and microplastics (consortium leader - Warsaw University and consortium member - Jan Kochanowski University, 2. MPWN: Multifunctional reference material of polish natural waters with microplastics (consortium leader - Warsaw University and consortium member - Jan Kochanowski University),
  - realization (2024-2027) interdisciplinary projects within "Regional Excellence Initiative" program (project no.: RID/SP/0015/2024/01).

TXRF 2025 conference

TXRF 2025, 20th anniversary International Conference on Total Reflection X-ray Fluorescence Analysis and Related Methods will take place in September 2025, in Institute of Physics at Jan Kochanowski University, Kielce, Poland!



ENFORCE XRF

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Holy Cross Cancer Center

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