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NAA W NAUKACH O ŻYCIU W LABORATORIUM FIZYKI NEUTRONOWEJ ZJEDNOCZONEGO INSTYTUTU BADAŃ JĄDROWYCH W DUBNEJ

Abstract: Experience in applying conventional and epithermal neutron activation analysis for some challenging areas of the life sciences is reviewed. For more than 25 years of its operation the radioanalytical complex REGATA at the IBR-2 reactor in Dubna has become a source of analytical data for the environmental studies, marine geology, bio-nano-technology and medicine, *etc.* In spite of competing non-nuclear analytical techniques (AAS, ICP-ES, ICP-MS, *etc*), the reactor neutron activation analysis (NAA) as a primary (ratio) method continues to be the most powerful multi-element analytical technique providing quantification of trace elements at ultra low levels. Combined with modern statistical data treatment of large arrays of data, GIS (geographic information system) technologies, electron scanning microscopy, tomography, and others, NAA serves to obtain practical results resumed in the review.

Keywords: epithermal neutron activation analysis, reactor IBR-2, environmental studies, biotechnology

Introduction

This review considers the contribution of a reactor *neutron activation analysis* (NAA) at Frank Laboratory of Neutron Physics (FLNP) of Joint Institute for Nuclear Research (JINR) to solution of the major problems in the studies of the chemical composition of objects in the biosphere with a view to understanding the role of various elements in the functioning of living organisms and ecosystems under anthropogenic effects on nature, often resulting in irreversible changes in the environment and human health.

These studies are concerned with water, atmosphere, soil, vegetation, microorganisms, animal, and human tissues. Their main goal is to elaborate a metrological and analytical

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approach to the description of the "noochemical" processes (Greek *noos*, *nous*, "mind") associated with the exposure of the environment to human activities [1].

According to the International Atomic Energy Agency in Dubna (IAEA) data [2], the toxic agents that are most harmful for human health are (i) naturally occurred toxins (bacterial toxins, aflatoxins, mycotoxins, *etc.*), (ii) organic pollutants (petroleum products, pesticides, dioxins, *etc.*), (iii) radionuclides, and (iv) toxic inorganic chemical elements and their compounds. Among the latter there are, first of all, heavy metals [3].

Since the early 1990s, neutron activation analysis based on the IBR-2 reactor (FLNP, JINR) has been widely used in biology, medicine, and ecology. The REGATA radioanalytical complex, with its state-of-the-art pneumatic system, makes it possible to perform mass multielement analysis of biological and environmental samples. Epithermal neutron activation in the low temperature irradiation channel with a cadmium shield is particularly effective for this class of samples.

The purpose of the review is retrospection and description of current trends in the use of neutron activation analysis at the IBR-2 reactor as an integral part of life sciences, in biomonitoring of atmospheric deposition of trace elements including heavy metals, lantanides and actinides, quality assessment of foodstuffs, biotechnology of new pharmaceuticals, monitoring of environment and human health, *etc.*

Analytical problems of chemical element analysis in the life sciences

In the context of the life sciences, the notion of *analytics* has acquired a new meaning. Analytics refers to more than only to physical or chemical methods for element analysis of a substance. In ecology, for example, this is primarily determination of objects under study exposed to any kind of pollution, choice of strategy and sampling methods, preparation and systematization of samples, choice of optimum conditions for analysis with the appropriate standards, processing of the analytical information using the systematic approach, searching for data correlations, construction of spatial distributions of pollutions, and finally provision of particular information and recommendations for undertaking appropriate environmental protection measures.

Analysis of biological and environmental samples is currently carried out using not only classical methods of analytical chemistry, but also nuclear and related physics techniques, such as: *atomic absorption spectrometry* (AAS), *X-ray fluorescence analysis* (XFA), *inductively coupled plasma-atomic emission spectrometry* (ICP-AES), *inductively coupled plasma-mass spectrometry* (ICMP-MS), synchrotron radiation, *neutron activation analysis* (NAA), *etc*. Nuclear and related techniques are found to be most sensitive. The NAA technique is particularly effective in solving environmental problems where multielement analysis of a great deal of samples is required.

As far back as the beginning of the 20th century, when analytical techniques were rather underdeveloped, V.I. Vernadskii believed that living systems contain all existing chemical elements [4]. At present, the number of elements that are determined in human tissues and biosubstrates is between 80 and 92. The main elements of which a living substance is built are H, C, N, O, and S. Their concentrations are g/kg. Macrominerals Na, Mg, P, Cl, K, and Ca, which are structural elements of cells, also occur in concentrations on the order of g/kg. Other elements of the periodic system, which occur in concentrations of mg/kg or μg/kg, are called trace elements. Some of the trace elements are essential for

functioning of living systems. The criteria according to which trace elements are divided into essential and non-essential have been formulated in [5].

Investigations have shown that, for each trace element, there is a concentration level corresponding to the adequate effect on the living system and that each element, even essential, can be potentially toxic in concentrations above the level of adequate effect [6-9]. The dependence of the biological function of a trace element in a living system on its concentration in the food or medium is schematically shown in the known Bertrand diagram (Fig. 1 [10]).

Heavy metals are believed to be the most active toxicants. They can be supplied to a living organism through food, air, water, and its outer surface. A high concentration of heavy metals in environmental objects, air, soil, vegetation, water bodies, *etc.*, is a serious risk factor for the population's health. Particularly harmful can be toxicants entering the organism with the air, the uptake of which is 3×10^4 times larger in volume and 20 times larger in mass than that of food and water and which directly affects the lungs and the gastrointestinal tract [11].

Correlations of air pollution and medical statistics confirm the results of investigations [11-14]. Recently, in connection with the development of analytical methods, rare earth elements (lanthanides, Sc, Y), which were earlier beyond the reach of analytical techniques, have been extensively studied in environmental objects from the point of view of their toxicity [15, 16].
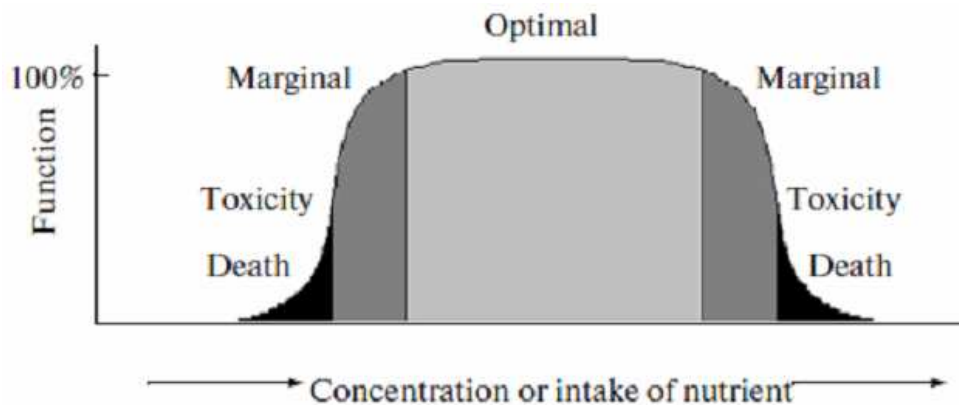

Fig. 1. Dependence of the biological function on the concentration of the nutrient in the tissue [10] (abscissas: concentration or intake of the nutrient)

Most elements associated with various industries belong to group I toxicants and are potentially hazardous for the environment and human beings. Biological research is currently being carried out in many areas with the concentrated efforts of scientists from many countries. The most important of these areas are investigation of environmental objects (soil, water, air, vegetation, *etc.*); analysis of trace elements in human and animal tissues; and investigation of the elemental composition of foodstuffs. The biomonitoring technique is widely used in various variants in this research. Depending on the character of

the particular investigation, the biomonitors are mosses and lichens, bacteria, fungi, animals, and human and animal tissues and substrates, *etc.*

The statistical approach to solution of environmental problems using biomonitors requires a multielement analysis of a great deal of samples, and in this respect non-destructive instrumental neutron activation analysis (INAA) surpasses other techniques.

Neutron activation analysis: merits and drawbacks

Powerful reactor fluxes ($10^{12} \div 10^{14}$ neutrons cm^{-2} s^{-1}); a combination of various versions of the technique (activation with epithermal neutrons, cyclic activation analysis); and the use of modern high-resolution (~2 keV) semiconductor detectors, measuring and computing equipment, and state-of-the-art computer technologies for data processing and interpretation are factors that determine the current level of NAA. Highly accurate determination of the impurity composition in solid matrices allowed NAA to be recognized in 2007 as a primary method of measurement to be used for metrology purposes and for certification of reference materials [17].

NAA features the following **merits**: high sensitivity to a majority of elements, especially to heavy metals with Z > 10; good selectivity due to specific nuclear physics characteristics of elements; a possibility of simultaneously determining a large number of elements; independence of the results on the form of chemical compounds; a non-destructive nature, which allows avoiding the risk of contamination of samples with reagents or their incomplete dissolution; easy procedure for preparation of samples for analysis; good accuracy, about $\pm 10 \div 15\%$, in determination of concentrations of the order of ppm (10^{-6} g); a possibility of eliminating systematic errors; a possibility of minimizing the effect of matrix elements of samples; ease of preparation of standards for comparison.

The **drawbacks** of NAA are, first of all, the necessity of using nuclear reactors and the problems arising from storage and disposal of nuclear waste [18].

In the 1980s, certified international standard samples and reference materials appeared especially for analytical purposes. The quality control of analytical measurements allowed the accuracy to be increased and systematic errors to be reduced to minimum. It became possible to perform interlaboratory comparisons of the analysis results and to compare data obtained by different analytical techniques.

Reactor epithermal neutron activation analysis

The energy spectrum of reactor neutrons (Fig. 2 [19]) can be conventionally divided into three parts: (i) fast fission neutrons with an energy from 0 to 15 MeV, the most probable value being 1 MeV; (ii) intermediate energy neutrons resulting from moderation, so called epithermal or resonance neutrons with energies from 1 eV to 1 MeV; and (iii) thermal neutrons that are in thermal equilibrium with the moderator atoms. The velocity of these neutrons is described by the Maxwellian distribution with the maximum value corresponding to the neutron kinetic energy E = 3/2kT (k is the Boltzmann constant, and T is the temperature). The most probable energy is 0.052 eV. As neutrons interact with substance, the following reactions can proceed: elastic scattering (n, n); inelastic scattering (n, n'); radiative capture (n, γ); particle producing reactions (n, α), (n, p), and (n, 2n); and fission (n, f).
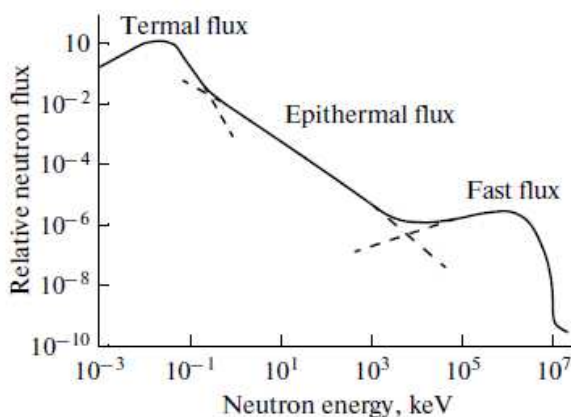
Fig. 2. Schematic representation of the nuclear reactor neutron spectrum [19]

Thermal neutron reactions (n, γ) and (n, n) have large cross sections of 0.1÷100 barn, while fast neutron reactions (n, p), (n, α), (n, n'), and (n, 2n) have cross-sections that are two to three orders of magnitude smaller. The reaction most often used in NAA is radiative capture, $n+^{A}Z \rightarrow ^{A+1}Z^{*} \rightarrow ^{A+1}Z + \gamma$, where ^{A}Z is the irradiated isotope, $^{A+1}Z^{*}$ is the compound nucleus in the excited state, and γ is the secondary gamma radiation.

In some cases, however, interfering reactions occur, eg, ^{27}Al (n,γ) ^{28}Al; ^{28}Si (n,p) ^{28}Al, ^{31}P (n,α) ^{28}Al or ^{26}Mg (n,γ) ^{27}Mg; ^{27}Al (n,p) ^{27}Mg. These reactions are a source of difficulties for determination of concentrations of elements and appreciably decrease the sensitivity and accuracy of the technique.

The INAA detection limit for some elements strongly depends on the composition of the samples under investigation. High background from Compton radiation of matrix elements often contributes to interference. To decrease this background, measurements are sometimes carried out after an appropriate delay time, which is beneficial if the main interfering radionuclides have relatively short half-lives [20].

In other cases, purely instrumental methods are used to suppress Compton radiation [21]. Practice has shown that the most effective technique for increasing NAA sensitivity is the resonance activation technique. At present, *epithermal neutron activation analysis* (ENAA) is successfully being used at many reactors, including IBR-2 (FLNP, JINR).

The NAA technique using epithermal neutrons with the energy $0.5 \div 10^{4}$ eV is based on selective activation of those isotopes for which the neutron caption cross-section σ does not obey the law 1/v (where v is the neutron velocity) and features pronounced resonance peaks.

The occurrence of resonances is associated with quasi-stationary states in intermediate compound systems (nuclide nucleus + neutron) with lifetimes that are much longer than the time of flight of the neutron through the nucleus. The metastability of these states stems from the Coulomb and centrifugal barriers, which delay decay processes, and from the complexity of the inner structure of the nucleus. If the neutron energy is such that the total energy of the system corresponds to one of the levels of the intermediate nucleus, the probability of its production is much higher than in the case in which the energy

corresponds to the gap between the energy levels. Then a resonance peak may arise on the curve of the energy dependence of the interaction cross section $\sigma(E)$.

Irradiation of these nuclides by epithermal neutrons allows selective activation to be carried out and specific element determination techniques to be developed. It also becomes possible to effectively suppress the induced activity of some matrix elements in samples of environmental and biological nature (^{28}Al, ^{56}Mn, ^{24}Na, ^{35}Cl, ^{45}Sc, and others) that have large cross-sections for thermal neutron absorption and reduce the number of competing reactions.

Thus, when there are no interfering elements, selectivity, sensitivity, and accuracy of the analysis increase and it becomes possible to determine small concentrations of trace elements and microelements in biological, environmental, geochemical, and other samples.

Epithermal neutrons for ENAA purposes are obtained using filters made of substances that have larger cross-sections for absorption of neutrons with energies below some specified values. In the practice of activation analysis, resonance absorbers are either permanently installed in the reactor irradiation channel or placed into transport containers (capsules) for irradiation of samples. Cadmium and boron are most often used as absorber materials.

In the reviews of ENAA investigations that had been conducted by the mid 1980s, Steinnes highlighted the most important advantage of the technique: the possibility of determining trace elements in environmental, biological, and geochemical samples [22, 23].

Analysis of many trace elements in concentrations down to 10^{-6} ppm in a large number of samples makes NAA one of the most effective methods in life sciences and determines the scale of its application.

H																H	He
Li ○	Be											B	C	N	O	F	Ne
Na ○□	Mg ○□											Al ○□	Si	P	S	Cl □	Ar
K ○□	Ca ○■	Sc ○■	Ti ○□	V ○□	Cr ○□	Mn ○□	Fe ○□	Co ○□	Ni ○□	Cu ○	Zn ○□	Ga ○	Ge ○	As ○■	Se □	Br □	Kr
Rb ○□	Sr ●□	Y ○	Zr ○□	Nb	Mo ○□	Te	Ru	Rh	Pd	Ag ○□	Cd ○	In	Sn ●□	Sb ○■	Te ○	I □	Xe
Cs ○□	Ba ●□	La ○□	Hf ○□	Ta ○■	W ○□	Re	Os	Ir	Pt	Au ○■	Hg ○□	Tl ○	Pb ○	Bi ○	Po	At	Rn
Fr	Ra	Ac															

Ce ●□	Pr ○	Nd ●□	Pm	Sm ○□	Eu ○□	Gd ○□	Tb ○□	Dy ○	Ho ○	Er ○	Tm ○□	Yb ○□	Lu ○□
Th ○■	Pa	U ○□	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Fig. 3. Elements determined by NAA (□) and ICP-MS (○) in plant and biological samples. Solid symbols (●, ■) denote the greatly preferable technique [25]

The 2000 review of the uses of NAA in environmental research [24] considers the main lines of this research: study of atmospheric deposition of heavy metals (analysis of aerosols and biomonitors - mosses, lichens, fungi); determination of trace elements in fuel and its refining products; investigation of pollution in soil and water concretions; and determination of trace elements in plants and animals.

To be effective, NAA requires standard samples and reference materials, and combination of NAA with other analytical techniques, such as AAS and ICP-MS/AES, allows the number of determined trace elements to be increased and fuller information on the state of environment to be obtained.

Figure 3 shows for which elements ENAA is more preferable as compared with the main competing technique, namely, ICP-MS, the major competitor of NAA, the in analysis of plant and biological samples [25].

The methodological NAA studies at the REGATA radioanalytical complex of the IBR-2 reactor using in the course of constructing the NAA and radiation investigations are described elsewhere [21, 26-31]. The questions of analytical *quality control* (QC) and sources of uncertainties are well summarized in [32, 33] and not considered in this review.

ENAA applications at the IBR-2 reactor (FLNP, JINR) for purposes of the life sciences

Atmospheric air monitoring with aerosol filters

NAA of several hundred micropore aerosol filters taken at various sites in a relatively clean North Polar region and polluted regions of Tyumen, Novosibirsk and Krasnoyarsk territory was used to solve some specific problems of atmospheric air monitoring in Siberia. A total of 39 elements were determined in these filters installed in a cascade impactor for aerosol particles of submicron size [34]. The statistical processing of the entire amount of the data allowed evaluating factors of enrichment of aerosols by particular metals and variations in their concentrations.
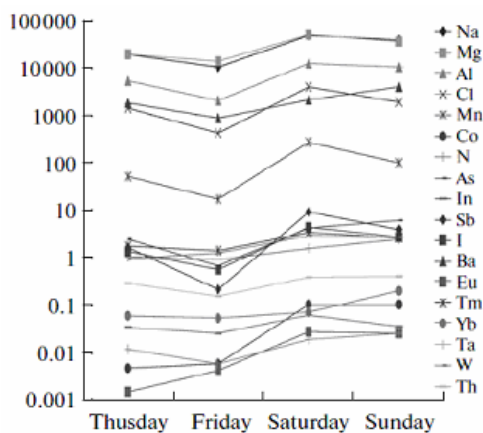
Fig. 4. Concentration of elements in the filters along the Greater Cairo Area [35]

Of particular interest is work on aerosol filters [35] carried out in collaboration with the National Center for Nuclear Safety and Radiation Control (Cairo, Egypt). Weekly cycles of pollutant elements in the air of the Greater Cairo Area were studied by NAA. These investigations, like those conducted in the United States and Canada [36], made it possible to reveal the so called Sunday effect, that is, a decrease in emission of air pollutants on the

weekends, when many factories were closed for days off and discharges decreased. The results are shown in the form of temporal and spatial distributions in Figures 4 and 5, respectively. It follows from Figure 4 that the minimum concentration of elements in the aerosol filters is observed on Islamic days off, Thursday and Friday, whereas in the aerosols on the Atlantic coast of America the minimum concentrations of elements are observed on Saturday and Sunday. The spatial distribution (Fig. 5) demonstrates a trend for concentrations of heavy metals, rare earth elements, and actinides to increase in the direction from the north to the south, which corresponds to the predominant motion of air streams along the Nile from the Sahara to the Mediterranean Sea.
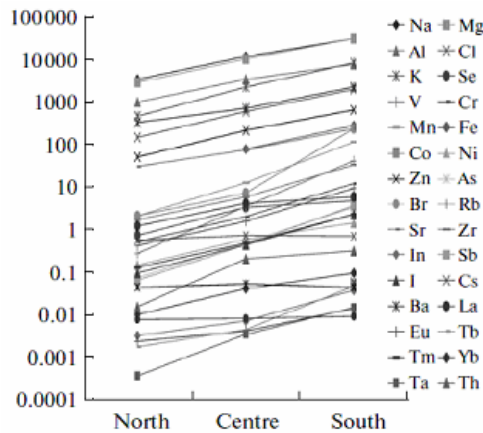
Fig. 5. Concentration of elements in the filters in relation to the day of the week [35]

The ENAA results for the Slovakian aerosol filters from Bratislava [37] were handled using the enrichment factor concept to reveal pollution sources other than the Earth's crust. The enrichment factor is the ratio

$$EF = \frac{\left(c(x) \middle/ c(Al) \right)_{Sample}}{\left(c(x) \middle/ c(Al) \right)_{Crust}}$$

where the numerator is the concentration of the element of interest $c(x)$ in the sample normalized to the concentration of a chosen element, eg, $c(Al)$, in the same sample and the denominator is the corresponding expression for the crust element. In the case under discussion, average concentrations of elements in the humus layer of the soil in Slovakia were used [38]. According to the criterion proposed for aerosols in [39], if $EF < 7$, the aerosol particles are of soil (crust) origin and, if $EF > 7$, the aerosol particles are of anthropogenic origin, such elements as Cu, Zn, As, Se, Cd, Sb, Hg, and Pb in the aerosol filters from Slovakia are of anthropogenic origin (Fig. 6).
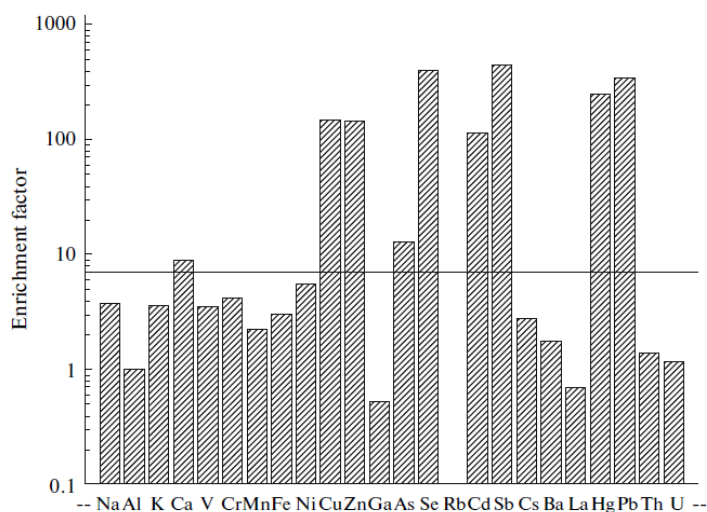
Fig. 6. Enrichment factor calculated for some elements determined by ENAA in the aerosol filters from Bratislava [36]

Biomonitoring of atmospheric deposition of trace elements

Passive biomonitoring. It is well-known that lichens, mosses, ferns, grass, bark and leaves of trees, conifer needles, *etc.*, are used as biomonitors of atmospheric deposition of trace elements and other organic toxic compounds [40]. In the investigations of atmospheric deposition of heavy metals at the IBR-2 reactor lichens and pine needles were used for biomonitoring in [41, 42]. Peat cores were also studied [43]. However, most of the investigative work was done with moss biomonitors.

Moss biomonitoring has been developing as a method for studying atmospheric deposition of heavy metals since 1960 [44, 45]. Mosses well meet all the requirements imposed on biomonitors. A developed surface of their aboveground parts is in good contact with the atmospheric air, and thus deposition and capture of aerosols is quite probable. In this respect moss biomonitors can be thought of as analogues of air filters. Surface properties of moss cuticles facilitate penetration of metal ions into cells and onto the places in which they are bound [46]. Since mosses have an underdeveloped root system, their contact with soil and, thus, uptake of metal with substrates can be considered negligibly small. Mosses are usually tolerable to even a high pollution level. Some species of mosses, such as *Hylocomium splendens* and *Pleurozium schreberi*, are acknowledged as the most suitable monitors for investigation of atmospheric deposition of heavy metals. These species widely occur in the moderate climate zone and can be encountered in the majority of European countries. Since *Hylocomium splendens* and *Pleurozium schreberi* have distinct annual growth segments, they can also be employed for studying temporal trends. Moss sampling is not a difficult procedure and can be done in summer over large regions. Mosses effectively concentrate most heavy metals and other microelements from the air and precipitation. Some types of moss (*Hylocomium splendens*, *Pleurozium schreberi*, *Hypnum cupressiforme*) frequently occur in a wide range of moderate climate zones, and their

growing part is such that the annular growth segment can be easily identified. It is easy to collect samples, and analysis of mosses is simpler than analysis of precipitation: the exposure period is easy to determine (3-year growth segments of mosses are usually taken for analysis).

The moss biomonitor method in combination with nuclear and related analytical techniques has been regularly used for the past three decades in Western Europe to study atmospheric heavy metal deposition, and in the past 15 years it has become widely used in Eastern Europe as well.

Concentration of heavy metals in mosses well correlates with atmospheric deposition, and passage to absolute values of the heavy metal content of the air through calibration in total precipitation amount is quite simple [47]. In 1998, 36 countries, including Russia, signed the United Nations Convention for control of emissions of heavy metals in the air using biomonitoring (the Aarhus Protocol).

The United Nations Organization established a special Economic Commission for Europe intended for shaping the scientific policy of the countries that signed the UN Convention in the field of research on the critical ozone levels and evaluation of atmospheric heavy metal deposition in Europe by the methods based on the collection and analysis of moss biomonitors (Task Force Meeting of the UNECE ICP Vegetation). Information on the activities of this commission is available from the website http://icpvegetation.ceh.ac.uk.

Beginning in 1995, the FLNP team at the JINR has made a contribution to the European program "Atmospheric Heavy Metal Deposition in Europe - Estimation Based on Moss Analysis" by submitting the results of moss analysis to the European Atlas. Simultaneous moss surveys were regularly carried out in 1995-1996 [48], 2000-2001 [49], and 2005-2006 [50].

Neutron activation analysis of mosses allows determination of as many as 45 elements: Ag, **Al**, **As**, Au, Ba, Br, Ca, Ce, Cl, Co, **Cr**, Cs, Dy, Eu, **Fe**, Hf, **Hg**, I, In, K, La, Lu, Mg, Mn, Na, Nd, **Ni**, Rb, Sb, Sc, Se, Sn, Sm, Sr, Ta, Tb, Ti, V, U, W, Yb, Zn, and Zr.

The environmentally important elements **Cd**, **Cu**, **Hg**, and **Pb** are additionally determined by the atomic absorption spectrometry technique. The above list of elements appreciably exceeds the number of elements included in the European Atlas (bold-faced). Not all of the above-listed elements are air pollutants, but their determination by the multielement analysis does not require any significant extra budget, and they can be used for tracing transboundary air mass transport.

NAA is carried out at the IBR-2 reactor of the FLNP (JINR, Dubna) using epithermal neutrons, as well as the full spectrum of neutrons for activation. Induced gamma activity is measured by the HPGe detector with the resolution of 1.9 keV for the ^{60}Co 1332 keV gamma line. A software package developed at the FLNP is used to process gamma spectra and calculate concentrations of elements. Element contents are calculated both by the relative method using the certified reference materials Lichen-336 (IAEA), DK1 (Danish moss) and Pine Needles (NIST) and by the absolute method using nuclear constants.

AAS of mosses was carried out by the unified method in Russia, at the analytical laboratory of the Geological Institute, Russian Academy of Sciences, where a Perkin-Elmer spectrometer was used for determination of Cd, Cu, Hg, and Pb by the flame AAS, and using the same methodic in the countries-counterparts. Analytical features of both methods are described in the works cited below.

The concentration determination uncertainty for most element falls within the range of 5÷10%, amounting to 20÷25% in only a few cases.

The first contribution to the 1995/1996 Atlas [48] was the results obtained from biomonitoring of atmospheric deposition of heavy metals and trace elements in the Eastern Carpathians [51].

Later, these investigations, supported by grants of the Plenipotentiaries of some JINR member states and IAEA programs, were carried out in some industrial regions of Central Russia (Tula region [52, 53], Tver and Yaroslavl regions [54], northeastern parts of Moscow region [55]), the Southern Urals [56-58], the Udmurt Republic [59], Norway [60], Poland [61-64], Bulgaria [65-67], Slovakia [68, 69], Romania [70-74], Western Ukraine [75], Belarus [76], Serbia [77-79], Macedonia [80], Croatia [81], and the European part of Turkey [82] in cooperation with specialists of those countries.

The contribution of the FLNP to the UNECE ICP Vegetation Program has been indicated in large international publications [83-85].

Similar pilot projects have been realized by the FLNP in Asia in cooperation with the scientists from China [86], South Korea [87], Mongolia [88], and Vietnam [89].

Biomonitoring of atmospheric deposition of heavy metals and other trace elements is carried out using not only multivariate statistical analysis (method of principal components, or factor analysis).

Modern GIS technologies allowing analytical information to be represented as geographical color maps of element distributions are also widely used.

To illustrate, Figure 7 shows black-and-white maps of factor score distributions over the territory of Croatia calculated with the programe STATISTICA-6 (factor analysis) [81].

To make raster contour maps of the distributions, the GRINVIEW program from the GISINTEGRO software package with raster and vector graphics was used. This package has interfaces for all international GIS standards: ARC-info, MAP-info, *etc.*

Active biomonitoring. An alternative to passive biomonitoring using ground mosses is active biomonitoring using moss transplants (preferably *Sphagnum girgensohnii* Russow collected in a clean background region) kept under exposure in moss bags for a certain time in areas of interest. This method allows estimation of the air pollution level in those regions where ground mosses do not grow because of heavy technogenic load in large cities and industrial regions, as well as in arid areas where mosses never grow at all. The results obtained by the JINR team from active monitoring in Serbia, Poland, Romania, Bulgaria, and Greece are reported in [90-95]. In [96], where the active biomonitoring method was further developed by employing wet mosses, it was shown that wet mosses, as compared with dry mosses, have a higher ability to accumulate such elements as Sr, Se, As, Zn, Ca, Mg, Te, Al, Cr, Sm, Si, Be, Ce, Co, Mo, Dy, La, Hf, Nb, Ti, and W.
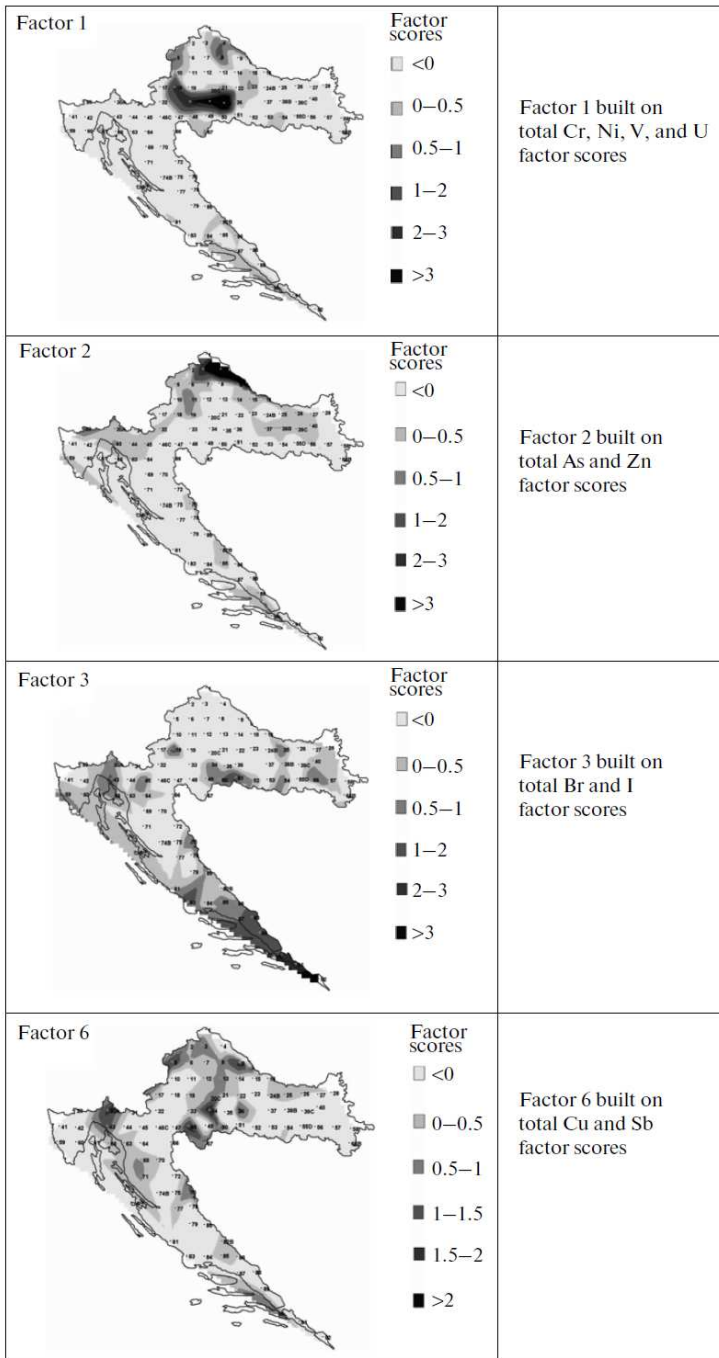
Fig. 7. Example of using GIS technologies for visualization of the multivariate statistical analysis results
in the form of maps showing distributions of factor scores [81]

Quality and safety of foodstuffs

Participation of the JINR team in the IAEA Coordination Program for the use of nuclear and related analytical techniques to study effect produced on human health by toxic elements taken in with food produced under conditions of industrial pollution (2002-2005) initiated development of a new direction in the investigative work of the team on quality and safety of foodstuff [97, 98]. Within the IAEA Technical Cooperation Project "Investigation of Health Effects on Children from the Consumption of Food Grown in Industrially Contaminated Areas of Russia, the Southern Urals, and Siberia using NAA and AAS" (2003-2005), a great number of food samples were investigated by the neutron activation technique. The work on studying bioaccumulation of chemical elements by vegetables was further continued in the technogenically contaminated area of Tula region [99].

Estimation of the technogenic contamination effect on the microelemental composition of basidial mushrooms in the European part of Russia was performed in [100]. The difference in the microelemental composition of edible wild mushrooms and artificially grown mushrooms was shown. NAA of 12 species of basidial mushrooms and soils on which they grew allowed the conclusion to be drawn that a high concentration of trace elements, including heavy and toxic metals, in mushrooms results from their high concentration in the soil in their mobile forms readily uptaken by mushrooms. In areas with intense technogenic contamination, the factors contributing to an increase in concentrations of mobile forms of metals in the upper soil horizon overlap and are piled up.

Workplace monitoring and personnel health

Due to the use of nuclear and related analytical techniques [101], it was shown for the first time that phosphate fertilizers are a source of contamination of the environment with rare-earth elements [102]. ENAA at the IBR-2 reactor in combination with the AAS and XRF methods made it possible to establish a relation between concentrations of pollutant elements (Cr, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Pb, REEs, Th, U, and others) at workplaces and in the raw material, by-product (phosphogypsum), and substrates (teeth, hair, nails) of the personnel involved in production of phosphate fertilizers at the Mineral fertilizer production association (Voskresensk, Moscow region). Analysis of the biosubstrates of the personnel and local inhabitants was carried out in parallel with a general assessment of environment in the vicinity of these factories using analysis of transporting and depositing natural media (soil, water, air), plants (grain crops), and foodstuffs [103-108]. The results of the studies showed that production of mineral nitrogen-phosphate fertilizers is a source of supplying F, Ca, Sr, and rare earth elements (REEs) to the environment, mainly by emission of these elements into the air. Elevated concentrations of F and REEs were observed in biosubstrates of the personnel and population. Recommendations were worked out for possible recycling of the production waste (compost from the factory's settling ponds and phosphogypsum) in agriculture to minimize damage inflicted to the environment by the factory.

Use of INAA for assessing negative effect of toxic elements on soil, bottom sediment, and aquatic biota

NAA proved to be good for studying scattered elements in the soil and vegetation of boreal forests in the European part of Russia [109, 110]. Profiles of trace elements in Al-Fe

humus podzolic soils subjected to air pollution from the apatite and nepheline production under varying lithogenic background were studied for the first time [111, 112]. Investigations of heavy metal contamination of soils around the lead-and-zinc works in Veles, Republic of Macedonia [113], carried out by ENAA at the IBR-2 reactor of the FLNP laid the basis for preparing and publishing the *Geochemical Atlas of Veles and Environs* [114]. In 2009 the international team of the authors of the Atlas was awarded the State Prize of the Government of Macedonia.

ENAA of the chemical composition of soils near some busy roads in Dubna (tunnel under the Moskva Canal) and Moscow (Shchelkovskoe and Minskoe highways) revealed the highest soil contamination level around the Shchelkovskoe highway [115]. It was shown that concentrations of Fe, Zn, Cr, Sb, and W exponentially decrease with increasing distance from the road. Data on the concentrations of Sb (group I toxicant) and W (hard metal) were obtained for the first time.

To assess the anthropogenic effect on the river Sestra, a tributary of the Volga, samples of water, bottom sediments, and biota were taken near the town of Klin (Moscow region) and investigated by NAA [116]. The results showed substantial technogenic contamination of the bottom sediments by a number of heavy metals (Fe >>> Zr > Mn > Zn > Cr > V > Ni = As > Co), and especially by cadmium, caused by textile and aircraft industry effluents.

Bottom sediment (BS) samples taken in the Danube delta were also investigated [117]. NAA was carried out using both epithermal neutrons (Dubna) and thermal neutrons (Bucharest, Romania).

The distribution of elements in unconsolidated bottom sediments sampled in unique cores from the depth of 600 m in the anoxic Black Sea area was studied [118-120]. Vertical distribution of seven macroelements (Na, Al, Cl, K, Ca, Ti, Fe) and 31 trace elements (Sc, V, Cr, Mn, Co, Ni, Zn, As, Se, Br, Rb, Sr, Zr, Mo, Sn, Sb, I, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Yb, Hf, Ta, W, Th, U), including anthropogenic pollutants (Zn, As, Mo, Sn, Sb), was determined in the sediment cores using INAA. The results of the investigations allowed better insight into the dynamics of both the process of environment pollution and the process of sedimentation in the Danube delta under the effect of soil erosion, navigation, and anthropogenic activity in the Danube basin.

The distribution of pollutants in water bodies (as illustrated by the Oka River basin) and their transport by underground waters in the European part of Russia was studied in [121, 122]. ENAA of bottom sediments (BS), bivalve mussels, and fish tissues from different parts of the Rybinsk Reservoir freshwater ecosystem (Central Russia) allowed revealing environment pollution sources, such as Rybinsk and Cherepovets, the town known for Europe's largest iron-and-steel works [123]. The concentrations of most elements in the BS are an order of magnitude larger than the concentrations of these elements in soft tissues of mussels (*dreissena*). The opposite trend is found only for calcium, cobalt, nickel, and zinc. Their concentrations in the *dreissena* body differed from their concentrations in the bottom sediment by no more than a factor of 2, and the concentrations of selenium and bromine in the *dreissena* body were an order of magnitude larger than in the bottom sediment. This not only means that most elements taken in by mussels are not accumulated, but excreted, from the organism [124], but also points to physiological significance of some essential macro- and microelements in the mussel organism. At the same time, it is known that, under considerable anthropogenic contamination, microelements can be accumulated in the organism in concentrations exceeding the physiologically necessary level. Under

these conditions the organism is incapable of controlling their uptake and excretion processes. Therefore, it is not impossible that high concentrations of zinc, cobalt, and nickel in the *dreissena* body are associated in our case with the influence of the Cherepovets iron-and-steel works, which is responsible for anthropogenic pollutants passing into the ecosystem.

ENAA in biotechnology of new pharmaceuticals and sorbents

One of the currently important fields in life sciences is scientific and applied biotechnology using the blue-green microalga *Spirulina platensis* (*S. platensis*), which has become one of the widely used objects since the 1990s. Spirulina biomass and products of its processing are used in various fields of the food, pharmaceuticals, and perfumeries industry; agriculture; medicine; and scientific research. This wide use of spirulina is due to its easy assimilation (85÷95%), high protein content (60÷70%), rich amino acid and vitamin composition, and a diversity of biologically active substances [125-127]. Spirulina is thought of as an effective stimulator and is able to produce antiviral and anticarcinogenic effect. It is often used in parallel with medical procedures applied to the organism for strengthening and purification purposes. Study has shown that *S. platensis* is able to biologically transform and endogenically add some essential trace elements, such as Se, I, Cr, Zn, and others. On the other hand, being a living organism, it accumulates elements in particular amounts depending on their concentration in the nutritional medium where *S. platensis* cells grow. Trace elements are important for acid metabolism. Metals like Fe and Cu form free radicals, whereas Zn and Se are able to reduce their effect. Selenium is a normal component of some enzymes, proteins, and nucleic acids. A low Se level causes such diseases as cardiomyopathy, cancer, endemic osteoarthropathy, anemia, vision dysfunction, *etc.* Added in specified doses to a diet, Se favors treatment of cardiological diseases, lowers the risk of cancer and AIDS, slows down aging processes, *etc.* [128].

Another important trace element, iodine, is part of all plants and living organisms. It is vitally important for development and growth of an organism, contributing to metabolism and oxidation-reduction processes and influencing the functioning of the thyroid, immune system, *etc.* Iodine deficiency adversely affects mental, emotional, and some physiological functions of the organism [129]. Chromium is an element that is rather peculiar in its effect on the human organism. It is vitally important, but may be toxic as well. With its differing valence (from +2 to +6), chromium has two stable states, non-toxic Cr(III) and toxic Cr(VI). The toxic form Cr(VI) easily penetrates tissue cells and may produce a carcinogenic and genotoxic effect, participating in oxidation-reduction reactions [130, 131]. The most important function of chromium is sugar blood regulation (glucose tolerance factor). Chromium deficiency may result in lipid and fat metabolism derangement, growth suppression, loss of weight, impaired coordination of movements, *etc.* ENAA investigations of interaction of trace elements with the blue-green microalga *S. platensis* were jointly carried out by the scientists of the JINR team and Georgian biophysicists from the E.L. Andronikashvili Institute of Physics, Georgian Academy of Sciences (Tbilisi, Georgia) [132, 133]. The objective of the investigations was to substantiate the possibility of developing new pharmaceuticals and food additives on the basis of the spirulina biomass where vitally important elements (Se, I, Cr, and others) occur in the bioactive form. A wide range of useful substances in the *S. platensis* biomass combined with the specified elements

in the necessary doses should produce an enhanced effect in prevention and treatment of various diseases. Biochemical experiments were carried at the Institute of Physics (Tbilisi, Georgia). The cultivation conditions and detailed description of the experimental procedure can be found in [134-137].

The elemental composition of the pelletized *S. platensis* biomass samples was investigated by ENAA at the IBR-2 reactor of the FLNP, JINR. The main results of those investigations are listed below. The background concentration level of macroelements, microelements, and trace elements in the *S. platensis* biomass determined by ENAA is shown in Figure 8.
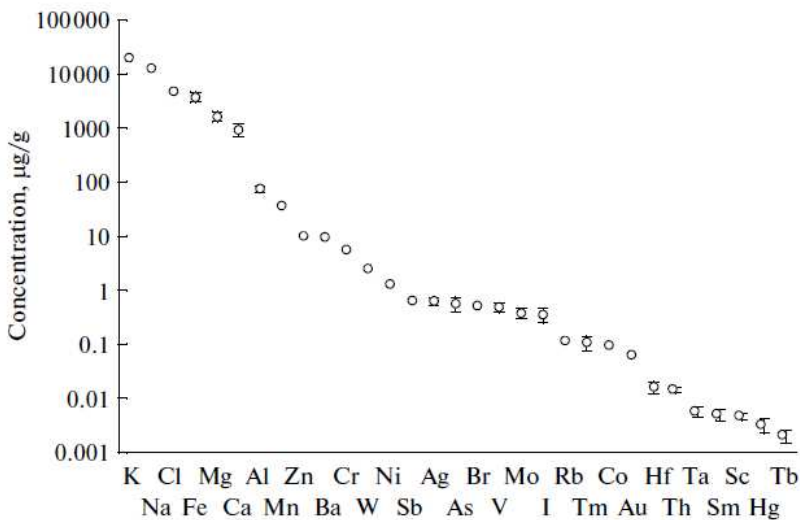
Fig. 8. Background concentrations of macroelements, microelements, and trace elements in the *Spirulina platensis* biomass [135]

Concentrations of 31 elements were determined within seven orders. It was found that the concentrations of such toxic elements as Hg, As, Cr, and Cd in the spirulina biomass are no larger than the tolerable values for the human organism (http://www.spirulina.com/SPBNutrition.html).

The ENAA was used to study accumulation of two chromium valence states, Cr(III) and Cr(VI), by spirulina which, unlike other living organisms, predominantly consumes essentially important Cr(III) from the nutritional medium while uptake of toxic Cr(VI) is much lower (approximately by a factor of 2). An increase in the chromium concentration in the nutritional medium does not lead to saturation, which makes it easier to determine the necessary doses with a satisfactory accuracy of 30÷100 µg/g for food additives and 200÷250 µg/g for treatment and prevention purposes [138, 139].

In all experiments simultaneous microscopic monitoring of the morphological state of spirulina cells was carried out and the protein content of the biomass was determined by the polyacrylamide gel electrophoresis technique. The investigations showed that under the chosen conditions of spirulina cultivation in a nutritional medium loaded with Se, I, and Cr

the quality and protein content of the biomass did not change. Thus, it was shown that pharmaceuticals could be made without losing the quality of the *S. platensis* biomass and the main regularities of accumulation of necessary trace elements by cells were determined.

The investigations also resulted in issuing two patents of the Russian Federation for methods for production of selenium- and chromium-containing preparations based on the biomass of the blue-green microalgae *S. platensis* [140, 141].

Features of the interaction of *S. platensis* with such a toxic metal as mercury were also investigated by ENAA. It was shown that, when Hg concentrations in the solution were about $100 \div 150$ μg/dm^3, the living spirulina biomass was capable of extracting mercury in the course of cultivation with adsorption dominating in a time interval of the order of minutes, being then followed by accumulation [142, 143]. Thus, the *Spirulina platensis* biomass can be used as a sorbent of heavy metals. Apart from the *Spirulina platensis* microalgae, another microorganism, bacteria *Arthrobacter oxydans*, was also investigated. The bacteria were isolated from basalt rocks and showed ability to reduce the toxic chromium form Cr(VI) to the non-toxic form Cr(III). It was shown that these bacteria could be successfully used for detoxification or immobilization of toxic agents [144-146]. Accumulation of chromium by bacteria *Arthrobacter globiformis* 151B in the presence of mercury was investigated in collaboration with the NAA group from Delft University (Netherlands) [147].

NAA in biotechnology for extraction of metals from poor ores, rocks, and production waste

NAA was also used to develop a method for bacterial leaching of some metals from poor ores, production waste, and rocks. As is known, microorganisms take an active part in geochemical processes of mineral formation, mineral diagenesis, and sedimentation both directly, by enzymatic action, and indirectly, by chemical interaction of their metabolism products with minerals. They act as agents favoring concentration, decomposition, or fractioning of mineral substances in natural cyclic processes of formation and destruction of rocks. The main mechanisms for bacterial separation of metals are biooxidation, bioreduction, acidolysis, bioaccumulation, biosorption, and complex formation. For each of these processes, there is a corresponding group of microorganisms capable of interacting in a particular way with some minerals or others. Natural organic mass of plant origin - peat of microbiological composition, including many aerobic and anaerobic forms - was used as a source of microorganisms. Peat suspension with the energy material for microorganisms and various additives stimulating their growth was kept under anaerobic conditions at the temperature $25 \div 28°C$ for 15-20 days. This allowed technically simple and economical activation of microorganisms capable of metabolism under oxygen deficiency. The solution obtained after filtration of the conditioned suspension was used in leaching experiments [148, 149]. The bacterial community of the peat suspension consisted of silicate, amonifying, manganese-reducing, spore-forming, and butyric acid bacteria and mold fungi. It was mainly behavior of rare, rare-earth, and radioactive metals that was studied in the leaching processes, but concentrations of such frequently occurring metals as Mn, Cu, Fe, K, Na, and many others were also determined for elucidating the nature and features of microbiological processes. The elemental composition of the samples was investigated by ENAA. The investigations showed that the peat suspension used as a growth solution

contained a variety of active microorganisms capable of interacting with metals. Trends toward removal and concentration of some rare, precious, and radioactive metals in the investigated rocks, ores, and production waste were established, which indicates that the proposed bacterial leaching method is a promising tool for applications with various types of bacteria depending on the particular purpose (waste purification, extraction of scarce metals, enrichment in a required element, *etc.*).

Conclusions

The review of the studies dealing with the use of neutron activation analysis in the life sciences has shown the performance capability of this technique for solving practical problems that sometimes cannot be solved by other analytical techniques. Commissioning of the upgraded IBR-2M reactor with the improved parameters for INAA and improvement of experimental equipment will allow mass analyses of samples within international projects of the JINR team to be carried out at a new level. Accreditation of the JINR analytical laboratory to the ISO standards will make it one of the world's best laboratories employing nuclear and related analytical techniques.

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NAA W NAUKACH O ŻYCIU
W LABORATORIUM FIZYKI NEUTRONOWEJ
ZJEDNOCZONEGO INSTYTUTU BADAŃ JĄDROWYCH W DUBNEJ

Abstrakt: Omówiono zastosowanie konwencjonalnej i epitermalnej neutronowej analizy aktywacyjnej w niektórych ważnych obszarach nauk przyrodniczych. Przez ponad 25 lat swojej działalności radioanalitycznej kompleks REGATA przy reaktorze IBR-2 w Dubnej stał się źródłem danych analitycznych do badania

środowiska, geologii morskiej, bionanotechnologii i medycyny itd. Mimo wielu konkurencyjnych, niejądrowych technik analitycznych (AAS, ICP-ES, ICP-MS, itp.) neutronowa analiza aktywacyjna (NAA) jest nadal najlepszą, podstawową wielopierwiastkową techniką analityczną zapewniającą ilościowe oznaczenie stężenia pierwiastków śladowych na bardzo niskim poziomie. W połączeniu z nowoczesnymi statystycznymi technikami przetwarzania dużych ilości danych, technologią GIS (system informacji geograficznej), elektronową mikroskopią skaningową, tomografią i innymi NAA pozwala na otrzymywanie ważnych w praktyce wyników, które zostały przedstawione w tym przeglądowym artykule.

Słowa kluczowe: epitermalna neutronowa analiza aktywacyjna, reaktor IBR-2, ochrona środowiska, biotechnologia