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## ВИКОРИСТАННЯ РЕНТГЕНІВСЬКОЇ ФЛУОРЕСЦЕНТНОЇ СПЕКТРОСКОПІЇ ДЛЯ ВИЗНАЧЕННЯ ЕЛЕМЕНТНОГО СКЛАДУ РЕЧОВИН ПРИ ВИВЧЕННІ БІОФІЗИКИ

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## THE USE OF X-RAY FLUORESCENCE SPECTROSCOPY TO DETERMINE THE ELEMENTAL COMPOSITION OF SUBSTANCES IN THE STUDY OF BIOPHYSICS

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## АНОТАЦІЯ

**Постановка проблеми.** Центральним питанням, що ставиться до обговорення в даному дослідженні, є ефективно включення новітніх технологій, зокрема рентгенофлуоресцентної спектроскопії, у навчальні курси вищої освіти, пріоритетно у сфері медицини та фармації. Основна мета дослідження полягає у відповіді на актуальне педагогічне запитання: як найкраще адаптувати навчальний процес для полегшення засвоєння та використання складних аналітичних методик, одночасно спонукаючи студентів до розвитку навичок критичного мислення та самостійного вирішення завдань.

**Матеріали та методи.** Матеріали: використовувалися зразки різноманітних матеріалів (у вигляді порошку, тверді та рідкі), рентгенофлуоресцентний спектрометр (модель EXPERT 3L), а також зразки ґрунтів для аналізу їхнього елементного складу. Методики: Теоретичне навчання: огляд теоретичних основ рентгенофлуоресцентної спектроскопії через комплексний підхід, що включає лекції, дослідницькі проекти та дискусії. Практична лабораторна робота: самостійна індивідуальна робота за допомогою рентгенофлуоресцентного спектрометра EXPERT 3L. Навчання аналізу даних: освоєння методик аналізу даних, отриманих із спектрометра, розвиток навичок якісного та кількісного аналізу. Самостійне навчання: стимулювання ініціативи, креативності та самостійності студентів через заохочення до самостійного вирішення задач.

**Результати.** Успішно розроблено та впроваджено лабораторний практикум в процес підготовки майбутніх медичних працівників, що підсилило залученість студентів та сприяло розвитку їхніх вмінь. Практичні заняття значно покращили активізацію інтелектуальної діяльності студентів, формування навичок логічного мислення, встановлення міжпредметних та міжпредметних зв'язків, а також розвиток їхніх творчих компетентностей. Експериментальний клас ефективно сприяє розвитку як академічних, так і професійних навичок, надаючи студентам потрібні інструменти для успішного старту їхньої майбутньої кар'єри.

**Висновки.** Інтеграція передових аналітичних методів, таких як рентгеновська флуоресцентна спектроскопія, у навчальну програму значно покращує взаємодію та розуміння студентів, сприяючи набуттю теоретичних знань та розвитку практичних навичок. Експериментальний підхід допоміг у встановленні міжпредметних і міжпредметних зв'язків, що має вирішальне значення для комплексного навчання в мультидисциплінарних галузях, таких як медицина та біологічна фізика. Результати підтверджують, що інтеграція практичних занять у навчальний процес, особливо тих, що використовують новітні методи, може допомогти подолати розрив між теорією та практикою, сприяючи глибшому осмисленню матеріалу. В майбутніх дослідженнях ми розглянемо інші освітні стратегії та технології, які зможуть покращити залученість студентів та їх здатність до аналітичного осмислення матеріалу.

**КЛЮЧОВІ СЛОВА:** експериментальне навчання; активне навчання; професійні компетенції; рентгеновська флуоресцентна спектроскопія; аналіз елементного складу; лабораторні практичні роботи; залучення студентів; міжпредметні зв'язки.

## ABSTRACT

**Formulation of the problem.** The central challenge addressed in this study is effectively integrating cutting-edge technologies, such as X-ray fluorescence spectroscopy, into higher education curricula, particularly within medical and pharmaceutical disciplines. The study seeks to address the pedagogical question: how can educational practices be optimized to facilitate the understanding and application of complex analytical techniques while simultaneously encouraging the development of students' critical, independent problem-solving skills?

**Materials and methods.** Materials: various material samples (powdered, solid, liquid), X-ray fluorescence spectrometer (EXPERT 3L model), and assorted soil samples for elemental composition study. Methods: Theoretical Instruction: Comprehensive teaching of the theoretical principles underlying X-ray fluorescence spectroscopy using a blend of lectures, readings, and discussions. Practical Laboratory Work: Encouraging hands-on, independent laboratory work using the EXPERT 3L X-ray fluorescence spectrometer, enhancing the understanding of practical applications. Data Analysis Training: Instruction on analyzing data from the spectrometer, improving both qualitative and quantitative analysis skills. Self-Directed Learning: Encouraging initiative, creativity, and self-sufficiency among students by promoting independent problem-solving activities.

**Results.** Successful development and implementation of laboratory practicals titled into the training of future medical professionals. Enhanced Student Engagement and Skill Development: The practical session had a significant impact on stimulating students' intellectual activity, fostering logical thinking skills, establishing inter-thematic and inter-subject connections, and promoting creative competencies. Formulation of Subject and Professional Competencies: The experimental class successfully molds both subject-specific and professional skills, equipping the students with the tools necessary for their future careers.

**Conclusions.** Integrating advanced analytical techniques, such as X-ray fluorescence spectroscopy, into the curriculum greatly enhances student engagement and understanding, contributing to theoretical knowledge and practical skill development. The experimental approach aided in establishing inter-thematic and inter-subject connections, vital for comprehensive learning in multidisciplinary fields like medical and biological physics. The results affirm that integrating practical exercises into the curriculum, especially those utilizing modern techniques, can help bridge the gap between theory and practice, fostering a deeper understanding of the subject matter. Future studies could explore other educational strategies or technologies that enhance student engagement and understanding in material analysis.

**KEYWORDS:** Experiential Learning; Active Learning; Professional Competencies; X-ray Fluorescence Spectroscopy; Elemental Composition Analysis; Laboratory Practical Work; Student Engagement; Interdisciplinary Connections.

## INTRODUCTION

**Formulation of the problem.** The primary pedagogical challenge presented in this study pertains to the evolution of educational paradigms in higher education. Specifically, the task is to transition from traditional, lecture-focused teaching towards a more hands-on, independent learning model.

In the evolving landscape of higher education, an immediate challenge lies in reshaping the educational content and enhancing the learning process by amplifying the proportion of students' independent work (from 1/3 to 2/3 of ECTS credit). The distinctive feature of this contemporary educational format is to prepare a specialist who can think creatively, deepen their knowledge independently, apply it pragmatically, and demonstrate capacities for self-growth, self-education, and innovative endeavors. Consequently, the primary objective of an educator in higher education is to facilitate productive, independent work among students rather than a mere reproduction of the course material.

Laboratory work is pivotal in students' independent work in natural science disciplines. The principal aim in a higher education context is to harmoniously meld lectures with independent work, create a scientific worldview in students through the application of experimental research methods, implement theoretical knowledge practically, and assess their knowledge, skills, and competencies in specific topics or sections of biophysics. The challenge involves balancing these two approaches to promote creative thinking, deepening knowledge, and practical application of theory in students. This necessitates increasing the proportion of students' independent work, a shift especially critical in the natural sciences, where laboratory work plays a significant role.

When teaching elemental composition analysis using X-ray fluorescence spectroscopy, issues like complex technical understanding arise. This subject is technically complex and may pose challenges for students, particularly those new to the topic. Understanding the principles of X-ray fluorescence and how it can be used to analyze elemental composition requires a solid grounding in physics and chemistry. Practical Application: Translating theoretical knowledge into practical applications is a significant hurdle. Students may understand the theory but struggle to apply it when using X-ray fluorescence spectroscopy in a laboratory setting. Interdisciplinary Integration: The topic straddles several disciplines, including physics, chemistry, and material science. Students may struggle to integrate knowledge from these various areas effectively. Student Engagement: Due to its technical nature, maintaining student engagement and enthusiasm can be challenging, particularly for those less inclined towards technical or scientific subjects.

Furthermore, the task of integrating lectures and independent work into a coherent whole is a complex one. The pedagogical problem, therefore, involves designing, implementing, and assessing laboratory work that encourages students to apply their theoretical knowledge in practice while also developing their problem-solving skills and fostering a scientific worldview. Creating effective methodical guidelines for teaching these subjects forms another aspect of the pedagogical problem this work seeks to address.

**Analysis of current research.** A literature review revealed that international and local researchers have dedicated considerable effort to addressing this issue. The pivotal role of physical experiments as a wellspring of knowledge and a benchmark for validating theoretical studies, along with the significance of laboratory workshops within the general physics curriculum, is demonstrated in the works of Oleksandr Kyianovskiy (2019), Nataliia Stuchynska, (Krotevych et al., 2023), Stepan Velychko, (Velychko & Siryk, 2017), K. Paige Brown, (Brown et al., 2019), Jaclyn E. Tetenbaum-Novatt, (Tetenbaum-Novatt et al., 2018), Faith Celeste B. Ole (2020), Neville, M. W., (Neville et al., 2015). Recommendations for the integration of foundational and clinical sciences throughout the pharmacy curriculum were made by Margarita DiVall (DiVall et al, 2020). Numerous researchers have extensively studied the main facets of laboratory teaching. Moreover, this problem of modern education is highlighted in the national doctrine of educational development for Ukraine in the 21st century. Issues of online education were revealed in the works of Hannah Park (Park, & Shrewsbury, 2016), Iwona Czaplinski, (Czaplinski & Fielding, 2020). The concepts of modern education and formation of professional competence were revealed in the works of Juha Nieminen (Nieminen et al., 2004), Julia Mazurenko (2020), Nataliia Stuchynska, (Stuchynska et al, 2020). The importance of developing practical skills for medical and pharmaceutical students at the early stage of education and practical application of X-ray fluorescence spectroscopy in medical practice was revealed in the works of Tracy M. Kosinski (Kosinski et al., 2017), Patric Zimmermann (Zimmermann et al., 2020), Roozbeh Ravansari (Ravansari et al., 2020), Hervé Rebiere (Rebiere et al., 2019), Alexandra Figueiredo (Figueiredo et al, 2016)

**The purpose of the article.** This article aims to delve into the effective incorporation of X-ray fluorescence spectroscopy in higher education, particularly in the context of student-led laboratory work. The paper seeks to develop clear, understandable methodological guidelines for practical training that help students understand the topic and broaden their scientific comprehension. Through this, the article intends to contribute to the evolution of teaching practices, enhancing the ability of graduates to meet the demands of rapidly evolving technologies and industries.

## THEORETICAL BASES OF THE RESEARCH

The basic theory of X-ray fluorescence analysis.

X-ray fluorescence spectroscopy (XRF) is an efficient, non-invasive, and eco-friendly analytical method noted for its high precision and reproducibility. This technique allows for qualitative, semi-quantitative, and quantitative identification of all elements ranging from beryllium to uranium in powdered, solid, and liquid samples.

The XRF method encompasses energy ranges or wavelengths from  $E = 0.11\text{--}60$  keV or  $\lambda = 11.3\text{--}0.02$  nm. Light, in addition to its wave properties, exhibits particle-like characteristics. Hence, we often use "photon" or "light quantum." We employ the quantum count as an intensity unit, with measures calculated per second in imp/s (impulses per second) or kimp/s (kilo-impulses per second).

The generation of X-radiation.

The generation of X-radiation, or X-rays, can occur when charged particles, especially electrons, lose kinetic energy due to any form of change. This energy loss can happen when an electron is decelerated, alters its direction of motion, or moves to a lower energy level within the atom's electron shell. Electron deceleration and their transitions from a particular energy level in an atom's electron shell to a lower one play a significant role in generating X-radiation.

The Bohr atomic model proves useful in explaining these processes. This model views an atom as a positively charged atomic nucleus orbited by electrons moving in designated spaces or shells. Electrons in different energy shells or levels possess varying energy due to their proximity to the atomic nucleus. Consequently, a distinct minimum amount of energy is necessary to remove an electron from an inner shell of an atom. The binding energy of an electron within an atom is inversely related to its distance from the nucleus, which can be understood as the amount of energy absorbed by the radiation emitted by the atom.

Characteristic radiation.

Characteristic radiation is a unique phenomenon associated with each element, defined by its atomic number ( $Z$ ) in the Periodic Table of Chemical Elements or by the count of its electrons in a neutral state. Because of the varying number of electrons (negative charge carriers) or positive charges ( $Z$ ) in the atomic nucleus (atomic number), the binding energy or energy levels are distinct for each element and typical to it.

Suppose an electron from the inner shell of an atom is ejected due to irradiation. In that case, the vacancy it leaves behind is filled by an electron from a higher shell, with the energy difference between the two levels being released. This energy is either emitted as X-ray quanta or transferred to another shell electron (through the Auger effect).

The likelihood of forming an X-ray quantum during this process is referred to as the fluorescence yield ( $\omega$ ), which depends on the element's atomic number and the shell where the vacancy originated. For lighter elements,  $\omega$  is very small (approximately  $10^{-4}$  for boron) but reaches 1 for the K shell of heavier elements like uranium.

However, the critical fact is that the energy or wavelength of the emitted X-radiation is characteristic of the element it originated from. This emission is referred to as characteristic X-radiation (see Fig. 1).

To induce X-radiation, a technique is needed that can eject electrons from an atom's inner shells (K, L) by transferring more energy to the inner electrons than their binding energy within the atom. Several methods can be used to achieve this:

- Irradiation with elementary particles of adequate energy (like electrons, protons,  $\alpha$ -particles) which, upon impact, convey the necessary energy to the electrons for their removal from the atom.
- Irradiation with X-rays or gamma rays.
- Irradiation with X-rays from an X-ray tube, which is technically the simplest and safest approach in terms of shielding against radioactive radiation.

An atom can emit excess energy as a photon of characteristic radiation. Given that the energies of the atom's initial state ( $E_1$ ) and final state ( $E_2$ ) are quantized, radiation of frequency  $\nu = (E_1 - E_2) / h$  is generated. All potential quantum transitions of radiation from an atom in its initial K-state form the most rigid (shortwave) K-series. Similarly, L-, M-, and N-series are formed.

In 1913, G. Moseley empirically established that the square root of the frequency  $\nu$  of an element's characteristic radiation spectral line is a linear function of its atomic number  $Z$ , expressed as  $\sqrt{\frac{\nu}{R}} = \frac{Z - S_n}{n}$ , where  $R$  is the Rydberg constant,  $S_n$  is the screening constant, and  $n$  is the principal quantum number (fig. 2). This law serves as a compelling demonstration of the proper arrangement of elements in the Periodic Table of Elements and aids in elucidating the physical meaning of  $Z$ .

The Moseley law points out that characteristic X-ray spectra do not exhibit the periodic patterns typical of optical spectra. This suggests that in the characteristic spectra of X-radiation, the inner electron shells of atoms across all elements have a similar structure. Subsequent experiments identified some deviations from the linear relationship for transition groups of elements related to changes in the sequence of the outer electron shell filling and heavy atoms due to relativistic effects.

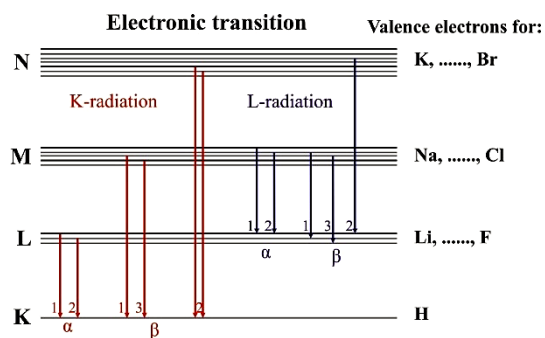


Fig. 1. Terminology for Energy Levels

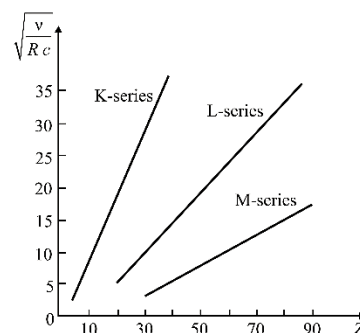


Fig. 2. Moseley diagram for K-, L-, and M-series characteristic X-radiation. The x-axis defines the ordinal number of the element  $Z$ , and the y-axis –  $\sqrt{\frac{\nu}{Rc}}$  ( $c$  is the speed of light in a vacuum)

The position of spectral lines in the Moseley diagram can fluctuate based on several factors, such as the number of nucleons in the nucleus (causing isotope shifts) or the state of the outer electron shells (leading to chemical shifts), among others. Investigating these shifts provides granular insights into the atom's structure and behavior. The relative intensities of lines within a single series are governed by the probability of quantum transitions and, consequently, by the corresponding selection rules. Apart from the most prominent lines of dipole electric radiation, characteristic X-radiation spectra can exhibit lines of quadrupole and octopole electric radiation and lines of dipole and quadrupole magnetic radiation.

The qualitative analysis hinges on the spectral position of the characteristic lines in the emission spectrum of the sample being tested. Rooted in Moseley's law, this analysis is executed based on the intensities of these lines. X-ray fluorescence spectral analysis can identify all elements with an atomic number  $Z$  greater than 12 and, in some cases, even lighter ones.

The X-ray fluorescence spectrometer

The X-ray fluorescence spectrometer, specifically the EXPERT 3L model, is depicted in Figure 3 in terms of its overall appearance and schematic layout. To stimulate the characteristic radiation of the element within the material being tested, the device utilizes both the braking radiation and the characteristic radiation emitted by the X-ray tube's anode material.

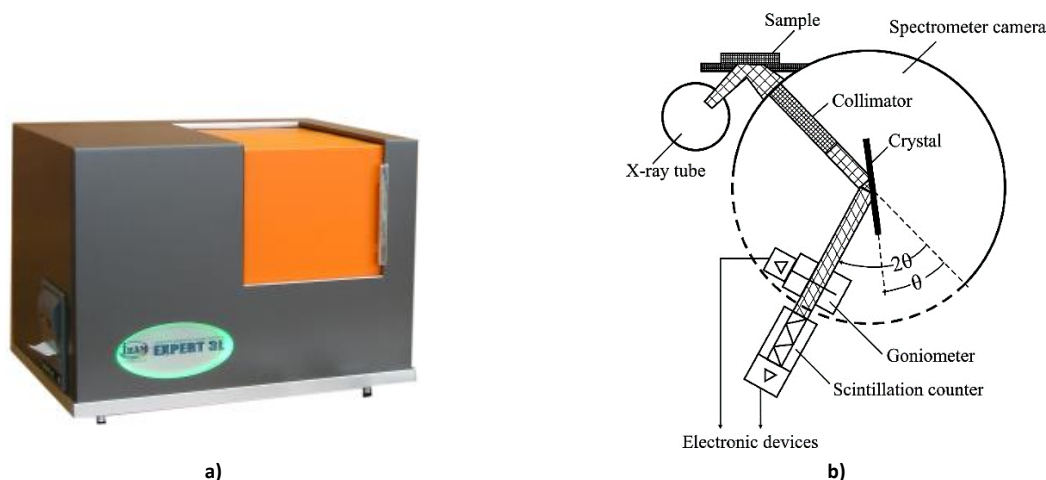


Fig. 3. General view (a) and schematic diagram (b) of an X-ray fluorescence spectrometer

Understanding that a chemical element within a sample can emit X-radiation when the energy of the excitation quanta surpasses the binding energy (absorption edge) of the element's internal electron is of vital significance. For instance, when a sample is irradiated with a 20 kV voltage, the maximum energy of the quanta released by the tube equals 20 keV. Hence, it is impossible to stimulate the K-radiation of elements with an atomic number  $Z > 43$  since the binding energy of their K-level exceeds

20 keV. The initiation of K-radiation of heavier elements occurs at a generator voltage beyond 60 V. Rhodium (Rh) is universally used as a standard anode material since its characteristic radiation is suitable for exciting both heavy and light elements.

Following the excitation of an element in the sample (by X-radiation), a series of element-specific wavelengths is discharged. This secondary radiation traverses the collimator and lands on the crystal, which is a spectrum analyzer. The constitution of crystals comprises atoms (or molecules) arranged periodically, thus forming a crystal lattice. In this particle configuration, numerous planes of different orientations pass through the crystal lattice's nodes (horizontal, vertical, oblique planes).

When X-rays encounter the plane of the crystal lattice, every particle situated on it behaves as a scattering center, releasing a secondary wave. These secondary waves amalgamate in the reflected wave. Identical waves are produced from planes parallel to the given plane, given the negligible absorption of X-rays within the interplanar distances  $d$ . All these reflected waves interfere; however, if the interference condition ( $\Delta = n\lambda$ ) is not perfectly met, the reflected waves interfere with attenuation. This condition is fulfilled only at a particular angle, known as the Bragg angle, for a specific wavelength and a certain interplanar distance.

A parallel, coherent X-radiation (1, 2) strikes the crystal with an interplanar distance  $d$  and is amplified and scattered at an angle  $\theta$  (1', 2') (fig. 4). The portion of radiation scattered on the 2nd plane will have a path difference  $ACB$  compared to the radiation scattered on the 1st plane.

From the definition of sine, we obtain  $\frac{AC}{d} = \sin\theta$  or  $AC = d\sin\theta$ .

The path difference  $ACB$  is doubled and equals  $ACB = 2d\sin\theta$ . The amplification condition is satisfied when the path difference equates to an integer multiple of the wavelength. This leads to the Bragg diffraction condition:  $2d \sin\theta = n\lambda$ , where  $n = 1, 2, 3, \dots$  is the order of reflection, often referred to as the Bragg equation.

Applying the Bragg diffraction condition makes it feasible to identify the wavelength  $\lambda$  of X-radiation by measuring the angle  $\theta$ , given a known interplanar distance  $d$ , and thus ascertain the corresponding chemical element. Spectrum analysis employs crystals such as lithium fluoride (LiF), indium antimonide (InSb), germanium (Ge), and multilayered structures.

X-ray detecting.

When measuring X-rays, we leverage their capability to ionize atoms and molecules; in other words, their energy is used to extract electrons from these particles. Certain materials utilized to detect X-rays produce pulses when subjected to X-ray influence. The amplitude of these pulses is directly proportional to the energy of the X-ray photons. Detecting the pulse amplitude provides information about the energy of the photons.

The count of photons during measurement, such as pulses per second (imp/s = impulses per second, kimp/s = kilo-impulses per second), is termed radiation intensity. This gives a preliminary approximation of the concentration of the radiating element in the sample. In contemporary X-ray fluorescence spectrometers, two types of detectors are employed: a gas proportional counter and a scintillation counter.

The gas proportional counter (as shown in Fig. 5) comprises a cylindrical metal tube, along whose axis a thin wire (the counting wire) is stretched. This tube is filled with an appropriate gas (for example, Ar + 10% CH<sub>4</sub>). A high voltage (+U) is applied to the counting wire. On the side of the tube is a hole or window covered with a material permeable to X-radiation.

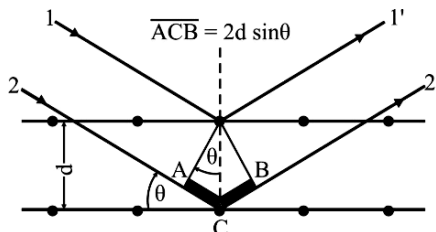


Fig. 4. Reflection of X-rays from the surface of the crystal

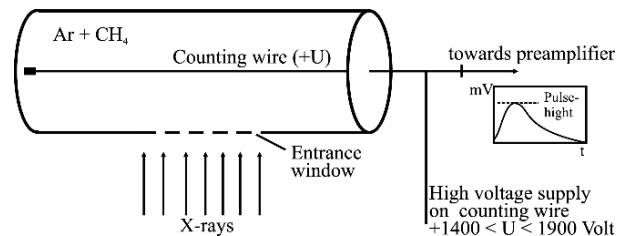


Fig. 5. Gas proportional counter

The scintillation counter (as shown in Fig. 6) used in the X-ray fluorescence analyzer consists of a sodium iodide crystal doped with thallium impurity - NaI (Tl).

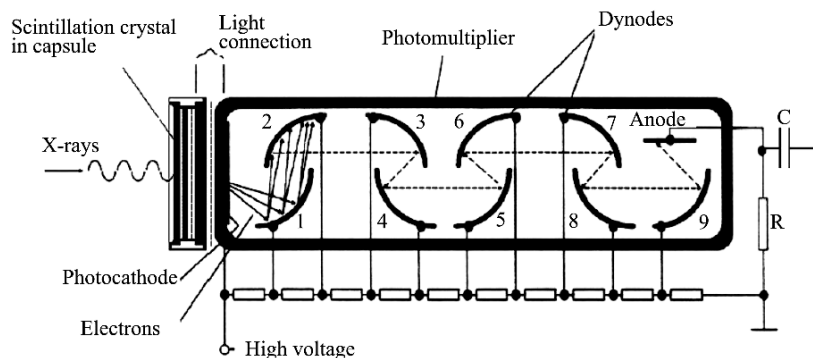


Fig. 6. Scintillation counter and photomultiplier

The crystal's thickness is substantial enough to absorb all the high-energy photons employed in the X-ray fluorescence analyzer. The energy of the X-rays penetrating the crystal gradually transfers to the crystal's atoms, which emit light. A composite of such light quanta forms a flash of light. The luminescent energy of these light flashes is proportional to the energy of the X-ray photon it absorbed from the crystal.

The resulting light radiation strikes the photocathode, triggering the emission of electrons. These electrons are then accelerated in the secondary electron multiplier, the photomultiplier, generating secondary electrons. The avalanche process produces a measurable signal at the output of the multiplier. Similar to the gas proportional counter, the amplitude of the created voltage pulses is proportional to the energy of the detected photon.

## METHODS OF THE RESEARCH

This work is both theoretical and practical. Methodologically, it is anchored in well-established laws of physics. Its primary scientific and practical outcomes were procured using photo documentation (screenshots) and digital technologies to process experimental results.

To measure the elemental composition of different samples, the method of X-ray fluorescence spectroscopy was used. It's based on the principle that individual atoms emit X-ray photons of a characteristic energy or wavelength when excited by an external energy source. In general, it works as follows:

1. **Excitation**: The sample is excited by an external X-ray radiation source. This high-energy radiation has enough energy to dislodge tightly held inner electrons from their atomic orbits in the sample.

2. **Emission**: This ejection creates a vacancy that an electron from an outer orbit must fill. As an outer electron moves to fill the vacancy, it loses energy. This energy is released in the form of a secondary X-ray photon, a process known as fluorescence.

3. **Detection**: These secondary or fluorescent X-rays are then detected, and their energies are measured. Each element produces a unique spectral pattern of fluorescence X-rays (i.e., X-rays of specific energies), and so the presence of these spectral lines allows us to identify which elements are present in the sample.

4. **Analysis**: The intensity of these fluorescent X-rays is directly related to the concentration of the elements in the sample, allowing us to quantify how much of each element is present.

X-ray fluorescence spectroscopy is a technique that developed along with the general understanding of X-ray physics, which dates to the late 19th century. The foundational principles for X-ray fluorescence spectroscopy were established early in the 20th century. X-ray spectroscopy was significantly advanced in the 1910s by scientists like Henry Gwyn Jeffreys Moseley, who developed a systematic method for identifying elements using X-ray spectra. As for XRF instruments themselves, the first commercial X-ray spectrometer was released in the early 1950s. These early instruments used gas flow proportional detectors and could perform only relatively simple tasks. Over the decades, with technological advancements, XRF instruments have become increasingly powerful, precise, and versatile in their applications.

X-ray fluorescence spectroscopy (XRF) does involve complex scientific concepts such as quantum mechanics, atomic structure, and the principles of X-ray production. Thus, from a certain perspective, it can be considered complex. However, while the underlying theory is advanced, first-year university students can learn and understand the practical application and operation of XRF instruments, particularly those studying fields such as chemistry, physics, or pharmacy. To understand the basics of XRF, a student needs knowledge of general chemistry and physics concepts, which are typically part of the first-year curriculum. This includes the structure of atoms, the nature of electromagnetic radiation (including X-rays), and some basic quantum mechanics principles. It is also worth noting that many modern XRF instruments are designed to be user-friendly and often come with software that automates much of the analysis. This allows users to focus more on understanding the results and less on the intricacies of how those results were generated.

## RESULTS OF RESEARCH

To enhance the practical application and understanding of lab work, students are given the opportunity to analyze soil samples from different suburbs of their city. The objective behind this choice is twofold. Firstly, students can learn the physical principles of XRF analysis and gain hands-on experience. Secondly, they receive valuable insights regarding the ecological condition of their living environment since soil composition and its microbiological state can significantly impact public health.

Knowing soil's chemical composition can help predict how environmental conditions might affect human health and anticipate potential health risks. This knowledge is particularly beneficial for aspiring medical professionals and pharmacists, enabling them to understand the broader context of environmental impacts on health. For instance, an excess of radioactive isotopes in the soil can lead to their accumulation in human tissues and bones, potentially causing radiation sickness. Other studies have demonstrated a direct correlation between soil contamination with pesticides, increased child morbidity rates, particularly during the neonatal period, and a higher incidence of birth defects.

Students are further encouraged to delve into individual research, such as preparing reports on environmental conditions and their connection with disease prevalence in specific regions.

We have proposed the following course of practical work:

The followings are the recommended steps for practical work and a sample method for processing experimental outcomes:

The procedure of the Task:

1. Familiarize yourself with the rules for operating the X-ray fluorescence spectrometer under the instructor's guidance.
2. Perform measurements and obtain the spectra of three samples for qualitative analysis.
3. Introduce a specific amount of a simple chemical as an external standard.
4. Carry out measurements and derive new spectra of the three samples for quantitative analysis.
5. Use an MS Excel spreadsheet to calculate the quantitative chemical composition of the compounds.

The outcome of the Task:

1. Three soil sample spectra, along with their comprehensive qualitative composition, were obtained:

Sample 1

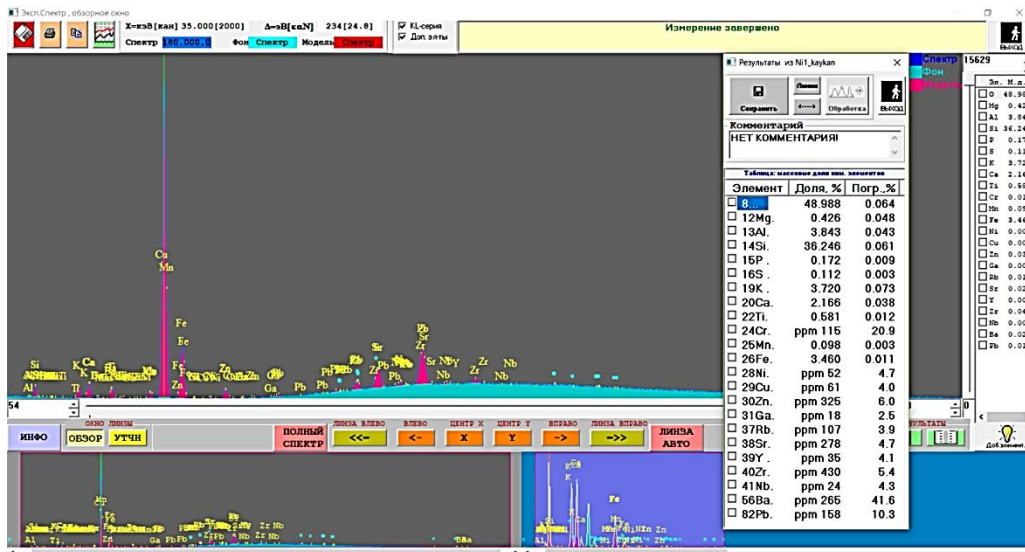


Fig. 7. Elemental (XRF) analysis of the Sample 1

Sample 2

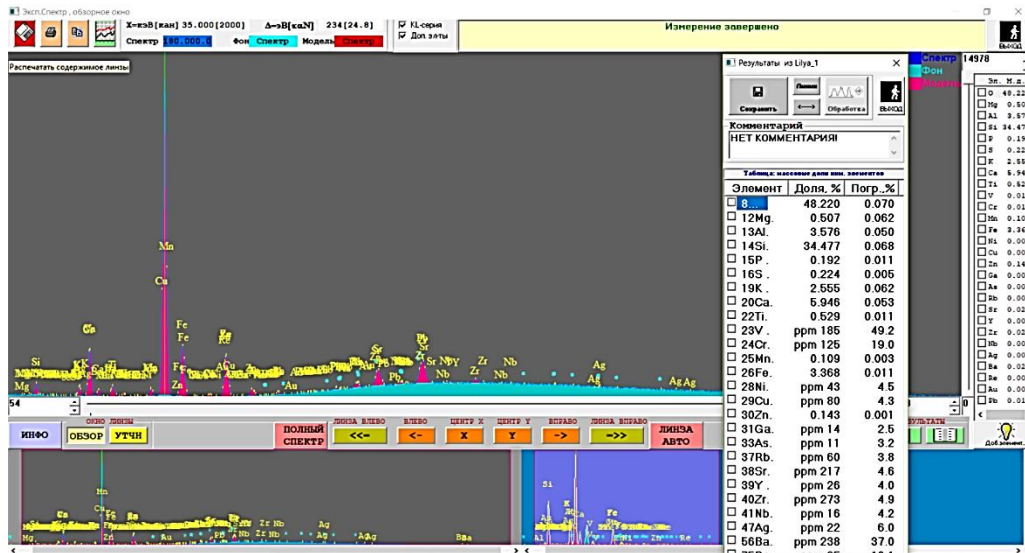


Fig. 8. Elemental (XRF) analysis of the Sample 2

Sample 3

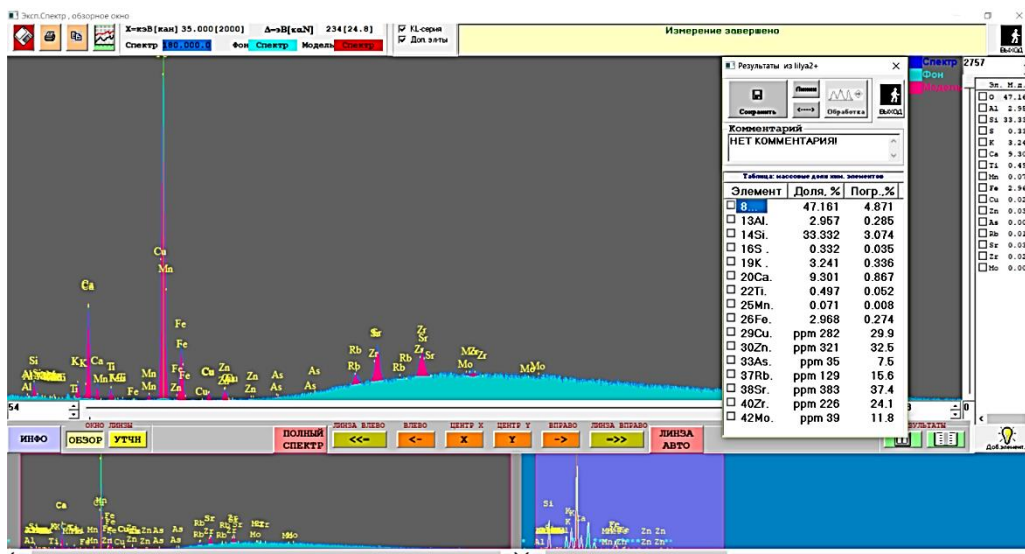


Fig. 9. Elemental (XRF) analysis of the Sample 3

2. An additional 0.2g of metallic copper was added as an external standard to each 0.5g sample.
3. Spectra of three soil samples along with their comprehensive qualitative composition were obtained with the presence of the external standard:

**Sample 1**

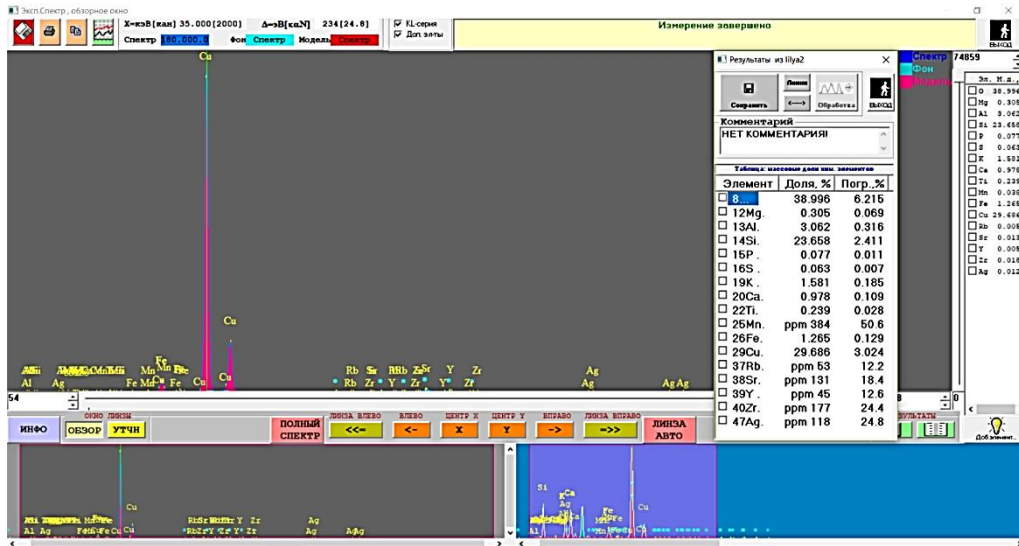


Fig. 10. Elemental (XRF) analysis of the Sample 1 after adding of 0.2g of metallic copper

**Sample 2**

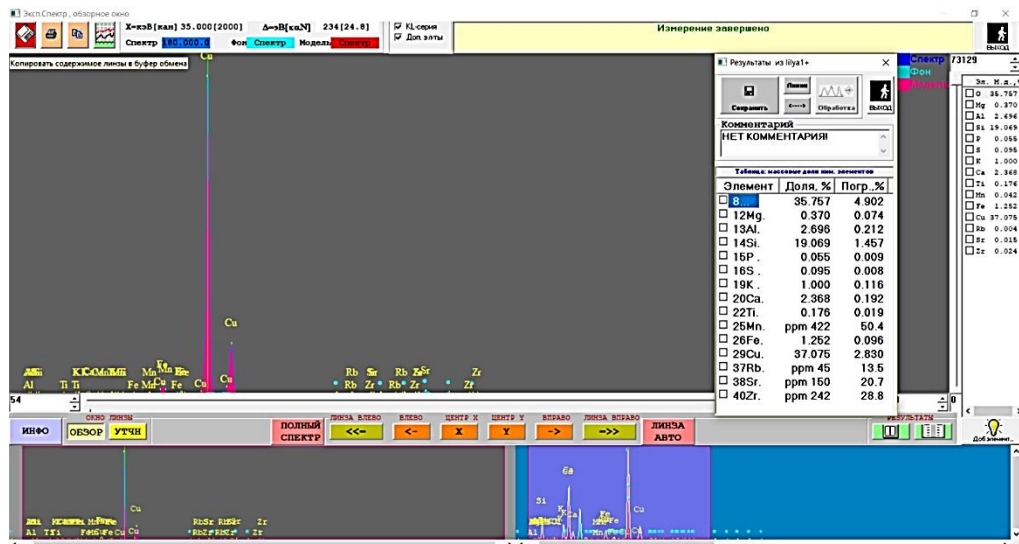


Fig. 10. Elemental (XRF) analysis of the Sample 2 after adding of 0.2g of metallic copper

**Sample 3**

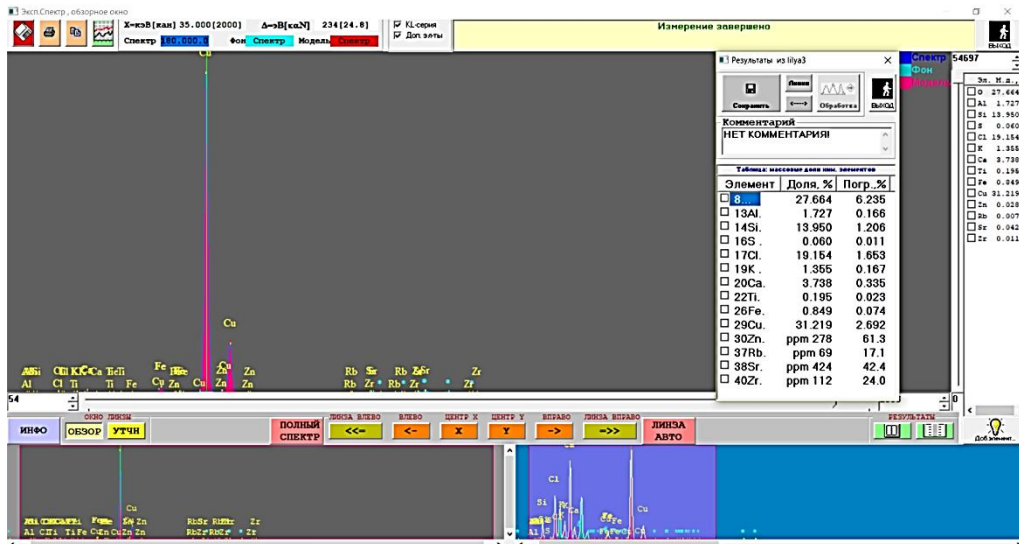


Fig. 10. Elemental (XRF) analysis of the Sample 3 after adding of 0.2g of metallic copper

4. The quantitative composition of individual chemical elements (with the highest radiation intensity) in the samples was calculated:

	Chemical element	Molar mass, g/mol	Mass fraction, %	Mass fraction (with the addition of Cu), %	Mass, g
Sample 1	Mg	24	0.426	0.305	0.002
	Al	27	3.843	3.062	0.021
	Si	28	36.246	23.658	0.159
	K	39	3.720	1.581	0.011
	Ca	40	2.166	0.978	0.007
	Mn	55	0.098	384 ppm	$2.6 \cdot 10^{-6}$
	Fe	56	3.460	1.265	0.009
	Cu	64	61 ppm	29.686	0.200
Sample 2	Mg	24	0.507	0.370	0.002
	Al	27	3.576	2.696	0.015
	Si	28	34.477	19.069	0.103
	K	39	2.555	1.000	0.005
	Ca	40	5.946	2.368	0.013
	Mn	55	0.109	422 ppm	$2.3 \cdot 10^{-6}$
	Fe	56	3.368	1.252	0.007
	Cu	64	80 ppm	37.075	0.200
Sample 3	Mg	24	0	0	0
	Al	27	2.957	1.727	0.011
	Si	28	33.332	13.950	0.089
	K	39	3.241	1.355	0.009
	Ca	40	9.301	3.738	0.024
	Mn	55	0.071	0	0
	Fe	56	2.968	0.849	0.005
	Cu	64	282 ppm	31.219	0.200

5. Formulate the conclusion.

Hence, X-ray fluorescence analysis enables the investigation of the qualitative chemical composition of various substances. Moreover, employing an external standard makes it possible to compute the actual mass proportion of chemical elements in the sample.

#### CONCLUSIONS AND PERSPECTIVES FOR A FURTHER RESEARCH

A well-executed and structured laboratory session not only stimulates students' intellectual engagement but also fosters logical thinking, effective comprehension of the material, and the ability to establish connections across various topics and subjects. It enhances creative skills and aids in developing subject-specific and professional competencies.

Implementing the laboratory practical work "The use of x-ray fluorescence spectroscopy to determine the elemental composition of substances in the study of biophysics" has demonstrated its effectiveness in fostering a deeper understanding of the subject matter and the development of practical skills. Through this approach, students were able to actively engage with the learning process, leading to enhanced cognitive skills, problem-solving abilities, and critical thinking. This practical work allowed students to connect theoretical knowledge and real-world applications, offering a comprehensive understanding of the concepts.

Future research could explore the long-term impacts of such laboratory practical work on student learning outcomes, knowledge retention, and career readiness. Studies could also focus on refining and enhancing this practical approach to improve its effectiveness in teaching and learning, perhaps by integrating technology or interdisciplinary elements. Comparative studies could be useful to evaluate the efficacy of this method against more traditional teaching approaches or in various educational contexts and different student populations. There is also potential to explore how this practical work could be adapted for other scientific subjects or concepts, broadening its applicability.

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