East European Journal of Physics. **3.** 53-58 (2022)
DOI:10.26565/2312-4334-2022-3-07 ISSN 2312-4334

COMPREHENSIVE ASSESSMENT OF BIOLOGICAL SUBSTRATES OF PROFESSIONAL SICK PERSON GROUP BY CHEMOMETRIC AND NUCLEAR PHYSICAL METHODS[†]

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Received July 3, 2022; accepted August 15, 2022

The article deals with the influence of negative factors of working conditions on the health status of NSC KIPT personnel when working with beryllium. Beryllium and its compounds render a general toxic, allergenic and carcinogenic effect on the organism. The high biological activity and toxicity of Be is due to its chemical activity and penetrating ability. The chronic professional disease such as berylliosis occurs as a result of prolonged systematic exposure on the organism of adverse factors. Elemental analysis of biosubstrates provides important information, that in combination with symptoms and other laboratory parameters, can help in the early diagnostics of physiological violations associated with metabolic disorders and exposure of toxic elements. The blood and hair samples were taken from 28 people, among which 5 patients were selected as a control group, and a group of 23 people were former employees of the beryllium production. The content of chemical elements in the biological substrates (blood and hair) of employees was determined by nuclear-physical methods. An elemental analysis was performed on the analytical nuclear physics complex appliance "Sokol". The methods based on registration of characteristic X-ray radiation of atoms and γ-radiation of nuclei excited by accelerated protons is used. After measurements, data arrays were obtained on the content of 14 chemical elements (N, Na, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Sr, Pb) in blood and hair. The processing of data arrays was carried out using the principal component method which is related to chemometrics technologies. As a result of the work, an analytical program was composed in MATLAB codes which were used to determine the content of elements in biosubstrates that are most sensitive to changes in external conditions. This made it possible to identify certain groups of patients who have different health state indicators, as well as to see the similarities or differences between patients depending on the different concentrations of chemical elements in the blood or hair.
Keywords: elemental analysis, biological substrates, berylliosis, characteristic X-ray radiation of atoms, chemometrics, principal component method.
PACS: 87.64.-t

Despite compliance with safety regulations, which practically exclude the interaction of personnel with harmful substances, the problem of professional diseases remains very relevant. Since the existence of the nuclear industry, NSC KIPT employees have been working with beryllium (Be). Be has high strength, heat capacity and heat resistance, high anti-corrosion properties, resistant to radiation, and has used as addition to metal alloys. Under production conditions, employees are exposed to soluble and sparingly soluble Be compounds, which differ significantly in their toxicological properties. The main intake of Be occurs by inhalation. With oral intake, sparingly soluble Be compounds are formed in the intestine. With the inhalation route of intake of soluble compounds, most part of Be remains in the lungs and tracheobronchial lymph nodes, a smaller part is distributed in the bones, liver, and kidneys.

Beryllium is determined in biological substrates both in practically healthy people who have worked in contact with the metal or its compounds, and in people who have undergone intoxication with its compounds, in patients with berylliosis and in people who lived in coal mining areas with a high Be content. Beryllium and its compounds can have a general toxic, allergenic and carcinogenic effect on the body. The high biological activity and toxicity of Be is due to its chemical activity and penetrating ability.

An essential toxicological feature of insoluble Be compounds is the lack of correlation between the dose of the affecting substance and the possible development of the disease. The development of berylliosis is often observed in people who worked with metal or its alloys, the content of which in the air did not always exceed the maximum allowable concentration. The occurrence of the disease is possible both after short-term contact with metal (from 6 hours to 2-3 weeks), and after long-term contact (within 10-20 years).

The chronic occupational disease such as berylliosis occurs as a result of long-term systematic exposure of the body to adverse factors. Berylliosis is characterized by diverse clinical symptoms with a predominance of signs of lung damage, a recurrent course of the disease, and damage to many organs.

For the correct diagnosis of professional disease, it is especially important to carefully study sanitary and hygienic working conditions, the patient's history, his "professional route", which includes all types of work performed by him from the beginning of his labor activity [1]. Currently, there is a group of professional patients with berylliosis among the former and current employees of the NSC KIPT.

[†] *Cite as*: M.F. Kozhevnikova, V.V. Levenets, O.P. Omelnyk, and A.O. Shchur, East Eur. J. Phys. **3**, 53 (2022), https://doi.org/10.26565/2312-4334-2022-3-07

Heavy metal compounds can enter the body in various ways such as inhalationly, orally, parenterally, as well as through the skin and mucous membranes. They are excreted most often very slowly, through the kidneys, liver, salivary and sweat glands, mucous membranes, which is accompanied by damage to these organs.

Recently, along with blood tests, there has been increasing interest in the study of hair to identify the state of microelement metabolism in the body and the toxic effects of individual heavy metals [2-6]. The available data show that the content of elements in hair reflects the elemental status of the organism as a whole, and hair samples are an integral indicator of mineralogical metabolism.

Spectral analysis of hair for trace elements is a study of the content of "useful" and "harmful" chemical elements in the human body by hair. Such chemical elements as K, Ca, Fe, Cu, Zn, Cr, Se are "useful" minerals that ensure the normal functioning of the body, but a toxic elements of industrial origin Hg, Cd, Pb, As are poisonous.

An elemental analysis of biosubstrates (blood and hair) provides important information that, in combination with symptoms and other laboratory parameters, can help in the early diagnosis of physiological disorders associated with metabolic disorders and exposure to toxic elements.

The aim of this work is a comprehensive assessment of the biological substrates of a group of professional patients by chemometric and nuclear physic methods.

SUBJECT OF STUDY AND INPUT DATA

The content of chemical elements in biological substrates (blood and hair) of employees was determined by nuclear-physical methods. Blood and hair samples were taken from 28 people, among which 5 patients were selected as a control group, and a group of 23 people were former workers in the beryllium production (9 women and 14 men). The age of the examined patients was from 52 to 78 years. Samples were prepared according to standard technology. The measurements were carried out at analytical nuclear physics complex "Sokol" of the NSC KIPT [7]. Methods based on the registration of the characteristic X-ray radiation of atoms (it is method1) and γ-radiation of nuclei excited by accelerated protons (it is method 2) were used. Elements with an atomic number of 16 or more were determined using the method 1, elements with an atomic number less than 16 were determined by the method 2. The spectra were measured at a proton beam energy of 1.7 MeV, a current of 20..50 nA, and a proton charge on the target of 100..150 μC. The radiation was recorded by Si (Li) and Ge (Li) detectors. After the measurements, data arrays were obtained on the content of elements in the blood and hair. In the range of elements from N to Pb, 14 elements (N, Na, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Sr, Pb) were selected for further processing.

SPATIAL ANALYSIS METHODS

Currently, when processing large data arrays, the possibilities of chemometrics are often used. Chemometrics is a scientific discipline that applies mathematical, statistical and other methods based on formal logic to construct or select optimal measurement methods and experimental designs, as well as to extract the most important information in the analysis of experimental data [8]. One of the main objects that chemometrics works with is chemical data. However, over time, chemometrics has become an independent discipline. Two circumstances contributed to this such as the complication of the mathematical apparatus used in chemometrics, and the emergence of numerous applications in which the chemometric approach is successfully applied in areas far from chemistry [9].

Chemometrics is closely related to mathematics. Therefore, chemometrics has found numerous applications in various fields related to and far from chemistry, for example, in multivariate statistical control of processes [10], in image analysis [11], and in biological applications [12]. It is used in physical chemistry to study kinetics [13], in organic chemistry to predict the activity of compounds based on their structure (QSAR – quantitative structure-activity relationship) [14], in polymer chemistry [15], in theoretical and quantum chemistry [16] .

Data is the main object that chemometrics works with. The simplest case is one-dimensional data, i.e. just one number. A more complex case is multidimensional, unimodal data, i.e. a set of results from several measurements related to the same sample. The next most common data type is bimodal data, which is represented by a 2D matrix, a table of numbers with I rows and J columns. Recently, much attention has been paid to three (or more) modal data. The main task of chemometrics is to extract the necessary information from the data. The concept of information is key in chemometrics. What is information depends on the purpose of the problem being solved. For example, in some cases it is sufficient to know that the desired substance is present in the system, while in others it is necessary to obtain quantitative values. All data contains noise, such as errors that hide useful information.

The principles of the chemometric approach to data analysis: 1) the use of a multidimensional approach in designing experiments and analyzing their results; 2) the definition of what is considered noise and what is information is decided taking into account the goal and the methods used to achieve it.

The traditional approach to processing measurement results is to identify individual, especially significant quantities. To select useful information in chemometrics, data compression methods are used. The idea behind this approach is to represent the original data using new hidden variables. In this case, two conditions must be met. Firstly, the number of new variables should be significantly less than the number of original variables, and, secondly, the losses from such data compression should be comparable to the noise in the data. Data compression methods allow you to present useful information in a more compact form that is convenient for visualization and interpretation.

The most popular method of data compression is principal component analysis (PCA), which underlies some other similar chemometric methods [17].

From a mathematical point of view, the method of principal component analysis is a decomposition of the original 2D matrix X, i.e. its representation as a product of two 2D matrices T and P [18]:

$$X = TP^t + E = \sum_{a=1}^{A} t_a p_a^t + E \,, \qquad (1)$$

where T is the score matrix; P is the load matrix; E is the matrix of residuals.

The transposition operation is denoted by the superscript t. The number of columns t_a in matrix T and p_a in matrix P is equal to the effective rank of matrix X. A is the number of principal components, and it is less than the number of columns in matrix X.

To illustrate a method PCA, a dataset should be considered. It is containing only two variables x_1 and x_2 that are strongly correlated. On Figure 1 (a) these data are presented in original coordinates. On Figure 1(b) the same data are shown in new coordinates. The load vector p_1 of the first principal component (PC1) determines the direction of the new axis along which the greatest data change occurs. The projections of all initial points onto this axis make up the vector t_1. The second principal component p_2 is orthogonal to the first, and its direction (PC2) corresponds to the largest change in the residuals by segments which are perpendicular to the p_1 axis (it is shown on Figure 1 (b)).

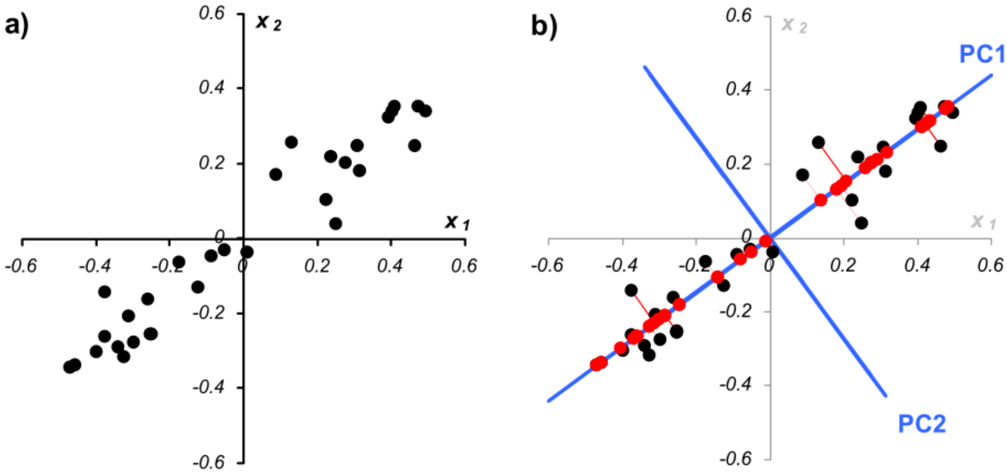

Figure 1. Graphical representation of the data set in the method of principal components: a) data in the initial coordinates of the variables x_1 and x_2; b) data in principal component coordinates [18]

An application of principal components analysis method is carried out sequentially, step by step. At each step, the residuals E_a are examined, and among them the direction of the greatest change is selected. The data are projected onto this axis, new residuals are calculated, and so on. This algorithm is called non-linear iterative projections by alternating least-squares (NIPALS) [19].

Principal component method can be interpreted as the projection of data onto a subspace of lower dimension. The residuals E arising in this case are considered as noise that does not contain significant information.

When examining data using the PCA method, special attention is paid to the graphs of accounts and loads. They carry information useful for understanding how the data is structured. On the score chart, each sample is depicted in coordinates (t_i, t_j), most often as (t_1, t_2). The proximity of two points means their similarity, i.e. positive correlation. Points located at right angles are uncorrelated, and diametrically opposite points are negatively correlated.

If the score chart is used to analyze the relationship of samples, then the load chart is used to study the role of variables. On the load graph, each variable is displayed as a dot in the coordinates (p_i, p_j), for example (p_1, p_2). By analyzing it in a similar way to a graph of accounts, one can understand which variables are related and which are independent. However, the most informative is the joint study of paired graphs of scores and loads [18].

RESULTS OF THE RESEARCH

The processing of data arrays obtained by nuclear-physical methods was carried out in order to determine the content of elements in human biosubstrates that are most sensitive to changes in external conditions. Based on the analysis of the obtained data, it was supposed to study the possibilities of using nuclear-physical methods for analyzing the composition of a substance for diagnosing certain types of diseases.

For a comprehensive assessment of the data obtained by nuclear-physical methods, the Analytica program has been developed, which implements the selection in a given factor space of the initial features of the m main components, or generalized features.

The Analytica program was created in MATLAB package codes. The process of data processing using the program includes the following steps:

1) a calculation of probabilistic-statistical values: mean, variance, deviation, variation;
2) a determination of the correlation matrix and correlation coefficients;
3) a calculation of eigenvalues for the correlation matrix;
4) using the NIPALS function, the obtaining a matrix of loads and a matrix of accounts;
5) a construction of graphs of accounts and loads.

When processing the obtained data, it was revealed that the hair is a more informative object, therefore, it is for this object the application of the principal component method is illustrated.

In the general case for biological objects it is rather difficult to determine the content of elements in the norm. Even when labeled, this value varies greatly for the control group as well. But the ratio of the contents of a number of elements is characteristic and is very constant. It is these relationships that can serve as an indicator of changes in the body's metabolism. In the process of data processing, various pairs of elements were selected, but normalization to the content of total N turned out to be the most informative. When processing the initial data for hair with normalization to N, a 28 × 13 matrix was compiled, where 28 is the number of patients, 13 is the number of chemical elements. Not included in the statistical processing of As and Cd, because the content of these elements is at the boundary and below the limits of detection.

According to the developed data processing algorithm, the eigenvectors (or load vectors) in the new coordinate system were determined. The coordinates of the samples for each component were calculated, which shows in what logic this or that component determines the structure of the samples and their position relative to each other.

The calculations were made for all components, allowing you to see how the samples relate to each other in one-dimensional (within one component) and two-dimensional spaces (planes formed by two components). The position of a single sample on a particular component was examined, as well as the percentage of features for the sample that explains the component. By varying the various main components, results were obtained linking the ratio of the content of separate elements in the hair with the presence of professional disease.

Considering the load vectors, we can conclude that in the first vector the load of element S is 86.2%, in the second vector the load Ca is 80%. Therefore, within the framework of component 1, the S index (it has the largest positive weight) acts as the most significant index. Within the framework of component 2 it is Ca, i.e. the following chemical elements predominate in the hair: S, Ca.

On Figure 2 shows the projection of the structure of hair samples in terms of elemental composition onto the plane of the principal components (1 comp and 2 comp).

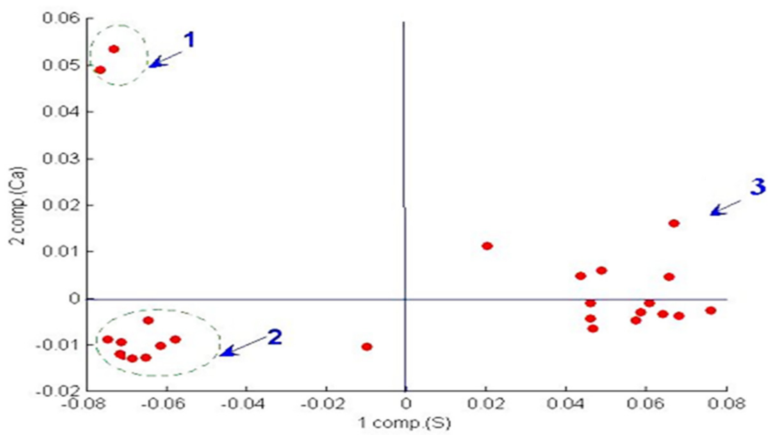

Figure 2. Chart of accounts for the data array on the content of chemical elements in the hair of 28 patients

On the given graph of scores, all points are located along the first principal component and form three groups. Group number 2 includes patients of the control group who do not have deviations in their state of health. They are characterized by a relatively low content of Ca and S. The first group of patients has a high content of Ca and low S and located apart. Based on the data set on the composition of the hair of these patients, it could be assumed that their hair was dyed (this was later confirmed). The third group includes patients with a certain professional disease. They are characterized by an increased content of S in the hair.

An application of the principal components method allows us to draw a conclusion about the microelemental state of the human body. Any pathology or any deviation in health is caused either by a deficiency of vital (essential) elements, or an excess of both essential and toxic microelements. Such imbalance of macro- and microelements has received its unifying name as microelementosis [20]. For example, it follows from the processed data that a group of patients with a professional disease is characterized by a higher content of N, K, Fe in the blood than in patients of the control group. The information [21] that an excess of Zn can lead to a deficit of Cu and Fe is confirmed, and, conversely, an excess of

Fe leads to a deficiency of Cu and Zn. For patients with a professional disease, there is an increased content of S in the hair than in patients of the control group.

The majority of patients (when examining hair samples) have approximately the same Ca/Mn ratio, in patients with an professional disease the Mn content is slightly increased. Ca content remains approximately the same as in patients in the control group. In the composition of the hair of patients with a professional disease, the content of Fe is increased, while the content of Ca is the same as in patients of the control group. It has been confirmed that an excess of calcium in the hair occurs when it is intensively excreted from the body [21].

CONCLUSIONS

The content of chemical elements in biological substrates (blood and hair) of 28 employees with a professional disease was determined by nuclear-physical methods. After the measurements, data arrays were obtained on the content of elements in the blood and hair. In the range of elements from N to Pb, 14 elements (N, Na, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Sr, Pb) were selected.

For complex data evaluation, the Analytica program has been developed, which implements the principal component method. The principal component method is effective in identifying the content of the main components of each sample and allows you to present useful information in a more compact form that is convenient for visualization and interpretation. Thus, with the help of graphs of accounts, certain groups of patients who have different indicators of health status are clearly identified. From the processed data, it follows that the group of patients with professional diseases is characterized by a higher concentration of N, K, Fe in the blood and an increased content of S in the hair than in patients of the control group.

It follows from the obtained results that hair is a more informative object than blood. Unlike blood, the chemical composition of hair is more stable. The study of hair shows the level of not only vital, but also conditionally essential, as well as toxic and potentially toxic trace elements.

The use of nuclear-physical methods and the mathematical apparatus of chemometrics made it possible to obtain information on the microelemental state of professional patients. As a result of the study, a conclusion was made about the possibility of using nuclear physics methods in the diagnosis of professional diseases.

Acknowledgements

This work has been supported organization and concrete peoples: SMSP №13, which has selected of bloods; Avramenko N.A. who has done targets; Glazunov L.S., Kuzmenko V.V., who has works on “Sokol”.

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КОМПЛЕКСНА ОЦІНКА БІОЛОГІЧНИХ СУБСТРАТІВ ГРУПИ ПРОФХВОРИХ ХЕМОМЕТРИЧНИМ ТА ЯДЕРНО-ФІЗИЧНИМ МЕТОДАМИ

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В роботі розглядається вплив негативних факторів виробничих умов на стан здоров'я персоналу ННЦ ХФТИ при роботі з бериллієм. Бериллій та його сполуки здійснюють загальнотоксичний, алергенний та канцерогенний вплив на організм. Висока біологічна активність та токсичність бериллію обумовлена його хімічною активністю та проникаючою здатністю. Хронічне професійне захворювання бериллiозу виникає як результат тривалого систематичного впливу на організм несприятливих факторів. Елементний аналіз біосубстратів надає важливу інформацію, яка в поєднанні із симптомами та іншими лабораторними показниками, може допомогти в ранній діагностиці фізіологічних порушень, пов'язаних із порушеннями обміну речовин та впливом токсичних елементів. Проби крові та волосся отобрано у 28 людей, серед яких 5 пацієнтів обрано в якості контрольної групи, а група з 23 людей – колишні працівники бериллієвого виробництва. Ядерно-фізичними методами визначено вміст хімічних елементів у біологічних субстратах співробітників (кров і волосся). Елементний аналіз виконано на аналітичному ядерно-фізичному комплексі «Сокол». Використовувались методи, засновані на реєстрації характеристичного рентгенівського випромінювання атомів і γ-випромінювання ядер, що збуджуються швидкими протонами. Після проведення вимірювань отримано масиви даних по вмісту 14 хімічних елементів (N, Na, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Sr, Pb) в крові та волоссі. Обробка масивів даних проводилась при використанні методу головних компонентів, який має відношення до технологій хемометрики. В результаті роботи була складена програма Analytica в кодах MATLAB, яка використовувалась для визначення вмісту в біосубстратах елементів, найбільш чутливих до зміни зовнішніх умов. Це дозволило виявити певні групи пацієнтів, які мають різні показники стану здоров'я, а також побачити схожість або відмінність між пацієнтами в залежності від концентрації хімічних елементів в крові чи волоссі.

Ключові слова: елементний аналіз, біологічні субстрати, берилліоз, характеристичне рентгеновське випромінювання атомів, хемометрика, метод головних компонентів.