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ZINICOVSCAIA INGA

IMPACT OF SOME METALS DETERMINED BY NEUTRON ACTIVATION ANALYSIS ON THE QUALITY OF THE ENVIRONMENT

145.01. ECOLOGICAL CHEMISTRY

Doctoral thesis in chemistry

Scientific adviser: **DUCA Gheorghe,** Doctor habilitate,
professor, academician

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ZINICOVSCAIA INGA

IMPACTUL UNOR METALE DETERMINATE PRIN ANALIZA DE ACTIVARE CU NEUTRONI ASUPRA CALITATII MEDIULUI AMBIANT

145.01. CHIMIA ECOLOGICĂ

Teză de doctor habilitat în chimie

Consultant stiinţific: **DUCA Gheorghe,** Doctor habilitat, professor universitar, academician

Autor: **ZINICOVSCAIA Inga**

CHIȘINĂU, 2022

CONTENT

ANNOTATION

Zinicovscaia Inga, Impact of some metals determined by neutron activation analysis on the quality of the environment. Thesis of doctor habilitate in chemistry, Chisinau, 2022

Structure of the thesis: Annotation (English, Romanian, Russian), Introduction, Five Chapters, Conclusions and Recommendations, Bibliography of 506 titles, 66 figures, 45 tables, and 212 pages of basic texts. The results of the thesis were published in 50 papers, including 24 articles with impact factor, 2 books and 4 book chapters.

Field of research: 145.01. Ecological Chemistry

Keywords: metals, neutron activation analysis, passive biomonitoring, active biomonitoring, soil, pollution, agricultural crops, elemental content, transfer factor, wastewater treatment, biosorption.

The scope and objectives of the thesis: The main objective of the thesis is the analysis of the content of some chemical elements using neutron activation analysis in order to determine the biological value of the environment, to assess the risk on human health and state of ecological systems, as well as to develop the technology of metals removal from industrial effluents using different types of sorbents.

The novelty and scientific originality: For the first time in the Republic of Moldova neutron activation analysis was used to determine the content of elements in mosses, soils, fruits, vines, medicinal plants. The originality of the thesis consists in the simultaneous determination of a considerable number of chemical elements and estimation of the state the environment on the basis of these data. The technology of metal removal from mono- and multicomponent systems was proposed.

The obtained result, which contributes to the creation of the new scientific direction: Application of neutron activation analysis in estimation of the biological value of the habitat for life based on the determination of the elemental composition of the different environmental samples.

The theoretical importance of the work. A new theoretical and practical approach in the field of ecological chemistry has been proposed, which allow based on the chemical composition evaluation of the non-carcinogenic risk, calculation of hazard coefficient for air, soil, agricultural crops polluted with metals and the values of transfer factor in soil-agricultural crops systems. The optimal physico-chemical conditions for metals maximum removal using different types of biological sorbents have been established.

The applied value of the work. The inclusion of the results of the biomonitoring study of air pollution in the Republic of Moldova in the „European Atlas of Heavy Metal Atmospheric Deposition” edited by „ICP Vegetation Programme Coordination Center” indicate the value of ang high appreciation of the performed research. Determination of the elemental composition of fruits, wines, and medicinal plants is extremely important for the assessment of their quality and effect on the consumers' health as well as identification of their origin. The results obtained for different sorbents can be used for the elaboration of the methods of metals removal with the purpose to reduce their concentration to maximum admissible levels.

The implementation of the results: The obtained results can be implemented in the training of the local producers of medicinal and aromatic plants, for analysis of the agricultural products and for the development of the new technique of metal removal using biological sorbents. The results were implemented at Public Institution “Central laboratory for testing alcoholic/non-alcoholic beverages and canned goods”, Moldovan Association of Aromatic and Medicinal Plant Producers "AROMEDA", Institute of Chemistry, and Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova.

ADNOTARE

Zinicovscaia Inga, Impactul unor metale determinate prin analiza de activare cu neutroni asupra calitații mediului ambiant, Teză de doctor habilitat în științe chimice, Chișinău, 2022

Structura tezei: Adnotare (în engleză, română şi rusă), Introducere, Cinci capitole, Concluzii si recomandări, Bibliografie cu 506 surse, 66 figuri, 45 tabele, 212 pagini de text. Rezultatele obţinute au fost publicate în 50 lucrări ştiinţifice, inclusiv 24 articole cu factor de impact, 2 monografii si 4 capitole în cărți.

Domeniul de studii: 145.01. Chimie ecologică

Cuvintele-cheie: metale, analiza prin activare cu neutron, biomonitoring pasiv, biomonitoring activ, sol, poluarea, culturile agricole, compoziția elementală, factor de transfer, tratarea apelor reziduale, biosorbția.

Scopul şi obiectivele tezei: Obiectivul principal al tezei este analiza conținutului unor elemente chimice prin metoda de activare cu neutroni cu scopul determinării valorii biologice a mediului ambiant, evaluarii riscului asupra sănătății și stării sistemelor ecologice, precum și elaborarea tehnologiei de indepărtare a metalelor din apele industriale folosind diferite tipuri de sorbenți.

Noutatea şi originalitatea ştiinţifică: Pentru prima data în Republica Moldova a fost folosită analiza prin activare cu neutroni pentru evaluarea conținutului de elemente chimice în muschi, soluri, fructe, vinuri, plante medicinale. Originalitatea lucrării constă în determinarea simultană a unui numar considerabil de elemente chimice și evaluarea pe baza aceestor date a stării mediului ambiant. A fost propusa tehnologia de indepărtare a metalelor din sistemele mono- și multicomponente.

Rezultatele obţinute care au determinat crearea unei noi direcţii ştiinţifice: Aplicarea analizei de activare prin neutroni în estimarea valorii habitatului pentru viață în baza determinarii compoziției chimice a diferitor obiecte din mediu.

Semnificaţia teoretică a tezei: A fost propusă o noua abordare teoretică și practică în domeniul chimiei ecologice pentru analiza chimica a mediului ambiant, care permite pe baza compozitiei elementale evaluarea riscului non-cancerogenic, calculul coeficientului de pericol pentru aer, soil, culturile agricole poluate cu metalele; si factorului de transfer a elementelor chimice în sisteme sol-culturile agricole. Au fost stabilite condițiile fizico-chimice optime pentru indepărtarea maxima a metalelor folosind diferite tipuri de sorbenți biologici.

Valoarea aplicativă a tezei. Includerea rezultatelor biomonitoringului poluării aerului din Republica Moldova în „European Atlas of Heavy Metal Atmospheric Deposition” editat de „ICP Vegetation Programme Coordination Center” susține valoarea și aprecierea înaltă a cercetărilor efectuate. Determinarea compozitiei elementale a fructelor, vinurilor, plantelor medicinale este extrem de importantă pentru estimarea calitatii lor și impactului asupra sănătății consumatorilor, precum și stabilirea provenienței lor; rezultatele obținute pentru diferiți sorbenți pot fi folosiți pentru elaborarea metodelor de îndepărtare a metalelor cu scopul de a reduce concentrațiile lor la limitele maxim admisibile.

Implementarea rezultatelor ştiinţifice: Rezultatele obținute pot fi implementate în procesul de instruire a producătorilor locali de plante medicinale și aromatice, pentru analiza produselor agricole și pentru elaborarea metodelor de îndepărtare a metalelor grele din apele uzate. Rezultatele au fost implementate la Instituţia Publică „Laboratorul central de testare a băuturilor alcoolice/nealcoolice şi a produselor conservate”, Asociaţia Cultivatorilor de Plante Aromatice şi Medicinale din Republica Moldova "AROMEDA", Institutul de Chimie, Institutul de Microbiologie si Biotechnologie, or. Chișinău, Republica Moldova.

АННОТАЦИЯ

Зиньковская Инга, Влияние некоторых элементов, определенных методом нейтронного активационного анализа на качество окружающей среды, диссертация доктора химических наук, Кишинев, 2022

Структура диссертации: Аннотация (на английском, румынском и русском языках), введение, пять глав, выводы и рекомендации, библиография из 506 источников, 66 рисунков, 45 таблиц и 212 страниц основного текста. Результаты диссертации отражены в 50 научных публикациях, включая 24 статьи в реферируемых журналах, 2 монографии и 4 главы в книгах.

Область исследования: 145.01. экологическая химия

Ключевые слова: металлы, нейтронно-активационный анализ, активный биомониторинг, пассивный биомониторинг, почвы, загрязнение, сельскохозяйственные культуры, фактор переноса, элементный анализ, очистка сточных вод, биосорбция.

Цель и задачи работы: основной целью диссертации является определение содержания ряда химических элементов методом нейтронно-активационного анализа в природных образцах для биологической оценки состояния окружающей среды; оценка риска для здоровья человека и состояния экологических систем; а также, разработка технологии извлечения металлов из промышленных сточных вод, используя различные типы сорбентов.

Научная новизна и оригинальность исследования: впервые в Республике Молдова нейтронно-активационный анализ был применен для определения содержания химических элементов во мхах, почвах, фруктах, винах и медицинских растениях. Оригинальность работы заключается в одновременном определении значительного количества химических элементов и оценке, на основе этих данных, состояния окружающей среды. Была предложена технология извлечения металов из моно- и многокомпонентных систем.

Полученные результаты, которые определили развитие нового научного направления: применение нейтронно-активационного анализа для оценки качества естественной среды обитания на основе определения элементного состава различных объектов окружающей среды.

Теоретическая значимость работы. Были предложены новые теоретические и практические подходы в области экологической химии, которые позволяют, на основе элементного состава образцов, оценить риски для здоровья человека, рассчитать значения коэффициента опасности загрязнения воздуха, почвы, сельскохозяйственных культур металлами; а также значения фактора переноса элементов в системах почва-сельскохозяйственные культуры. Были установлены оптимальные физико-химические параметры для максимального извлечения ионов металлов различными типами биологических сорбентов.

Практическая значимость работы. Включение результатов по оценке качества воздуха в Республике Молдова в „European Atlas of Heavy Metal Atmospheric Deposition” издаваемый „ICP Vegetation Programme Coordination Center” указывает на значимость и высокую оценку проведенных исследований. Определение элементного состава фруктов, вин, медицинских растений критически важно для оценки их качества и влияния на здоровье потребителей, а также определения их происхождения. Результаты, полученные для разных сорбентов, могут быть использованы для разработки методов извлечения металлов из сточных вод с целью снижения их концентраций до уровня ПДК.

Внедрение полученных результатов: Полученные результаты могут быть использованы для инструктирования местных производителей медицинских и ароматических растений, для анализа качества сельскохозяйственной продукции и для разработки новых методов извлечения металлов из промышленных сточных вод, используя биологические сорбенты. Полученные результаты были применены в Центральной испытательной лаборатории тестирования алкогольных/безалкогольных напитков и консервов, Ассоциации производителей ароматических и медицинских растений в Республике Молдова "AROMEDA", Институте химии, Институте Микробиологии и Биотехнологии, Кишинев, Молдова.

List of Tables

List of Figures

List of abbreviations

AAS - atomic absorption spectrometry

ADD$_{derm}$ - daily exposure amount of metals through dermal contact

ADD$_{ing}$ - daily exposure amount of metals through ingestion

ADD$_{inh}$ - daily exposure amount of metals through inhalation

AS - Academy of Science main building

AS- Average Soil

BG- Botanical garden

DA- Discriminant Analysis

DIM- daily intake of metals

FA - Factor analysis

FTIR - Fourier-transform infrared spectroscopy

H. cupressiforme - Hypnum cupressiforme

HI - Hazard index

HQ - Hazard Quotient

ICP-MS- inductively coupled plasma mass spectrometry

ICP-OES - inductively coupled plasma optical emission spectrometer

LC$_{50}$ - 50%-lethal concentrations

MAC maximum admissible concentrations

MPC - maximum permissible concentrations

NAA - neutron activation analysis

PCA - Principal component analysis

PL- Pedological Limits

RAF - Relative accumulation factor

REE- rare earth elements

RM -Republic of Moldova

ROS - reactive oxygen species

RP -Reference plant

S. cerevisiae - *Saccharomyces cerevisiae*

S. girgensohnii - *Sphagnum girgensohnii*

S. platensis – *Spirulina platensis*

S. xiamenensis - *Shewanella xiamenensis*

SD – standard deviation

TDI - tolerable daily intake

TF - transfer factor

TPP - thermal power plant

UCC- Upper Continental Crust

WHO- World Health Organization

XRF - X-ray fluorescence spectrometer

INTRODUCTION

Background and importance of the topic discussed

Today, environmental pollution has become increasingly prominent in many countries of the world. Traditionally, pollutants such as sulfur dioxide and carbon dioxide, have been put under control, however, pollution of different environmental compartments (air, soil, and water) with heavy metals, which leads to ecosystems deterioration and possess a serious threat to food security and human health, have yet to gain policymakers' attention [1]. Since the environment is the habitat of humans, plants, animals and microorganisms it is very important to maintain its chemical composition to be adequate to the biologic value of habitation. This is possible by the monitoring of the state of the environment and suggestion of the way of reduction of contaminants level in order to prevent environmental pollution and to ensure sustainable development. The solution to this problem is the exceptional prerogative of ecological chemistry, which is focused on the urgent need for economical development and appropriate environmental impacts, which need to be addressed [2].

Environmental pollution with heavy metals is associated with rapid development in industry and agriculture, as well as disturbance of the natural ecosystem due to the enormous growth in the world population [3]. At the same time, the knowledge on emissions and emissions sources of heavy metals is still limited and incomplete concerning the information on emissions source categories and emission generating processes [4]. Every year, air pollution causes nearly 500 000 premature deaths and annual urban levels of PM_{10} monitored in over 1790 cities in 42 countries generally exceed the World Health Organization guidelines value [5]. Nowadays, there are around 5 million sites of soil pollution covering 500 million ha of land, in which the soils are contaminated by different heavy metals or metalloids in concentrations significantly overpassing the geo-baseline or regulatory levels. Heavy metal pollution in soil has a combined worldwide economic impact estimated to be over US$10 billion per year [6]. In high-income and upper-middle-income countries of the WHO European Region, about 30% and 60% of urban wastewater, respectively, is released to the environment without preliminary treatment, leading to contamination of natural water bodies and soil with harmful compounds, especially heavy metals and creation of unbalanced ecosystems.

Even some of the heavy metals, iron, cobalt, copper, manganese, molybdenum, and zinc, serve as cofactors of enzymes that are essential for various biochemical and physiological processes and in small amount are important micronutrients required by living organisms, the majority of heavy metals are toxic even at low concentrations [7]. The danger of environmental pollution with heavy metals is determined by their non-biodegradability and longer biological half-lives [8,9].

Although heavy metals are naturally occurring elements that are found throughout the earth’s crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, domestic and agricultural use of metals and metal-containing compounds, metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation, and paper processing plants [10–12].

Nowadays, in many countries, norms and regulations attempt to ensure that adverse impacts of environmental pollution are minimized, starting with the planning stage, choice of location, and adoption of the best available technologies. This, however, is not always the case, especially when old technologies continue to operate in populated areas or when norms and standards are weak or not complied with and significant emissions occur [13]. Thus, the United Nations Economic Commission for Europe (UNECE) has signed in 1998 a protocol on heavy metals (www.unece.org/env/lrtap/), targeting the emissions of three key metals, Pb, Cd, and Hg in the atmosphere. According to data presented by different countries the content of these elements in the period 1990-2000 decreased by ~40%, ~30%, and ~ 65%, respectively. In the Republic of Moldova, the level of Cd, Pb, and Hg in the indicated period was reduced by 79, 98, and 90%, respectively [13].

To assess the air quality on the large territories, one of the important tasks of ecological chemistry, the mosses have been proposed and successfully used as biomonitors, since they obtain elements and nutrients mainly from precipitation and dry deposition [14]. The analysis of elemental concentrations in mosses is easier and cheaper than conventional deposition analysis and allows the determination of up to 50 chemical elements. Therefore, a much higher sampling density can be achieved [15]. The European moss biomonitoring network was established in 1990 and has since then been repeated at five-yearly intervals. The latest survey was conducted in 2010 with 25 countries reporting concentrations of twelve heavy metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, Zn, Al, and Sb [14–19]. In 2015 the Republic of Moldova, for the first time, participated in the “moss survey” in the framework of the International Cooperative Programme on effects of air pollution on natural vegetation and crops with heavy metals in Europe (UNECE ICP Vegetation) to assess the quality of the air on the entire territory of the country using moss-biomonitors and neutron activation analysis. The effect of metal ions in determining concentrations on human health was estimated as well. The obtained results were included in the

„European Atlas of Heavy Metal Atmospheric Deposition" edited by „ICP Vegetation Programme Coordination Center".

Currently, air pollution is one of the most serious problems in the Republic of Moldova and particularly in Chisinau. The quality of air in Chisinau is determined by two main pollution sources: mobile (vehicles) and stationary (the power and heat generation sector, as well as industry) [20]. According to the national reports, in air samples mainly the concentrations of particulates, sulfur dioxide, carbon monoxide, nitrogen dioxide, phenols, and formaldehyde are determined [21,22]. At the same time, information concerning the determination of heavy metals is very limited. The assessment of air quality in many cities in low- and middle-income countries, which are subjected to deteriorated environmental and urban air conditions is often problematic due to the lack of the capacity to adequately monitor air quality and the absence of naturally grown bioindicators of air pollution, such as mosses and lichens [23]. Active moss biomonitoring, predominantly using the "moss bag technique" was proposed as an effective method of assessments of air quality in urban microenvironments [23–25]. Begu [26] used lichens to monitor the ecological situation in Chisinau. The concentration of six elements (Cu, Pb, Zn, Cd, Cr, and Ni) determined by the atomic absorption spectrometer showed their significant accumulation during the exposure time of the lichen samples. For the first time "moss bag" technique was applied to assess the quality of the air in the Chisinau during five months' period.

Along with air, the soil is another media, rigorously investigated using approaches of ecological chemistry, significantly affected by anthropogenic activities, such as wastewater irrigation, fertilizers, pesticides, and sludge applications, solid waste disposal, mining, smelting, vehicular exhaust, and domestic and agricultural use of metals and metallic compounds [8]. The soil contains almost all stable elements of the periodic tables in a concentration varying between tens of percent such as Al, Si, Ca, and Fe to less than μg/kg elements of the platinum group. Often pollutants enter primarily the atmosphere and then are distributed in soil and water. Soil acts as a sink and also as a source of pollution with the capacity to transfer pollutants to groundwater and the food chain, and then to humans and/or animals [27]. Anthropogenically derived heavy metals into the soil and could be easily transported via the root system into the plants where they accumulate into their different parts including fruits [28]. Contaminated vegetables, fruits, medicinal plants can cause serious clinical and physiological problems for humans, especially when consumed in large quantities [29]. It is also the case of wine grapes as a result, the concentration of metals in wines increases, which, in the case of continuous consumption may cause chronic poisoning [28]. The contamination and accumulation of trace metals in vegetables, fruits, wine, and medicinal plants have been reported by several researchers [27,30–32]. Environmental and food safety are increasingly attracting the attention of the public, and there is greater interest in the analysis of the elemental content of soil and agricultural products and to assess elements transfer from soil to crops [29]. Neutron activation analysis proved to be a very useful tool for studying the minor and rare earth elements in soil and agricultural crops and the assessment of possible health risks of contamination of food chains with the above elements. In soil analysis neutron activation analysis is considered the irreplaceable technique, since it does not require sample digestion.

For the first time, the quality of soil, fruits, wines, and medicinal plants in the Republic of Moldova was assessed by neutron activation analysis, and the risk on human health of elements considered as environmental pollutants was estimated. The transfer of elements from soil to leaves, fruits, the wine was estimated to determine the main sources of elements in fruits. The daily intake of metals and the hazard quotient indices were calculated.

The applications of metals in different industrial processes and inappropriate wastewater treatment resulted in a significant increase in their environmental levels relative to the normal background [33]. The presence of heavy metal ions in wastewaters represents a hazard to the aquatic ecosystem and raises many risks for human beings [34]. Since wastewater often are used for irrigation, they have a negative impact on the soil and agricultural products as well. Thus, it is important to reduce the level of heavy metals to maximum admissible levels before their discharge. The inappropriate wastewater treatment is often associated with limitations of conventional methods: high cost, secondary pollution of water, regeneration of hazardous sludge, not effective either economically viable for the treatment of low concentrations of heavy metals. Often traditional techniques do not cope with the increasing volume of wastewater and their complex chemical composition [35].

One of the directions within ecological chemistry is ecological biotechnology focuses on resolving environment protection tasks using living organisms [2]. The application of microorganisms can be considered as a cost-effective alternative technology for the treatment of metal-contaminated wastewater. Biosorption for metal removal is found to be an attractive technology due to being inexpensive, with high efficiency and specificity, less chemical or sludge formation, no additional nutrient requirements, regeneration of biosorbents, and eco-friendly [36,37]. Various types of readily available and inexpensive biomass for the removal of metals have thus been considered [38–40]. Methodic of metal removal from single and complex batch systems and industrial wastewater using different types of sorbents was proposed. For the first time, the ability of *Spirulina platensis* biomass to act as a renewable sorbent was explored.

The results obtained for different sorbent can be used for the elaboration of the special methods of metals removal from industrial wastewater to reduce their concentration to maximum admissible levels with the possible application of effluents in the irrigation.

It is evident that the pollution of one of the environments' compartments with heavy metals in a consequence will result in metal migration into the other media and would significantly increase the burden on the environment and society. Thus, it is important to monitor the level of air, soil, and water pollution with heavy metals, to identify sources of pollution, and develop the approaches of metal concentration reduction to admissible levels. These are the specific tasks of ecological chemistry

The aim of the thesis

The aim of the present thesis was to determine the content of some chemical elements using neutron activation analysis in different environment' compartments in the Republic of Moldova in order to define the biological value of the environment, to assess the risk on human health as well as to develop the technology of metal removal from industrial effluents using different types of biological sorbents.

The research objectives

To achieve this purpose, it was necessary to solve the following objectives:

- to perform passive and active biomonitoring studies in the Republic of Moldova and to determine the elemental composition of moss samples;
- to apply statistical tools and GIS technologies to pinpoint possible air pollution sources;
- to calculate the relative accumulation factor and non-cancer health risk of air pollution with elements, considered as environmental pollutants on human health;
- to determine the content of elements in soil, fruits, leaves, juice, wine, and medicinal plants samples collected in the Republic of Moldova
- to establish the relation between metal concentrations in soil, fruits, juice, and wine samples;
- to calculate the hazard index values for elements in fruits and medicinal plant in order to establish their impact on consumers' health;
- to determine the metal sorption capacity of different type of biosorbents as a function of different physico-chemical parameters and to evaluate adsorption character using different kinetics models, models of adsorption isotherms as well as to calculate the thermodynamic of the process.

- to determine optimal conditions for effective metal ions removal from industrial effluents.

The hypothesis of the research

Since the anthropogenic pressure on the environment has increased in the recent decades, environmental protection become a priority task for many countries around the world. It is supposed that the current level of heavy metals emission by vehicles, industrial and agricultural sector in the Republic of Moldova has a negative effect on the quality of the environment and human health.

The main result of the thesis consists in determination of the elemental composition of the environmental compartments and assessment of its conformity to biological value of the habitat. Neutron activation analysis proved to be an efficient technique for simultaneous determination of the considerable number of chemical elements.

The synthesis of the research methodology and justification of the research methods

The concept of the research was developed following the ideas presented by acad., prof. Gheorghe Duca in “Ecological Chemistry” (2002). Air quality in the Republic of Moldova was monitored by two techniques passive and active moss biomonitoring. The idea of using mosses to estimate atmospheric heavy metal deposition was developed in the late 1960s by Rühling and Tyler (1968). The application of mosses in biomonitoring studies is determined by their ubiquity presence, lack of rood system, high surface to volume ratio, and high cation change capacity. The advantages of this method are (i) the simplicity of sample collection, (ii) strongly reduced contamination problems, and (iii) the possibility to apply different analytical techniques for the analysis of various elements.

To assess air quality in urban areas active biomonitoring using the ‘‘bags technique’’ developed by (Goodman and Roberts 1971) was applied. This technique has several advantages: well-defined exposure time, known background concentrations of elements in the moss, flexibility in site selection and number of sampling sites to be chosen, uniformity of entrapment surface, etc.

To determine the elemental content of moss samples, soil, agricultural crops, wine neutron activation analysis at the IBR-2 reactor was used. It is a high-sensitive, non-destructive technique, which allows simultaneous determination of more than 40 elements in samples, without their preliminary chemical pretreatment. The main features of the method as well as technical parameters of the used technique are presented in (Frontasyeva 2011; Frontasyeva and Pavlov 2000).

To assess the quality of the air and soil several pollution indices (Geo-accumulation index; Enrichment factor, Contamination factor, Pollution load Index) were calculated. To characterize the relationship between the elements in soil, leaves, fruits, wine the transfer factor introduced by Kiekens and Camerlynck (1982) was used. For fruits, the daily intake of metals and the hazard quotient indices were calculated.

Metal removal from wastewater was performed through biosorption and bioaccumulation processes. The application of biological objects for metal removal can be explained by their abundance, simplicity of the experiments, high removal efficiency, and environmental safety. Atomic absorption spectrometry was used complementary to neutron activation analysis to assess the efficiency of metal removal from single-component solutions.

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Summary of the thesis chapters

Chapter I comprises the state-of-the-art on the issues discussed in the thesis. The most important metal emission sources are highlighted and the effect of the metal ions on human health is described. Monitoring the air quality is a complex technical task, and techniques for tracing atmospheric contamination, active and passive biomonitoring, were described in detail. Examples of the application of these techniques in different countries are given. The calculations of non-cancer toxic risk are presented. The sources of soil and agricultural crops pollution with heavy metals are emphasized. The indexes used to assess soil pollution and calculate the non-carcinogenic risk of heavy metals are presented. Examples of research on the assessment of metal content in soil and agricultural crops as well as metal bioaccumulation in soil-agricultural crops systems are presented. The main sources, which generate wastewater, containing heavy metals indicated. The parameters which affect metals biosorption are described in detail. The equations used to describe isotherms of the sorption, kinetics, and thermodynamics of the process are presented.

Chapter II contains *a* description of *the* main chemicals and standard reference materials used in the study. The procedure of moss collection and preparation for analysis is described. The details of soil samples and crops collection and preparation for analysis are given. The design of sorption experiments is described. The chemical composition of wastewater used for analysis is given. The techniques (neutron activation analysis, atomic absorption spectrometry, Fourier Transform Infrared Spectroscopy) are used in the thesis, and the main parameters of the measurements are presented.

Chapter III presents the results of passive and active biomonitoring studies. Passive biomonitoring was performed on the entire territory of the Republic of Moldova. Using neutron activation analysis and atomic absorption spectrometry it was possible to determine more than 35 elements in analyzed moss. A total of 37 elements were determined in collected moss by NAA and 3 elements by AAS. Using statistical tools (factor analysis) and GIS technologies the main sources of air pollution in Moldova were identified. To quantify the anthropogenic influence on the environment, the contamination factor, the geo-accumulation index and pollution load index were calculated. Health risk assessment of elements considered as environmental pollutants was evaluated. Active biomonitoring was performed in Chisinau, where moss bags were exposed at three different sites (with different pollution loading. Relative accumulation factor values were calculated to assess the element accumulation in the exposed moss. To highlight any association of accumulated chemical elements factor analysis was used and the risk on human health was evaluated.

Chapter IV is devoted to the assessment of the quality of soil and agricultural crops collected in the Republic of Moldova. The elemental composition of soils, fruits collected in the four different regions of the country, soil, and wine from two wineries were determined by neutron activation analysis. The main characteristics of the soil, as well as its origin, were estimated. To quantify the anthropogenic influence on the soil, the contamination factor, the geo-accumulation index, pollution load index, and Enrichment factor were calculated. To assess metal bioaccumulation from the soil in fruits, leaves, juice and wine Transfer factor values were calculated. The risk of consumption of analyzed products was evaluated.

Chapter V contains the result of the application of different sorbents for metal removal from batch systems and real wastewater. The effect of different physico-chemical parameters:

pH, metal ions concentration, sorbent dosage, the temperature on the sorption was evaluated and the optimal conditions for metal removal were determined. The equilibrium data obtained for metal sorption were described using Langmuir, Freundlich and Temkin equilibrium models. Four models, namely pseudo-first-order, pseudo-second-order, Elovich model, and the intra-particle Weber and Morris diffusion model were applied in the present study to describe experimental data. The thermodynamics of the sorption process was evaluated. Possibility of application of cyanobacteria as renewable sorbent was tested.

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1. BIBLIOGRAPHICAL INVESTIGATIONS

An increasing continuous impact on the human and natural environment of chemicals, including natural substances which in many cases are not less hazardous and toxic, was observed during the first half the 20th century and has caused the establishing of systematic research in this area [41]. A pollutant is considered any substance in the environment, which causes negative effects, impairing the environment, reducing the quality of life, and consequently leading to death [42]. Although several chemical elements, including iron, cobalt, copper, manganese, molybdenum, and zinc, serve as micronutrients used (1) for redox-processes, (2) to stabilize molecules through electrostatic interactions, (3) as components of various enzymes, and (4) for the regulation of osmotic they can induce various diseases at high concentrations [42,43]. Nowadays, toxic levels of chemical elements in air, soil, and water is a serious problem for many countries.

1.1 Chemical elements sources in the environment, their toxicological effects and analytical techniques used for the determination of the elemental composition

Chemical elements occur naturally in the earth's crust and are found in the atmosphere, soil, rocks, water bodies, and living organisms with natural background concentrations [42,44]. The origin of metals in the environment can be natural or anthropogenic. Rocks and soils are the principal natural sources of heavy metals in the environment. Other natural sources of chemical elements are physical and chemical weathering, volcanic eruptions, forest fires, dust [45–47]. Although chemical elements are naturally occurring elements that are found throughout the earth’s crust, most environmental contamination and human exposure result from anthropogenic activities [12]. The quantities of metal ions released in the environment may range from hundreds to millions of tonnes annually. The most important metal emission sectors include:

- metals industry and mining (Al, As, Cd, Cr, Cu, Fe, Pb, Zn, Ni);
- other manufacturing industries and construction (As, Cd, Cr, Hg, Ni, Pb);
- electricity and heat production (Cd, Hg, Ni, V, U, As);
- road transportation (Cu and Sb from brake wear, Pb, V, Zn from tires);
- petroleum refining (Ni, V);
- phosphate fertilizers and pesticides in agricultural areas (As, Cd, Cu, Pb, Si, U, Zn) [18,42,48];
- combustion of fuels and gasoline (As, Cd, Cr, Ni, and Pb) [4];

- wastewater generated by different industrial processes (the full spectrum of elements);
- waste disposal (As, Cd, Cr, Cu, Pb, Hg, Zn) [42].

The release of metal ions from both natural and anthropogenic sources leads to contamination of air, water, soil, and foodstuff products. Chemical elements enter into the environment mainly *via* three routes: deposition of atmospheric particulates; disposal of metal-enriched sewage sludge and wastewaters; and by-products from metal mining processes [49]. Humans may directly get in contact with chemical elements by consuming contaminated food, sea animals, and drinking water, through inhalation of polluted air as dust fumes, or through occupational exposure at the workplace [7].

Although several heavy metals, including iron, cobalt, copper, manganese, molybdenum, and zinc, serve as micronutrients used (1) for redox-processes, (2) to stabilize molecules through electrostatic interactions, (3) as components of various enzymes, and (4) for the regulation of osmotic they can induce various diseases at high concentrations [42,43].

In living organisms heavy metal toxicity involves several mechanisms, that is, disruption of enzyme structures and functions by binding with thiol and protein groups, or by replacing co-factors in prosthetic groups of enzymes, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as proteins [50,51]. Chemical elements can cause oxidative stress, lipid peroxidation, damage of nucleic acids, and the inhibition of essential processes, like respiration, carbon metabolism, and photosynthesis [52].

For example, Cr(III) may change the structure and activity of enzymes by reacting with their carboxyl and thiol groups. Copper ions could catalyze the production of ROS via Fenton and Haber-Weis reactions, which will act as soluble electron carriers. This can cause severe injury to cytoplasmic molecules, DNA, lipids, and other proteins. Cadmium and lead pose a deleterious effect on microbes, damage cell membranes, and destroy the structure of DNA. This harmfulness is generated by the displacement of metals from their native binding sites or ligand interactions [50]

At low levels of exposure to metals, clinical signs in the human body are not manifested immediately and their effects can be observed only at the physiological or biochemical level [53]. Long-term exposure can lead to gradually progressing physical, muscular, and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Repeated long-term exposure to some metals and their compounds may even cause cancer. Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition, and other important organs [54]. For example, exposure to cadmium, lead, and methylmercury compounds are especially dangerous during prenatal development and infancy, as it causes irreversible changes in the central nervous system. Arsenic has carcinogenic, neurotoxic (hearing disorders), and genotoxic effects, and causes cardiovascular diseases, peripheral vascular disorders, anemia, and dysfunctions of the reproductive system [53].

Therefore, it is important to monitor the levels of chemical elements in natural environments using cheap and effective techniques and to ensure that they correspond to biological value of habitation. It is critical to develop environmentally friendly technologies for the prevention of chemical elements release in the environment and programs for the education of the population.

Nowadays, there is a large number of available analytical techniques allowing determination of the elemental composition of environmental and biological samples such as X-ray fluorescence spectrometry (XRF), atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometer (ICP-OES), neutron activation analysis (NAA), etc.

Atomic spectrometric techniques are very sensitive and can be used to measure the total element content within a sample; however, the accuracy of these techniques can be affected by the matrix of the sample. Routinely, the determination of elemental content has been carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), electrothermal atomic absorption spectrometry (ETAAS), and flame atomic absorption spectrometry (FAAS) [55–58]. AAS is most often used for single measurements of particular elements; AAS and ICP, for analysis of liquid and gaseous samples; and XFA, for analysis of large and medium concentrations of matrix elements [59].

ICP-MS is the most widely used method today for the determination of metal concentrations in both organic and inorganic samples. The main advantages of technique are: high sensitivity, multi-element capability, wide linear dynamic range, low detection limit, high sample throughput, low sample volume, relatively simple sample preparation, and ability to discriminate between isotopes [60,61]. Modern ICP-MS systems are capable of analyzing most elements in the periodic table simultaneously at levels of 10 pg/ml with a mass resolution of <1 amu [62]. Besides the very high expenses the main disadvantage of these methods of analysis of solid samples is the sample pretreatment, which is often time-consuming and problematic (e.g., incomplete dissolution, precipitation of insoluble analyte, loss of elements during heating, contamination) step and easily causes the inputs of cross-contamination among samples [55,63]. Moreover, ICM-MS is strongly affected by complex matrix effects. The ICP-MS is routinely used in diverse research fields such as earth, environmental, life and forensic sciences, food, material, chemical, semiconductor and nuclear industries.

Nowadays, due to high cost of ICP-MS techniques ICP-OES is widely used in routine analysis of environmental samples. ICP-AES is a method of optical emission spectrometry that uses the emission spectra of sample molecules to identify and quantify the elements in samples. ICP-AES can be used to measure almost all the elements of the periodic table with detection limits in the range of micrograms per liter to milligrams per liter, however technique is not suitable for determination of noble gases, halogens, or light elements such as H, C, N, and O [64]. ICP-AES instruments are limited to the analysis of liquids only, that require additional reagents and equipment. At the same time conversion of samples in a liquid form relieve from the necessity to use standards matched to the matrix of the original sample [65].

Neutron activation analysis, the main competitor of the ICP-MS, is a method of elemental analysis in which non-radioactive elements are converted to radioactive ones by neutron bombardment, and the elements of interest are determined from resulting radioactivity [59,66]. The NAA technique has numerous advantages, among which need to be mentioned: non-destructive nature, possibility of performing bulk analysis, sensitivity and wide applicability, especially for trace elements; the virtual absence of an analytical blank; negligible influence of the matrix; possibility of application in a self-validating manner [67]. Elements such as C, H, N, O and Si, often found as major components of many matrices, do not activate well and contribute little or no gamma-ray activity. The method is suitable for measurement of masses in the order range of 10^{-6}–10^{-9} g and less, depending on the element to be measured [68]. Further advantages of the technique are that minimal sample preparation and possibility of examination of very small samples [69]. There are about 60 elements, which can be determined by NAA with various detection limits, depending on nuclear characteristics of their isotopes and a neutron fluence rate available for activation [70]. In NAA problems related to the decomposition of the sample and separation (dissolution, contamination, and losses) are avoided. This aspect is very important when unique or valuable materials must be analyzed, e.g., archeological and art objects, or in the case of materials that are difficult to dissolve, e.g., silicon-based samples or ceramics [71].

NAA is less widely applied than other analytical techniques due to necessity of having access to a nuclear reactor [71]. The equipment needed for the analysis is rather expensive and requires special laboratories and a highly qualified staff [72]. The neutron activation analysis can be applied in a broad range of disciplines such as archeology, environmental monitoring, geology, biomedicine, materials science, nutrition, industrial products, nanotoxicology, etc.

[71,73,74]. The examples of the application of NAA in environmental studies will be presents below.

1.2 Monitoring of air quality using moss as biomonitors and analytical technique

The atmosphere is a mobile part of the biosphere, consequently any negative impact on it often gains a global character [41]. Monitoring of the air quality is a complex technical task, and techniques for tracing atmospheric contamination have been the objects of intensive research in past decades [75]. For the effective management of air quality, great importance is given to the identification of the sources, which emit pollutants. Physico-chemical techniques used today to control air quality are mainly used to measurements concentrations of CO, SOx, NxOy, PAHs, and particulate matter, while analysis of heavy metals in the atmosphere is a very expensive task [76].

Chemical elements in the atmosphere result from processes both natural and anthropogenic. The main natural sources of metal emission are dust re-suspension, foliage emissions, volcanic activity, and bubble bursting from water bodies, while anthropogenic sources include mining, industrial and agricultural activities, domestic emissions, as well as roadway emissions, and stationary power generation. In developing countries, the uncontrolled emissions from brick kilns, waste incineration, and cement plants are important sources of metals to communities close to these facilities [49,77,78].

Once chemical elements enter the atmosphere, they can be rapidly deposited close to the source of emission or transported over long distances through the atmosphere. Atmospheric deposition is considered as a major mechanism for metal input to plants and soils [48]. Chemical elements deposition on the earth’s surface may occur via dry or wet deposition. Dry deposition is mainly characteristic of arid and semiarid regions where removal by wet deposition is low due to limited precipitation. Wet deposition consists of metal removal together with rain, snow, and hail [79].

Information on air pollutants can be obtained by classical methods such as dispersion modeling (source-orientation, a priori knew emission sources) or by field measurements of the emission (receptor/effect orientation). However, large-scale application of these techniques is limited by their cost, sensitivity, the necessity for technical installations, and a small number of samples that can be collected on a large area of investigation [56,80].

Two types of biomonitoring using moss as a bioindicator to evaluate atmospheric contamination are differentiated in the literature: (i) passive biomonitoring, using moss that grows naturally in a particular area, and (ii) active biomonitoring, by transplanting moss from other locations in the areas of interest. Passive biomonitoring is more appropriate for extensive studies in large areas (i.e. regional or national studies), while active biomonitoring for studies in smaller areas (i.e. urban or industrial areas) [76,79].

1.2.1. Passive biomonitoring

The moss biomonitoring technique, introduced in Scandinavian countries in the seventies by Ruhling and Tyler [81], has proven to be an excellent technique for studying the atmospheric deposition of heavy metals and trace elements [82]. Several factors promote the application of mosses as bioindicators of air pollution with heavy metals: their ubiquity, morphology (lack of roots), high surface to volume ratio, and high cation change capacity, and last but not least simplicity and low cost of the technique [16,83]. Biomonitoring with moss can provide a good preliminary overview of the state of the air quality because dense sampling networks can be covered [76].

The use of the moss biomonitoring technique allows researchers to distinguish between contaminated and uncontaminated sites, to identify small scale pollution sources, and to map spatial and temporal patterns of the heavy metals in the atmosphere [84]. The factors that determine the final concentrations of elements in moss tissues in passive biomonitoring studies can be divided into two main groups: “avoidable” and “unavoidable” factors. As unavoidable factors are considered sources of pollutants other than atmospheric deposition, for example, physicochemical characteristics of pollutants, moss biology, and different environmental factors. Avoidable factors mainly include sample collection, processing, and chemical analysis [79].

The program of heavy metal deposition monitoring (in five-year intervals) using mosses was originally established as a Swedish initiative in 1980. The first European moss survey was conducted in 1990/1991. Since 2001 the moss survey program is coordinated by the ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK [14,18]. From the beginning, the European moss survey has provided data on concentrations of ten heavy metals (arsenic, cadmium, chromium, copper, iron, lead, mercury, nickel, vanadium, and zinc) in naturally growing mosses. Since 2005, the concentration of aluminum (a good indicator of geogenic pollution), antimony (a good indicator of anthropogenic pollution since it is present in very low concentrations in the earth’s crust), and nitrogen were also determined [18]. For biomonitoring studies are recommended several species of mosses namely, *Hypnum cupressiforme*, *Pleurozium schreberi*, *Brachythecium rutabulum*, and *Homalothecium sericeum* [82]. The metal concentrations in moss samples are determined by a range of analytical techniques, under the broad headings of AAS, ICP spectrometry (both ICP optical emission spectrometry and ICP mass spectrometry), fluorescence spectrometry, NAA, and advanced mercury analysis [18].

Today, the passive biomonitoring technique is widely used in many European countries [14,16,17,19,82]. For example, 330 moss samples were collected in Romania in the summer and autumn of 2010. Data obtained by NAA and ASS for aluminum, cadmium, chromium, copper, iron, lead, nickel, vanadium, and zinc have shown that the median concentrations of cadmium, 1.20 mg/kg, and lead, 30.8 mg/kg were higher compared with other European countries. The spatial distribution maps of elements suggest high metal concentrations, especially for the related elements of anthropogenic origin, Zn, Cu, Cd, and Pb, in the north and northwest Romania due to mining operations associated with the flotation and smelting [19]. Moss biomonitoring technique was applied to air pollution studies in the Republic of Macedonia. The presence of 47 elements was determined by NAA, AAS, and ICP-MS. Distribution maps were prepared to point out the regions most affected by pollution. Three reactivated lead-zinc mines have been shown to contribute to a high content of lead and zinc in the eastern part of the country. The cities of Veles, Skopje, Tetovo, Radoviš, and Kavadarci were determined to experience particular environmental stress [16]. On the next stage moss technique was used to investigate air deposition and to explore the natural distribution and contamination with potentially toxic elements in the Kumanovo Region, North Macedonia. It was found that the atmospheric deposition for chemical elments in the moss samples in specific parts of the study area is influenced by anthropogenic and urban activities [85].

Zinicovscaia and co-authors prepared a review devoted to moss biomonitoring technique application in former Soviet Union countries. The air pollution sources in each country were identified. The mean concentration of elements considered as environmental pollutants were compared in order to detect the most polluted countries on the post-soviet space [86].

Three moss surveys were performed in the Moscow region in 2004, 2014 and 2020. Results obtained by NAA showed that the situation with air pollution in Moscow region during 10 years has not changed significantly. Industrial activity, thermal power plants, and transport still have the largest anthropogenic impact on air pollution in the studied region, mainly in satellite towns [87]. The third study of atmospheric deposition of trace elements in the Moscow region and the first covering the territory of the whole region, without including Moscow, using moss biomonitoring technique, was performed in 2020. Comparison of the results obtained for the limited number of samples collected in the Moscow region in 2014 and 2020 revealed a significant decrease in the content of the main part of elements in 2020, indicating the improvement of the environmental situation mainly in the northeast part of the region. The main sources of air pollution in the Moscow region in 2020 can be considered industrial activity, transport, mining, and construction. According to contamination factor values, the environmental status of the region may be characterized as unpolluted or moderately polluted [88].

The air quality studies performed in Albania indicate moderate pollution throughout the country. Examination of the potential ecological risk found that As, Cd, Cr, Hg, Ni, and Pb pose the highest potential ecological risks particularly in the areas with high metal contents. Factor analysis applied to identify the possible sources of metals in the environment suggested that Al and Fe likely originated from natural sources. As, Cd, Hg, Pb, Cu, Zn, Ni, and Cr likely originated from anthropogenic source [89]. The moss biomonitoring technique was used for assessment of air pollution in the central part of Georgia, Caucasus. Principal Component Analyses allowed identification of four factors, of which two are of geogenic origin (Factor 1 including Na, Al, Sc, Ti, V, Cr, Fe, Co, Ni, Th, and U and Factor 3 with As, Sb, and W), mixed geogenic–anthropogenic (Factor 2 with Cl, K, Zn, Se, Br, I, and Cu) and anthropogenic (Factor 4 comprising Ca, Cd, Pb, and Br) [90].

Moss *Pleurozium schreberi* (Brid.) Mitt. were used to indicate the relative levels of air pollution by trace elements in nine Polish national parks. The concentrations of most elements (As, Ba, Ca, Cd, Cl, Cr, Cu, Mo, Pb, Sr, and Zn) were higher in mosses collected from highland and mountainous parks than in lowland parks, with the opposite relationship only for Mn. A comparison of data obtained from Polish national parks in 1970s and 1990s showed a significant decrease in the concentrations of heavy metals [91].

In Hristozova et al. [92] the changes in atmospheric deposition trends in Bulgaria, studied using the moss biomonitoring technique since 1995 were investigated. Hierarchical clustering on principal components of the clr-transformed data illustrated the similarities between the datasets and proved to be effective in determining the decreased atmospheric pollution loads in the area of the Kardzhali lead-zinc plant, where the production processes ceased in 2011. The results were consistent with decreased industrial output in the country, increased coal combustion and transport pollution, and construction of roads. A biomonitoring study using terrestrial mosses was performed in the vicinity of an Integrated Iron and Steel plant near the Czech–Polish border. It was demonstrated that not only the distance from the industrial source but also the sampling season and PM_{10} concentrations significantly affect the elemental content in mosses; the association of the emissions from the source and the determined concentrations of elements in moss samples were more evident outside the heating season (October). The analyses of transformed data revealed the association of Fe, Cr, V, As and Al with the coarse particles and their dominant spatial distribution depending on the prevailing wind directions. The spatial distribution of Mn, Zn and Cd, which are carried by fine particles, appears to depend more on atmospheric dispersion and long-range transport, and, thus, these metals should be considered weak markers of the pollution load in the close surroundings of an industrial source [93].

1.2.2 Active biomonitoring

Passive moss biomonitoring is not always applicable to trace heavy metals deposition. In this case, interest represents the “moss bag” technique, which allows overcoming the problem of the lack of native moss in areas (i.e. industrial and urban areas) affected by relatively high pollutant levels or unsuitable climatic and environmental conditions. This technique has many advantages: well-defined exposure time; known original concentration of elements in the biomonitor, the uniformity of entrapment surface, and flexibility both in site selection and in the number of stations that can be chosen [24,76,94]. Using active moss biomonitoring techniques, the high degree of variability in the uptake of contaminants by native moss within the same sampling site is reduced. When moss bags are used, the initial concentration and exposure period are known [76]. For active biomonitoring, moss samples are collected from relatively unpolluted habitats; they are then cleaned, selected, and pre-treated before being exposed in a different environment.

Sphagnum species have been considered especially suitable for the monitoring of heavy metal pollution due to their high cation-exchange capacity of cell walls, large area/volume ratio, high permeability of tissues to water and elements, and high water retention capacity [95].

Several studies using active biomonitoring were performed in Belgrade, Serbia. In 2004 the moss genera *Brachythecium* sp. were collected in the urban area of Belgrade to assess atmospheric deposition of heavy metals. In 2005 moss bags with and without irrigation were exposed at three different sites with the same scope. From 29 to 36 elements were determined in moss samples using the NAA technique. Obtained data have shown that the main pollution sources in Belgrade are transport and fossil fuel combustion [24,96]. The moss-bag transplant and NAA techniques were used to investigate the kinetics of the accumulation of 38 elements in *Sphagnum girgensohni* moss samples in the highly polluted municipality of Baia Mare, Romania. Accumulation of elements considered as environmental pollutants indicates a certain degree of contamination of the Baia Mare air due to the existing non-ferrous metallurgic industry [97]. Transplants of the mosses *Hypnum cupressiforme* and *Pseudoscleropodium purum* were used as active biomonitors of airborne trace elements at Trieste, Italy. Comparing the accumulation capacity of moss species, the author has shown that both species may be used in active biomonitoring of trace elements. However, *Pseudoscleropodium purum* showed similar or higher accumulation and lower loss of almost all elements and it can be considered as a suitable bioaccumulator in moss bag surveys, especially for local, particulate emissions [98]. The Moss bags technique was employed in Baku. A total of 37 elements were determined in *Sphagnum girgensohni* exposed for three months and subjected to two complementary analytical techniques: INAA and AAS. The main air pollution sources in Baku can be considered cement, paint manufacturing, and oil and gas production [99]. Active biomonitoring for the first time was applied in Moscow, at the state museum-reserve Tsaritsyno. The obtained results showed an increase in the concentration of elements considered as environmental pollutants, Sb, Cr, V, etc., in moss samples exposed at different sites in Tsaritsyno park [25].

Three moss species: *Sphagnum fallax*, *Pleurozium schreberi* and *Dicranum polysetum* were used to study the effect of traffic and car workshop activity on the air quality. The results showed that the concentrations of individual elements in mosses and thus in the atmospheric aerosol depend on the site of exposure. The difference in analyte concentrations between outdoor and indoor environments depends on the type of element and its source. The mosses exposed in the workshop were good bioaccumulators of elements such as Al, Cr, Fe and Ba, whose concentrations were higher inside than outside and their emission sources related to the activities of the car workshop were defined. Each moss species used proved to be a good biomonitor for individual elements, with *Sphagnum fallax* being the best under the given outdoor and indoor environmental conditions [100]. Active moss monitoring with non-indigenous mosses was used to monitor several volcanoes for trace element emissions. For all volcanic areas, highest accumulation was found for S, followed by Fe and Mg, Sr, Ba, Pb, Cr, Li, then Co, Mo and the volatile elements As, Sb, Se, Tl, Bi. For most elements, open conduit volcanoes (Etna, Stromboli, Nyiragongo) showed higher moss accumulation rates than more quiescent hydrothermal areas (Vulcano > Nisyros > Yellowstone National Park) and a correlation of S, Fe, and Pb from eruptive ash and lava emissions [101]. The moss-bag approach was applied to investigate the pattern of air dispersed elements in 12 coupled indoor/outdoor exposure sites, all located in urban and rural residential areas of the in the Campania region, Italy. Of the 53 analyzed elements, 15 (As, B, Ca, Co, Cr, Cu, Mn, Mo, Ni, Sb, Se, Sn, Sr, V, Zn) were enriched in moss exposed outdoor, whereas a subset of 7 elements (As, B, Cr, Mo, Ni, Se, V) were enriched also in indoor moss samples. As derived from outdoor and indoor sources, B, Mo and Se were enriched mostly in outdoor sites; Ni, Cr and V were specifically enriched in most indoor samples [102].

Three moss (*Pleurozium* spp., *Polytrichum* spp., and *Rhytidiadelphus* spp.) and two lichen (*Hypogymnia physodes* and *Pseudevernia furfuracea*) were exposed for six weeks at 0-, 50-, 100-, 150- and 200-m distances from Slovinky tailing pond, in the main wind direction (down the valley). Accumulation ability of heavy metals increases in the order of Polytrichum spp.<H. physodes <*Pleurozium* spp.< *Pseudevernia furfuracea* < *Rhytidiadelphus* spp. *Rhytidiadelphus* spp. was found to possess the significantly highest ($P < 0.01$) ability to accumulate Cd, Zn, Ni, Mn and Fe. The highest relative accumulation factor values of Pb, Zn, Ni and Fe were determined in samples exposed at 200-m distance from pollution source [103]. Two moss species *Ceratodon purpureus* and *Brachythecium campestre* was applied to assess the environmental pollution in the Donetsk region. The relative accumulation factor values indicate that the most prevalent elements in *Brachythecium campestre* and *Ceratodon purpureus* were Na, Al, Ca, Fe, Ti, V, Cr, Mn, Co, Ni, Zn, Ba, Sr, Pd, and Cd. The main air pollution sources in the region are the Zuivska power station (Zuivska TES), Donetsk Metallurgical Plant, and Yenakiieve Iron and Steel Works [104].

1.3 Evaluation of elemental profile of soil and agricultural crops

Soil is a link between the atmosphere, hydrosphere, lithosphere, and living organisms. Soil moisture, formed from the atmospheric precipitates, determines the chemical composition of surface, river, lake and even sea water. As soil is not mobile, it is considered as a biosphere structural-functional component [41]. The determination of the elemental composition of soil and of soil and crops growing on it has drawn significant attention due to several reasons one of the most important being the nutritional and toxicological values of these elements or their compounds. The consumption of fruits, wine, and herbal tea, may significantly contribute to the daily dietary intake of elements such as K, Ca, Mg, Cr, Co, Fe, F, I, Cu, Mn, Mo, Ni, Se, and Zn by a human [105]. Some elements, such as Ca, K, Mg, and Na, are required in macronutrient amounts, others occur in trace or ultra-trace quantities Cu, Fe, Ni, Zn, and Mn are at the top end of this trace scale and play an important role in biological systems. Several metals and metalloids, such as Cd, Pb, Sn, Hg, and As, are known to be potentially toxic even in trace amounts and pose a threat to human health [106].

At the same time, the knowledge of certain elements content in wines/fruit is of special interest not just because of their toxicity in case of excessive intake but also due to the negative impact they may have on the organoleptic properties, especially of wine. For example, Cu, Fe, and Mn cause an organoleptic effect and also contribute to the haze and taste of wines [107].

A great number of natural and anthropogenic factors such as soil characteristics, water quality, area of growth, environmental conditions, industrial processes, mining activities, fertilizers, inorganic pesticides, road traffic, application of additives, and storage conditions have a significant influence on the final composition and concentration of minerals in agricultural crops [106,108,109]. In the case of wine wine-making process and storage conditions could significantly influence the concentration of major as well as trace elements in the final product [27,110]. For example, climatic conditions influence necessary fungicide treatments of fruits and berries that determine the level of copper in the final product. The two major sources of lead in fruits/wine are natural (soil) and anthropogenic (equipment, treatment, atmospheric aerosols rich in industrial fumes) [111]. Another important source of heavy metals in fruits and vegetables is irrigation with wastewater, which is a common practice in the world [106]. Mahmood and Malik [112] showed that wastewater irrigation has changed the physical and chemical properties of the soil and led to heavy metal uptake by plants, mostly vegetables.

Consumption of fruits and beverages contaminated with heavy metals is one of the main ways in which these elements enter the human body and lead to their accumulation in different tissues causing both chronic and acute health outcomes, including kidney dysfunction, polycythemia, bone fracture, respiratory illness, memory deterioration, asthma, heart problems, and various kinds of cancers [113].

The monitoring of metals (both essential and potentially toxic) content in fruit, wine, and medicinal plants is of great importance mainly due to the control of the quality of the products, metals' bioavailability, and toxicity [107]. In different countries, different acceptable levels for heavy metal content in foodstuffs were adopted [106,114]. At the same time to prevent metal accumulation in food products national and international regulations on food quality have lowered the maximum permissible levels of toxic metals in human food [114].

1.3.1 Soil - the major source of elements in agricultural crops

Soil is considered one of the main source of metals accumulation in different plants. The sources of trace elements in the soil can be natural and anthropogenic. Natural sources include volcanic eruption, weathering of rocks, and erosion. The main anthropogenic sources are wastewater irrigation, use of fertilizers, pesticides, and sludge applications, industrial activities, solid waste disposal, mining, smelting, transport [8,115]. Excessive accumulation of metals in agricultural soils results in their contamination and consequently affects food quality and safety [115].

Metals are firstly absorbed by roots and then translocated to different parts of the plant through various pathways [8]. The dynamics of heavy metals accumulation in the soil and their uptake by plants depend on soil properties (pH, temperature, texture, cation exchange capacity, organic matter, humidity), plant species, time of crop harvesting, translocation and transpiration rate, and source and form of metal [8,106,116]. Kabata-Pendias [116] claimed that acidic soil pH is considered to be the most important factor influencing the increased absorption of heavy metals by vegetables. In alkaline soils (pH within the range of 7.1–8.1), a risk of metal ions leaching and their bioavailability to plants is lower, and the presence of organic matter can inhibit metals uptake from the soil solution [106].

The excessive intake of metals from the soil has two consequences i) the harvested crops get contaminated, thus affecting health, and ii) a decline in crop yield due to the inhibition of metabolic processes [8].

Ding et al. [29] assessed the quality and safety of agricultural products from metal mining areas. Analysis of soil and crops showed a high transfer of Cd, followed by Zn, Cu, As, and Pb from the soil in plants. Pb, Cd, Cu, Zn, and As accumulation in six vegetable types showed the highest ability of elements accumulation by leafy vegetables and the lowest one by melon vegetables [117]. Measurement of selected metals (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Sr, and Zn) in the fruits of eight medicinal plants (*Carrisa opeca, Phyllanthus emblica, Solanum nigrum, Zizyphus nummularia, Zizyphus mauritiana, Physalis minima, Opuntia dillenii, and Phoenix dactylifera*) and relevant soil samples showed high accumulation of Co (0.813–1.829) and Pb (0.060–2.350) from the soil to the plants [118]. Murtić [119] studyng heavy metal dynamics in the soil-leaf-fruit system in an intensive apple cv. 'Idared' planting located in the Municipality of Goražde showed that, excepting zinc, in the soil-leaf-fruit system was characterised by relatively high total levels of heavy metals in the soil and a very low degree of their accumulation in the leaves and in particular the fruits. Studying accumulation of cadmium, lead, nickel, cobalt, zinc, copper, and manganese in soil, coriander, onion, and tomato collected from agricultural fields of Jhansi city, India the authors showed that significant accumulation of Zn (1.04) and Mn in coriander (1.42), and in onion (1.32) [120].

Mahmud et al. [121] assessed the ecological risk of heavy metals such as nickel, copper, arsenic, lead, and manganese in agricultural soil from Bangladesh and transfer status to rice grain. Transfer Factor of arsenic (0.018–0.032 mg/kg) and manganese (0.059–0.155 mg/kg) was higher from soil to rice grain. On the other hand, transfer factor of lead was found negligible that is a good sign of improvement. The levels of heavy metals (Cr, Cd, Zn, Fe, Pb, As, Mn, Cu, Hg, Ni and Co) in soil and commonly consumed vegetables from Mojo area in central Ethiopia have been determined. It was observed that leafy vegetable (cabbage) has accumulated heavy metals to greater extent compared with tomato. The total cancer risk analysis revealed the potential adverse cancer risk induced by As, Cd, Hg, and Ni from the consumption of both tomato and cabbage [122].

1.3.2 Evaluation of elemental content of fruits, berries, and juices

Fruits are berries are important components of the human diet since they are rich sources of vitamins, carbohydrates, proteins, fibers, and also take on dependable anti-oxidative effects [114,123]. They are also considered a source of minerals since they can accumulate metal in their edible parts. Fruits and berries are consumed in fresh form or as, juices that are made from the extraction or pressing (mechanically and technologically) of one or mix of mature, fresh, chilled, or frozen fruits [124].

Many studies focused on the bioaccumulation of heavy metals, mainly in vegetables and lesser in fruits in different countries around the world. Several examples are presented below. Thus, Roba et al. [114] determined the concentration of Zn, Cu, Pb, and Cd in several vegetables and fruits cultivated in the Baia Mare mining area (Romania). The concentration order of heavy metals in the analyzed vegetable and fruit samples was Zn>Cu>Pb>Cd and the accumulation of heavy metals was higher in vegetables. The content of Cd, Hg, Sn, Al, Pb, and As was determined in 72 samples of fruits and juice, marketed in Tehran, Iran, by ICP-OES. The level of heavy metals measured in different kinds of fruit juices was ranked as Al > Sn > Pb > As > Cd > Hg, and for fruits canned this rank was Pb > Al > Sn > As > Cd > Hg. The concentrations of heavy metals in fruits canned were higher than in fruit juices [124]. The rate of heavy metal pollution of some fruit samples growing at the roadsides in Turkey was determined by ICP-AES. B, Zn, Cu, Cr, Cd, Ni, Pb, and Se content in all Cornelian cherry and plum fruits was higher compared to other samples. However, the levels of Cu, Cd, and Cr in most of the analyzed samples do not exceed the permissible levels [125]. Eleven chemical elements were determined in 57 orange samples grown in organic and conventional systems. In the organically grown samples, a high concentration of Cu, Mn, Cs, Fe, and Si were determined, while conventionally grown samples were characterized by high concentrations of Al, Rb, Sr, Br, and Zn [126]. Mitic et al. [127] analyzed three varieties of sour cherry and three table grape cultivars using ICP-OES spectrometry. The elements: Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Cr, Cd, Co, Pb, and Ni were detected in all samples. The concentration of macro, micro, and trace elements in citrus fruit varieties was determined. The elemental content of citrus fruits was found to be largely dependent on the type of analyzed samples. Thus, they were found to be good sources of potassium, calcium, zinc, and manganese, while the concentrations of toxic elements (Pb, Cd, As, Al, Hg) were very low [128].

Due to the importance of the minor and major elements present in soft drinks, several studies have been carried out to determine their levels. Cindrić et al. [129] used ICP-AES to determine the minor and major elements present in apple juices. The obtained results showed that apple juice contains significant amounts of the essential elements Ca, Cr, Fe, K, Mg, Mn, Na, and Zn. The concentrations of zinc, copper, iron, chromium, manganese, cobalt, and nickel in apple, orange, and mango fruit juice samples of 15 different brands widely consumed in Saudi Arabia were determined. The daily intake of these metals was well below the recommended/tolerable values [130]. The elemental content of Sicilian red orange juices and the bioaccessibility of elements of particular nutritional interest was evaluated using several analytical techniques. Among determined elements, only manganese showed high bioaccessibility. The low bioaccessibility found for iron, zinc, and copper is likely due to the presence in the orange juice of ligands such as polyphenols and organic acids [131].

The levels of Cd, Cr, Cu, Pb, Zn, Sn, and Fe of 37 samples of 6 types of fruit juices (orange, mango, guava, pineapple, peach, and mixed fruit) marketed by different brands and of easy access in Sana'a food stores, Yemen were evaluated using ICP-OES technique. Traces of chromium were detected in two fruit juices and cadmium in seven juices. High level of tin, iron and zinc were detected in some fruit juices. It should be noted that etal content in some fruit juices sold on the Yemeni market exceeded the permissible limits set by health organizations for drinking water [132]. The levels of Cd, Hg, Sn, Al, Pb and As of 72 samples of fruits juices and fruits canned of three different brands including of Peach, Orange, Cherry, and Pineapple marketed in Tehran, Iran, were evaluated using ICP-OES technique. The level of heavy metals measured in different kinds of fruit juices was ranked as Al > Sn > Pb > As > Cd > Hg, and for fruits canned this rank was Pb > Al > Sn > As > Cd > Hg. The lead (Pb) concentration in 97.22 % (35 out of 36 samples) of fruit juices samples surpassed Codex limit (0.05 mg/kg) [133]. The level of Cd, Cr and Ni and Pb, Zn and Cu in water, soil, and vegetables (swiss chard, lettuce, cabbage, collard green, tomato, green pepper and carrot) irrigated with waste water in Gamo, Ethiopia wasa assessed usiong AAS. The mean levels of Cd in most vegetables and Cr and Pb in some vegetables were higher than the maximum recommended limits set by WHO/FAO. Cabbage was maximally contaminated with potential toxic elements followed by Swiss-chard, carrot, tomato, collard green, green pepper and lettuce [134]. Yaqub at al. [135] estimated the level of Cd, Zn, Ni, Co, Cu, Cr, and Mn in groundwater, soil, fruits, and vegetables within the cement industry and its vicinity. According to obtained data vegetable samples were contaminated with metals except for Ni and Mn, whereas, in fruit samples, Mn and Cd were not present. The highest Ni concetration was determined in fruits, while of Zn in vegetables.

1.3.3 Evaluation of elemental content of wine samples

Wine is a beverage of great social and economic significance widely consumed around the world. For this reason, the determination of the elemental content of wines to survey the concentration of certain metals was intensively investigated [27,108]. The sources of elements in wine can be both natural and anthropogenic. The first, natural source, is a transfer of metals from the soil *via* the roots to the grapes and finally to wine, providing the bigger part of ions present in wine, which is the result of the weathering of parent rocks and the atmospheric deposition of airborne particulate matter [108,136]. The second source of inorganic elements in wine arises from the anthropogenic source like fertilizers, inorganic pesticides, pollution of the surrounding environmental industries, which can be introduced during the whole wine process, from grape growth, harvest, and wine-production [27,108]

Potassium and calcium are natural components of the grape and their concentration in wine is a reflection of the grapevine status in the final stages of berry ripening. A high potassium level could also be the result of the precipitation of potassium hydrogen tartrate. The natural content of calcium in wine can be affected by the addition of $CaCO_3$ for deacidification and an elevated level of this metal also can lead to the onset of calcium tartrate precipitation [136]. Aluminum is a natural element in the wine, however, its content in wine also depends on the way of wine storage. Thus, the mean Al concentration for red wine preserved in oak barrels is around 915.5 μg/L, whereas for those preserved in cement barrels only 425.6 μg/L [137]. The long contact of wine with materials such as aluminum, brass, glass, stainless steel, wood, etc., used to build pipes, casks, and barrels is the usual source for Al, Cd, Cr, Cu, Fe, and Zn. The wines from vineyards close to the coast show a relatively high concentration of Na whereas high concentrations of Pb or Cd are found in wines from vineyards located close to road traffic or industrial areas [57]. The agrochemical treatments in the vineyard may generate: Ca, Cu, Cd, K, Mn, Na, Pb, Zn, while the application of fining agents, such as bentonite, copper sulfate (Al, Ca, Cd, Cr, Cu, Fe, Zn) [138].

Fingerprint techniques, based on chemical composition and multivariate statistical analysis, can be used not just for the determination of product quality but also origin, variety, type, and other features [109]. Thus, ICP-OES, in combination with different statistical approaches, has been used to verify the origin of different red wine samples from the Utiel-Requena, Jumilla, Yecla, and Valencia regions, Spain. Results of Principal component analysis (PCA) clearly show that Utiel-Requena and Jumilla red wines are very easy to discriminate from the wines of the other regions [109].

Most studies have focused on the quantification of metals in wines produced in countries such as Spain, Italy, Argentina, Turkey, Macedonia, Romania, etc. Examples of wine analysis in the different counties are presented below.

Lead and cadmium were determined in Macedonian white wines. The lead and cadmium concentrations were ranged between 2–28.5 and 0.4–5.69 μg/L, respectively. The levels were below the maximal allowed concentration for wine (Ivanova-Petropulos et al. 2015). The Cu, Fe, and Zn contents in twenty selected wine samples produced in the South-East region of Serbia were determined by FAAS. The Cu concentrations varied from 0.07 to 0.57 ppm in wines, and the Fe concentrations fluctuated from 2.93 to 36.2 ppm, while the Zn levels were in the range from 0.21 to 0.67 ppm. Obtained values showed that wines from this part of the world could serve as good dietary sources of the essential trace metals [139]. The content of aluminum, cadmium, calcium, chromium, copper, iron, nickel, lead, and zinc was determined in twenty white and red wine samples from the province of Mendoza in Argentina by ETAAS and USN-ICP-OES. The aluminium, cadmium, calcium, copper, iron, lead, zinc, chromium concentrations were between 17.0–18.0 μg/L, 1.0–4.7 μg/L, 10.0–15.0 μg/L, 23.0–28.0 μg/L, 480–790 μg/L, 50–90 μg/L, 24–130 μg/L, and <0.2–6.25 μg/L, respectively. The levels were comparable well with the data reported for similar wines from some other parts of the world [57]. Eleven elements were determined in 31 wine samples and 137 soil samples from the three regions in Argentina. The authors suggested that 86% of metal contents in wines could be explained by the composition of vineyard soil. The remainder of 14% could be attributed to other factors, like climate, agricultural practices, pollution, grape maturity at harvesting, wine-making techniques, etc. [108].

The elemental composition of wine and soil from two major wine-producing areas in Southeast Romania (‘Valea Calugareasca’ and ‘Murfatlar’) and the region of Moldova (Eastern Romania) were analyzed by ICP-MS. The results show that the differentiation of Romanian wines according to their provenance is based on the following main elements: Ni, Ag, Cr, Sr, Zn, and Cu for Valea Calugareasca, Rb, Zn, and Mn for Murfatlar, and Pb, Co, and V for Moldova [27]. The concentration of 17 elements (Al, As, B, Ba, Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, Zn) in Portuguese wines were determined by ICP-MS. Factor analysis (FA) allowed distinguishing elements in four groups: the lithophile elements Ba, Mg, and Mn; elements related to fertilization practices and/or winemaking procedures - B, K, and P; metal mainly derived from the use of phytosanitary products and/or stainless steel or brass materials – Zn and As mainly derived from phytosanitary products [140]. NAA was applied to determine the elemental composition of Macedonian wine. According to obtained data, the content of major and trace elements in wine samples from different Macedonian vineries was below the maximally allowed concentrations according to the Office International de la Vigne et du Vin [141].

The concentrations of 44 elements (Na, Mg, P, K, Ca, Cu, Co, Cr, Zn, Sn, Fe, Mn, Li, Be, B, V, Sr, Ba, Al, Ag, Ni, As, Sn, Hg, Pb, Sb, Cd, Ti, Ga, Zr, Nb, Pd, Te, La, Sm, Ho, Tm, Yb, W, Os, Au, Tl, Th, U) in 90 white and red wines from six different regions in Greece was determined using ICP-MS technique. The statistical analysis of data obtained has shown that a compensatory differentiation of the wine samples can be achieved concerning their multi-element composition from different varieties and cultivated regions [142]. The concentration of Cu, Fe, Mn, Cd, Zn and Pb the white wines (Feteasca Alba, Feteasca Regala, Babeasca Gri, Sarba) and red wines (Merlot, Feteasca Neagra, Cabernet Sauvignon) were assesed. According to obtained results grapevine cultivars for red wine recorded the highest concentrations of Cd and Pb and the grapevine cultivars for white wines recorded the highest concentrations of Cu, Zn, Fe and Mn [143].

Samples from the different processing stages in the elaboration of sparkling wine (cava) - including must, base wine, and sparkling wine - of Pinot Noir and Xarel·lo grape varieties have been analyzed by ICP techniques. Among determined elements, Cu and K are abundant in musts and their concentrations progressively decrease through the cava production process. S levels due to the addition of sulfites as preserving agents was higher in base wine and sparkling wine. The concentrations of Na, Ca, Fe, and Mg increase from the first fermentation due to the addition of clarifying agents such as bentonite [144]. Major, minor and trace elements of 34 AOC Rioja wines (red and white) were determined by ICP-MS. The content of chemical elements in the wines allowed their differentiation according to several factors: geographical zones (Sr, Ca, Mg, and Mn), soil types (Sr, Ba, Ni, and Cu. Cs and Pb), wines aged in oak barrels (Na and Cs) [145]. The level of Fe, Cu and Zn in 41 wine samples have been determined using Flame Atomic Absorption Spectroscopy (FAAS). The concetration of determined elements in all wine samples, except Rhine Riesling, were below the limit set by the EU directive EC 606/2009 and International Office of Vine and Wine. In white wine Rhine Riesling Cu concentration exceeds several times the permissible values [146].

1.3.4 Determination of the elemental composition of medicinal plants

The use of medicinal plants is an old practice dating back to ancient times. They are used worldwide and very common in many countries. According to the World Health Organization (WHO), for more than 70% of the global population, traditional medicines, including herbal medicines, are the main source of cure of different diseases and, sometimes, the only source of care [147,148].

The health properties of medicinal plants are determined by the content of different pharmacologically and therapeutically bioactive substances of varying structures and properties, including, alkaloids, fibers, organic acids, phenolic compounds, phytates, pigments, polysaccharides, saponins, tannins, vitamins, as well as important microelements [147]. Trace element plays an important role in chemical, biological, metabolic, and enzymatic reactions in the living cells of plants, animals, and human beings [149]. Soil is the primary source of elements in medicinal plants, additionally, they can accumulate elements from the surrounding aerial environment [147]. However, the release of trace metals through human activities into the environment has increased over the years and lead to the accumulation of excess metal in the plants [149]. Thus, pollution of soil with heavy metals from polluted irrigation water, atmospheric dust, transport industrial exhausts, pesticides, and fertilizers play important roles in the contamination of medicinal plants [150].

The quantitative estimation of various trace element concentrations is important for determining the effectiveness of the medicinal plants in treating various diseases and also to understand their pharmacological action. Moreover, trace element analysis of medicinal plants needs a thorough investigation to prevent elevated concentrations of heavy metals reaching the consumer [150,151].

The concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, and Pb elements in thirty medicinal herb samples widely consumed in Kayseri, Turkey were determined by using ICP-MS. The concentrations of Pb in all samples do not exceed 10 μg/g recommended for medicinal plants [150]. The toxic elements including Al, Pb, As, and Cd were determined by ICP-MS in five different medicinal plants collected in the city of Madina, Saudi Arabia. Al levels of all elements were the highest in Cymbopogon, however, none of the plants exceeded the daily guideline set by the WHO [148]. The uptake and translocation pattern of trace metals from two different plant species *Datura stramonium* and *Amaranthus spinosus* from a waste dump site in Pretoria, South Africa was traced by ICP-OES. The trace metal content was determined in different parts of these plants (leaf, stem, and root). The concentrations of trace metals from the leaves and stems of both plants changed in the same order Fe > Mn > Zn > Cu > Cr > Ni > Pb and in different ways from roots. In both plants, the concentrations of trace metals were in the order roots > leaves > stems [149]. The metallic element concentrations of medicinal plants (coriander, dill, Echinacea, lavender, chamomile, mint, and plantain, used for phytopharmaceutical products), cultivated in the unpolluted region of Romania was assessed by

NAA. The level of toxic or potentially toxic elements was higher in the plants from spontaneous flora: As (chamomile), Al (coriander and plantain), Ba (chamomile, coriander, and dill), and Cr (chamomile and dill), an exception was the Al from lavender [152]. Twenty-one chemical elements were identified by NAA in the samples of *Mentha pulegium*, Algeria. The essential and toxic element concentrations in all samples were well under the estimated permissible limits set by FAO/WHO for human consumption, except the case of iron [153]. The levels of copper, zinc, iron, manganese, nickel, and cadmium in 27 medicinal plant species from Ghana were studied to evaluate their health implications. Zinc, copper, and cadmium were present in all the analyzed species, while the content of other elements varied from species to species. The levels of iron in some species were higher than the maximum permissible level of 1000 μg/day. The results also highlighted the differences in the content of minerals in plants obtained from different locations in Ghana [154].

The concentrations of cadmium, lead, arsenic, mercury and copper were investigated using ICP-MS with 1773 herbs samples around the world. The over-limit ratio for Pb was 5.75% (102), Cd at 4.96% (88), As at 4.17% (74), Hg at 3.78% (67), and of Cu, 1.75% (31). For exposure assessment, Pb, Cd, As, and Hg have resulted in higher than acceptable risks in 25 kinds of herbs. The maximal Estimated Daily Intake of Pb in seven herbs, of Cd in five, of Hg in four, and As in three exceeded their corresponding Provisional Tolerable Daily Intakes. In total 25 kinds of herbs present an unacceptable risk as assessed with the Hazard Quotient or Hazard Index [155]. The quantitative evaluation of lead, cadmium, zinc, and copper content in *Mentha piperita* L. and *Zataria multiflora* Boiss. frequently sold in the markets of Iran was done. The concentration of heavy metals in analyzed medicinal plants was not more than the standard concentration reported for similar plants [156].

The mineral contents of roots, leaves, stalks, and inflorescences of the aromatic plant species *Anethum graveolens* L., *Coriandrum sativum* L., *Lavandula angustifolia* Mill., *Levisticum officinale* W.D.J. Koch, and *Salvia sclarea* L. were studied by means of neutron activation analysis. Among major and minor elements, K with a content in the range of 9230–59,600 mg/kg and Fe in the range of 69–3420 mg/kg were the most abundant elements in the studied plants. The content of the toxicant As ranged between 0.14–0.79 mg/kg; however, in the leaves (1.3 mg/kg) and inflorescences (1.0 mg/kg) of *Lavandula angustifolia* there was found to be about 1 mg/kg, equal to the guideline maximal level recommended for food by the WHO. High contents of the elements Al, Hf, Se, Sc, Na, Ta, Th were determined in all studied plants [157]. Using NAA, 18 major and minor elements, the contents of which fell within the value ranges reported for other medicinal herbs of this genus, were identified in the plant species

Teucrium polium, *Teucrium hircanicum*, *Teucrium botrys*, *Teucrium chamaedrys*, *Teucrium flavum*, *Teucrium orientale*, and *Teucrium scordium* of Moldovan origin. In all samples, the most abundant major element was K and minor element Fe. The mass fractionof As did not exceed the limit of 1 mg/kg recommended for medicinal plants by the WorldHealth Organization [158]

1.4. Water pollution and the elaboration of the techniques of wastewater treatment

The problems of water chemistry, biology and physics are of paramount interest for Ecological Chemistry and associated with the disastrous ecological situation worldwide. The contemporary world is facing the most acute problem of ecological systems degradation and gradual exhaustion of natural resources, especially of water resources [41]. Chemical elements widely applied in different industrial processes (mining, burning of fossil fuels, nuclear weapons and nuclear fuel production, manufacturing of fertilizers, metal plating and alloying, and wood preservation) lead to the generation of large volumes of wastewater, containing metal in both cationic and anionic forms [159] and pose a serious danger for human health and water ecosystem. Today, global wastewater discharge reaches 400 billion m^3/year, polluting ~5500 billion m^3 of water [160]. Present-day schemes of wastewater treatment include a variety of chemical and physical methods of metal removal, such as ion exchange, lime precipitation, chemical oxidation or reduction, electrochemical treatment, and filtration, etc. However, these techniques require high capital investment and operation costs are often inefficient at low pollutants concentrations in effluents, and some of them create a large amount of sludge, which leads to secondary contamination of the environment [39,161]. Thus, often inappropriately treated or untreated wastewaters are discharged into the environment or used for irrigation leading to contamination of soil and crops with heavy metals.

The discharge of wastewater, containing metal ions in natural water bodies may result in numerous physical, chemical, and biological responses. These can be separated into two groups: (i) effects of the environment on the metal, and (ii) effects of the metal on the environment [162].

Traditionally the assessment of natural water quality is done by measurement of physicochemical characteristics, but they are not very efficient for largescale management of catchments or for assessing whether water ecosystems are being protected. The use of biological methods to assess water quality is of particular importance since organisms show an integrating response to their environment [163]. All metals can be potentially toxic for living organisms, their toxicity is dependent on the absorbed dose, the route and the duration of exposure, properties of metals, the bioavailability of chemicals, and physicochemical parameters of the aquatic environment [51,164]. A range of biological assemblages has been used to monitor and assess environmental contamination or long-term changes. Cyanobacteria are considered useful bioindicators since they can be found in a vast array of habitats and tolerate a great range of environmental conditions including extreme environments that usually exclude eukaryotic microorganisms [163].

The toxic effect of six heavy metals, silver, copper, mercury cadmium, lead, and zinc on cyanobacteria *Synechocystis aquatilis* was studied by [165]. At the concentration used, 1, 10, and 100 μM, the cyanobacteria showed to be more sensitive to silver, copper, and mercury than to cadmium, lead, and zinc metals. The influence of copper and cadmium on the growth and ultrastructures of cyanobacterium *Anabaena flos-aquae* grown at three different temperatures: 10°C, 20°C, and 30°C was investigated. Both metal ions inhibited the growth of the tested cyanobacterium. However, copper at all temperatures tested was proven to be more toxic than cadmium leading to phycobilins and total protein destruction [166].

A study performed by [167] has shown that low lead concentration (5 μg/ mL) in the medium stimulated *Spirulina platensis* biomass growth but it was drastically reduced (up to 78%) at high lead concentrations (30, 50, and 100 μg/mL). The pigment contents (chlorophyll α and β carotene) were decreased in a dose-dependent manner. The highest reductions (67% and 53% respectively in chlorophyll α and β carotene) were observed at cyanobacteria treatment with 100 μg/ mL Pb. Effect of Cr(VI) in concentrations 0.1-8.0 ppm on the growth rate, doubling time, biomass productivity, Chl A content, carbon assimilation, and metal uptake by *Anacystis nidulans* and *Spirulina platensis* was studied. Significant reductions in the growth rate, biomass productivity, Chl A content, and carbon assimilation were observed for both the species [168]. Experiments on *Synechocystis aquatilis* cultures showed that exposure to copper at concentrations of 0.01–0.05 mg/L not only has a direct toxic effect but also significantly modifies the copper tolerance of cyanobacteria for repeated exposure [169]. Thus, it is evident that metal ions even at low concentrations have a negative impact on living organisms. Due to the noxious of metals, there are growing environmental and public health concerns, and a consequent need for increasing awareness to remediate the heavy metal polluted environment [51]. Since new technological processes are continuously developed often traditional techniques do not cope with increasing volume and complex wastewater chemical composition. A new cheap, efficient, and eco-friendly techniques are required to be used for metal removal from wastewater.

Today, in many countries, especially developing ones, treated or untreated wastewaters are often used for irrigation. The application of wastewater, which contains different nutrients can reduce the use of fertilizers and consequently reduce the cost of the agricultural practice.

Unlike clean water, wastewater flows do not vary with seasons, climatic conditions, or precipitation levels, thus allowing farmers to grow crops throughout the year [160]. In some countries, the reuse of reclaimed wastewater for crop irrigation could contribute to mitigate/decrease water shortage, support the agriculture sector, and protect groundwater resources [170]. At the same time, it should be mentioned what irrigation using wastewater poses serious risks to human health and the environment. The presence of parasitic organisms and heavy metals can lead to the spread of different diseases as well as metal accumulation in soil and foodstuff [160].

Three species *Colocasia esculentum*, *Brassica nigra*, and *Raphanus sativus* grown on the soil irrigated with wastewater accumulated a significant amount of Cd (TF > 1). Even the wastewater contains low levels of heavy metals (Fe, Mn, Pb, Cd, and Cr), they tend to accumulate in soil and plant samples [171]. Treated wastewater were used in the Alsukhna area in Jordan to grow vegetables. The use of wastewater leads to an increase of Zn, Fe, Pb, and Ni, content in vegetables by 116-6980% [172]. The soil in Morocco was irrigated with wastewater containing zinc, copper, lead, and cadmium. The trend of heavy-metal concentrations in all samples was $Zn > Pb > Cu > Cd$. The Health Risk Index was >1.0 for Cd and Pb indicating a potential health risk [173].

To prevent the negative impact of heavy metals on the aquatic organisms and their accumulation in the soil it is necessary to treat wastewater and reduce the concentrations of metals below the maximum admissible level. In recent years, research attention has been focused on biological methods for the treatment of effluents, some of which are in the process of commercialization. Among developed and proposed techniques, the biological methods, bioaccumulation and biosorption have been demonstrated to possess the good potential to replace conventional methods for the removal of heavy metals [164]. The application of microorganisms for the removal of metal has been recommended as a simple, economic, efficient, and environmentally friendly technique [174]. Compared with plants, microorganisms can resist environmental stress through rapid mutation and evolution. They can develop resistance systems to overcome the stress from toxic heavy metal ions. Additionally, some microorganisms can remediate heavy metal ions by transforming the active heavy metal ions into inactive forms, which can be used in the bioremediation of contaminated areas [175].

Below the biological processes of metal removal and prevention of their release in natural water, bodies are described in more detail.

1.4.1 Metal removal from wastewater via biosorption

Biosorption can be considered as a competitive and cost-efficient alternative to conventional methods resembling conventional adsorption or ion exchange. It has been exploited based on a favorable combination of such features as a reduced requirement for chemicals, low operating costs, high efficiency at low concentrations of pollutants, possibilities for metal recovery, and biosorbent regeneration, that allow its use for many cycles [33,176]. Biosorption is a process of concentration of sorbate (metals) on the surface of the sorbent (biological matrix). There can be emphasized several ways of metal ions interaction with sorbent: electrostatic attraction caused by ion exchange, physical adsorption, and chemical adsorption or chemosorption [176–178]. Physical adsorption is a reversible process that occurs through involving intermolecular interactions (van der Waals forces). Chemical adsorption is an irreversible process that occurs with the formation of chemical bonds. Ion-exchange occurs through the replacement of mobile ions by metal ions [179]. For example, Cochrane and co-authors [180] studying the copper biosorption by *Fucus vesiculosus* have shown that metal biosorption was 77% due to ion exchange with calcium, magnesium sodium, and potassium ions. Depending on the type of biosorbent mechanisms described above may occur simultaneously, complementing each other and ensuring the efficiency of accumulation of metal ions in solution or parallel [179].

A wide range of biomaterials (microbial biomass and its derivative products) available in nature can be employed as biosorbent for heavy metal removal [176]. As biosorbents can be used microorganisms collected directly from the environment or growth in the laboratory, as well as wastes of different biotechnological processes.

Metal ions removal by biosorbents is affected by several factors, including the type of the biomass and its concentration, physical and chemical treatments, the surface area to volume. Apart from these, physico-chemical factors such as pH, metal ions concentration, the presence of other anions and cations, metal speciation, pollutant solubility and form, time of contact, and temperature may also influence the removal efficiency [177].

pH Solution pH is an important parameter in biosorption experiments since it affects the chemistry of the metal ions and the activity of functional groups on the biomass surface [181]. Low removal of positively charged metal ions at low pH is determined by protonation and deprotonation of functional groups and competition of H^+ and H_3O^+ and cations for binding sites on the cell surface [182,183]. With the increase in pH, functional groups such as amine, carboxyl, and hydroxyl are exposed by deprotonation, thus increasing the attraction of metal ions in cationic form due to negative charges on the biomass surface [182]. Usually, the maximum removal of metal ions occurs at a pH range of 4.0-6.0. For example, the highest zinc removal by *Scenedesmus obliquus* was achieved at pH 6.0–7.0 [33]. Biosorption capacity of *Pseudomonas aerugi*nosa AT18 for Cr^{3+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} in water increased with increasing pH in the range 5.5–7.7 [184]. Han et al. [185] showed maximum copper removal by beer yeast at pH 6.0, while in Nascimento et al. [186] study the best biosorption efficiency of yeast for copper was found at pH 5.0. Thus, maximum removal of Er(III) of 70% by *Spirulina platensis* took place at pH 3.0 [187]. Maximum zinc biosorption by bacteria strain XZN4 occurred at ph 5.0 [188]. The maximum removal of cobalt by bacteria (*Bacillus haynesii, Bacillus aerius)* and fungi (*Aspergillus foetidus*, *Aspergillus parasiticus*, and *Penicillium oxalicum)* was achieved at pH 6.5 [189]. Al-Fakih et al. [24] reported that pH plays a major role, where maximum Co(II) biosorption capacities using fungal biomasses were 6.20 ± 0.32 mg/g at pH 7 [190]. At pH > 7.0 metal removal usually decreases due to the formation of hydroxide anionic complexes and metal precipitation [182].

At the same time, acidic pH is favorable for the removal of metal ions present in solutions in anionic form. Gokhale and co-authors [191] have shown high efficiency of Cr(VI) biosorption by *Spirulina* biomass at pH 1.5. Maximum chromium uptake by *Phanerochaete chrysosporium* occurred at a pH of approximately 3.0 [192]. Aksu and Gulen [193], showed that optimum pH for the biosorption of Cr(VI) and Fe(III) ions by *Rhizopus arrhizus* was 2.0. Zinicovscai et al. [194] studied rhenium removal from multicomponent sytems by *Saccharomyces cerevisiae* as function of pH. Maximum rhenium removal in all analyzed systems was achieved at pH 2.0: 84% in Re system, 76% in Re-Cu system, 80% in Re-Mo system, and 75% in Re-Cu-Mo system. Along with rhenium, molybdenum ions were better sorbed from solution at low pH values. The highest efficiency of molybdenum removal in both Re-Mo and Re-Mo-Cu systems was 85%. Removal of copper was more favorable at pH 3.0–5.0 in the Re-Cu system (66–68%) and pH 6.0 in the Re-Mo-Cu system.

Time of interaction. Time has a great influence on metal biosorption. Metal sorption by biological sorbents usually can be described as a two-stage process, the first rapid stage is associated with maximum metal removal and it lasts from 15 min to several hours, depending on the sorbent. High metal ions sorption in the first minutes of biosorbent interaction with sorbate is due to the availability of a large number of binding sites. At the second stage, the equilibrium stage, no more sorption of metal ions occurs due to saturation of biomass and lack of binding sites. The optimum time for zinc removal by *Spirulina platensis* was found to be 15 min [195], and 300 min for Fe (II), Mn (II), and Zn (II) ions removal by Ca-alginate beads with immobilized *Chlorella vulgaris* biomass [196]. Zinc uptake by *Scenedesmus obliquus* dropped rapidly within the time interval 5–15 min, with an apparent equilibrium being established after 30 min [33]. Chromium uptake by *Phanerochaete chrysosporium* increased from 42.4 mg/g at 15 min to 149.6 mg/g at 180 min [192]. The time taken for maximum manganese biosorption of 19.34 mg/g by *Aspergillus niger* and 18.95 mg/g by *Saccharomyces cerevisiae* was 60 and 20 min, respectively [197].

In multicompenent systems containing rhenium along with copper and molybdenum ions the maximum percentage of metal removal *Saccharomyces cerevisiae* ranged from 70–75% for rhenium in 45–60 min, 90–94% for molybdenum in 45 min, and 45–46% for copper in 45 min [194]. The equilibrium for bacteria (*Bacillus haynesii*, *Bacillus aerius)* and fungi (*Aspergillus foetidus*, *Aspergillus parasiticus*, and *Penicillium oxalicum)* in cobalt removal was found after 90 min [189]. The removal of Eu(III) by *Spirulina platensis* was very rapid in the first 3 min of sorbent-sorbate interaction, when 74% of Er(III) was removed from solution. Further increase in contact time did not influnce significantly the rate of Er(III) removal and the equlibrium was attained [187]. XZN4 strain had the fastest zinc removal between 0 and 10 min when the removal rate reached 57.2%. At 30 min, cognitive aspiration occurred. Between 30 and 60 min, the removal process slowed down, and basically reached a state of equilibrium after 60 min. At 60 min, the removal rate of zinc reached a maximum of 80.9% [188].

Biosorbent dosage. Metal biosorption is strongly dependent on the sorbent amount. At low biosorbent dosage, the rate of metal biosorption is usually very high, as a result of more intercellular space and less crowding allows for increased contact. The use of a high amount of biosorbents leads to cell agglomeration, thus reducing metal biosorption. Biosorption of manganese increased with a rise in the concentration of *Aspergillus niger* and *Saccharomyces cerevisiae* biomass in solution from 0.25 to 1%, respectively [197]. Thus, *Spirulina platensis* in the concentration of 1-2 g/L effectively absorbed cadmium from solution with a metal concentration of 100-200 mg/L, and 4 g biomass was effective to the metal concentration of 600-800 mg/L [198]. The removal of Cu(II) ions from aqueous solution by *Bacillus subtilis*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* was carried-out at biosorbent doses 0.2-1.0 g. The maximum removal of Cu(II) ion was attained at an adsorbent dosage of 0.2-0.4 g and a slight decrease of metal removal was noticed with an increase of adsorbent dosage [199].

Metal concentration. The initial concentrations of metal ions have a strong effect on the biosorption capacity of various biosorbents [176]. An increase in metal concentration in solution leads to an increase in the amount of metal ions retained on the biosorbent. At the same time, it should be mentioned that with an increase of metal ions concentration in solution, their removal efficiencies usually decrease, indicating saturation of all the binding sites on the microbial surface beyond a particular concentration [200,201]. For example, Cu removal by *Spirulina platensis* decreased from 90.6% at an initial copper concentration in solution 100 mg/L to 65.7% at the copper concentration in solution 200 mg/L [202]. The rise in manganese concentration from 25 to 200 mg/L resulted in an increase in its uptake by *Aspergillus niger* and *Saccharomyces cerevisiae* from 2.46 to 19.34 mg/g (9-fold) and from 1.54 to 18.95 mg/g (18-fold), respectively [197].

The effect of rhenium concentration on the yeast biosorption capacity was investigated by changing its concentration in solution from 10 to 100 mg/L, and the concentration of copper and molybdenum in solution was maintained as constant. An increase in rhenium concentration in solution promotes its sorption by yeast biomass. In three systems, namely Re, Re-Cu, and Re-Mo, biosorption capacity increased on average from 0.6 to 5.0 mg/g as the rhenium concentration rose, while in the Re-Mo-Cu system the increase in sorption capacity was lower, ranging from 0.7–2.9 mg/g. Increase of rhenium concentration led to a decrease of copper and molybdenum removal [194]. At the lowest concentrations (15 mg/L), the La (92%) was preferably biosorbed by *Bacillus subtilis* than Sm (71%). The increase in lanthanide ions concentration caused an increase in biosorption, which reached 99% at 85 mg/L (La and Sm) [203]. An increase in Co(II) ions concentration resulted in a decrease in removal percent and an increase in the removal capacity when bacteria (*Bacillus haynesii, Bacillus aerius)* and fungi (*Aspergillus foetidus*, *Aspergillus parasiticus*, and *Penicillium oxalicum)* were usaed. The highest removal capacity was achieved at 750 ppm for an immobilized thin film of *Bacillus haynesii* and thin-film of dry *Aspergillus foetidus* [204].

Temperature is an important parameter in the biosorption study because it influences the stability of metal ions in a solution and the stability of metal-cell complexes. Biosorption removal of the main part of pollutants is endothermic since the rise of temperature usually results in the increase of biosorbent surface activity and kinetic energy. At the same time, high temperatures can also cause physical damage to the biosorbent; thus, the temperature in the range from 20 to 35ºC is usually desirable for the biosorption processes [205,206].

Increase in temperature from 20 to 50 ºC lead to the decrease in rhenium removal by *Saccharomyces cerevisiae* in single and multi-metal systems by 11–20% on average. The most relevant decline was noticed in Re system (by 20%). Copper removal in the Re-Cu system was favored by the increase in the temperature, which resulted in the increase in its removal from 35 to 59%, indicating that the removal process was endothermic. The efficiency of copper removal in the Re-Mo-Cu system did not depend on the temperature change. Removal of molybdenum in both analyzed systems was not affected by temperature increase [194]. The highest removal percent of cobalt was up to 25°C for immobilized *Aspergillus parasiticus* and up to 37°C for immobilized *thin film of dry Aspergillus foetidus* [189]. It was found that, temperature in the range of 20 -50 ºC almost did not affect the rate of Er(III) removal by *Spirulina platensis*. An increse of temperature from 20 to 50 ºC resulted in the decrease of spirulina biosorption capacity by 7%, from 68 to 61% [187]. The removal rate of zinc by XZN4 increased and then decreased with temperature. When the temperature was between 15 and 20 °C, the removal rate increased significantly, then at the temperature between 20 and 35 °C the removal rate did not change significantly, ranging from 65 to 74%. When the temperature reached 30 °C, the removal rate of zinc reached a maximum of 84.5% and at further temperature increased up to 40 °C the removal rate decreased gradually [188].

Effect of co-ions Compared to batch solutions, industrial effluents contain various pollutants including the target, one of interest. Co-ions presented in wastewater can influence biosorptive removal of a target pollutant by competing with the adsorbate for binding sites. The inhibitory effects of iron(III) ions on the chromium(VI) uptake by *Rhizopus arrhizu* was observed [206]. Cations (Na^+, K^+, $Mg2^+$, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}) had no significant effects on vanadate uptake by the *Halomonas* sp. GT-83, while anions and oxyanions, competitively reduced the uptake. Vanadate uptake was reduced by oxyanions as follow: sulfate > chromate > nitrate > selenite > molybdate > tungstate > perrhenate. Sulfate highly affected the sorption and induced a 52.5% reduction in sorption [207]. Biosorption of nickel, cobalt, zinc and copper ions by *Serratia marcescens* strain 16 in the presence of monometallic solutions turned out to be higher than in multimetallic solutions which can be explained considering that in multimetallic solutions competition is established between different metal cations for binding sites on the biomass [208].

The possibility of processed rice husk and saw dust to remove Cr, Ni, Cu, Cd and Zn from the single, binary and multi-component aqueous solutions was examined. A substantial lesser removal of metal ions was observed in the presence of co-ions. Although, a higher concentration of one metal ion than others in effluents increased its removal due to a greater number of ions as compared to other for the biosorption, yet presence of other ions influences the uptake of individual ions [209]. Studying the effect of Cu(II), Zn(II), Cd(II), Ni(II) and Cr(III) on Pb(II) biosorption it was shown that the simultaneous presence of metal ions in solution reduced their adsorption capacities. Pb(II) contributed to 70–77% of the total adsorption capacity, and the % change in adsorption capacity (Δqm) was negative [210].

Surface modification. As the biosorption process primarily occurs on the surface of the biomass, improvements in the biosorptive capacity of a biosorbent can be obtained through enhancement or modification of its functional groups [205]. Chemical modification procedures include pretreatment, binding site enhancement, binding site modification, and polymerization [164]. A series of adsorption experiments using *Saccharomyces cerevisiae* was carried out using raw biomass and the biomass pretreated with different chemical methods to investigate the effect of chemical modifications on biosorption capability and to identify chemical groups responsible for metal binding. The modification of biomass with methanol and formaldehyde resulted in the decrease of the biomass adsorption capacity, which led to the conclusion that the carboxyl and amino groups existing on the cells of *Saccharomyces cerevisiae* play a more important role in the biosorption of copper [210]. Chen and co-authors [192] have shown that modification of *Saccharomyces* sp. with formaldehyde improves biosorption capacity by approximately 20%. Sar et al. [211] observed that the metal (Cu^{2+} and Ni^{2+}) uptake capacity of lyophilized *Pseudomonas auruginosa* cells was enhanced when pretreated with NaOH, NH_4OH or toluene; whereas, oven heating (80 ºC), autoclaving, acid, detergent and acetone treatments had an inhibitory effect on metal removal.

1.4.2 Mechanisms of metal ions biosorption

The main mechanisms involved in the biosorption process include chemisorption, complexation, adsorption–complexation on surface and pores, ion exchange, microprecipitation, heavy metal hydroxide condensation, and surface adsorption [212]. Often heavy metals are taken up by the biosorbent through several mechanisms. In addition, mechanisms of metals removal using biological sorbents are not fully understood in detail due to complexations in the biological nature of the biosorbents [213]. Several factors can control the mechanisms of metals biosorption, those include the chemical and coordination characteristics of the metal and characteristics of the biosorbent (the number of binding sites, their accessibility, type of binding sites and the affinity between the binding site and metal ions).

Studying Cr(III) removal by Spirulina the authors have shown that binding of Cr(III) by Spirulina might be of ion-exchange character [214]. Ion-exchange was one of the mechanisms of Cu(II) and Pb(II) sorption on waste beer yeast [185].The microbial cell wall is the first component that comes into contact with metal ions where the solutes can be deposited on the surface or within the cell wall structure. Since the mode of solute uptake by inactive cells is extracellular, the chemical functional groups of the cell wall play vital roles in biosorption [164]. It has been reported that upon binding of copper and manganese to hybrid nanofiber with *Spirulina platensis* an enhanced release of calcium, magnesium and sodium was observed due to ion exchange mechanism [216]. Thus, biosorption of zinc ions on *Spirulina platenis* may occur due binding of zinc ions to functional groups from the cellular wall such as carboxylic (–COOH), hydroxyl (–OH), amide (–NH_2), thiol (–SH), and phosphate (PO_4^{3-}) [38]. Removal of Cr(VI) and Zn(II) kaolin-supported bacterial biofilms of Gram-negative *E. coli* and Gram-positive *Staphylococcus epidermidis* occurred *via* metal ions binding to phosphodiester and amine groups [217]. Carboxyl and amino groups were involved in Cr(VI) removal by yeast strain of *Saccharomyces cerevisiae* [218]. Carboxyl, phosphate, sulfhydryl, hydroxyl and nitrogen-containing groups of yeast biofilm formed on gravels participated in the removal of Zn(II) ion [219]. Amino and carboxyl groups, and nitrogen and oxygen of the peptide bonds are also available for coordination bonding with metal ions such as lead(II), copper(II) or chromium(IV) [220]. The complexation by carboxyl, phosphonate and amine ligands was the main mechanims of chromium(III) removal by *Chlorella miniate* [221]. Precipitation and complexation are be the main mechanism responsible for iron ions removal by *Rhizopus arrhizus* at pH values higher than 2.5 [193].

1.4.3 Metal removal from wastewater via bioaccumulation

Bioaccumulation in comparison with biosorption is a more complex process since it deals with living organisms. Accumulation of metals can occur either by metabolism-independent (passive) sorption or by intracellular, metabolism-dependent (active) uptake [222]. Thus, in bioaccumulation, it is possible to reach a lower residual concentration of metal ions because cells offer binding sites on the surface and inside the cell [223]. Living cells have developed several mechanisms of resistance to toxic metals such as bioreduction of toxic metals to their nontoxic forms, chelation by a ligand, and compartmentalization of the ligand-metal complex, intracellular precipitation, nanoparticles formation, and methylation [224]. Some microorganisms produce organic ligands such as siderophores and biosurfactants, which enhance the solubility and accumulation of the metals [179].

As well as biosorption, bioaccumulation is highly affected by the operational conditions: pH, temperature, the chemical composition of the effluent, available moisture, substrate characteristics, climatic factors, which frequently also influence the metabolic functions of any organism [225,226]. Different aspects of microbial metal accumulation were studied mainly in the single-component system. Single-component systems have the advantage of providing excellent information on the metal-binding properties of microorganisms but cannot directly be applied to metal behavior in the heterogeneous systems that real wastewater constitutes [220].

At the same time, the complex nature of effluents and high metal concentrations can inhibit the growth of cells and also bioaccumulation itself [223]. High metal removal efficiency during the bioaccumulation process can be achieved using adapted microorganisms or species isolated from polluted environments. De and his coauthors [227] used mercury-resistant bacteria such as *Alcaligenes faecalis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, and *Brevibacterium iodinium* for the removal of cadmium and lead. *Pseudomonas aeruginosa* and *Alcaligenes faecalis* removed 70 % and 75 % of cadmium in about 72 h. *Brevibacterium iodinium* and *Bacillus pumilus* remove greater than 87 % and 88 % of lead in 96 hours

Nalimova et al. [228] reported bioaccumulation of copper and zinc by *Spirulina platensis* biomass. It was found that bioaccumulation was dependent on the initial concentration of metal ions and the culture growth phase. The bioaccumulation capacity of *Rhizopus arrhizus* for the selective removal of chromium, copper, and nickel ions during the growth was investigated by Preetha and Viruthagir [229]. A maximum percentage uptake yield of 93, 95, and 64% were found for 25 mg/L of initial metal ion concentrations of chromium, copper, and nickel, respectively. Molnár et al. [230] investigated bioaccumulation of four microelements Fe(III), Cu(II), Zn(II), Mo(VI) by *Chlorella vulgaris* and *Spirulina platensis*. Both cyanobacteria proved to be efficient in terms of metal accumulation in two-week-long experiments. Except for molybdenum, the bioaccumulation capacity of chlorella exceeded that of spirulina biomass. The four resistant indigenous bacteria strains were used as a mixture to remove heavy metals and reduce the organic load of wastewater effluent. They were able to resist 275 mg Cu/L, 320 mg Cd/L, 140 mg Co/L, and 29 mg Cr/L respectively [231]. *Coprinopsis atramentaria* was able to bioaccumulate 76 % of Cd at a concentration of 1 mg/L Cd, and 95% of Pb, at a concentration of 800 mg/L Pb [232]. Viable *Spirulina platensis* was used for zinc removal from effluent obtained after the galvanic process. The amount of accumulated zinc in the biomass exceeded that of the native biomass by a factor of 1498–1650. Besides zinc, spirulina was able to remove from solution iron, arsenic, and tungsten [38].

1.5. Conclusions to Chapter 1

1. Chemical elements released into the environment by different natural sources and as the result of industrial activity, transport, and agricultural practices may lead to environment pollution and negatively affect biologic value of habitation.
2. Neutron activation analysis, along with a variety of analytical techniques, has proven to be an efficient technique to determine more than 40 elements in different type of samples. The main advantages and limitations of the technique were emphasized.
3. The level of air pollution with chemical elements. can be efficiently assessed using mosses, which are cheap and reliable biomonitors. The “passive” and/or “active” biomonitoring allows investigation of the state of the environment on the extended territories, including highly polluted zones. Today, these techniques are accepted and actively implement by

many countries of the world. Examples of active and passive techniques application by different countries were presented in the Chapter.

4. Contamination of soil with chemical elements influence the living conditions of soil organisms and leads to their accumulation in agricultural crops. The main sources of soil and agricultural crops with heavy metals were presented. Examples of the evaluation of the elemental profile of soils, fruits, wines, and medicinal plants were described.
5. Water pollution with chemical elements is a critical problem nowadays. Untreated or purely treated wastewater, containing heavy metals can be released in natural water bodies or used for irrigation leading to the water, soil, and crop contamination. Thus, it is extremely important to remove heavy metals from the wastewater before their discharge into natural water bodies. Microorganisms present attractive opportunities as a low-cost means of protecting the environment from pollution. They offer efficient and environmentally friendly technology for the treatment of metal-containing wastewater. The main parameters which affect metal biosorption and mechanisms of metal removal using biological sorbents were discussed.

2. MATERIALS AND METHODS

2.1 Material used for experiments performing

The following chemicals were used in the present work: $Pb(NO_3)_2$, $AgNO_3$, $Zn(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$, CrO_3, NH_4ReO_4, $UO_2(NO_3)_2$, Na_3VO_4, $La(NO_3)_3 \cdot 6H_2O$, $K_2Cr(SO_4)_2$, K_2CrO_4, $ZnCl_2$, $CuSO_4 \cdot 5H_2O$, $FeCl_3$, $CoCl_2 \cdot 6H_2O$ and K_2CrO_4, HNO_3, HCl, H_2O_2, NaOH. All the chemicals were purchased from Sigma-Aldrich and were of analytical grade.

National Institute of Standard and Technology (NIST, Gaithersburg, MD, USA) certified reference materials: SRM 1633b/SRM 1633c (Constituent elements in coal fly ash), SRM 2709 (San Joaquin soil), SRM 2711/SRM 2710 (Montana soil), SRM 1570a (Spinach leaves), SRM 1575a (Pine needles), SRM 1547 (Peach leaves), and Institute for Reference Materials and Measurements (IRMM, Geel, BE) standard reference material BCR-667 (Estuarine sediment) were used to calibrate the neutron activation analysis and atomic absorption spectrometry measurements. The difference between certified and measured content of elements usually varied between 1 and 10%.

2.1.1 Biological sorbents used for metal removal

Several types of biological sorbents were used in the present study. Two types of *Spirulina platensis* biomass were used as biosorbent for a wide range of metal removal from batch systems and industrial effleutns: one purchased from “Biosolar MSU” company (Moscow, Russia) and the second one obtained from the Institute of Microbiology and Biotechnology (Chisinau, RM). The sorbents obtained were homogenized in a homogenizer at 600 rpm for 10 min and afterward used in the experiments.

The yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is one of the economical biosorbents obtained as by-product of fermentation industry, which is widely applied for metal removal from aqueous solutions [233,234]. Application of *S. cerevisiae* as biosorbent is attributable to yeast safety, availability in large quantities at a very low cost, high metal uptake capacity [235,236]. The process of chromium-containing synthetic effluents treatment using *S. cerevisiae* was investigated. As biosorbent, the yeast *S. cerevisiae* obtained from Efes Vitanta Moldova Brewery (Chisinau, Republic of Moldova) company was used. The yeasts were dried in oven at 105 ^{0}C for 48 h, then the biomass was homogenized at 400 rpm for 10 min and afterwards was used in experiments.

The biosorbent obtained was used for metal removal from complex chromium [237], zinc [238], and nickel-containing effluents [239].

For the first time bacteria, *Shewanella xiamenensis* (*S. xiamenensis*) immobilized onto the natural zeolite was tested for metal removal from metal-containing solutions. Zeolite was chosen as support due to its abundance in nature, low cost, high chemical stability, and good adsorption properties. Zeolite clinoptilolite of the Chola deposit used as support in the present study was purchased from “Zeolite-Trade” company and represents isometric aggregates of 3-5 mm, consisting of microaggregates of micron size with thin isometric pores and elongate channels and has the following chemical composition: Al_2O_3 – 12.9-13.2%; SiO_2 – 66.2-78.3%; K_2O – 4.0-4.8%; Na_2O – 1.8-2.2%; CaO – 1.8-2.4%; Fe_2O_3 – 0.8-1.2%; H_2O – 10-12%. The density of zeolite was 2.2-2.6 g/cm^3, specific surface area – 10.1 m2/g; bulk weight – 1.02-1.2 g/cm. Zeolite was ground up and sieved and the fraction with size 300-100 μm was used for further experiments.

Bacteria strain *S. xiamenensis* DCB2-1, isolated from metal-contaminated environment [240] was cultivated aerobically in the medium with the following composition, (g/L): K_2HPO_4 – 1.5; KH_2PO_4 – 0.75; NH_4Cl – 0.3; NaCl – 5.0; $MgSO_4 \cdot 7H_2O$ – 0.1; KCl – 0.1; $CaCl_2$ – 0.02 at pH 7.0. As source of carbon and electrons donor glucose and sodium acetate were used. On the third day of the biomass growth, 50 g of zeolite were added in the cultivation medium, and bacteria growth continued until the 7th day. At the end of the experiment zeolite with biofilm was separated from the cultivation medium by filtration, freeze-dried, and used for further biosorption experiments [241].

2.1.2 Wastewater types and their chemical composition

Wastewater containing chromium(IV), nickel, and zinc in different concentrations were obtained from electroplating units of the Scientific Production Association “Atom” (Dubna, Russia), producer of a large volume of construction steel parts, dedicated tanks, and vessels, including those operated under pressure and in an aggressive environment. Industrial effluents were collected directly after the electroplating process. Two industrial effluents, containing rhenium were taken from different stages of leaching from polymetallic ore plants (Moscow, Russia). The chemically complex wastewater was obtained from electroplating units of the Tactical Missiles Corporation (Dubna, Russia). Characteristics of the wastewater used in the work are given in Table 2.1.

Table 2.1 Industrial effluents elemental composition

Company	pH	Metal of interest	Concentration, mg/L	Other elements
“Atom”	4.0	Cr(VI)	9.4	Fe, Ni
“Atom”	5.5	Ni(II)	14.1	Fe, Ba, Zn
“Atom”	6.5	Zn(II)	45	-
“Atom”	4.0	Cr(VI)	34	-
“Atom”	6.0	Ni(II)	117	-
Polymetallic	6.8	Re(VIII)	0.7	Fe, Cu, Mo, U
Polymetallic	2.0	Re(VIII)	20	Cu, Cl, Mg, Na
“Atom”	1.7	Cr(VI)	2.9	
		Cu(II)	54.8	
		Fe(III)	18.7	
		Ni(II)	4.3	
		Zn(II)	45.2	
“Atom”	6.9	Ni(II)	14.3	
“Atom”	8.9	Cr(VI)	73	
		Ni(II)	1.7	
		Zn(II)	4.2	
“Atom”	6.0	Sr(II)	0.34	
		Ni(II)	0.84	
		Cu(II)	0.06	
		Zn(II)	49.8	
		Ba(II)	0.035	
“Atom”	7.0	Sr(II)	0.3	
		Mo(V)	2.3	
		Cr(VI)	0.06	
		Ni(II)	143	
		Cu(II)	0.03	
		Zn(II)	0.5	
		Fe(II)	0.7	
Tactical Missiles Corporation	6.0	Al(III)	0.2	
		Cr(VI)	0.1	
		Fe(III)	3	
		Ba(II)	0.1	
		Zn(II)	0.2	
		Sr(II)	0.5	

2.2 Methods applied for elemental analysis

2.2.1 Neutron activation analysis

To determine the elemental composition of the samples, neutron activation analysis at the radioanalytical complex REGATA of the IBR-2 reactor (Dubna, Russia) was applied. The method is based upon the conversion of stable atomic nuclei into radioactive nuclei by irradiation with neutrons and subsequent measurement of the radiation released by these radioactive nuclei. The process of neutron capture by a target nucleus followed by the emission of gamma rays is illustrated Fig. 2.1.

In the experiments we used the main advantages of NAA which consisted in high sensitivity to a majority of elements, especially to heavy metals, good selectivity due to specific nuclear physics characteristics of elements; a possibility of simultaneous determining of 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, an easy procedure for preparation of samples for analysis, good accuracy, about ±10÷15%, in the 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.

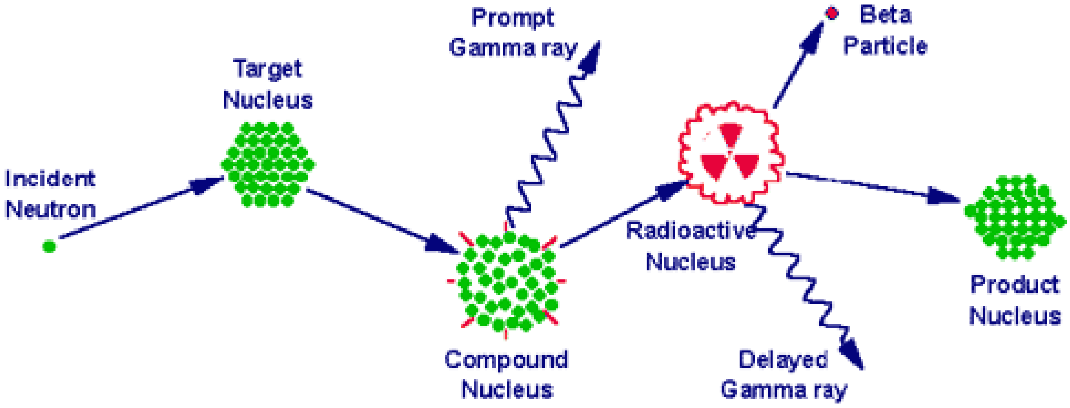


Fig. 2.1 Diagram illustrating the process of neutron capture by a target nucleus followed by the emission of gamma rays [242]**.**

The limitations of NAA are, first of all, the necessity of using nuclear reactors, the problems arising from storage and disposal of nuclear waste, and the time required for analysis

[59,242, 243]. The general scheme of NAA performing at the REGATA facility [244] is presented in Figure 2.2.

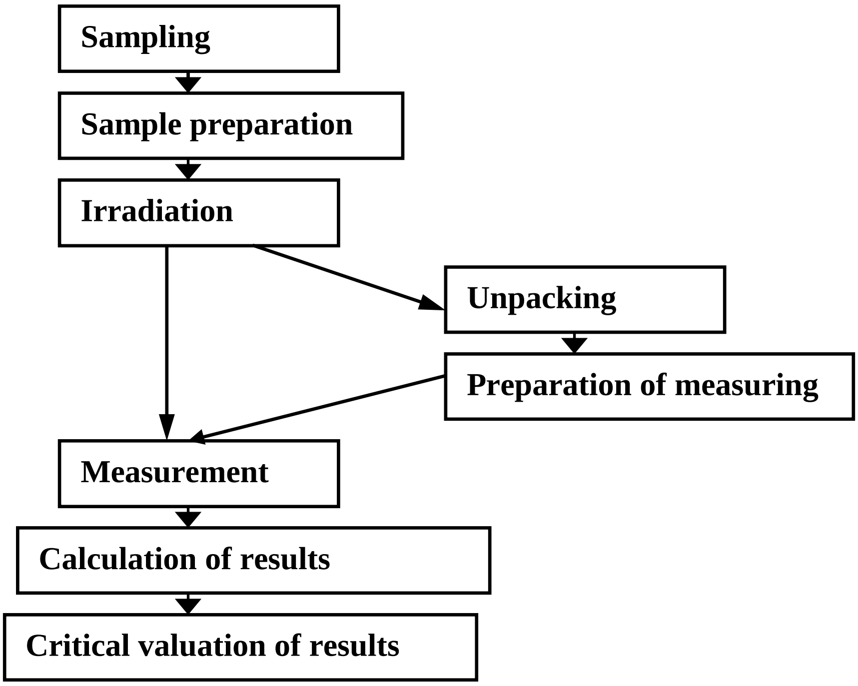


Fig. 2.2 Scheme of procedures for NAA [244]

Radioanalytical complex REGATA **(**Fig. 2.3) consists of four channels for irradiation (Ch1-Ch4), the pneumatic transport system (PTS), and spectrometric circuits based on pure germanium detectors with a resolution of 1.8-2.0 keV for total-absorption peak 1332 keV of the isotope ^{60}Co and Canberra spectrometric electronics. The main parameters of the irradiation channels are presented in Table 2.2.

Table 2.2 The main characteristics of the irradiation channels [242]

Irradiation channel	Neutron flux density (n/cm^{2}s)$^{\times}$10^{12}			T^0 C	Channel diam., mm	Channel length, mm
	Thermal	Resonance	Fast			
Ch1	Cd-coated	3.31	4.32	70	28	260
Ch2	1.23	2.96	4.10	60	28	260
Ch3	Cd-coated	7.5	7.7	30-40	30	400
Ch4	4.2	7.6	7.7	30-40	30	400

The irradiation channel Ch1 is Cd-coated to “cut off” neutrons with energy lower than 0.55 eV. Low temperature (not higher than 60-70 ºC) in cadmium screened channel of the IBR-2 reactor allows irradiation of biological samples without their damage [59, 242].

Channel Ch1 is used for the determination of elements based on long-lived isotopes. Samples are irradiated for 3–5 days, repacked, and measured two times after delay for 4 and 15–20 days. The measurement time varies from 1.5 to 10 h.

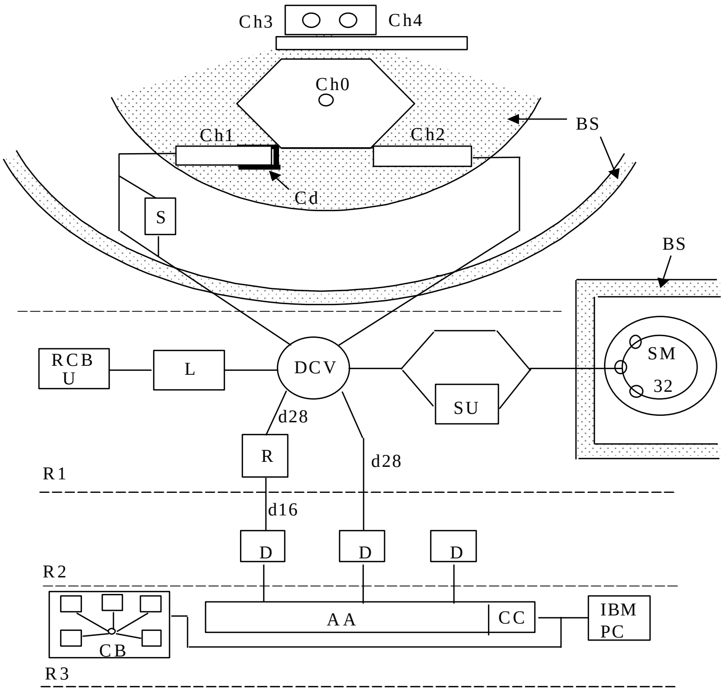


Fig. 2.3 The scheme of the REGATA experimental setup [242]

Ch1-Ch4 – irradiation channels, S- intermediate storage, DCV– directional control valves, L–loading unit, RCB – radiochemical glove-cell, U – unloading unit, SU – separate unit, SM – storage magazine, R – repacking unit, D – Ge(Li) detector, AA – amplitude analyzer, CB – control board, CC – CAMAC controller, R1 – R3 – the rooms where the system is located.

Elements with short-lived isotopes are determined in channel Ch2. The samples are irradiated for 1-20 min and measured directly after irradiation. The time of sample measurement depends on the sample matrix and obtained induced activity.

The samples are irradiated in transport containers made of polyethylene, Teflon, and aluminum. Up to 8 containers can be simultaneously loaded in each channel for long irradiation. The neutron flux density is controlled by monitors (Au, Zr, *etc*.). The pneumatic system transports containers by compressed air (at 3–s atm. pressure). All its units are located far from the reactor core (50–60 m). The polyethylene containers are transported to the irradiation site for 7–20 *s*, the aluminum containers fly faster – 3–7 *s*.

The intermediate storage (S) (for up to 15 containers) is used to reduce the activity of aluminum containers after long irradiation. It is located between two rings of the biological shield of the reactor. There are three hot cells for material sciences studies equipped with a set of instruments and devices to measure the mechanical characteristics of samples.

To provide radiation safety the unloading unit is placed into a glove-cell. All devices of the pneumatic system are equipped with photo sensors and end-switches for an indication of the container position in the system and the correct operation of all mechanisms. Acoustic detectors, placed on the «flight pipe» of irradiation channels behind the first ring of a biological shield, allow to determine the time of container arrival and departure accurately. There are many noise pulses during the flight of a container in the «flight pipe». The personal computer controls the pneumatic transport system and amplitude spectrometers.

Samples preparation for analysis

The information about sample collection for analysis is given in Chapters 3-5. In the laboratory, samples are firstly cleaned from different kinds of impurities, dried till the constant weight and if necessary homogenized. Then, samples are weighed using the analytical balance and packed. The weight of vegetation, wine, fruits, and biological samples usually varies between 200-300 mg, while the weight of soil, zeolites is around 100 mg. Soil and zeolites in the performed experiments were used as a powder, while vegetation and biological samples were pelletized before weighting. After weighing the samples are packed in polyethylene bags for short-term irradiation and in aluminum cups for long-term irradiation, signed, and send to the reactor

Samples irradiation at the REGATA facility

To determine short-lived isotopes (Ti, Cu, Ca, Cl, V, I, Mg, Al, Mn, and Dy), the samples are irradiated for 1 min (soils) and 3 min (moss, biosorbents, crops) under a thermal neutron flux of approximately 1.6×10^{12} n cm^{-2} s^{-1} and measured for 15 min. To determine elements with long-lived isotopes: Na, K, Sc, Cr, Fe, Co, Ni, Zn, As, Se, Rb, Sr, Zr, Mo, Sb, Cs, Ba, La, Re, Ce, Sm, Eu, Tb, Hf, Ta, W, Th, and U, the cadmium-screened channel 1 was used. The samples were irradiated for 3-5 days under a resonance neutron fluency rate of approximately 3.31×10^{11} n cm^{-2} s^{-1}, repacked, and measured using high-purity germanium detectors twice (after 4–5 days and 20–23 days of decay, respectively).

The analysis of the spectra was performed using the Genie2000 software from Canberra, with the verification of the peak fit in an interactive mode. The concentration calculation was carried out using the software "Concentration" developed in FLNP [244]. The software automatically proposes to create the so-called group standard sample (calibrator) from all reference materials irradiated in one container for calculation of concentrations in the analyzed samples. This group standard includes the values of radionuclide activities determined with the lowest uncertainty. Then, using the obtained group standard, the program allows to check the quality of analysis by calculating the concentrations in each of irradiated reference materials and automatically comparing the obtained values with the certified ones [74].

The quality control of NAA results was ensured by simultaneous analysis of the examined samples and standard reference materials. The measured concentrations are as rule in good agreement with the recommended values. An example of a comparison of reference materials used at the irradiation of medicinal plants with the passport data is given in Table. 2.3

Table 2.3 Certified and experimental values for the used reference materials

Element	Obtained values, µg/g			Certified value, µg/g			Z-score
Na	1707	±	90	1707	±	60	0
Mg	1164	±	56	1060	±	315	-0.31
Al	252	±	12	249	±	7	0.19
Cl	347	±	30	360	±	19	-0.35
K	22140	±	2050	21100	±	1120	0.45
Ca	14899	±	350	15600	±	200	-0.17
Sc	37	±	0.7	37.6	±	0.6	0
Mn	260	±	8	246	±	7	1.32
Cr	198.2	±	4.7	198	±	4.9	0
Fe	100126	±	4900	104900	±	3880	-0.76
Co	40.5	±	2.4	42.9	±	3.4	-0.57
Zn	241	±	8	235	±	14	0.35
As	696	±	14	626	±	35	1.7
Br	99.7	±	3.1	99.7	±	2.5	0
Rb	124	±	19	120	±	36	0.09
Sr	901	±	80	901	±	56	0
Mo	19	±	5.7	19	±	5.7	0
Sb	34.8	±	1.7	38.4	±	3	-1.03
Cs	9.39	±	0.3	9.39	±	0.2	0
Ba	707	±	51	729	±	58	0.7
La	29.6	±	1.1	27.8	±	0.9	0
Ce	56.7	±	2.5	56.7	±	5.0	0
Sm	19.4	±	3	19	±	5.7	0.06
Hf	3.2	±	0.9	3.4	±	0.3	0.02
Th	22.6	±	1.2	23	±	4	-0.09
U	2.26	±	0.15	2.3	±	0.12	0.003

2.2.2 Atomic absorption spectrometry

Atomic absorption spectrometry (AAS) was applied complimentary to NAA to determine Cu, Pb, and Cd concentration in moss samples as well as the metal concentration in solution in some of the biosorption experiments (it is discussed in details in Chapter 5).

For AAS approximately 0.3 g of moss was placed in a Teflon vessel and treated with 5 mL of concentrated HNO_3 and 1 mL of H_2O_2. The moss material was introduced in a microwave digestion system (Mars; CEM, USA) for complete digestion. Digestion was performed in two steps: (1) ramp: temperature 160 °C, time 15 min, power 400 W, and pressure 20 bar; (2) hold: temperature 160 °C, hold time 10 min, power 400 W, and pressure 20 bar. Digests were quantitatively transferred to 100-mL calibrated flasks and made up to the volume with bi-distilled water. Metal concentrations in supernatant obtained after sorption experiments were determined directly without any sample pretreatment.

Table 2.4 Main resonance lines of the elements and type of spectrometer used for measurements

Element	Resonance line, nm	Device type
Pb	217.0	KVANT-2a
Ag	328.1	AAC
Zn	213.9	KVANT-2a
Ni	232.0	KVANT-2a
Cr	357.9	AAC, iCe 3000 series
Al	309.3	AAC
Ba	553.6	AAC
Fe	248.3	AAC
Sr	460.7	AAC

The concentration of Cd, Cu, and Pb in moss samples was determined using atomic absorption spectrometry (Thermo Scientific iCE 3400, USA) with electrothermal atomization. The calibration solutions were prepared from a 1 g/L stock solution (AAS standard solution; Merck, Germany). In case of biosorption the experiments data were obtained using several spectrometers. The element, resonance line, and type of devices used for biosorption experiments are presented in Table 2.4. The quality control of AAS results was ensured using the same standard reference materials as for NAA analysis.

2.3 Evaluation of the experimental data

2.3.1 Indices used to evaluate air pollution with heavy metals

Several indices can be used to evaluate the impact of heavy metals on the environment. Some of them are presented below.

Contamination factor (C_F) is a tool that can be used to determine the pollution status of a given environment over time and is defined as:

$$C_F = \frac{C_M}{C_B} \tag{2.1}$$

where C_M is the measured content of the metal at any given site and C_b the background level for that metal [245].

Fernández and Carballeira [246] have established a scale that allows the categorization of sampling sites in terms of the C_F values for each element, taking into account the method of dispersion of contaminants in the atmosphere. The scale is presented as follow: $C_F < 1$ no contamination; 1–2 suspected; 2–3.5 slight; 3.5– 8 moderate; 8 –27 severe, and > 27 extreme.

Index of geo-accumulation I_{geo}, which is widely used to evaluate the degree of metal contamination or pollution in different environments can be calculated using the following formula (2):

$$C_F = \frac{C_M}{1.5C_B} \tag{2.2}$$

where C_M/C_B is a contamination factor. The factor of 1.5 is introduced to minimize the effect of possible variations in background [247].

The degree of metal pollution is assessed in terms of seven contamination classes based on the increasing numerical value of the index as follows: $I_{geo} <0$ means unpolluted; $0\leq I_{geo} <1$ means unpolluted to moderately polluted; $1\leq I_{geo} <2$ means moderately polluted; $2\leq I_{geo} <3$ means moderately to strongly polluted; $3\leq I_{geo} <4$ means strongly polluted; $4\leq I_{geo} <5$ means strongly to very strongly polluted; $I_{geo} \geq 5$ means very strongly polluted [248].

The calculation of an environmental index consists of two fundamental steps: (a) calculation of the sub-indices for the analytical parameters to be considered: (b) aggregation of the sub-indices into an overall index [245]. While both I_{geo} and C_F regard individual elements, the PLI offers a more global characterization as it represents the n order geometric mean of an entire set of C_F regarding the contaminating elements as follows:

$$PLI = \sqrt[n]{\prod_{i=1}^{n} C_{F,i}} \tag{2.3}$$

where n equals the total number of contaminating elements.

For PLI the following classification was proposed: PLI = 0 (background concentration); 0 < PLI ≤1 (unpolluted); 1 < PLI ≤2 (moderately to unpolluted); 2 <PIL ≤ 3 (moderately polluted); 3 < PLI≤ 4 (moderately to highly polluted); 4 <PLI ≤5 (highly polluted); PI >5 (very highly polluted) [249].

In the active biomonitoring Relative accumulation factor (RAF) is used to assess the element accumulation in the studied moss using the following formula (4):

$$RAF = \frac{(C_x - C_0)}{C_0} \tag{2.4}$$

where: C_0 is the initial metal concentration, mg/kg and C_x denotes the metal concentration in exposed moss, mg/kg.

RAF values > higher than 0.5 indicated slight elemental enrichment in the moss, and the values higher than one indicated considerable elemental enrichment [23].

2.3.2 Assessment of the health risk associated with air pollution

Calculations of human exposure to metals are done according to the model developed by the Environmental Protection Agency of the United State. According to the Exposure Factors Handbook [250], the average daily dose (ADD) (mg/kg/day) of a pollutant *via* ingestion, dermal contact, and inhalation as exposure pathways can be estimated using equations (5-7).

$$ADD_{ing} = \frac{c \cdot R_{ing} \cdot CF \cdot EF \cdot ED}{BW \cdot AT} \tag{2.5}$$

$$ADD_{inh} = \frac{c \cdot R_{inh} \cdot EF \cdot ED}{PEF \cdot BW \cdot AT} \tag{2.6}$$

$$ADD_{derm} = \frac{c \cdot SA \cdot CF \cdot SL \cdot ABS \cdot EF \cdot ED}{BW \cdot AT} \tag{2.7}$$

where ADD_{ing} is daily exposure amount of metals through ingestion (mg/kg/day); ADD_{inh} is daily exposure amount of metals through inhalation (mg/kg/day); ADD_{derm} is daily exposure amount of metals through dermal contact (mg/kg/day), c concentration of the contaminant in the sample, mg/kg; R_{ing} ingestion rate of the soil, mg/day; EF exposure frequency, days/year; ED exposure duration, years; BW average body weight, kg; AT the average time, days; CF conversion factor, kg/mg; R_{inh} inhalation rate, m^3/day, PEF particle emission factor, m^3/kg; SA surface area of the skin that contacts the dust, cm^2, SL skin adherence factor for dust, mg/cm, ABS dermal absorption factor (chemical specific) [251].

After the ADD for the three exposure pathways is calculated, a Hazard Quotient (HQ) based on noncancer toxic risk can then be calculated by dividing the daily dose to a specific reference dose (RfD).

$$HQ = \frac{ADD}{RfD} \tag{2.8}$$

The *RfD* (mg/kg/day) threshold determines the possibility of the occurrence of health effects during a lifetime due to a particular pollutant. If ADD < RfD, the HQ value will be lower than 1.0. HQ < 1.0 suggests that there is no possibility of occurrence of any adverse health effect due to the specific pollutant. If the HQ > 1.0, most probably the exposure pathway will cause adverse human health impacts [77,251].

The hazard index (HI) is the sum of HQs. HI is used to assess the cumulative non-cancer risk from a single metal [77].

$$HI = \sum_{i=1}^{3} HQ_i \tag{2.9}$$

if HI < 1.0, it is considered that there is no significant risk from non-carcinogenic effects. If HI > 1.0, there is a possibility for non-carcinogenic effects to occur, with the probability tending to increase as HI increases. Calculations of human exposure to metals are done according to the model developed by the Environmental Protection Agency of the United State.

2.3.3 Evaluation of the elements transfer in soil-agricultural crops systems, risk assessment of fruits and medicinal plants consumption

Transfer factor is the index used to assess the bioaccumulation of metal by plants and is defined as the ratio between the metal concentration in the considered plant compartment (stem, leaf, fruit, whole plant, etc.) and the metal concentration in the topsoil [252]. The transfer factor (*TF*) is defined by the following equation (10):

$$TF = \frac{C_{plant}}{C_{soil}} \tag{2.10}$$

where C_{plant} is the concentration of an element in the plant material (dry weight basis) and C_{soil} is the total concentration of the same element in the soil (dry weight basis) where the plant was grown. The higher the value of the *TF*, the more mobile/available the metal is.

To assess the degree of anthropogenic influence on soil quality are used several indices. Some of them, such as *CF*, I_{geo}, and *PLI* were described in section 1.3.3. Besides presented indices Enrichment factor (*EF*) [253] is a widely used metric for determining how much the presence of an element in a sampling media has increased relative to average natural abundance because of human activity [254].

The *EF* for the element *i* is defined as (11):

$$EF = \frac{M_x S_x}{M_b S_b} \tag{2.11}$$

where M_x and Sc_x are the soil sample concentrations of the heavy metal and scandium considerer a natural element, without any anthropogenic origin, and Sc, while M_b and Sc_b are their background concentrations of the same elements considered as reference.

Calculation of oral daily intake of metals (DIM) from the soil through fruits could be done using the following formula (12):

$$\mathrm{DIM} = \mathrm{DFC} \cdot \text{mean fruit concentration} \tag{2.12}$$

where DFC represents the average daily fruit consumption, assumed to be 300 g per person, mean fruit content is expressed in mg/day – fresh weight

The risk to human health by the intake of metal-contaminated fruits can be characterized using HQ, which is determined by the following equation (13):

$$\mathrm{HQ} = \frac{\mathrm{DIM}}{\mathrm{R_fD}} \tag{2.13}$$

where R_fD is the oral reference dose for the metal (mg/day), assuming 70 kg bodyweight. An index under 1.0 is assumed as safe. Although the HQ does not quantitatively estimate the probability of negative health effects, it indicates the health risk.

Since medicinal plants are one of the most important sources of essential nutrients and toxic elements it is very important to determine the tolerable daily intake (TDI) of potentially toxic elements. The TDI values for metals can be calculated taking into account data given by US EPA and assuming an intake of 10 g (dry weight) of plant leaves ration per person.

2.3.4 Evaluation of the biosorption data

In biosorption experiments the amount of heavy metals retained on the mass unit of biomass (q, mg/g) can be obtained from formula (14):

$$q = \frac{(c_i - c_f)V}{m} \tag{2.14}$$

while the efficiency of heavy metals removal (R, %) from equation (15):

$$R = \frac{C_i - C_f}{C_i} \cdot 100 \tag{2.15}$$

where *q* is the amount of metal ions adsorbed on the biosorbent, mg/g; *V* is the volume of solution, L; C_i is the initial concentration of metal in mg/L, C_f is the final metal concentration in the solution, mg/L, and *m* is the mass of sorbent, g.

Biosorption kinetics models. Biosorption kinetics demonstrates the rate of solutes bonding on the surface of the biological materials. Kinetics studies provide important information about the possible mechanism of biosorption that involves the diffusion (bulk, external, and intraparticle) and chemical reactions [176]. Among kinetics models used to describe experimental data the most frequently used are of metal Lagergren pseudo-first-order and Ho pseudo-second-order models. In many studies, Elovich and intra-particle diffusion models were applied as well. The pseudo-first-order kinetic model describes the rate of sorption which is proportional to the number of unoccupied binding sites of biosorbent [200]. This model is expressed by the equation (16):

$$q = q_e \left(1 - e^{-k_1 t}\right) \tag{2.16}$$

and linear form:

$$\log(q_e - q) = log q_e - \frac{k_1}{2.303} t \tag{2.17}$$

where q_e and q_t are the amounts of metal ions (mg/g) adsorbed at equilibrium and at *t* (min) time, respectively, and k_1 (1/min) is the rate constant of pseudo-first-order [255].

The pseudo-second-order model is based on the assumption that the rate-limiting step of the adsorption may be chemical adsorption or chemisorption involving valency forces through sharing or exchange of electrons between sorbent and sorbate [256]. The model is expressed by the following formula:

$$q = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \tag{2.18}$$

and linear form:

$$\frac{t}{q} = \frac{1}{K_h q_e^2} + \frac{t}{q_e} \tag{2.19}$$

where k_2 (g/mg·min) is the rate constant of the second-order model [255].

Elovich kinetic model is applied widely to describe the chemisorption of metal ions on sorbents and is suitable for systems with heterogeneous adsorbing surfaces [257]:

$$q_t = \frac{1}{\beta} \ln(1 + \alpha\beta t) \tag{2.20}$$

and linear form

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t) \tag{2.21}$$

where α and β are the Elovich equation constants.

Weber and Morris intraparticle diffusion model is based on the assumption that the step that limits the rate of biosorption is related to the diffusion of molecules: (a) transfer (diffusion) of the sorbate molecules to the border film of the sorbent, (b) diffusion of the molecules into the inside of the pores of the sorbent, (c) binding of the molecules of the sorbate to the active sites of the sorbent [176]:

$$q = k_{diff} \cdot t^{0.5} + C_i \tag{2.22}$$

where k_{diff} is the rate parameter of the *i* step (mg/g·min$^{1/2}$), C_i is an intercept of *i* step, giving an idea about the thickness of the boundary layer.

Equilibrium modeling. The equilibrium of sorption is one of the important physico-chemical aspects for the evaluation of the sorption process as a unit operation. A variety of models have been used to characterize biosorption, of which the Langmuir and Freundlich models are probably the most widely used. The Langmuir isotherm model is based on the following assumptions: the sorption is limited to monolayer coverage; all surface sites are alike and can only accommodate one adsorbed species; the ability of a species to be sorbed on a given site is independent of its neighboring sites occupancy; sorption is reversible [200,205].

This equation of the Langmuir isotherm is expressed as follows*:*

$$q_e = \frac{q_m bC_e}{1 + bC_e} \tag{2.23}$$

and the linear form:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{bq_m C_e} \tag{2.24}$$

where q_e (mg/g) is the amount adsorbed at the equilibrium concentration C_e, q_m (mg/g) is the Langmuir constant representing the maximum monolayer adsorption capacity and *b* (L/mol) is the Langmuir constant related to the energy of adsorption [255].

The dimensionless separator factor (R_L) is the essential characteristic of this model, which is defined by equation (25):

$$R_L = \frac{1}{1 + bC_i} \tag{2.25}$$

where b is the Langmuir constant and C_i is the initial concentration of metal in solution. The value of R_L indicates the shape of the isotherms to be either irreversible (R_L=0), favorable ($0<R_L<1$), or unfavorable (R_L >1).

The Freundlich isotherm, an empirical equation, defines adsorption to heterogeneous surfaces, i.e. surfaces possessing adsorption sites of varying affinities [177]. The mathematical expression of the Freundlich isotherm model is presented by formula (26):

$$q_e = K_F\, C_e^{\frac{1}{n}} \tag{2.26}$$

and the linear form:

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \tag{2.27}$$

where q_e is the amount of metal adsorbed at equilibrium (mg/g), C_e is the concentration of metal ions in aqueous solution at equilibrium (mg/L); K_F, and n are Freundlich constants that include factors that affect adsorption capacity and adsorption intensity, respectively [255].

The Temkin isotherm is usually applied for heterogeneous systems. The derivation of the Temkin isotherm is based on the assumption that the decline of the heat of sorption as a function of temperature is linear rather than logarithmic, as implied in the Freundlich equation [205].

The Temkin isotherm model is given below:

$$q_e = \frac{RT}{b}\ln\,(a_T C_e) \tag{2.28}$$

and the linear form:

$$q_e = \frac{RT}{b_T}\ln K_T + \frac{RT}{b_T}\ln C_e \tag{2.29}$$

where $1/b_T$ indicates the sorption potential of the sorbent, a_T is Temkin constant, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), and T is the temperature (K).

Similar to Freundlich isotherm, Dubinin-Radushkevich isotherm is used to describe adsorption on the heterogeneous solid surfaces and is expressed by equation (30):

$$q_e = q_m \exp\,(-K_{DR}\varepsilon^2) \tag{2.30}$$

where K_{DR} is a constant related to adsorption energy (mol^2/kJ^2), q_m is a constant that indicates the sorption capacity of sorbent (mg/g).

Polanyi potential, ε, can be calculated from the equation (31):

$$\varepsilon = RTln(1 + \frac{1}{C_e}) \tag{2.31}$$

The free adsorption energy (E_s) can be calculated using the following expression (32):

$$E_s = (-2K_{DR})^{-1/2} \tag{2.32}$$

Thermodynamics of the biosorption process. The adsorption characteristics of a material can be expressed in thermodynamic parameters such as ΔG° (Gibbs free energy change), ΔH° (enthalpy change), and ΔS° (entropy change). These parameters can be calculated by using the thermodynamic equilibrium coefficient obtained at different temperatures and concentrations [205].

The Gibbs free energy, (ΔG°), of specific adsorption, can be calculated using the well-known equation (33):

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{2.33}$$

The values of enthalpy (ΔH°) and entropy (ΔS°) can be obtained from the slope and intercept of lnK_d vs. 1/T plots, which are calculated by a curve-fitting program.

$$lnK_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{2.34}$$

where ΔH°, ΔS°, T, and R are the enthalpy, entropy, temperature the gas constant, respectively.

The ΔG value is the fundamental criterion of spontaneity if its value is negative, then adsorption would take place, indicating the spontaneity of the reaction [205]

2.4 Conclusion to Chapter 2

1. The main reagents, biological sorbents and type of wastewater used in the study were presented.
2. The main advantages and drawbacks of the neutron activation analysis as well as main characteristics of the REGATA facility at the IBR-2 reactor, and procedure of environmental samples preparation and irradiation were presented. The procedure of samples preparation and analysis using an atomic absorption spectrometer was described.
3. The formulas used to evaluate the level of air and soil pollution, health risk assessment, and accumulation of elements in the system soil - agricultural crops were given. The models applied to describe equilibrium, kinetics, and thermodynamics of the sorption process were described in detail.

3. ASSESSMENT OF AIR POLLUTION IN THE REPUBLIC OF MOLDOVA

3.1. Assessment of heavy metal pollution in the Republic of Moldova using passive biomonitoring

Air pollution is considered an acute worldwide problem. Although significant efforts were undertaken to reduce emissions, air pollution in the Republic of Moldova (RM) continues to pose the threat for human health and the environment [258]. In the RM three main groups of air pollution sources: mobile (vehicles), stationary (the power and heat generation sector, as well as industry), and transboundary pollution can be highlighted [258]. According to the national reports, in the RM in air samples mainly the concentrations of particulates, sulfur dioxide, carbon monoxide, nitrogen dioxide, phenols, and formaldehyde are monitored [21,258]. At the same time information, concerning air pollution chemical elements is very limited. Thus, the concentrations of calcium, magnesium, ammonium, sulfate, and chloride are monitored in the urban area [258].

Over the past several decades, the moss biomonitoring has been developed and implemented as a valuable tool for air pollution assessing in many European counties, due to its low price and high effectiveness, as well as the diversity of mosses habitats (they grow in urban, industrial, and unpolluted areas), their structural simplicity and rapid multiplication rate [259].

The first attempt to apply the moss biomonitoring technique in the RM was done in 2001 by Cucu-Man [260] in the framework of the NATO project, but covered only a limited area, mostly along the Prut river. The concentrations of elements: V, Cr, Ni, Cu, Zn, As, Mo, Cd, In, Sn, Tl, Pb, and Bi were determined in the thirteen collected samples by ICP-MS. Factor analysis (FA) revealed three main groups of elements. Factor 1, which included V, Cr, Ni, As, In, and Tl was associated with the thermal power station, the second factor included Cu and Zn, elements emitted due to the agricultural practice and Factor 3 with the dominant elements Mo and Cd, which main source is considered the coal combustion.

In 2015, the RM for the first time joined the moss survey in the framework of the International Cooperative Programme on the effects of air pollution on natural vegetation and crops with heavy metals in Europe (UNECE ICP Vegetation). 33 samples of moss *Hypnum cupressiforme* (*H. cupressiforme*) were collected in May 2015 throughout the territory of the country (Fig.3.1). As the usually preferred carpet-forming moss *Pleurozium schreberi* was found only in some parts of the country, *H. cupressiforme* was chosen for this study. The map of sampling points is shown in Fig. 3.1. The moss sampling and further preparation for elemental analysis were done following the CLRTAP [261] manual for moss sampling. The main criteria regarding the sampling were: 3 to 10 m away from the nearest projected tree; at least 300 m from main roads, villages, and industries, and at least 100 m away from smaller roads. Each sample was collected within the area of about 30 km^2. Longitude, latitude, and elevation were noted for every sampling location using the global positioning system. Around 500 g of fresh moss was collected at each sampling point, consisting of five to ten sub-samples of the same moss species. A separate set of polyethylene gloves was used for the collection of each sample. Collected samples were stored in special bags from air-permeable material. In the laboratory, moss samples were cleaned of soil particles and other contaminants. The material obtained was dried at 40 ^{0}C to constant weight and prepared for NAA and AAS analysis [262].

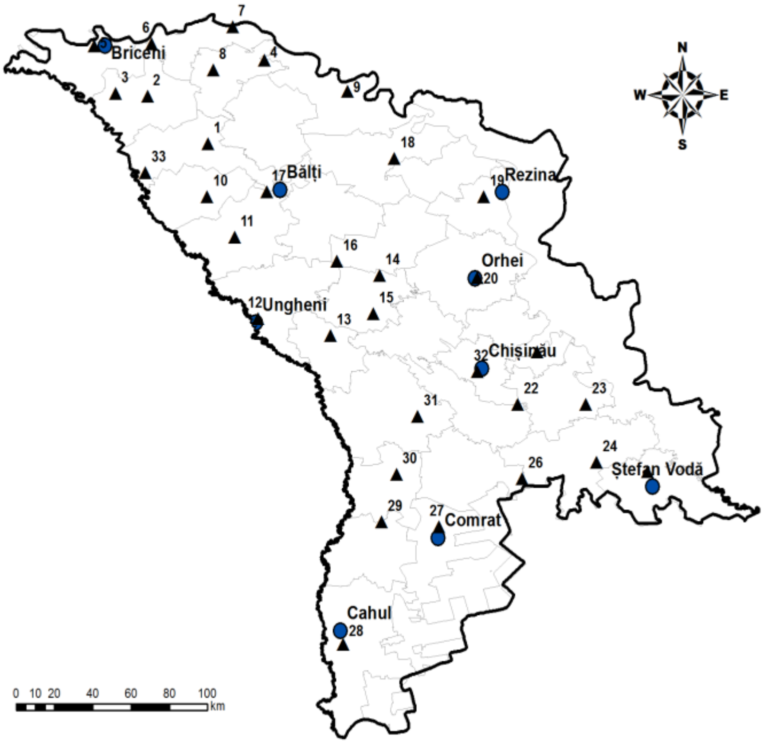


Fig. 3.1 Location of sampling points in the RM during the moss survey 2015/2016

A total of 37 elements were determined in the collected moss samples by NAA and 3 elements by AAS. Table 3.1 summarizes the results regarding the content of 41 elements, mean, median, minimum, maximum, coefficient of variation (CV%), the first and third quartiles as well as values for “reference plant” (RP) [263]. The content of the main part of elements determined in the moss collected in the RM, except of Mg, Cl, K, was higher than the “reference plant” values, confirming the impact on natural and/or anthropogenic activities on elements emission in the atmosphere.

Table 3.1 Descriptive statistics of measurements for moss samples (mg/kg)

Element	Max	Min	Md	X_a	X_g	P_{10}	P_{90}	St.	CV	RP
Na	2110	151	308	454	365	184	883	370	81	150
Mg	905	182	328	412	368	198	683	203	49	2000
Al	17200	1560	3120	5620	4220	1990	12640	4450	79	80
K	9670	2610	7110	6620	6280	3480	9000	1950	29	19000
Cl	373	46	104	129	108	54	243	87	67	2000
Ca	17200	5320	9880	10130	9800	7290	12980	2700	26	10000
Sc	3.3	0.3	0.6	1.1	0.9	0.4	2.5	0.8	75	0.02
Ti	1300	103	232	410	305	122	912	332	81	0.05
V	29	2.9	5.5	9.5	7.2	3.2	22	8.1	80	0.5
Cr	33	2	7.2	10	8.1	3.8	20	7.4	72	1.5
Mn	401	42	120	134	116	59	222	76	56	200
Fe	9200	1010	2130	3300	2600	1280	6950	2380	72	150
Ni	17	2.3	4.7	6.2	5.2	2.6	12.1	3.9	62	1.5
Co	4.7	0.4	0.8	1.3	1.08	0.5	2.7	1	74	0.2
Zn	79	20	37	39	37	25	55	13	33	50
Cu*	28	5.9	14.7	15	14	9.9	20	5.02	33	10
As	4.06	0.4	0.8	1.2	1.03	0.6	2.4	0.8	66	0.1
Se	0.4	0.2	0.3	0.3	0.3	0.2	0.4	0.06	21	0.02
Br	9	1.6	4.7	4.7	4.4	2.2	6.6	1.6	35	4.0
Rb	31	4.3	9.8	12	10	4.9	24	7.6	61	50
Sr	125	21	41	48	43	26	86	23	48	50
Zr	126	5.0	14	23	16	7.3	45	23	102	0.1
Cd*	0.95	0.2	0.39	0.4	0.38	0.2	0.6	0.2	40	0.05
Sb	1.1	0.1	0.2	0.3	0.3	0.2	0.5	0.2	69	0.1
Ba	137	25	61	68	63	34	113	31	45	40
Cs	1.4	0.15	0.3	0.5	0.4	0.2	1.2	0.4	73	0.2
La	11.7	0.9	2.1	3.3	2.5	1.2	7.1	2.6	79	0.2
Ce	27	1.9	4.4	6.8	5.2	2.5	14.4	5.5	82	0.5
Nd	10.7	0.5	1.9	3.01	2.1	0.8	6.5	2.6	88	0.2
Sm	1.6	0.1	0.3	0.6	0.4	0.2	1.2	0.4	80	0.04
Eu	0.4	0.02	0.07	0.1	0.09	0.03	0.3	0.1	84	0.008
Gd	1.5	0.04	0.2	0.4	0.2	0.05	0.9	0.4	92	0.04
Tb	0.3	0.02	0.05	0.08	0.06	0.03	0.2	0.07	82	0.008
Tm	0.1	0.01	0.03	0.04	0.03	0.01	0.1	0.04	85	0.004
Yb	0.9	0.06	0.2	0.3	0.2	0.09	0.6	0.2	81	0.02
Hf	3.3	0.17	0.47	0.7	0.5	0.2	1.6	0.7	92	0.05
Ta	0.3	0.03	0.06	0.1	0.08	0.03	0.2	0.08	80	0.001
W	1.1	0.1	0.2	0.4	0.2	0.1	0.5	0.2	70	0.2
Pb*	27	5.4	12	14	13	8.5	21	5.1	37	1.0
Th	3.8	0.27	0.65	1.1	0.8	0.4	2.3	0.9	81	0.005
U	1.25	0.1	0.23	0.3	0.2	0.1	0.6	0.2	75	0.001

∗- determined by AAS; n - number of samples; min - minimum; max - maximum; Md - median; Xa - arithmetical mean; Xg - geometrical mean; P10 -10 percentile; P90 - 90 percentile; St. Dev. - standard deviation; CV - coefficient of variance [262].

According to the data presented in Table 3.1, elements Na, Al, Sc, Ti, V, Zr, La, Ce, Nd, Sm, Eu, Gd, Tb, T, Yb, Hf, Ta, Th, and U show high values of variability (CV% > 75%) and indicate the influence of several pollution sources of these elements content in moss samples. Thus, the soil can be considered as one of the main sources of these elements. For the other elements the CV value was less than 75%, indicating the low spatial variability and thus likely dominant influence of the regional source types [87]. The median values on Al, Sc, Ti, V, Cr, Fe, Ni, Co, As, Rb, Sr, and rare earth elements were much lower than P_{90} and maximum values. This suggests that the distribution of elements is not equal - there are large variations, due to the increased content of these elements in the air [264].

The content of elements in moss (minimum-maximum and median values), collected in the RM was compared with data reported by Cucu-Man (Simona-Maria Cucu-Man 2006) for the RM, by Harmens et al. [18] for Romania (whole country and Moldova province), and by Steinnes et al. [265] for Norway, considered s pristine territory (Table 3.2) [266]. The median values for V, Cr, Fe, Ni, As, Br, La, Ce, Th, U obtained for the RM were comparable with the data obtained for the province of Moldova (Romania) and Romania as a whole country, but higher than the data presented for Norway. The maximum content of Mg, Mn, Fe, Ni, Co, Zn, Cu, Rb, Cd, and Ba in RM was lower in mosses sampled in Moldova. Lower values for the main part of elements in comparison with Romania can be explained by the reduced industrial activity in Moldova. A comparison of the data obtained in 2001 [260] with present ones (Table 3.2) showed that the content of Ni, Zn, Cu, As, Cd, and Pb in moss samples remained almost unchanged. The increase of V content from 4.3 to 5.5 mg/kg and of Cr from 3.1 to 7.2 mg/kg was observed. It should be noted that different techniques were used to determine metal content in moss samples: ICP-MS in Cucu-Man [260] thesis and NAA in the present work. It is well known that NAA determines the total content of elements in the samples, whereas ICP-MS and AAS determine only the fraction soluble in mineral acids. Since the industrial sector in Moldova did not show considerable development over the past 11 years, it is highly probable that the apparent increase in Cr and V may be explained by moss contamination with mineral particles [262].

The data processing was performed using software the Statistica (Stat-Soft version 9). Correlation analysis was applied to investigate the association degree of the chemical elements in the moss samples (Table A1.1, annex). The Pearson correlation coefficient shows the correlation degree between two random variables or sets of random variables. If r values are between 0.5 and 0.7, good association between the elements is assumed, whereas the values between 0.7–1.0 show the strong association of elements.

Table 3.2 A comparison between the experimental values obtained in the present study and literature data

	Moldova (present work)		Moldova [215]		Romania, 2010 [18]		Romania, 2010 [18]		Norway [220]	
n	33		13		78		330		464	
Element	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range
Na	308	151-2110	-	-	350	127-5880	510	102-6150	123	11–864
Mg	328	182- 905	-	-	372	86-3980	461	51-3980	1335	502–3128
Al	0.31	0.16-1.72	-	-	0.23	0.03-2.7	0.33	0.02-3.44	0.03	0.005–0.46
Cl	104	46-373			342	72-2070	263	17-2860	-	-
K	0.71	0.26-1.16	-		0.84	0.52-1.54	0.88	0.35-2.57	0.39	0.18–0.87
Ca	0.99	0.53-1.70	-	-	0.87	0.26-0.78	0.75	0.09-4.07	0.28	0.09–0.85
Sc	0.6	0.3-3.3	-	-	0.4	0.05-5.3	0.6	0.05-11	-	-
Ti	232	103-1300	-	-	238	29-1840	307	19-3490	25	4–260
V	5.5	2.9-29	4.3	6.4-29	3.8	0.4-42	4.6	0.4-49	1.4	0.3–25
Cr	7.2	2.0-33	3.1	1.1-18	4.3	0.7-35	5	0.7-62.2	0.6	0.2–49
Mn	120	42-401	-	-	240	39-699	202	15-1000	292	19–2653
Fe	0.21	0.10-0.92	-	-	0.14	0.03-1.43	0.17	0.03-2.95	0.03	0.003–2.47
Co	0.8	0.4-4.7	-	-	0.6	0.1-7.4	0.8	0.1-13	0.2	0.03-39
Ni	4.7	2.3-17	2.9	2.0-16	2.9	0.8-17.8	3.1	0.4-36	1.2	0.15–856
Cu	15	5.9-28	10.7	8.0-25	15	4.2-161	18	0.2-627	4	1.4–443
Zn	37	20-79	38	20-141	33	4.6-80	42	0.6-1440	31	7.4–368.
As	0.8	0.4-4.1	0.9	0.6-2.4	0.6	0.15-6.7	0.7	0.1-51	0.01	0.02-4.8
Rb	10	4.3-31	-	-	9.1	2.5-57	13	2.5-84	13	1.3–72
Sr	41	20-125	-		28	7.7-102	33	6.6-128	15	1.9–72
Cd	0.39	0.2-0.95	0.3	0.2-0.9	0.6	0.3-2.3	1.2	0.13-24	0.08	0.009–1.9
Sb	0.2	0.1-1.1	-	-	0.16	0.04-0.5	0.2	0.01-16	0.06	<0.001–1.1
Cs	0.3	0.15-1.6	-	-	0.25	0.05-4.4	0.3	0.01-16	0.15	0.01–1.7
Ba	61	25-137	-	-	63	14-334	72	12-488	25	4-325
La	2.2	0.9-11.7	-	-	1.5	0.2- 14	1.9	0.2-70	0.3	0.043–5.6
Ce	4.4	1.9-27	-	-	2.5	0.3-25	3.4	0.3-141	0.6	0.09–9.5
W	0.2	0.1-1.1	-	-	0.12	0.04-0.7	0.17	0.003-1.7	0.2	<0.003–4.9
Pb	12	5.4-27	10.8	5.3-30	21	2-55	31	2.2-120	1.5	0.33–21
Th	0.65	0.27-3.8	-	-	0.4	0.06-4.9	0.5	0.05-21	0.1	<0.02–1
U	0.23	0.1-1.25	-	-	0.12	0.01-1.1	0.15	0.01-6.7	0.02	<0.001–0.37

All contents expressed in mg/kg excepting Al, K, Ca and Fe whose content is expressed in % dw). In an absence of confident data regarding a normal distribution, we have used instead of the mean value the average value. For a better comparison, only the elements reported in the literature are presented.

The correlation matrix shows the high correlation between the elements Na, Al, Fe, Ni, Co, As, La, Ce, Sm, Eu, Gd, Tb, Yb, Hf, Ta, W, Th and U. Elements showing low or negative correlation such as K, Ca, Se, Br, Cd, Pb and Cu were not included in the correlation matrix. High correlation between the abovementioned elements may indicate the common source of their emission.

Further analysis of the obtained set of data was performed using the FA, which explains the variance of observed variables using the smaller number of potential variables. The results of the factor analysis are given in Table 3.3. Three factors were identified, explaining a total of 79% of variability in the data set. Factor 1 (F1) is the strongest factor representing 53% of total variability, Factor 2 (F2), and Factor 3 (F3) include 10% and 16% of the total variance, respectively. Application of the linear algebra methods and in particular of Moore–Penrose inverse has reduced the number of factors from three to one, which included the main part of the determined elements. However, this technique needs approval using the large number of data. Since FA was widely used in evaluating the moss biomonitoring data, the results of this technique will be discussed further.

Table 3.3 Matrix of the first three factors loading

Element	F1	F2	F3	Element	F1	F2	F3	Element	F1	F2	F3
Na	0.76	0.25	0.52	Cr	**0.92**	0.13	0.34	Sr	0.1	**0.71**	0.39
Mg	**0.93**	0.11	0.08	Fe	**0.95**	0.1	0.25	Cd	-0.25	-0.27	**0.48**
Al	**0.96**	-0.07	0.08	Co	**0.88**	0.21	0.40	Sb	0.55	0.13	**0.62**
Cl	-0.14	**0.79**	-0.06	Ni	**0.86**	0.08	0.46	Cs	**0.96**	0.09	0.20
K	0.48	0.32	-0.14	Cu	0.54	0.25	0.36	La	**0.90**	0.16	0.32
Ca	0.48	0.44	0.59	Zn	0.27	0.14	**0.77**	Pb	0.26	-0.13	**0.69**
Ti	**0.97**	-0.05	0.03	As	**0.86**	0.14	0.42	Th	**0.9**	0.18	0.36
V	**0.95**	-0.09	-0.02	Se	-0.14	**-0.68**	0.07	U	**0.82**	0.24	0.45

*Boldface numbers indicate significant ($p<0.05$) loading factors

The maps showing the geographical distribution of selected elements were built using the ArcGIS software. Although the mosses are mainly used to reflect the atmospheric inputs, it was shown that the elements are also supplied by the sources other than atmospheric anthropogenic deposition: mineral particles, mostly windblown soil dust, and root uptake by vascular plants with subsequent leaching [267]. Factor 1, which includes elements such as Al, As, Fe, Ti, V, Cr, Co, Ni, Cs, La, Th, and U, can be considered as a combined geogenic/anthropogenic association of elements.

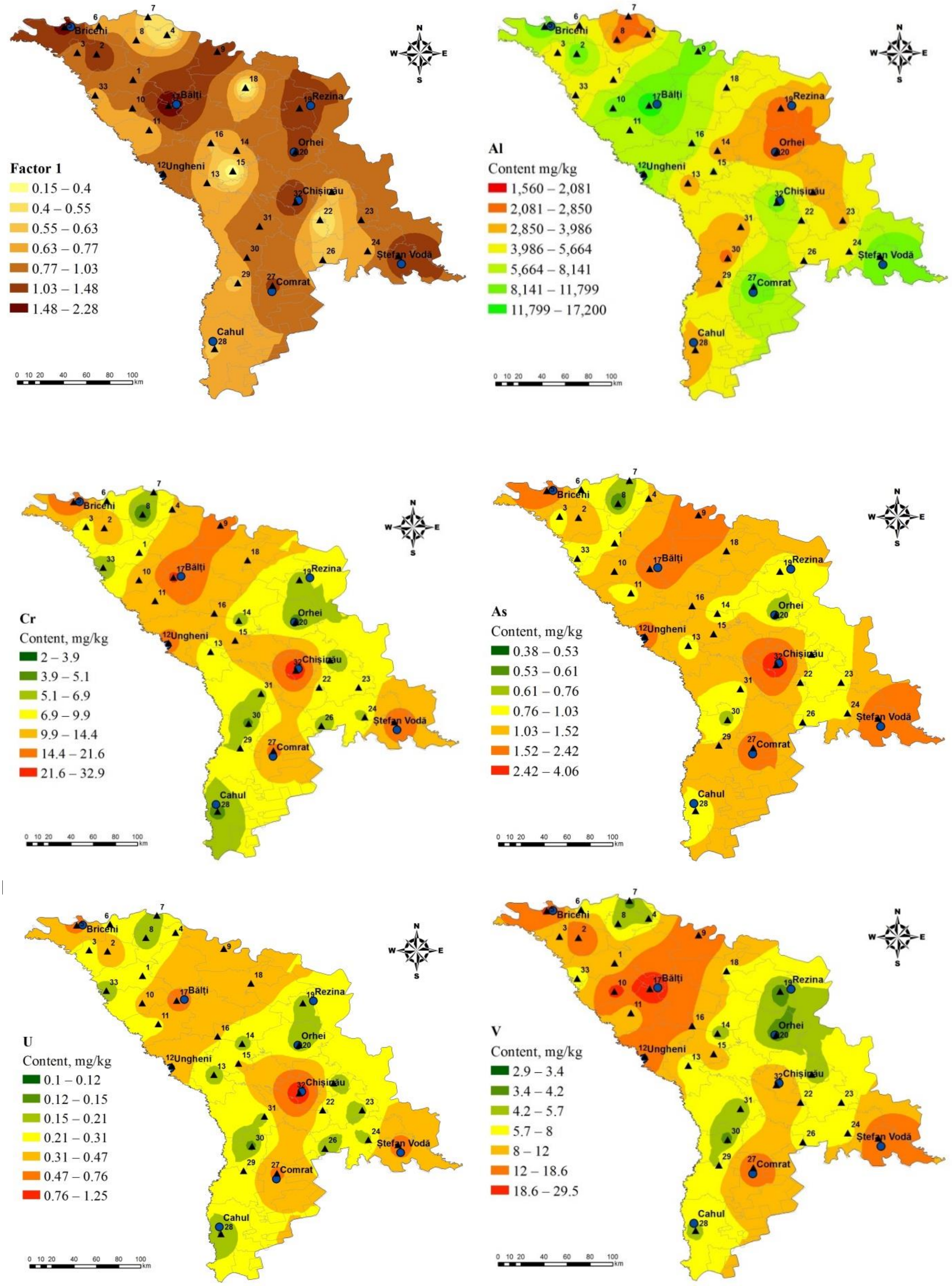


Fig. 3.2 Spatial distribution of Factor 1 scores and atmospheric deposition patterns for Al, Fe, Cr, As, U, and V

The geographical distribution of factor 1 is shown in Fig. 3.2. The elements (Al, Fe, Ti, Cs, La, Th, U) are typical crustal elements and their content in the mosses can be associated with soil particles release into the atmosphere by wind, mainly in the area with dry climate and low rainfall [264]. The presence of Al confirms this assumption, since the main source of Al found in biological systems is considered soil [268]. In the RM around 50% of agricultural lands are represented by arable land, which substantially contributes to the distribution of mineral particles and subsequent accumulation by moss species. The high concentrations of some elements (Fe, Cr, As, V, and U) near Chisinau and Balti also indicate the possible influence from anthropogenic sources. Chisinau and Balti characterized by high traffic density and high emissions of industrial pollutants. High content of As and U were determined near Chisinau where the Combined Heat Power Plants No.1 and No. 2 are operated. The main source of these elements is considered to be the coal combustion. High levels of V and Ni, near Balti, can be associated with the burning of heavy fuel oil for heating and electricity production at the North Combined Heat Power Plant. Iron (Fe) and Cr emissions near Chisinau and Balti may be associated with the of SA “Moldagrotechnica” in Balti, SA “Moldovahidromas” in Chisinau and other small industrial enterprises [56]. Relatively high content of V, As and U almost on the whole territory of the country can be explained by use of coal and wood for heating in rural area [262].

The second factor F2 (Fig.3.3), includes elements Cl, Se, and Sr. The high content of the above-mentioned elements is explained by their leaching from higher plants or penetration from the soil. According to Lucaciu et al. [269], this transfer is facilitated by the absence of a protective litter/humus layer between the moss and the mineral soil. At the same time, it should be mentioned that the important source of Se can be coal burning. Again, the Chisinau area is the most polluted as here the human influence on the environment is significant [262].

Factor 3 is an exceptionally clear-cut anthropogenic component with high values for Pb, Sb, and Zn (Fig.3.4). Higher Pb content have been found in the densely populated and industrialized parts of the RM (Chisinau, Rezina, Ungheni, Stefan Voda). Lead is a local pollutant resulting mainly from the combustion of leaded fuel. More than half of the anthropogenic emissions of Pb in Europe in 2000 arrived from the combustion of gasoline and 75% of lead in gasoline is emitted to the atmosphere during the gasoline combustion [4].

Some contributions are also made by cement production and waste disposal. The highest Pb values were registered in the zone Causani-Stefan-Voda. Antimony is a toxic trace element, commonly enriched in coals, and fossil fuel. Sb is also used in alloys, ceramics, glasses, plastics, and synthetic fabrics production [270]. The highest Sb values were determined in Chisinau and

Balti. The highest levels of Zn found in Rezina town can be explained by the influence of the metallurgical industry located in Ribnita [262].

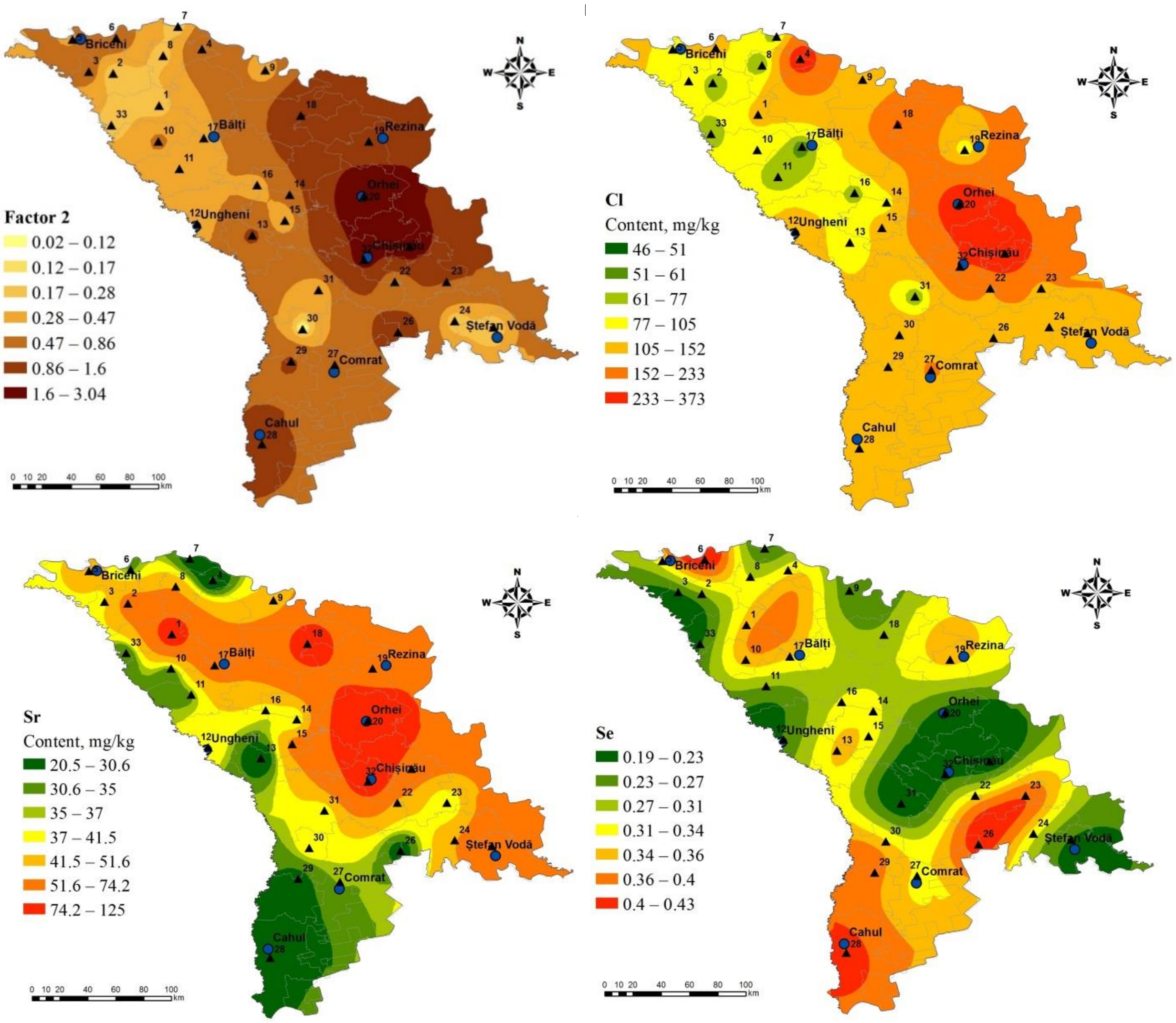


Fig. 3.3 Spatial distribution of Factor 2 scores and atmospheric deposition patterns for Cl, Sr and Se

The values obtained for some elements, namely As, Cd, Cr, V, Pb and Sb were compared with respective values reported by the other European countries, which participated in the moss survey 2016/2016 (Fig. 3.5) [271]. Content of As, Cd, Cr, and Sb in the RM was amongst the highest within the countries taken for comparison, while the concentration of V was the highest. Vanadium is a constituent of nearly all coal and petroleum crude oils [272].

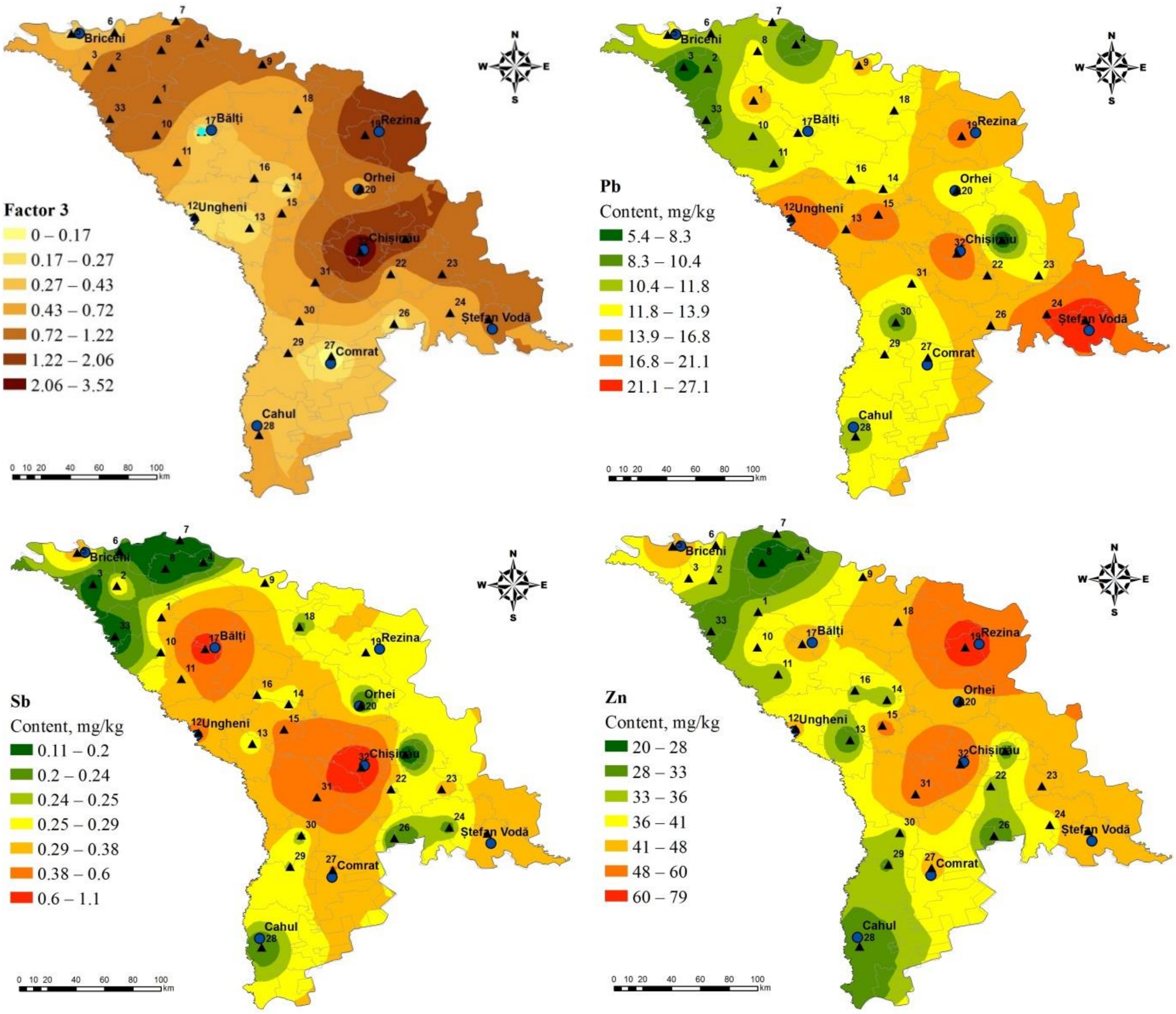


Fig. 3.4 Spatial distribution of Factor 3 scores and atmospheric deposition patterns for Pb, Zn, and Sb

In the RM the main sources responsible for the release of these elements are thermal power plants. The highest content of these elements was found in the vicinity of the cities of Edinet, Briceni, Glodeni, Ungheni, Balti, Chisinau, and Stefan-Voda [262]. Arsenic is released into the air mainly from the coal combustion and pesticide application. According to Jigau et al. [273], the soils in RM are characterized by high arsenic content which exceeds the allowable concentration limits. As in the case of V, the highest concentrations of As were determined near the towns, where the thermal power plants are operated. Chromium compounds have numerous industrial uses, such as steel hardening, chromate pigments in dyes, paints, inks, and plastics; chromates added as anticorrosive agents to paints, primers, and other surface coatings; pigments, abrasives, refractory materials, in electric semiconductors, and as photographic chemical [274]. The highest concentrations of Cr in the RM were detected near Chisinau (32.9 mg/kg) and Balti (24.6 mg/kg). The incineration of wastes is one of the main sources of airborne Cd. In the RM median value of

Cd was very close to values reported for Ukraine and Bulgaria. Since in Moldova Cd concertation was almost the same in all collection points it can be assumed that its sources are soil particles. At the same time, the level of Pb in the RM was one of the lowest among the compared countries, on the level of Norway and Sweden.

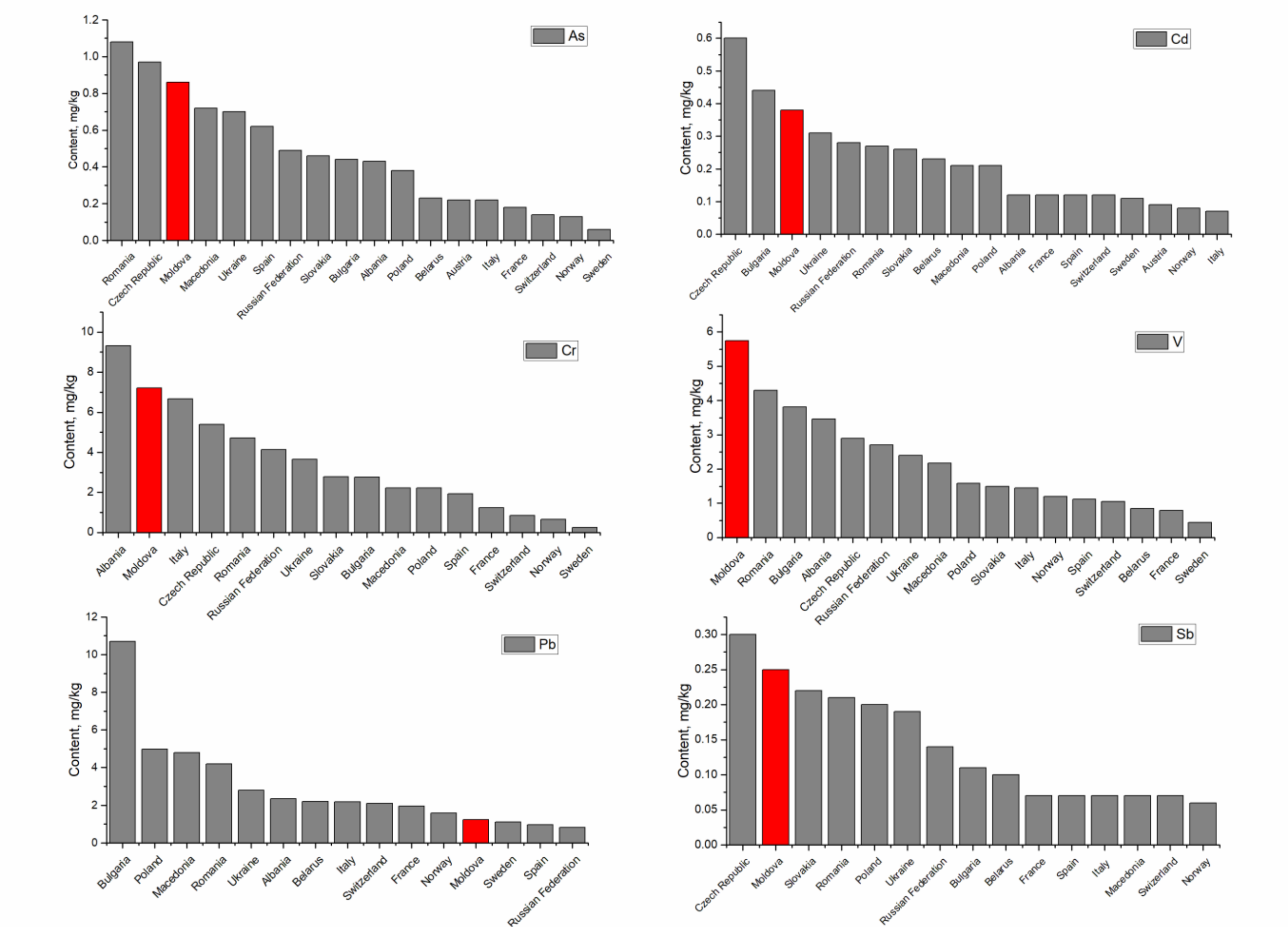


Fig. 3.5 Comparison of the median values for selected elements in Moldova with corresponding data from other European countries

To quantify the anthropogenic influence on the environment, C_F, I_{geo}, and PLI values were calculated according to the formula presented in Chapter 1 (Section 1.3.3). All these indices, starting from the local values of the contamination factor C_F proposed different criteria to assay the anthropogenic influence on the environment.

The indices were calculated for the whole set of data covering the entire RM territory as well as for Chisinau and Balti separately (Table 3.4). Ten elements were taken into account, which can be emitted by the industrial activity and are considered the environmental pollutants.

According to data presented in Table 3.4, the C_F values obtained for all elements for the entire country lied between 1 and 2 indicating the moderate pollution of the territory with these elements. C_F values for Chisinau ranged from 0.52 for Cd to 9.1 for U and for Balti from 0.33 for Cd to 8.6 for V. The increased content of U in the vicinity of Chisinau could be attributed not only to its release from the soil but also to power plants in the process of coal burning. The highest C_F value in Balti was obtained for V, the main source of which can be considered fuel combustion.

Table 3.4 Average values of the CF, Igeo and PLI for entire Republic of Moldova, Chisinau and Balti municipalities

Element	Moldova		Chisinau		Balti		Element	Moldova		Chisinau		Balti	
	C_F	I_{go}	C_F	I_{go}	C_F	I_{go}		C_F	I_{go}	C_F	I_{go}	C_F	I_{go}
Al	1.5	0.58	5.1	Zn	7.2	2.85	Zn	1.2	0.26	2	1.00	1.6	0.68
V	1.2	0.26	4.1	Sb	8.6	3.1	Sb	1	0.00	4.7	2.23	3.5	1.81
Cr	1.7	0.77	7.6	Pb	5.8	2.54	Pb	2	1.00	1.9	0.93	1.1	0.14
Fe	1.6	0.68	6.7	U	6.5	2.70	U	1.7	0.77	12.5	3.64	1.5	0.58
As	1.3	0.34	6.7	*PLI*	2.7	1.43	*PLI*	1.44		4.89		3.38	

I_{geo} values were significantly lower in comparison with C_F values. Its values for the entire country were ≤ 1.0, except U, indicating that mosses were unpolluted to moderately polluted with analyzed metals. Uranium values of (1.5) indicated the moderate country pollution and the strong environment pollution around Chisinau with I_{geo} value 3.6. For Chisinau, all I_{geo} values, except Cd and Pb, were higher than 1.0. Thus, the values for Zn (1.0) and Cu (1.51) showed moderate pollution and for other elements, pollution varied from moderate to strong. According to I_{geo} values for Pb (0.14), Cd (0.22), U (0.58), Zn (0.68) and Cu (0.94) in Balti there is no significant contamination with these elements. For other elements, pollution can be classified as moderate.

The PLI classification similarly indicated moderate to severe pollution, especially in Chisinau where the main contribution comes from U (Fig. 3.6) [86,262].

It is already proved that the elemental composition of mosses and lichens reflects the chemical composition of their environment and they are considered important bioindicatios of the air pollution level in the investigated region. High content of chemical elements may have a negative impact on bioindicators' vital function, indicating the worsening of the condition of their habitation. According to Basile et al. [275] at Zn content in moss in the range of 224-588 mg/kg and of Cu in the range 53-324 mg/kg no severe ultrastructural changes occurred in dependent of the metal content in the moss. The toxic effect of Cd and Pb at their concentration in moss of 360 and 1070 mg/kg, respectively was expressed by alteration of chloroplast shape, disorganization of thylakoid arrangement and increase of plastoglobules number. The chlorophyll concentrations of the mosses exposed in the field was not affected negatively at Zn concentration in moss up to 500 mg/kg, of copper 68 mg/kg and of lead 25 mg/kg. In the laboratory experiments, the reduction of the chlorophyll concentrations in moss was observed at copper content in moss 286 mg/kg [276]. At Cu content in moss of 195 mg/kg total chlorophyll content in mosses decreased by 22-47%, indicating the toxic effect of copper, which can be associated with the co-factors substitution of various enzymes, degrading their activities. Zinc and Pb toxicity was manifested at higher concentrations [277].

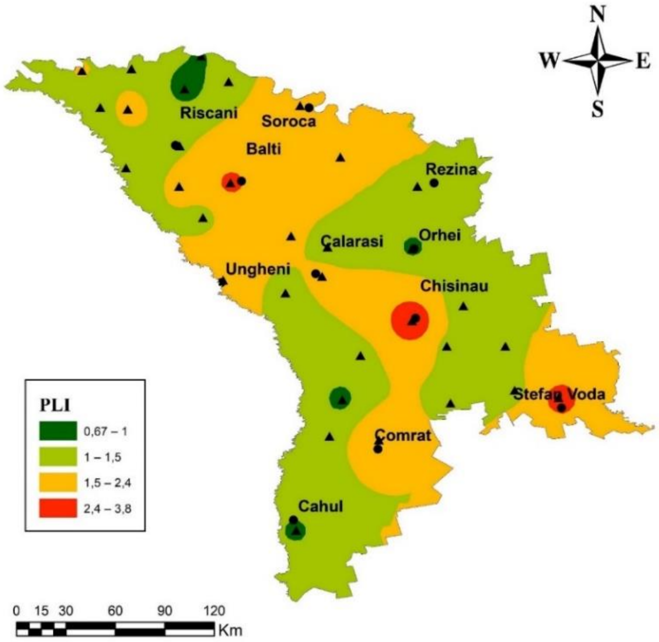


Fig. 3.6 Pollution load index calculated for V, Cr, Fe, Zn, As, Cd, Pb, Sb, Cu and U

The damage endured by the membrane lipid peroxidation was the physiological indicator depicted by the diversity of epiphytic lichens at Zn content in lichens 26-32 mg/kg, V content 2.4-2.7 mg/kg and Pb content 2.0-3.4 mg/kg [278]. The high degree of cell membrane damage in lichens was observed at As content in lichens 0.2-0.25 mg/kg, Cd 0.08-0.12 mg/kg, Co 0.28-0.4 mg/kg, Cr 5-8.7 mg/kg, Cu 6.5-9.4 mg/kg, Ni 2.5-3.5 mg/kg, Pb 6.8-9.7 mg/kg, Sb 0.31-0.44 mg/kg, V 1.4-1.7 mg/kg and Zn 30-53 mg/kg [279]. However, Dzubaj and co-authors did not observe typical stress responses in lichens at mean Al content 1620 mg/kg, Cd 1.9 mg/kg, Cu 28.7 mg/kg, Fe 2213 mg/kg, Mn 128 mg/kg, Pb 23.7 mg/kg, 46.1 mg/kg, and Zn 100 mg/kg [280]. Comparing the values ontained for Moldova with concentration, which provoke toxic effect in organisms biomonitors moses and lichens it can be concluded that at given metal concentrations vital processes in mosses are not affected. At the same time level of concentrations of determined elements may provoke toxic effects in lichens. It should be mentioned that mosses possess high tolerance to high metal concentration and the effect of such levels of concentrations on the human health can be different.

3.1.1 Health risk assessment evaluation

Important task of ecological chemistry is not only determination of the chemical composition of the environment, but also to estimate the risk of pollution and its impact on human health. To assess the negative effect of elements considered as environmental pollutants, which can find their way in the human body *via* ingestion, dermal contact and inhalation the ADD values were calculated for ten elements according to formula presented in Chapter 1 (Section 1.3.4) and obtained values are are listed in Table 3.5. According to calculated data, the ADD of ingestion for all metals was significantly higher than those of inhalation and dermal absorption [281].

The values of HQ and HI, calculated according to the formula presented in Chapter 1 (Section 1.3.4), for ten elements for the different sampling sites are presented in Table 3.6 for the three daily doses. The values of reference doses (RfD) were taken from [251,282] studies. It is considered that more than 95% of contribution to the overall human health risk is through the ingestion pathway. The highest calculated average values of HQ, were found to be through the ingestion pathway and decrease in the order of ingestion>dermal contact>inhalation. The previous studies that investigated the influence of atmospheric dust on human health have shown the similar results [77,251]. According to calculated data for all elements, except V for children, HQ, and HI values were lower than 1.0, indicating that there is no significant health risk associated with these elements. The orders of HI of metals decreased in the order: V>As>Fe>Cr>Sb>Pb>Cd>Cu>U>Zn for children and V>Cr>Pb>Zn>U>Fe>As>Cd>Cu>Sb for adults [281].

For children, HI values of V were higher than 1.0, indicating its potential danger for children's health. Vanadium (V) in the atmosphere mainly originates from the man's activities. The children can have the elevated contaminant exposures because they exhibit behavior that increase the indirect ingestion by the hand-to-mouth activities and mouthing of various dust-contaminated objects and ingest dust at the rates that are greater than for adults on a body-weight basis [283].

Vanadium and its compounds are widely applied in ceramic and pigments production of steel, machinery, batteries, and semi-conductors, and in catalyst-based recycling processes. Petroleum refinery, coal combustion, smelting, welding, and cutting of V-rich steel alloy, the cleaning, and repair of oil-fired boilers are other important sources of V in atmosphere [284].

Table 3.5 Daily dose in three models: ingestion, dermal contact, and inhalation

			ADD_{ing}		ADD_{inh}		ADD_{derm}	
Element			**children**	**adult**	**children**	**adult**	**children**	**adult**
V	max	2.88	3.7E-04	5.06E-05	7.14E-09	7.66E-09	3.39E-06	2.53E-06
	min	29.5	3.68E-05	4.94E-06	6.97E-10	7.48E-10	3.31E-07	2.47E-07
	mean	9.57	1.2E-04	1.64E-05	2.31E-09	2.48E-09	1.10E-06	8.20E-07
Cr	max	2.02	4.2E-04	5.64E-05	7.965E-09	8.55E-09	3.78E-06	2.82E-06
	min	32.9	0.3E-04	3.46E-06	4.89E-10	5.25E-10	2.32E-07	1.732E-07
	mean	10.3	01.3E-04	1.77E-05	2.502E-09	2.68E-09	1.18E-06	8.86E-07
Fe	max	1010	0.118	0.015	2.76E-10	2.39E-06	1.3E-04	7.00E-04
	min	9200	0.013	0.0017	2.66E-11	2.62E-07	1.1E-04	8.66E-05
	mean	3302	0.042	0.005	7.34E-11	8.58E-07	3.8E-04	2.8E-04
As	max	0.379	5.19E-05	6.96E-06	9.83E-10	1.05E-09	4.67E-07	3.48E-07
	min	4.06	4.84E-06	6.50E-07	9.17E-11	9.85E-11	4.36E-08	3.25E-08
	mean	1.2	1.57E-05	2.11E-06	2.98E-10	3.19E-10	1.41E-07	1.05E-07
Zn	max	20.2	1.0E-03	1.3E-04	1.91E-08	2.05E-08	9.11E-06	6.79E-06
	min	79.2	2.6E-04	9.38E-07	4.89E-09	5.25E-09	2.32E-06	1.73E-06
	mean	39.	5.0E-04	5.15E-05	9.48E-09	1.01E-08	4.50E-06	3.36E-06
Sb	max	0.11	1.45E-05	1.95E-06	2.76E-10	2.96E-10	1.31E-07	9.77E-08
	min	1.14	1.40E-06	1.88E-07	2.66E-11	2.85E-11	1.26E-08	9.43E-09
	mean	0.30	3.87E-06	5.20E-07	7.34E-11	7.87E-11	3.48E-08	2.60E-08
Pb	max	5.4	3.4E-04	4.64E-05	6.56E-09	7.04E-09	3.11E-06	2.32E-06
	min	27.1	6.90E-05	9.26E-06	1.30E-09	1.45E-09	6.21E-07	4.63E-07
	mean	13.7	1.75E-05	2.34E-05	3.31E-09	3.55E-09	1.57E-06	1.17E-06
U	max	0.101	1.59E-05	2.14E-06	2.44E-11	3.24E-10	1.43E-07	1.07E-07
	min	1.25	1.29E-06	1.73E-07	3.026E-10	2.62E-11	1.16E-08	8.66E-09
	mean	0.31	3.94E-06	5.29E-07	7.47E-11	8.01E-11	3.55E-08	2.64E-08
Cd	max	0.22	1.21E-05	3.77E-07	2.30E-10	2.46E-10	1.09E-07	8.14E-08
	min	0.95	2.81E-06	1.62E-06	5.32E-11	5.71E-11	2.53E-08	1.88E-08
	mean	0.41	7.64E-05	6.97E-07	9.84E-11	1.05E-10	4.67E-08	3.48E-08
Cu	max	5.97	3.6E-04	4.85E-05	2.34E-10	7.34E-09	3.25E-06	2.42E-06
	min	28.2	5.19-06	1.02E-05	5.32E-11	1.55E-09	6.87E-07	5.12E-07
	mean	15.2	1.9E-04	2.62E-05	9.84E-11	3.97E-09	1.76E-06	1.31E-06

Table 3.6 Average Hazard Quotient (HQ) and Hazard Index (HI) values for ten heavy metals based on three daily dose models via ingestion pathway, dermal contact pathway, and inhalation pathway

Metal	**$\mathrm{RfD}_{\mathrm{ing}}$**	**$\mathrm{RfD}_{\mathrm{inh}}$**	**$\mathrm{RfD}_{\mathrm{derm}}$**	**$\mathrm{HQ}_{\mathrm{ing}}$**		**$\mathrm{HQ}_{\mathrm{inh}}$**		**$\mathrm{HQ}_{\mathrm{derm}}$**		**HI**	
				children	**adult**	**children**	**adult**	**children**	**adult**	**children**	**adult**
V	7.00E-05	-	9.00E-3	1.71E+00	2.34E-01	-	-	1.22E-04	9.11E-05	1.71E+00	2.34E-01
Cr	5.00E-03	2.86E-05	2.5E-04	2.60E-02	3.54E-03	8.75E-05	9.37E-05	4.72E-03	3.54E-03	3.08E-02	7.17E-03
Fe	0.7	-	-	6.00E-02	7.14E-03	-	-	-	-	6.00E-02	7.14E-03
Zn	3.00E-01	3.00E-01	6.00E-02	1.67E-03	1.72E-04	3.16E-08	3.37E-08	7.50E-05	5.60E-05	1.75E-03	2.28E-04
As	3.00E−04	3.00E−04	3.00E−04	5.23E-02	7.03E-03	9.93E-07	1.06E-06	8.98E-03	3.50E-04	6.13E-02	7.38E-03
Sb	3.5E-4	-	8.00E-06	1.11E-02	1.49E-03	-	-	4.35E-03	3.25E-03	1.55E-02	4.74E-03
U	3.00E-3	-	-	5.30E-03	7.13E-04	-	-	-	-	5.30E-03	7.13E-04
Pb	3.5E-03	3.52E-03	5.25E-04	5.00E-03	6.69E-03	9.40E-07	1.01E-06	2.99E-03	2.23E-03	7.99E-03	8.92E-03
Cd	1.00E-03	1.00E-03	5.00E-05	0.00519	6.97E-04	9.84E-08	1.05E-07	9.34E-04	6.96E-04	6.12E-03	1.39E-03
Cu	3.7E-02	4.02E-02	1.9E-03	5.14E-03	7.08E-04	2.45E-09	9.88E-08	9.26E-04	6.89E-04	6.07E-03	1.40E-03

Vanadium compounds can cause a variety of toxic effects such as hematological and biochemical changes, neurobehavioral injury, or morphological and functional lesions in the liver, kidneys, bones, spleen, and leukocytes. Inhalation of vanadium may cause rhinitis, pharyngitis, chronic productive cough, tracheobronchitis, and bronchopneumonia [285].

3.2. Active biomonitoring in an urban area: Chisinau case study

According to the significant number of scientific papers published on the subject, the mosses adsorb the elements mainly from the atmosphere. Based on the passive biomonitoring data Chisinau and Balti are characterized by the increased pollution in comparison with the entire country. Since the concentrations of pollutants in Chisinau were the highest it was agreed to perform an active biomonitoring study in the city in order to identify the main air pollution sources in an urban area. The first attempt to carry out the active biomonitoring study in Chisinau was done by Begu in 2009 [26]. The author used lichens to monitor the ecological situation in the city for 9 months. The concentration of six elements: Cu, Pb, Zn, Cd, Cr, and Ni determined in lichen samples by AAS showed their significant accumulation during the exposure time in lichen samples.

To examine the ecological situation in Chisinau, the active biomonitoring using the “moss bag technique” was applied. Moss bags were recommended to be prepared primarily with species of the genus Sphagnum, which is characterized by large surfaces and numerous surface binding sites, which increase the adsorbent efficiency [272]. Moss *Sphagnum girgensohnii* (*S. girgensohnii* Russow) was collected in 2016 from a pristine wetland located in the Tver region, Russian Federation. Moss samples were cleaned manually from soil particles, plant remains, and epiphytes. Approximately 2.0 g of the unwashed, cleaned, and air-dried moss was packed in 10 × 10 cm nylon net bags, and these ‘‘moss-bags’’ were suspended using a T-system at three different sites in Chisinau: the thermal power plant (TPP), and the Academy of Sciences main building (AS) as potentially polluted places, and in the Botanical Garden (BG) considered as an unpolluted site (Fig. 3.7) [286].

Moss samples have not been washed before the exposure. It was shown in Aničić et al. [96] study that the difference in the element content of washed and unwashed samples of *S. girgensohnii* was insignificant. At each site, fifteen bags were suspended from October 2016 to March, 2017 (the period considered heating season in Chisinau). Every month three of them were collected and kept in tightly closed paper and plastic bags. A part of the unexposed moss material was kept in the laboratory and further used as a reference sample [286]. The procedure of moss preparation for NAA and AAS analysis is described in the Chapter 2.

Fig. 3.7 Exposure sites of the *S. girgensohnii* moss bags: I (TPP), II (BG) and III (AS)

The results of the analysis of the exposed moss samples are summarized in Table A1.2 (Annex). The concentrations of the main part of elements in the exposed moss samples were higher than in the control sample indicating that the sites, where the moss bags were exposed, can be contanimated by the elements in question. The mosses, due to their anatomy and the specific nutrition type, easily absorb substances contained in atmospheric precipitation. On their surface, in crevices and bends, they accumulate dust containing macro-and micro-nutrients, which under the favorable conditions dissolve in water that wets the thallus and penetrates into its structure [287]. At the TPP site the continuous increase of Na, Cr, Fe, Co, Br, Sr, Sb, Br, Sr, La, Ce, Hf, Th, U, Cu, and Pb content was observed. The behavior of other elements changed differently. At the BG site, the content of almost all elements increased in the first month of exposure and it did not change significantly during the next four months. At the SA site Na, Mg, Cl, Br, Ce and Cu accumulation in mosses reached the maximal value after four months of exposure following the period when their slight decrease was noticed [286].

The significant decrease of K and Rb content after two months of exposure can be explained by their replacement with other metal cations. It is known that mosses can accumulate metals above their physiological needs due to the absence of cuticle in their tissues and the abundance of sites with exchangeable cations in their cell wall [259]. The high ion-exchange capacity is connected to the high concentration of un-esterified pectates of poliuronic acids mainly in cell walls of moss [288].

The change of the content of elements, considered as environmental pollutants in the exposed moss samples V, Ni, As, Sb, Pb, Fe, Cu, and Cr is presented in Fig. 3.8. The most pronounced increase of V content was observed at the TPP place, confirming that the combustion of fossil fuels is one of the main air pollution source in Chisinau. Vanadium is a constituent of nearly all coal and petroleum crude oils. The content of V in moss increased by 3.7-20 times. A maximum amount of V was accumulated in moss after 4 months of exposure. In the fifth month, its content decreased threefold. The high content of V in January and February can be explained by the low outside temperatures and consequently, more intense heating. At the same time, high metal accumulation is typical for the months with low precipitation. Vanadium content almost did not change at the BG site, its content in moss increased by 1.4-1.7 times. The increase of V content at AS site by 1.5-4.1 times can be explained by windblown dust and proximity to the TPP site [286].

The coal combustion is also the main source of Ni emission in the atmosphere. Content of Ni increased at all exposure sites: by 2-5.9 times at TPP, 1.5-4.5 times at BG, and 2-4 times at AS. Its highest level was observed at the TPP site, with the maximum amount accumulated in November (4.7 mg/kg). It was shown in Aničić et al. [24] studies that V and Ni accumulation in the moss in the wintertime was 2-3 times higher than in the summer time, and for As and Fe 1.5 times higher. The continuous increase of As content at the TPP site by 1.1-1.4 times in comparison with control was detected. The main arsenic pollution sources are the high-temperature oil and coal burning, as well as the operation of power plants [289]. At the BG site (except the first month) and at the AS site the As content was lower than in the control sample. A significant increase of As in the first month at the BG site (1.7 times) can be connected with its accumulation from soil particles. It should be mentioned that the trend of As and Ni accumulation in moss samples was approximately the same indicating a common source of pollution.

The significant increase of Sb at TPP site (8.3-33 times) can be explained by coal burning and traffic emissions, a key tracer of non-exhaust traffic, i.e., deterioration of tires, brakes, engines and vehicle components [290]. High accumulation of Sb at the BG (3-10 times) and AS (15-43 times) sites could be associated with transport and vehicle components as well as its natural release from the earth's crust [286].

As it was previously mentioned all three exposure sites are surrounded by the road network, which led to the accumulation of other elements, for example, Pb in the exposed moss samples. Continuous accumulation of Pb moss at all three sites indicates on use of lead-loaded gasoline in the RM. The emissions from traffic, besides Pb, contain many toxic heavy metals such as Cd, Sb, Ba, and Zn. Lead content in moss exposed at TPP increased 9.8-15 times, at BG site 5-14 times, and at AS site 6-17 times [286].

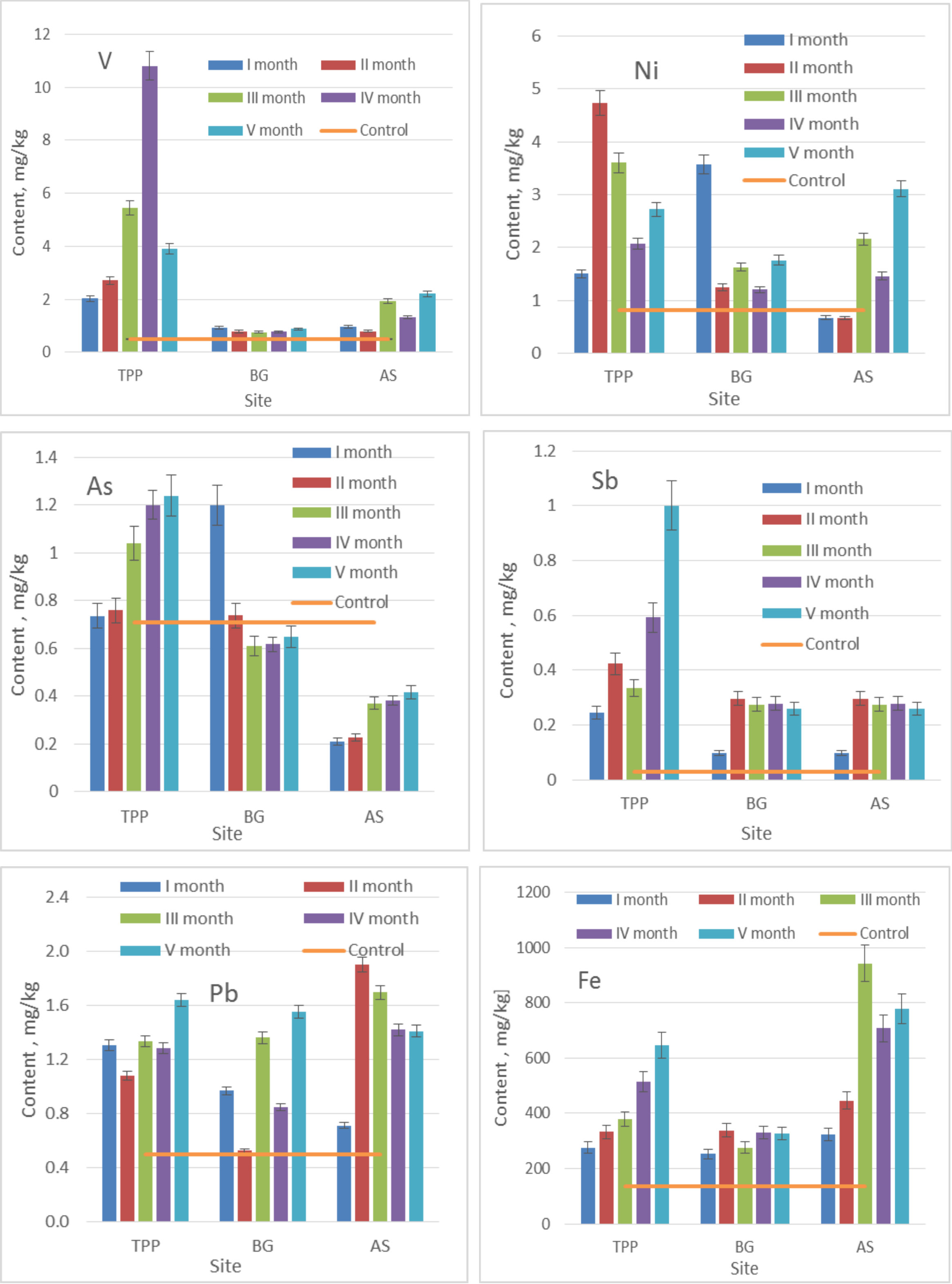


Fig. 3.8 Concentrations of the elements in moss bags exposed for 5 months at three sites: TPP, BG, and AS

Iron is generally considered as typical geological marker element. However, in urban areas, it often originates from the metallic wear parts of vehicles, industrial activity, and road dust re-suspension [96]. The highest accumulation of Fe was noticed at AS site 7 times higher in comparison with control, at TPP the increase was 2-5 times higher and 2-3 times higher at the BG site [286].

Continuous increase of Cu and Cr content at the TPP site was observed: 4-14 times and 2-3 times in comparison with control, respectively. At AS site the maximum amount of both elements was accumulated in January (4 mg/kg of Cr and 8.6 mg/kg of Cu), then their content was decreased. Chromium is emitted in the atmosphere due to coal and oil combustion, especially from diesel-fed vehicles, which refuses incineration. The main sources of Cu in atmosphere are diesel engines, unleaded gasoline, Cu-containing fungicides, and electroplating materials [45]. At BG site Cr content increased by 1.5-3 times, while Cu by 6-9 times.

A sudden decrease in the accumulation of some elements can be explained by the rapid decrease of temperature in the exposition period. The occurrence of ground frost could lead to the freezing of the moss material and thus cell walls rupture causing the leakage of already accumulated elements [291]. The statistical analysis data (min, max, mean, median) and relative accumulation factors (RAF) values are summarized in Table 3.7 [286].

Vanadium and nickel are two trace metals emitted in large amounts during the oil combustion [4,96]. The elements such as Sb, As, U, and Cr are considered as indicators of emission from fossil fuel combustion processes. Besides the fuel combustion the vehicles are important source of Ca, Fe, Co, Zn, Mo, Sb, and Ba [96,292].

The thermal power plant zone is also surrounded by the roads with intensive traffic. Road traffic is one of the primary sources contributing to total PM mass concentrations in urban areas. Thus, the transport can be considered as a second air pollution source in the TPP area. Some of the elements in moss, namely La, Ce, Hf, and Th may originate from the re-suspension of soil and road dust.

According to the RAF values, at BG site mosses mainly accumulated La (10.6) and Mg (10.3), then in descending order Sb > Th > U> Sc > Hf > Ba > Na> Fe>Ba > Ni. For the rest of the elements, RAF value was lower than 1.0 [286]. The main sources of the abovementioned elements are soil and road dust (Na, Mg, Ba, Hf, Th, Sc, U, and Sr) [293] and leaching of living or dead tissue of other plants (Mg) [263]. The Botanical Garden is situated in the vicinity of the city and is surrounded by the national roads. The main road, which connects the city with the international airport Chisinau passes close to BG.

Table 3.7 Min, max, mean, median (in mg/kg) and RAF values of 30 elements as determined by NAA and AAS techniques

Site	TPP				BG				AS			
Element	**Min**	**Max**	**Md**	**RAF$_{md}$**	**Min**	**Max**	**Md**	**RAF$_{md}$**	**Min**	**Max**	**Md**	**RAF$_{md}$**
Na	269	2720	608	3.5	258	673	348	1.6	217	1530	786	4.9
Mg	n.d.	1040	0	–	887	1120	945	10.3	639	1170	949	10.3
Cl	394	2430	568	-0.02	157	559	376	-0.4	171	1360	740	0.3
K	2440	6890	5020	-0.5	2750	4400	3470	-0.7	816	3880	2330	-0.8
Sc	0.057	0.174	0.09	2.7	0.0	0.08	0.08	2.2	0.1	0.3	0.2	6.3
Ca	2980	9680	6500	1.4	4180	5880	4910	0.8	6310	11000	10700	3.0
Ti	31	110	72	-0.08	0	39	7.7	0	-1	32	96	63
V	2.0	10.8	3.9	6.2	0.8	0.9	0.8	0.5	0.8	2.2	1.3	1.4
Cr	0.7	2.3	2.0	1.6	1	2.05	303	0.4	1.9	4.0	2.9	3.0
Mn	239	390	295	0.2	232	357	1.6	0.2	192	359	264	0.1
Ni	1.5	4.7	2.7	2.4	1.2	3.57	327	1.0	0.7	3.1	1.5	0.8
Fe	276	646	378	1.8	253	338	0.3	1.4	324	942	707	4.3
Co	0.3	0.5	0.3	1.3	0.19	0.27	49.6	0.7	0.30	0.57	0.53	2.7
Zn	43	88	54	1.0	44.3	54.5	0.7	0.8	46	67	61	1.2
As	0.7	1.2	1.0	0.5	0.61	1.2	3.9	0	0.2	0.4	0.37	0
Br	3.5	7.1	5.9	0.7	3.17	4.38	16.8	0.1	5.0	9.5	6.9	1.0
Sr	10	35	24	2.9	11.2	25.5	16.1	1.8	24	52	41	5.8
Rb	11	31	26	-0.55	12	21	17	16	-0.7	3.2	16	10.4
Mo	0.11	0.22	0.17	-0.15	0.09	0.13	0.12	0.12	-0.4	0.1	0.3	0.2
Sb	0.25	1	0.4	13.5	0.10	0.30	23.3	8.4	0.5	1.3	0.9	29
Ba	18	29	28	1.8	14.9	26.9	0.3	1.3	16	31	27	1.7
La	0.2	0.6	0.3	11.3	0.18	0.34	0.5	10.6	0.3	0.7	0.6	29
Ce	0.3	1.0	0.7	1.4	0.26	0.56	0.0	0.5	0.3	1.5	1.0	2.4
Hf	0.03	0.11	0.05	2.7	0.03	0.07	0.1	1.7	0.04	0.14	0.09	5.2
Th	0.04	0.16	0.07	2.9	0.04	0.09	0.06	2.5	0.07	0.17	0.17	8.6
U	n.d.	0.06	0.03	3.1	0.01	0.03	3.6	2.3	0.05	0.15	0.08	9.2
Cu	2.5	6.9	4.2	0.3	3.37	3.85	1.0	0.1	4.9	8.6	5.4	0.7
Pb	1.1	1.6	1.3	1.6	0.53	1.55	0.2	0.9	0.7	1.9	1.4	1.8
Cd	0.2	0.3	0.2	0.6	0.17	0.26	1.1	0.9	0.14	0.19	0.16	0.5

Thus, the transport can be considered as the main sources of Ni, Fe, Sb, and Cd. The elements such as Fe, Cu, Ba, Pb, and Zn, have been observed in particles related to brake linings, while Cd, Cu, and Pb are also utilized in tire manufacturing and thus may be emitted from tire-pavement abrasive emissions [293].

At the AS site, the highest accumulation of Sb and La was observed, RAF values constituting 29 for both elements. The main source of La is considered dust (mainly soil), while Sb can be emitted also by transport and industrial activity. The AS is located in the city center, thus the RAF values were higher than one for elements characteristic for the road traffic Ca, Cr, Fe, Co, Zn, Br, Sb, and Ba [286]. The increase of Fe, Cr, Sc, Th, and U content is also an indication of the input of terrigenous dust to the moss [294].

The RAF values for Pb were higher than one for all three places of samples exposure. The main source of Pb emissions is the use of leaded gasoline combustion [292]. For the physiologically active elements, the negative RAF values were obtained: Cl (TPP and BG), K, and Rb (all sites). The decrease in their concentration can be explained by their participation in ion-exchange. The process of cation sorption, based on the ion exchange between the moss thallus and a solution that wets the thallus, is the most known [287].

To highlight any association of chemical elements as well as to decrease the number of variables for the obtained data the FA was used. The results of FA are presented in Table 3.8. Three factors explaining a total of 81% of the variability were identified in the data set. The first factor, which elucidates most of the variance (49%), has high loadings for Cr (0.87), Fe (0.94), Co (0.92), Sr (0.92), Sb (0.96), U (0.89), and Cu (0.90).

Table 3.8 Factor loadings after Varimax rotation for the elements determined in exposed moss samples

Element	**F1**	**F2**	**F3**	Element	**F1**	**F2**	**F3**
Al	0.43	**0.87**	0.05	**As**	-0.27	**0.83**	0.19
Cr	**0.87**	0.10	0.05	**Sr**	**0.92**	-0.06	0.06
V	0.15	**0.86**	0.13	**Sb**	**0.96**	0.08	-0.10
Ni	-0.04	**0.43**	0.56	**U**	**0.89**	-0.17	-0.12
Fe	**0.94**	0.13	0.10	**Cu**	**0.90**	0.10	-0.08
Co	**0.92**	0.19	0.11	**Pb**	**0.70**	0.08	0.27
Zn	0.48	0.17	**0.76**	**Cd**	-0.14	0.05	**0.93**
Prp.Totl	0.49	0.18	0.14				

This factor represents a combination of geogenic and anthropogenic associations of elements. Iron (Fe), Sr, Sb, and U are typical crustal elements, and their content in the mosses can be associated with mineral particles released into the atmosphere mainly by wind erosion. However, the high values for Cr, Fe, Cu, and Pb also indicate the influence of anthropogenic sources, mainly transport. The second factor, with 18% of the total variance, shows high loading for Al, V, and As pointing to the contribution from thermal power plants [286]. The third one accounts for 14%, with the pronounced contribution from Cd (0.93) and Zn (0.76), which might be attributed to traffic [292].

3.2.2 Health risk assessment evaluation

As in case of passive biomonitoring, the risk of human exposure to the determined levels of elements content was assessed. The ADD values were calculated according to the formulas presented in Chapter 1 (Section 1.3.4) and are listed in Table 3.9.

The values for ADD of ingestion for all metals were higher than those of inhalation and dermal absorption. The values of HQ and HI, calculated according to the formulas given in Chapter 1 (Section 1.3.4) for ten elements for three sites of exposure are presented in Table 3.10 for the three daily doses. The values of reference doses (RfD) were taken from [251,282] studies.

The highest average values of HQ were found to be through the ingestion pathway followed by the dermal contact and inhalation. As in the case of passive biomonitoring for all elements, except of V, HQ and HI values were lower than 1.0, indicating that there are no significant health risks from these metals.

Table 3.9 The daily dose in three models: ingestion, inhalation and dermal absorption

	Site	**Ni**	**Sb**	**As**	**V**	**Pb**	**Fe**	**Cu**	**Cr**
Mean content, mg/kg	TPP	2.57	0.52	0.88	4.97	1.33	429	4.25	1.69
	BG	1.70	0.24	0.54	0.82	1.05	305	3.59	1.21
	AS	1.48	0.24	0.32	1.44	1.43	639	6.50	2.90
ADD_{ing}	TPP	4.4E-05	8.9E-06	1.5E-05	8.5E-05	2.2E-05	7.3E-03	7.2E-05	2.9E-05
	BG	2.9E-05	4.1E-06	9.3E-06	1.4E-05	1.8E-05	5.2E-03	6.2E-05	2.1E-05
	AS	2.5E-05	4.1E-06	5.5E-06	2.5E-05	2.4E-05	1.1E-02	1.1E-04	5.0E-05
ADD_{inh}	TPP	6.7E-10	1.3E-10	2.3E-10	1.3E-09	3.5E-10	1.1E-07	1.1E-09	4.4E-10
	BG	4.4E-10	6.3E-11	1.4E-10	2.1E-10	2.7E-10	7.9E-08	9.3E-10	3.2E-10
	AS	3.8E-10	6.3E-11	8.4E-11	3.7E-10	3.7E-10	1.7E-07	1.7E-09	7.5E-10
ADD_{derm}	TPP	2.2E-06	4.5E-07	7.5E-07	4.3E-06	1.1E-06	3.7E-04	3.6E-06	1.5E-06
	BG	1.5E-06	2.1E-07	4.7E-07	7.0E-07	9.0E-07	2.6E-04	3.1E-06	1.0E-06
	AS	1.3E-06	2.1E-07	2.8E-07	1.2E-06	1.2E-06	5.5E-04	5.6E-06	2.5E-06

At the same time, it should be mentioned that HI and HQ values higher than 1.0 for V were only at the TPP site, confirming that the combustion of fuel (coal, oil) is the main source of air pollution with V in Chisinau. The orders of hazard indexes of metals were V>Sb>As>Cr>Fe>Pb>Ni>Cu at TPP site, V>Sb>As>Cr>Fe>Pb>Cu>Ni at BG site and V>Sb>As>Cr>Fe>Pb>Cu>Ni at AS site. Thus, it can be seen that V, Sb, and As are the main air pollutants in Chisinau.

Table 3.10 Average HQ and HI values for ten heavy metals based on three daily dose models *via* the ingestion pathway, dermal contact pathway, and inhalation pathway

	Metal	**Ni**	**Sb**	**As**	**V**	**Pb**	**Fe**	**Cu**	**Cr**
RfD	RfD_{ing}	0.02	3.5E-04	3.0E-04	7.0E-05	3.52E-03	0.7	0.037	05.0E-03
	RfD_{inh}	0.02	-	3.0E-04	-	3.52E-03	-	0.0402	2.86E-05
	RfD_{derm}	0.001	0.8E-05	0.0003	9.0E-03	5.2E-045	-	1.9E-03	2.5E-04
HQ_{ing}	TPP	2.21E-03	2.54E-02	5.03E-02	1.22E+00	6.51E-03	1.05E-02	1.97E-03	5.81E-03
	BG	1.46E-03	1.19E-02	3.10E-02	2.01E-01	5.14E-03	7.46E-03	1.66E-03	4.16E-03
	AS	1.27E-03	1.19E-02	1.84E-02	3.53E-01	6.99E-03	1.57E-02	3.01E-03	9.95E-03
HQ_{inh}	TPP	3.34E-08	-	7.62E-07	-	9.81E-08	-	2.75E-08	1.54E-05
	BG	2.21E-08	-	4.70E-07	-	7.75E-08	-	2.75E-08	1.54E-05
	AS	1.92E-08	-	2.79E-07	-	5.20E-07	-	4.20E-08	2.64E-05
HQ_{derm}	TPP	2.21E-03	5.56E-02	2.51E-03	4.74E-04	2.17E-03	-	1.92E-03	5.81E-03
	BG	1.46E-03	2.59E-02	1.55E-03	7.83E-05	1.71E-03	-	1.62E-03	4.16E-03
	AS	1.27E-03	2.59E-02	9.20E-04	1.37E-04	2.33E-03	-	2.93E-03	9.95E-03
HI	TPP	4.41E-03	8.11E-02	5.28E-02	1.22E+00	8.68E-03	1.05E-02	3.89E-03	1.16E-02
	BG	2.92E-03	3.78E-02	3.26E-02	2.01E-01	6.86E-03	7.46E-03	3.28E-03	8.32E-03
	AS	2.54E-03	4.96E-02	1.93E-02	5.55E-01	9.32E-03	1.57E-02	5.95E-03	1.83E-02

3.3. Conclusions to Chapter 3

1. The moss biomonitoring combined with the analytical techniques and statistical analysis proved to be an effective approach to assess the air quality in the Republic of Moldova.
2. The neutron activation analysis along with the atomic absorption spectrometry allowed to determine 30-40 elements in mosses collected in the passive and active biomonitoring studies.
3. In passive biomonitoring factor analysis revealed the association between three groups of elements, indicating their geogenic, vegetative and anthropogenic origin. According to the results obtained, the main air pollution sources in the Republic of Moldova can be considered the suspended soil particles, transport, thermoelectric plants and industrial activity.
4. The values of ecological indices point at the moderate to severe air pollution, which is mainly localized around the major urban and industrial centers – the municipalities of Chisinau and Balti.
5. Comparing the concentrations of elements in moss samples with values considered as toxic for organisms biotests it can be concluded that determined levels of concentrations are not toxic for mosses but can provoke toxic effects in lichens.

6. In the active biomonitoring study, the accumulation of such pollutants as V, Cr, As, Ni, Cu, Fe, Sb at all exposure sites was noticed, supporting the hypothesis that the primary sources of air pollution in Chisinau are thermal power plants, vehicles and industry. The most pronounced accumulation of elements took place in the mosses exposed at the thermal power plant.
7. Assessment of the determined elements impact at the given concentrations on the environment and human health has shown that V is the only element which can present risk, especially for children’s health. Thus, emissions of V require special attention of the national authorities. It should be mentioned that the content of V in Moldova was the highest among the European countries, which participate in biomonitoring studies.
8. The results obtained in the thesis were included, for the first time, in the Atlas “Mosses as biomonitors of air pollution: 2015/2016 survey on heavy metals, nitrogen and POPs in Europe and beyond. Report of the ICP Vegetation Moss Survey Coordination Centre, Joint Institute for Nuclear Research, Dubna, Russian Federation” and will serve as the basis for further moss biomonitoring studies.

4. ECOLOGICAL CHEMISTRY OF SOIL AND FOODSTUFF PRODUCTS

In spite of the fact that chemical elements are naturally present in the soil, human activity can significantly increase their concentrations in soil and consequently in plants and other products consumed by humans. Thus, any environmental disturbance in soil ecosystems finds the way to reach man via food.

4.1 Analysis of the elemental composition of soil and wine from Cricova and Romanesti and their classification according to provenance

The RM is a well-known producer of world-class wines, which play a critical role in the economy of the country. Moldova holds a rich tradition in winemaking dating back thousands of years [295]. The main wine-growing zones in Moldova are Balti (Northern zone), Codru (Central zone), Purcari (South-Eastern zone), and Cahul (Southern zone), where the soil consists of typical for Eurasian steppe chernozem.

Wine is a beverage with a complex matrix, which, besides water, sugar, and alcohol, contains a great variety of organic as well as inorganic components. Wines typically contain the major elements such as Na, K, Mg, Ca ($c > 10$ mg/L), micro-elements such as Fe, Cu, Zn, Mn, Pb ($c > 10$ μg/L), and trace elements such as Cr, As, Cd, and Ni ($c < 10$ μg/L) [27]. Consumption of wine in moderate quantities can contribute to the daily requirements of the human organism for essential elements, such as Ca, Cr, Co, K, Se, Zn. At the same time presence of Al, Cu, K, Fe, Mn, Zn in excessive amounts can have a negative effect on organoleptic properties of the wine, contributing to haze formation and even color, aroma, and taste defects, and is also directly related to its destabilization and oxidative evolution, while As, Cd, and Pb are toxic even in ultra-small amounts [139,296]. Besides enological and toxicological implications, the elemental analysis is increasingly becoming an area of interest for chemical differentiation of wines and identification of their geographical origin [296].

Twenty-four samples of red and white wine obtained directly from two major wine-producers: S.A. Romanesti and S.A. Cricova (Fig. 4.1) were analyzed to determine their elemental composition as well as to use these data in an attempt to differentiate them by region and by types. Both vineyards belong to the Central (Codru) wine region. From the Romanesti company 13 samples were analyzed: Cabernet, Regent, Pinot Noir, Nero, Syzar, Merlot, Malbec, Sauvignon, Riesling, Sauvignon, Pinot Gris, Muscat, UniBlanc and from Cricova company - 11 samples: Pinot, Chardonnay, Cabernet, Chardonnay, Pinot Frank. For NAA, wine samples were evaporated in ultrapure quartz vials at 80^0C for 24 hours and then dried in an oven until constant weight [297,298].

Fig. 4.1 The schematic map of Moldova showing the location of Romanesti and Cricova vineyards

Wine multi-elemental composition is strongly influenced by the solubility of inorganic compounds of the soil and in principle, the pattern of wine will reflect the geochemistry and the level of soil pollution [296]. Thus, 18 samples of soils were collected from the corresponding vineyard and following the grapevine type at depths varying from 10 to 20 cm to avoid the topsoil pollution arising from the surrounding environment. The samples were dried at 40 ºC and homogenized in the agate mortar [297,298]. The samples preparation for NAA and irradiation procedure are described in Chapter 2 (Section 2.2).

4.1.1 Major and trace element distribution in the soil of two Moldavian vineyards

An important source of metal content in wine comes from the vineyard soil *via* grapevine roots, being influenced by the soil geochemistry and vine rootstock, among the other factors [32]. Applying NAA it was possible to determine 35 elements in analyzed soil: seven major, rock-forming elements: Na, Mg, Al, Ca, K, Mn and Fe and 28 trace elements: Sc, Ti, V, Cr, Co, Ni, Zn, As, Br, Rb, Sr, Zr, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Gd, Tb, Tm, Yb, Hf, Ta, Th and U [299]. The procedure of samples preparation for analysis is given in Chapter 2. The data obtained, as well as corresponding data for the Upper Continental Crust (UCC) [300], Average

Soil (AS) [301] are presented in Table A1.3 (Annex). Principal Component Analysis (PCA) performed to differentiate soil samples from two vineyards showed no significant differences between their chemical composition (Fig. 4.2). The similarity in the elemental composition of soil can be explained by the vicinity of two vineyards, which are located at a distance of about 15 km from each other. Since the elemental composition of the soil was similar in further discussion the average values of all analyzed soils are given [299].

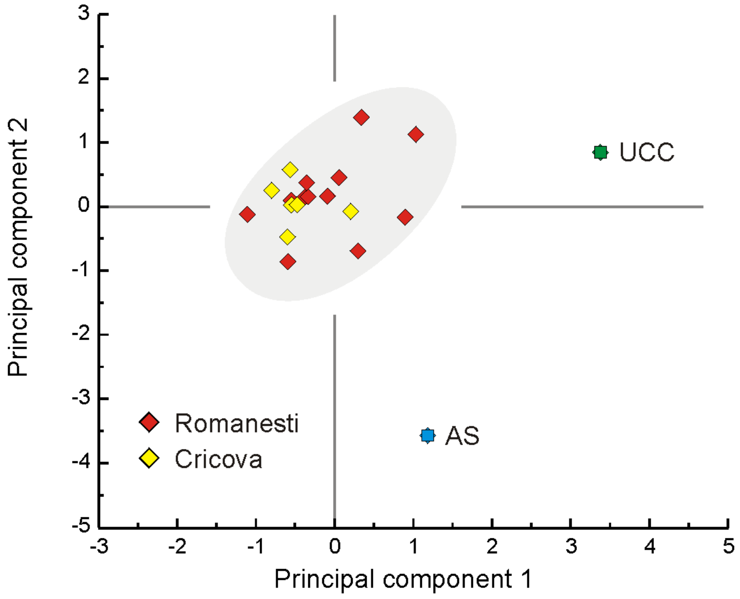


Fig. 4.2 The bi-plot illustrating the homogeneity of the soil samples

Comparison of the content of major elements with those of the UCC and AS (Fig. 4.3) showed that the Na content in the analyzed soil was approximately 6 times lower than UCC and 2-3 times lower than AS. The content of Mg and K was higher than the corresponding UCC and AS values. Aluminum was almost on the level of UCC and AS. Calcium content in several soil samples from Romanesti vineyard was on the level of AS, while for the main part of samples collected on both vineyards it was significantly lower than the literature values. Titanium content was almost the same as the UCC and AS values, while Fe content was on the level of UCC and considerably higher than AS. Increased content of Mg in the soil suggests the presence of dolomite [299].

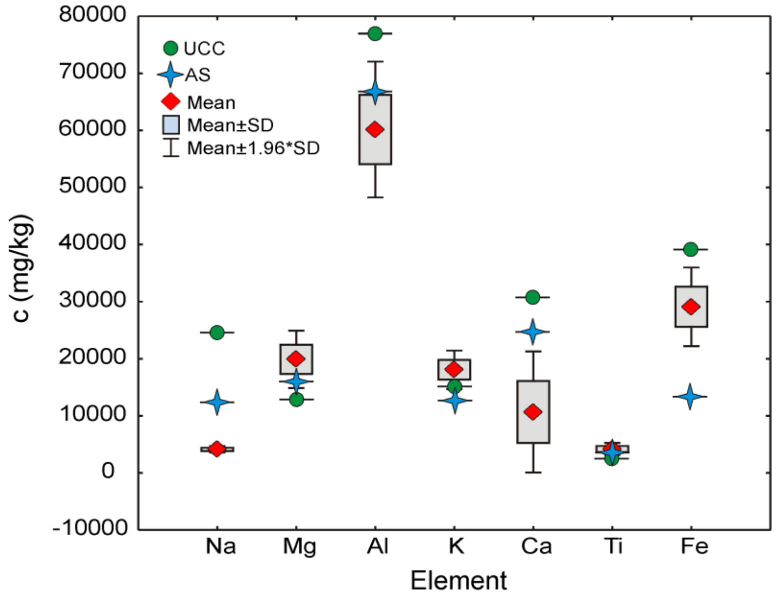


Fig. 4.3 The box-and-whiskers plot illustrating the content of major elements (in mg/kg) of both vineyards soil

The content of the 28 trace elements normalized to UCC is illustrated in Fig. 4.4. The content of the main part of elements, except As, Br, Sr, Zr, Sb, and Hf was on the level of UCC values.

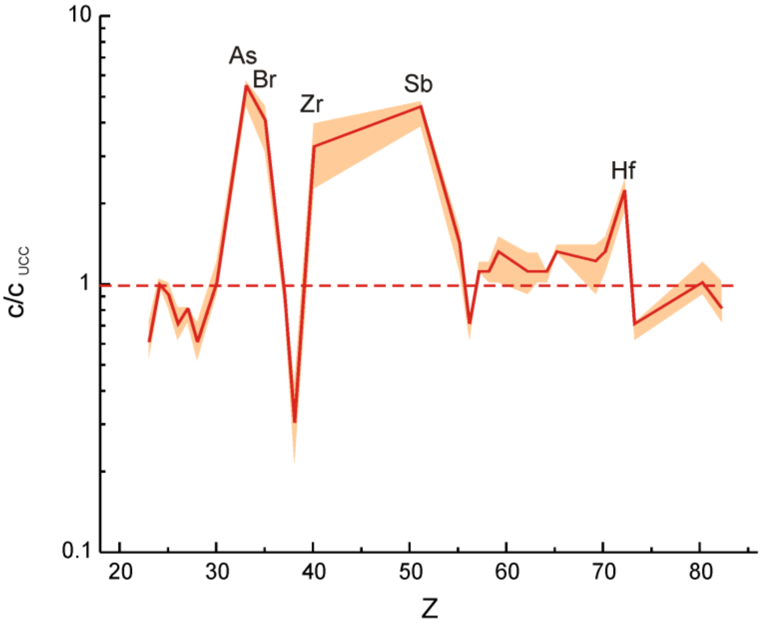


Fig. 4.4 The distribution of the trace element contents normalized to UCC

Elemental analysis of the soil provides the important information about: (i) the soil geochemistry and (ii) possible anthropogenic contamination [299].

Strontium and Rb are two alkaline elements whose ratio can give an important information about the soil constitution. The ratio between Rb and Sr is useful in identifying the material provenance and indicating the intensity of chemical weathering. Strontium, due to its ionic radius close to Ca substitutes it in the Ca-rich minerals such as limestone, dolomite, plagioclase, hornblende, etc. Rubidium mainly coexists with Na and K – rich minerals such as plagioclase, K-feldspars, different variety of micas, etc. Ca-bearing minerals are easy to breakdown compared with the K-bearing minerals, respectively the K-Rb pair and Ca-Sr pair are easy to fractionate during natural processes [299]. The chemical weathering can leach the Ca-Sr much easier than K-Rb, which leads the residue enriched in K-Rb but depleted in Ca-Sr [302]. The higher the Rb/Sr ratio is the higher is the degree of soil weathering. Rb/Sr ratio calculated from the values presented in Table A1.3 of the Annex resulted in the value equal to 1.11 ± 0.34, significantly higher than the UCC one of 0.26 [303]. A high Rb/Sr ratio indicates the significant chemical weathering which, in its turn suggests presence of mature soil.

The presence of Zr and Hf is mainly due to the mineral zircon, which due to its resilience to weathering and lack of reactivity persists in various sedimentary deposits including soils. For this reason, the Zr content is regarded as a proxy for weathering and material recycling (McLennan et al, 1993). The Hf/Zr ratio of 1.43 ± 0.04 is very close to 2.07 reported for the UCC (Rudnick and Gao, 2004) (see Table A1.3, Annex).

One of the most commonly used approaches in using of the trace element geochemistry for provenance studies is to plot the concentrations of three geochemically distinct elements in ternary diagrams. In the present study, to get more information regarding soil origin the relative distribution of three incompatible and immiscible elements Sc, La, and Th [304] were reproduced by the means of a ternary discriminating diagram (Fig. 4.5). All the points are concentrated around the UCC which indicates a primary continental origin of the soil material. La/Th radio for soil from Romanesti was 3.0±0.25 and for soil from Cricova 2.82±0.23 [299]. The values obtained fall into the range of AS and UCC values: 2.52 and 2.73, respectively. The ratio of La/Th in the range 1.7 - 10.6, suggests a mixed source of soil [305].

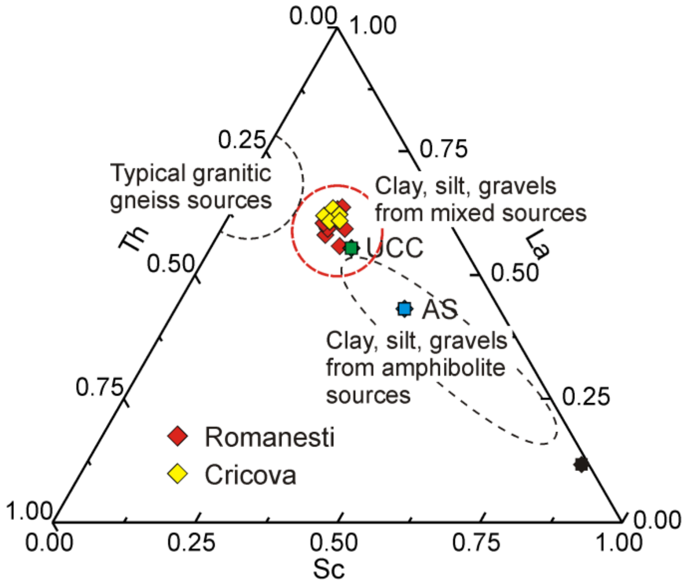


Fig. 4.5 The Sc-La-Th discriminating ternary diagram

The same conclusion is sustained by the chondrite-normalized REE diagram (Fig. 4.6) which shows a good similarity with the UCC distribution [299]. Chondrite-normalized data were obtained by dividing the data obtained in the present study into the chondrite values presented in [306].

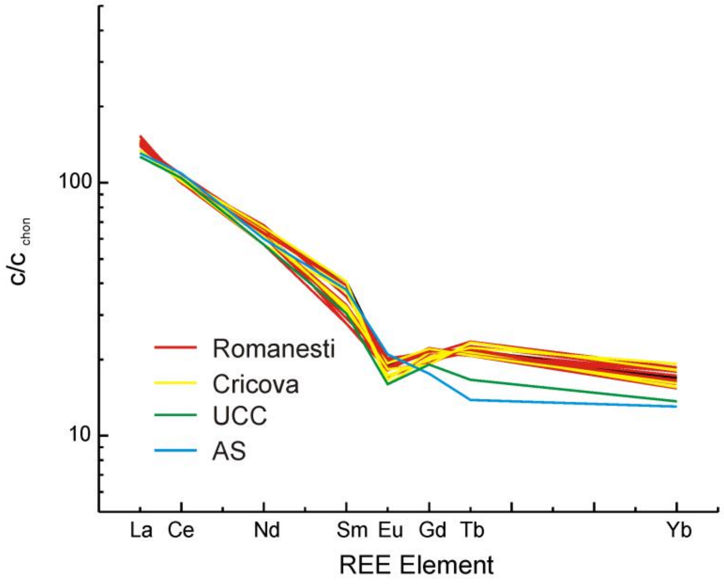


Fig. 4.6 Chondrite-normalized REE values for studied soil samples

Eu/Eu* anomaly, which concentration in a mineral is either enriched or depleted relative to the chondrite, was calculated using the following formula:

$$Eu_{anom} = Eu/Eu^* = Eu_N(Sm_N+Gd_N)^{0.5}$$

where N refers to chondrite normalization of concentrations. If Eu/Eu* is > 1 the anomaly is positive (Eu concentration in the mineral is enriched relative to the other rare-earth elements) and the if the ratio is <1 the anomaly is negative (Eu is depleted relative to the other REEs). In the present study, the Eu/Eu* anomaly presented in all soil samples is characterized by the average value of 0.46 ± 0.06, very close to the UCC and AS values of 0.45 and 0.5 respectively [299].

To evaluate the pollution of soil collected in vineyard area the data obtained for elements considered as possible environmental pollutants, Cr, Mn, Co, Ni, Zn, Sb, and As, were compared with the maximum admissible concentrations established by the National Authorities of Moldova [273] and neighboring counties: Romania [307] and Russian Federation [308] (Table 4.1).

As can be seen from Table 4.1, except for As, whose content in the soil of all the ineyards was about 8 mg/kg, the content of all possible industrial pollutants felt in the limits established for the RM and other countries.

Table 4.1 The average values of the content of elements in the analyzed samples well as the corresponding maximum admissible limits

Element	Analyzed soil	Moldova [273]	Russian Federation [308]	Romania [307]
	Concentration, mg/kg			
Cr	91 ± 9	90	6*	30 - 100
Mn	678 ± 56	1500	1500	900 - 1500
Co	13 ± 1	--	5	15 - 30
Ni	35 ± 7	45	20 - 80	20 - 75
Zn	72 ± 10	68	55 - 220	100 - 300
Sb	0.9 ± 0.1	-	4.5	5-12.5
As	8.7 ± 1.1	5.6	2 - 10	5 - 12.5

According to Jigau et al. [273] the increased content of As represents a general peculiarity of Moldavian soils and is not a result of a certain industrial process. According [309] As content in European soil range between < 7.5–20 mg/kg, with a median of 6 mg/kg. Besides the natural sources of arsenic, there are a large number of industrial and agricultural sources of arsenic in the soil such as coal fly ash, glass manufacturing, pesticide application, wastewater sludge, pharmaceutical waste, and phosphate fertilizers [310].With respect to the UCC [303] and AS [301] Co, Ni, and Zn content in the analyzed soils were close to the abovementioned values. Antimony also considered a toxic element had an average content of 0.9 ± 0.1 mg/kg, higher than the UCC value of 0.2 mg/kg and 0.7 mg/kg for AS. At the same time, this value was lower than officially established maximum admissible concentrations. Antimony has a wide range of uses including the manufacture of semiconductors, diodes, flameproof retardants, lead hardeners, batteries, small arms, tracer bullets, automobile brake linings, and pigment [311]. Bromine (Br) is the last element whose presence, in high amounts, could be related to human activity. In this case, the Br content of 8 ± 2 mg/kg, although overpass four times the UCC value of 2 mg/kg [299], is still compatible with the world soil values ranging between 7.9 and 130 mg/kg for different types of soil [116] and was significantly lower than the 50 mg/kg, the alert threshold as defined in Romanian Norms. The main anthropogenic source of Br is its release in automobile exhaust, but some Br used agriculturally as a soil fumigant (methyl bromide) and as a component of K fertilizers may also add to the total soil Br [116].

Pollution indices are widely considered and useful tool for the comprehensive evaluation of the contamination degree. *EF*, I_{geo}, and PLI (Table 4.2) were calculated using the formula presented in Chapter 1.

Table 4.2 Average numerical values of the EF, I_{geo}, and PLI of the investigated vineyards soils

Element	EF ± SD	I_{geo} ± SD	PLI± SD
Cr	1.29 ± 0.12	-0.62 ± 0.15	
Mn	1.18 ± 0.13	- 0.75 ± 0.12	
Co	1.01 ± 0.06	- 0.97 ± 0.13	
Ni	0.84 ± 0.10	- 1.24 ± 0.24	
Zn	1.33 ± 0.14	-0.58 ± 0.22	
As	7.14 ± 0.47	1.85 ± 0.16	
			1.15 ± 0.11

A common approach to estimate the anthropogenic impact on soil is to calculate a normalized *EF* for metal concentrations above uncontaminated background levels. To identify anomalous metal contents, the geochemical normalization of the metal data to conservative elements such as Al or Fe is employed. In the present study, Fe has been used for the calculation of the *EF*. Iron usually has a relatively high natural concentration and is therefore not expected to be substantially enriched from anthropogenic sources [253]. UCC values were used as reference values in this study because the regional geochemical background values for these elements are not available. Calculated EF values indicate the minor soil contamination. The I_{geo} values for all elements, except As, were less than 1.0, indicating an uncontaminated environment. The value obtained for As (1.85 ± 0.16) is characterized as a moderately contaminated medium. PLI value (1.15 ± 0.11) suggests weak soil pollution [299].

The high content of chemical elements can have also a negative impact on soil organisms. Thus, according to Bekuzarova et al. [312] at As content in soil of 25 mg/kg nodule bacteria had lost the amount of leghemoglobin, that lead to the bacteria disruption. At As concentration in soil of 13.2 mg/kg the considerable disturbance in the ground beetles *Calosoma chlorostictum*, such as a rupture and distortion of microvilli, destruction of the columnar and regenerative cells, large separation between epithelial cells, and stretching of the cellular axis, cytoplasmic vacuolization, disruptions, and vacuolation of mitochondria were detected [313]. The mortality of earthworms adopted as standard organisms for ecotoxicological testing by the European Union was higher than 50 % in soils containing 300 mg As/kg [314]. The reproduction of earthworm *Eisenia andrei* was significantly reduced at concentrations of 100 mg Cr/kg, while growth was only significantly reduced at the highest concentration tested (1000 mg Cr/kg). Zinc significantly reduced the reproduction at soil concentrations of 560 and 1000 mg/kg, and induced the production of malformed cocoons [315]. Marked cellular damage to the spermatozoa of worms exposed to Mn in concentration of 4.3 μmol/g, included the breakage and loss of nuclear and flagellar membranes, thickening of membranes, malformed acrosomes and loss of nuclear material [316]. Nickel caused a significant toxic effect on earthworm *Eisenia veneta* at soil concentrations above 85 mg/kg. Survival of adults was only reduced at concentrations above 245 mg/kg, while adult and cocoon wet weight were not affected by soil nickel concentrations up to 700 mg/kg [317]. The lethal effect of Sb on earthworm *Perionyx excavates* was observed only at Sb concentrations higher than 1000 mg/kg [318].

Metal concentration determined in the Moldavian soils were significantly lower than values which can provoke the negative effect in living organisms serving as bioindicators of soil quality, consequently they in the determined range they will not upset the balance in the environment.

4.1.2 Determination of major and trace elements in wines from Cricova and Romanesti

NAA allowed the determination of 18 elements in wine from Romanesti and 19 elements in wine from Cricova. The values obtained were comparable with the data obtained for other countries: Spain [137], Macedonia [141], Argentina [57], Croatia [58], Romania [27], Brasília [319], and Turkey [320]. The average ± standard deviation NAA data concerning both major: Na, Mg, Ca, Cl, K, Mn, Zn, and Fe and trace elements: Al, Co, Ni, As, Br, Rb, Sr, Sb, Cs, Ba, and U for the analyzed wine samples are summarized in Tables 4.3 and the whole set of data is presented in Tables A1.4 and A1.5 (Annex).

From the data presented in Tables 4.3, it can be seen that from 18 determined elements the highest values were obtained for K, its values in wines varied from 253 to 843 mg/L. The highest values were determined in Syzar and Malbec wine samples (Romanesti company). Potassium is the most abundant of the inorganic constituents of wine, comprising about 75% of total cation content of wines [105]. Its concentration in wines is influenced by soil, climate, grape variety, time of harvest, the temperature of fermentation, storage conditions, percentage of alcohol, pH, ion-exchange resins, and fining agent [321]. It should be mentioned that K content in all the white wines was lower than in red ones, which can be explained by a shorter period of white wine contact with the skins during the fermentation process. A certain amount of K can be added in wine as the result of the use of potassium metabisulfite as an antioxidant, but according to the EU Commission Regulation (EC) No. 606/2009, the maximum amount of K introduced in wine as potassium metabisulfite should be 96 and 135 mg/L for red and white wine, respectively. As both values are significantly lower than the results of our determinations, it can be supposed that the major source of K in wine is soil [297,298].

Sodium content in analyzed wine samples varied from 8 to 32 mg/L with the highest concentrations coming from Cabernet, Regent, and Uniblanc wines. The sources of sodium in wine can both natural and anthropogenic [321]. Usually, the concentration of K in grapes significantly overpass the concentration of Na, and this relationship remains relatively unaltered in wine. In the analyzed wines, concentration of Na was approximately 20 times lower than that of potassium [297,298].

Magnesium, which content in wine is affected by the grape variety, the winemaking process, wine storage, and the use of ion-exchange resins [257] in the studied wines varied between 65 and 132 mg/L [321]. Calcium and Mg are natural constituents of musts and wines, necessary for normal course of alcoholic fermentation. As main sources of Ca in wine can be listed soil, the treatment of the musts with calcium salts, and ion exchange treatment [321]. Calcium concentration in studied wines lies within the range of 29-121 mg/L [297,298].

Iron content in wines varied from 0.15 to 8.8 mg/L, with the maximum values in Cabernet, Pinot Noir, and Malbec wines (in these wines the concentration of iron was higher than 5 mg/L). Iron content in wines is partly due to the natural (soil) and partly due to the technological processes (fermentation, corrosion of wine making equipment, and steel containers used for the transfer of berries or must) [321,322].

Table 4.3 The mean experimental values (± σ) of the concentrations of 18 elements in investigated wines as determined by the NAA as well as values established by OIV [323]

Element	**Romanesti**	**Romanesti**	**Cricova**	**Cricova**	**OIV**
	red	*white*	*red*	*white*	
Concentration, mg/L					
Na	19±9	16±1	11±2	11±3	80
Mg	104±12	87±12	77±5	92±21	
Al	1.4±1	1.9±0.5	1.4±1	1.5±0.7	
K	690±80	380±230	460±80	550±100	
Ca	54±14	75±24	66±13	68±21	
Cl	14±5.9	4.5±3.7	2.3±1.15	6.3±3.2	
Mn	1.35±0.1	1.22±0.25	0.93±0.1	1±0.2	
Fe	3.5±3.3	2±1.9	1.1±0.7	0.7±0.4	
Zn	0.44±0.2	0.55±0.04	0.92±0.4	0.49±0.08	5
Rb	1.7±0.2	1.1±0.4	1.6±0.3	1.6±10.3	
Sr	1.01±0.2	0.64±0.2	0.84±0.3	0.75±0.15	
Concentration, µg/L					
Co	6.3±3.6	9.8±1.4	4.2±1.6	3.2±0.7	
Ni	39±10	32±11	18±9	20±5	
As	-	-	0.29±0.2	0.3±0.1	200
Br	280±90	92±92	69±25	104±44	1000
Sb	0.48±0.2	0.41±0.13	0.59±0.4	0.53±0.26	
Cs	3.3±0.6	3.1±2.5	7.5±2.8	6.1±1.1	
Ba	276±270	110±30	70±41	87±27	
U	0.12±0.06	0.20±0.06	0.13±0.04	0.13±0.01	

OIV rules do not define limits on the concentration of iron in wines. However, at iron concentrations greater than 10 mg/L, Fe(III) creates an insoluble suspension with tannin and phosphates which are known as hazes or “casses” [296]. Manganese compounds play an important role in the production and storage of wine since they are involved in the redox processes during the wine maturation [324]. The Mn amount in wines depends on several factors: soil composition, the subsequent absorption by the vine, atmospheric contamination, use of herbicides, the process of winemaking, and fining agents added [325]. In analyzed wines, its concentration varied from 0.7 to 1.6 mg/L with the highest value for Pinot Noir (Romanesti). Zinc effect on wine depends on its concentration, thus, thus low Zn concentrations in wines play a vital role during the fermentation, whereas its high concentrations negatively influence the organoleptic properties of wine. The data obtained for Zn does not exceed values recommended by OIV and lies between 0.2 and 1.3 mg/L [297,298]. Traces of Zn are naturally presented in must and wine. Anthropogenic sources of Zn in wine originate from fungicides, insecticides, and winemaking equipment [321,325,326]. Zinc concentrations in wine range from 0.14 to 4.0 mg/L [326]. Application of pesticides, fungicides, and fertilizers containing As, Cd, Cu, Mn, Pb, and Zn compounds leads to the increase in the amounts of these elements in soil, grape, and wine [58].

Among the microelements, whose concentration was expressed in μg/L, the most abundant were Ba and Al. Barium is the element whose content in wine is not affected by the winemaking process and it originates mostly from the soil [327]. In the analyzed wines, Ba concentration ranged from 13 to 323 μg/L. The concentration of Al in wines was in the range from 0.8 to 2.5 mg/L [297,298]. The values determined were lower than the recommended value for Al, which should be not exceed 3 mg/L [323]. The presence of Al in wines can be explained by their storage in aluminum containers. Contamination of wines with aluminum may result in their spoilage due to the haze formation and the creation of undesirable and unpleasant metallic taste [297,298,325].

Arsenic, a toxic element, was present in an insignificant concentration only in the wines from the Cricova company (0.16-0.63 μg/L). Determined values were significantly lower than limits set by OIV (0.2 mg/L) [297,298]. The concentration of arsenic in wines depends on a variety of factors including aerosol deposits on the grapes, grape variety, climatic conditions, soil composition, use of pesticides, and also the winemaking process and storage conditions [327]. If As concentration in wine is higher than 1.0 mg/L wine is considered unfit for consumption. Nickel's main sources in wine can be considered the use of stainless steel in wineries, while of Co its migration from bottles into the wine [328]. The main source of such elements Sr, Rb, U, Sb, Sm in wine is soil.

The matrix of the ANOVA Mann-Whitney U test (probability of the same median value) as well as of the Spearman's rank ρ correlation coefficient calculated on the base of the numerical data reproduced in Tables A1.4, A1.5 (Annex). The data otained data have shown that, concerning the content of all 18 elements, all the investigated varieties of wine are closer, with a probability varying between 0.718 and 0.986, the last figure as in the case of Cricova wines (Table 4.4). The same finding was confirmed by the corresponding matrix of the Spearman's rank correlation coefficient ρ whose values were greater than 0.950 at $p < 0.01$ (Table 5.4). According to the U test, the Cricova wines seem more homogenous [297]. The lower diagonal matrix element represents the U-test probabilities the element distribution has the same median while the upper diagonal consists of Spearman's rank correlation coefficients.

Table 4.4 The matrix of the ANOVA Mann-Whitney U test as well as of the Spaerman rank correlation coefficient calculated on the base of the experimental data

	Romanesti Red Wine	Romanesti White Wine	Cricova Red wine	Cricova White wine
Romanesti - Red Wine	----	0.985	0.963	0.956
Romanesti - White Wine	0.809	----	0.971	0.956
Cricova - Red Wine	0.770	0.796	---	0.975
Cricova - White Wine	0.718	0.863	0.986	----

Besides the determination of the wine elemental content, the Discriminant Analysis (AS) was used to differentiate the investigated wines by sort and by location. The results obtained are illustrated by the discriminating Root 2 x Root 1 bi-plot reproduced in Fig. 4.7.

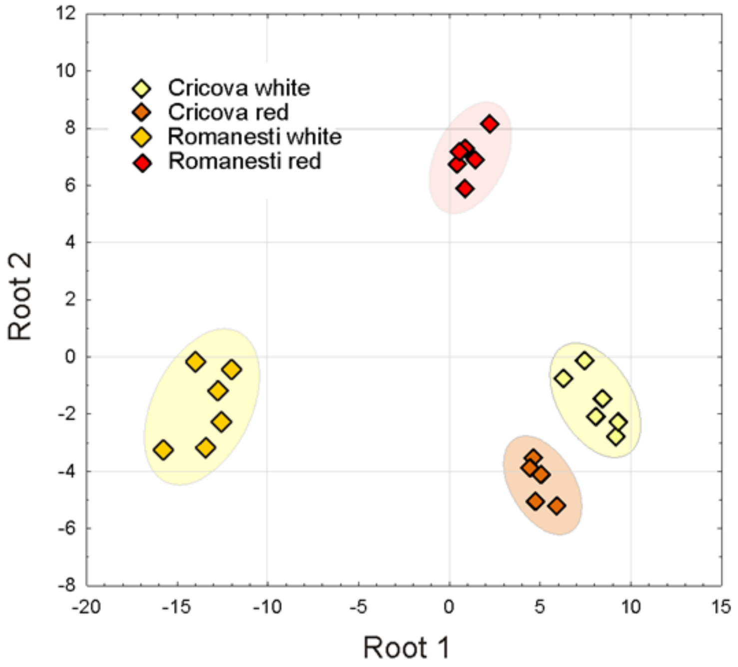


Fig. 4.7 The discriminating Root2 vs. Root 1 bi-plot illustrating the result of Discriminate Analysis of the 24 sets of wines

The Root 1 allowed the complete discrimination between the Romanesti white wine and all other ones while Root 2 better evidenced the Romanesti red wines. At the same time, from this point of view, the Romanesti wines seem to be more different between them with respect to the type while Cricova ones form a most compact group. Regardless of these considerations, the DA allowed the simultaneous discrimination of all types of wine on varieties: red and white as well as on vineyards: Romanesti and Cricova [297].

The ability for wines discriminating by the regions through their trace element patterns suggests that the elements are mainly regulated by their movement from soil to grape and them to wine. It is considered that the pattern of a wine reflects the geochemistry of the provenance soil [296].

4.1.3 The relationship between the elemental composition of soils and the corresponding wines

The number of trace elements evidenced in wine was almost twice lower than the total number of trace elements found in soil from the same locations. Another peculiarity consists in the fact that all the trace elements found in wines are more or less soluble elements while the insoluble trace elements such as Sc, rare earth elements, Hf, and Th were not identified, although the NAA allows this. The same remark is valid for Al, whose content in wine was almost everywhere around 1 mg/L, although its content in soil was about 6 % (60 g/kg). From this point of view, the U case is the most representative. In soil, its content was three times lower than those of Th, but traces of U were found in all wines while Th could not be evidenced in any sample, despite the NAA sensitivity is almost the same for both elements [297].

To characterize the relationship between the soil and the wine as the final product of the grape-wine, the best descriptor seems to be the *TF*, calculated according to the formula presented in Chapter 1. The higher the value of the *TF* is, the more mobile the element. In Table 4.5 the *TF* numerical values of all elements are presented whose presence was evidenced by NAA both in wine and soil samples.

The values of *TF* for Na, Mg, K, Ca, Mn, Zn, Rb, Sr, and Br were higher than 1.0. The highest *TF* values belonging to K were found in all wines: 36 ± 5 for Romanesti red wine and 32 ± 6 for Cricova white wine. High *TF* was also obtained for Br (35 ± 12 mg/kg Romanesti red wine) and Rb: (17 ± 3 Cricova red and white wines). High *TF* for Br could be explained by the fact that Br is an almost essential element for vineyard development, in the case of Rb one possible explanation could be related to its similarity with K. Arsenic, which was found only in the Cricova wine has a very low *TF* of 0.03 ±0.01 mg/L [297]. The calculation of elements transfer from soil in wine is quite caddish, since several links are missed (grapes, leaves, must). To get more information regarding the transfer of elements it was decided to study in more detail the elements transfer in the system soil-leaves -grape-must.

Table 4.5 The numerical values ± combined uncertainty of the *TF* for all analyzed wine samples

Element	Romanesti – red wine			Romanesti – white wine			Cricova – red wine			Cricova – white wine		
Na	4.5	±	2.2	3.9	±	0.3	2.7	±	0.6	2.7	±	0.8
Mg	5.3	±	1.0	4.6	±	0.9	3.6	±	0.6	4.3	±	1.2
Al	0.02	±	0.02	0.03	±	0.01	0.02	±	0.02	0.03	±	0.01
K	36	±	5	21	±	14	27	±	5	32	±	6
Ca	5	±	2	6	±	4	7	±	2	7	±	3
Mn	2.0	±	0.3	1.8	±	0.4	1.3	±	0.2	1.4	±	0.3
Fe	0.1	±	0.1	0.1	±	0.1	0.04	±	0.03	0.03	±	0.02
Co	0.5	±	0.3	0.8	±	0.1	0.4	±	0.1	0.3	±	0.1
Ni	1.2	±	0.3	0.9	±	0.3	0.5	±	0.3	0.6	±	0.1
Zn	6	±	3	8	±	1	16	±	7	8	±	1
As	-	-	-	-	-	-	0.03	±	0.02	0.03	±	0.01
Br	35	±	12	13	±	14	8	±	3	12	±	5
Rb	16	±	2.0	11	±	5	17	±	3	17	±	3
Sr	12	±	4	7	±	4	7	±	3	6	±	2
Sb	0.5	±	0.2	0.5	±	0.2	0.7	±	0.5	0.7	±	0.3
Cs	0.6	±	0.2	0.6	±	0.5	1.7	±	0.6	1.4	±	0.3
Ba	0.7	±	0.7	0.3	±	0.1	0.2	±	0.1	0.2	±	0.1
U	0.05	±	0.03	0.10	±	0.03	0.05	±	0.02	0.05	±	0.01

4.2. Assessment of metals accumulation in soil-leaves-grapes- must system

To trace the links of metal bioaccumulation the grapes type – Ialovenschii Ustoicivii was selected for the study. Fruits and leaves of grape sampling were carried out in August-September 2017 in the Danceni village, Ialoveni district (the Central zone of the country). The amples of leaves and fruits were collected from three to five cranes. For NAA, green leaves and fruit samples were washed with distilled water and dried at 105 ^{0}C (convectional weighing) until the constant weight. After that, the samples were burned in a muffle furnace at 400^{0}C. Soil samples were collected from the same vineyard area at the depths varying from 10 to 20 cm to avoid topsoil pollution arising from the surrounding environment. The samples were dried at 40 ^{0}C, homogenized in the agate mortar, and prepared for NAA analysis. In the studied areas chernozem soil of brownish grayish color with a pH 5.96±0.02 predominates [329]. The samples preparation for NAA and irradiation procedure are described in Chapter 2 (Section 2.3).

4.2.1 Determination of the soil elemental content

Elements in soils are derived from natural components as well as from anthropogenic sources A total of 30 major and trace elements were determined in soils taken at the root of the vine (Table 4.6). The table also contains data for the UCC [303] and AS [301].

Table 4.6 Elemental content of studied soils (average ± standard deviation) as well as the corresponding values of the UCC and the AS

Element	Grape	UCC	AS	Element	Grape	UCC	AS
	Major elements, %						
Na_2O	0.69	3.27	0.85	Zn	91	67	50
MgO	3.61	2.5	1.0	As	10	5	5
Al_2O_3	10.95	15.4	13.5	Br	9.3	2.1	5
SiO_2	66.00	66.6	70.6	Sr	90	320	300
CaO	1.43	3.6	1.9	Rb	110	84	60
TiO_2	0.64	0.64	0.77	Mo	2.4	1.1	1.1
K_2O	1.90	2.8	1.5	Sb	1.1	0.4	0.2
MnO	0.10	0.1	0.11	Ba	380	624	500
FeO	4.5	5.6	5.4	Cs	6.3	4.9	0.2-26
	Microelements, mg/kg						
Cl	<60	370	100	La	36	31	40
Sc	11.7	14	7	Sm	6.0	4.7	-
Cr	95	92	200	Ta	0.9	0.9	1.5
V	110	97	100	W	1.7	1.9	-
Ni	51	47	40	Th	11.4	11	6
Co	13	17	8	U	2.4	2.8	1

The content of major elements, Si, Ti, Mn, and Fe was very close to both values, while the content of Na, Ca, and Mg was closer to AS values. The content of the main part of microelements corresponded to UCC and AS values. Zinc, As, Br, Sb and Rb concentrations were significantly higher, while concentrations of Sr and Ba notably lower than UCC and AS values. The low Sr content in the studied soils can be explained by the high solubility of strontium carbonate, which ensures higher mobility of this element. With the intensification of soil erosion, the Sr content in the soil increases [329]. At the same time, it should be marked, that concentrations of microelements and trace elements (Mn, Ni, Cr, V, Ba, Zn, and La) in the soil taken at the root of vine corresponds to their content in common chernozems, characteristic for the RM [330].

As it was presented in Section 4.1.1 the concentration of heavy metals in the analyzed soil samples are lower than the values reported in the literature, which may the provoke toxic effects in the organisms indicatiors of soil pollution.

4.2.2 Determination of the elemental content of grapes and leaves

The main sources of trace elements in plants are their growth media. At the same time, the bioavailability of trace elements from aerial sources through the leaves has also a significant impact on plant contamination [331]. Using the NAA a total of 25 major and trace elements were determined in the grape samples and 26 elements in leaves samples [329]. In the leaves, besides the elements determined in fruits also Ba was determined. The elemental composition of the studied leaves and grapes, the RP values [263] as well as data reported by the other researchers are presented in Table 4.7.

Determination of cations, such as potassium, sodium, and calcium, in fruits, is important due to their dietary significance (Dehelean and Magdas 2003). Among the determined major elements, K was the most abundant element in grapes, its content constituted 14500±725 mg/kg. Potassium is highly mobile in plants, it is involved in enzyme activation, protein synthesis, osmoregulation, stomatal opening and closing, photosynthesis, and cell expansion [252]. The content of other major elements was changed in the following order Ca>Cl>Ma>Na. Among the trace elements, the highest content in grapes was determined for Fe followed by Zn, Br, Mn, Cr, Mo, and Co. The content of trace and toxic elements was significantly lower than *RP* values [329].

The content of almost all the elements determined in leaves was significantly higher than in grapes, which can be explained by their larger horizontal surface that lead to the accumulation of more aerosols [332]. Calcium was the most abundant element in leaves with a concentration of 33400±1670 mg/kg, which can be linked to the rich limestone soil in Moldova. The content of magnesium was significantly higher in the leaves samples, which can be explained by the magnesium accumulation in chloroplasts, tied for plastocyanin, which participates in the process of photosynthesis [116].

Table 4.7 Elemental content of analyzed leaves and grape samples determined by NAA (in mg/kg)

Element	**Concentration, µg/g**						**RP**
	leaves			***grape***			
	Present study	***[292]***	***[291]***	***Present study***	***[293]***	***[294]***	
Na	400±20	-	224 ± 10	90±5	34	1.7	150
Mg	3690±180	2890	1310 ± 10	150±70	446	134	200
Cl	540±15	-	-	730±30	193	-	2000
K	9250±460	6580	-	14500±725	10754	2469	19000
Sc	0.054±0.003	-	--	0.06±0.003	-	-	0.02
Ca	33400±1670	28200	3005± 93	4780±240	594	299	10000
Cr	0.8±0.04	5.3	-	0.3±0.02	0.14	-	1.5
V	0.6±0.03	5	-	0.8±0.04	-	-	0.5
Mn	130±6.5	-	-	1.1±0.05	3.7	1.3	200
Ni	0.9±0.05	-	9.47 ± 0.75	0.5±0.02	<0.26	--	1.5
Fe	250±10	180	137 ± 4.1	5.6±0.2	13.9	1.53	150
Co	1.1±0.05	1.8	0.47 ± 0.07	0.18±0.01	0.4		0.2
Zn	23±1.1	15.2	25.20 ± 1.37	4.0±0.2	2.7	0.46	50
As	0.84±0.04	-	-	0.14±0.06	0.5	0.4	0.1
Br	4.4±0.2	-	-	2.7±0.1		-	4
Sr	250±12.5	74.9	--	26.2±1.3	2.31	-	50
Rb	35±1.7	-	-	5.1±0.3	-	0.89	50
Mo	0.5±0.02	-		0.2±0.01	-	-	0.5
Sb	0.026±0.001	-	-	0.004±0.0002	-	0.002	0.1
Ba	37±1.8	40.6	-	n.d	-	-	40
Cs	0.029±0.001	6.3	-	0.007±0.0004	-	-	0.2
La	0.2±0.008	4		0.026±0.001	-	0.4	0.2
Sm	0.026±0.001	-	--	0.004±0.0002	-	0.086	0.04
Ta	0.005±0.0002	-		0.005±0.0003	-	-	0.001
Th	0.049±0.002	1.3	-	0.057±0.002	-	0.003	0.005
U	0.016±0.0008	1.0	-	0.004±0.0002	--	-	0.01

Relatively high Ca and Mg content in the leaves in comparison with fruits indicate their low mobility. Barium was determined just in the leaves samples. Ba is usually found in the different trees and shrubs grown in the area of arid climate [102]. According to Kabata-Pendias [102], trace elements taken up by leaves can be translocated to other plant organs. Data obtained in the present study were in good accordance with the values given in the literature (Table 4.7).

4.2.3 Determination of elemental content of the grape juice

Due to the fact, that the main part of grapes is industrially processed into beverages (wine, clarified, or combined juices) a particular interest represents the elemental content of juice as well as the transfer of elements from soil and fruits in juice. The elemental composition of juice was also compared with available literature data. According to the data presented in Table 4.8, the juice obtained is characterized by high K content, which can be associated with its extraction from the peel during the grapes processing. Potassium was followed by Ca, Cl, Na, and Mg. At the same time, it should be mentioned that the content of Na and Cl in the juice was higher than in grapes, while the content of K, Ca, and Mg was almost on the same level. The content of microelements was changed in the following order: Fe>Br>Zn>Mn>Cr>Mo. The levels of rare earth elements and toxic metals (Sb) in all the samples were less than 0.01 mg/kg [329].

Table 4.8 Elemental content of analyzed juice samples determined by NAA

Elements	**Concentration, mg/kg**			**Element**	
	The present study, mg/kg	Miele et al.. [333], mg/L	Velimirović et al, mg/kg		The present study, mg/kg
Na	370±18	2.23±2	-	Br	5.8±0.2
Mg	125±7.5	77.7±15.5	-	Sr	32±3
Cl	545±20	-	-	Rb	7.9±1.1
K	18700±200	1436±388	32-57	Mo	0.3±0.03
Sc	0.08±0.008	-	-	Sb	0.007±0.0007
Ca	3800±410	-	-	Cs	0.02±0.002
Cr	0.4±0.04	0.023	0.33-0.58	La	0.02±0.001
V	0.6±0.06	0.01	-	Sm	0.004±0.0004
Mn	0.9±0.03	1.25±0.63	75-88	Ta	0.006±0.0006
Ni	0.5±0.05	0.016	101-114	Th	0.007±0.0007
Fe	7.6±0.5	2.15±1.1	144-156	U	0.001±0.0001
Co	0.3±0.02	0.005	0.2-0.3		
Zn	5.4±0.2	0.5±0.2	-		
As	0.2±0.02	0.025	-		

4.2.4 Metal bioaccumulation in the soil-leaf-fruit-juice systems

The values of *TF* were calculated to reveal the accumulation of elements in the following systems: $TF_{L/S}=C_{leaves}/C_{soil}$, $TF_{F/S}=C_{fruits}/C_{soil}$, $TF_{F/L}=C_{fruits}/C_{leaves}$. The results obtained are shown in Figs. 4.8-4.9. According to the calculated values, leaves of the vines accumulate from soil predominantly Ca ($TF_{L/S} = 3.3$), Sr ($TF_{L/S} = 2.8$) and U ($TF_{L/S} = 1.0$) (Fig. 4.8). The other micro- and macro-elements were accumulated in the considerably lower amounts. The grapes accumulated K from soil with *TF* values 0.92. For the other elements, TF was less than 1.0, which indicates the lack of their accumulation in grapes. From leaves in grapes are accumulated Cl, K, Sc, V, Ta, and Th.

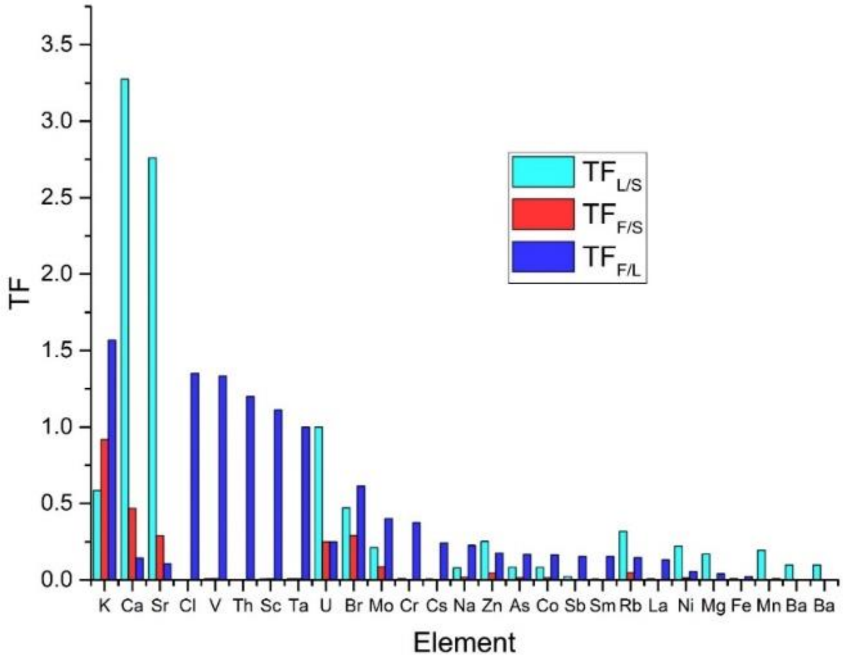


Fig. 4.8 Transfer factors values in system soil-leaves-grape

The values for $TF_{juice/soil}$ and $TF_{juice/fruits}$ are presented in Fig. 4.9. As in the case of grapes, in juice predominantly accumulated K from soil. For the main part of elements, $TF_{J/F}$ has values close to 1.0, indicating the lack of micro- and macro-elements losses in the technological process of industrial processing of grapes. The highest values were obtained for Na, Cs, Br and Co 4.2, 2.4, 2.1, and 1.8, respectively [329]. For all studied systems, the accumulation of rare earth elements and elements, which can be considered as environmental pollutants was very low. It is known, that the plants act as a barrier to the accumulation of pollutants in the food chain.

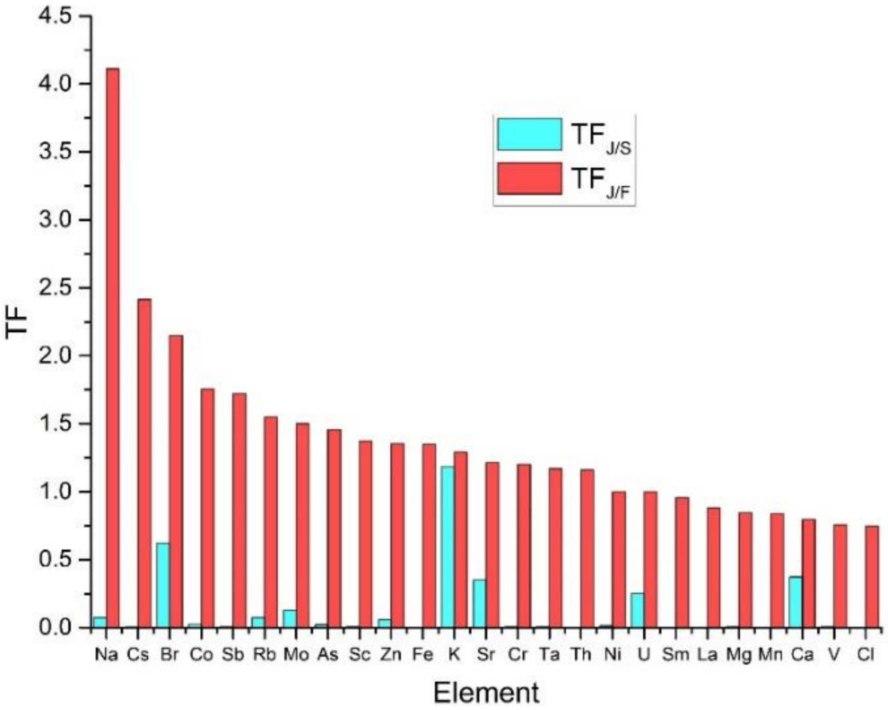


Fig. 4.9 Transfer factors in system soil-grape-juice

The low level of uptake and accumulation of some elements in grapes and juice can be also explained by the low level of available forms of these elements in the soil, as well as the slightly acid to neutral soil pH (in present case pH=5.96±0.02), which is unfavorable in terms of their uptake [119].

4.3 Major and trace elements determination in the fruits collected in Ialoveni district and assessment of their quality

The RM, due to favorable climatic conditions and fertile soil has gained a good reputation as a supplier of high-quality wine. fruit and vegetable products in south-eastern Europe [334]. In Moldova, three main agricultural zones can be evidenced: -i. Northern zone, where mainly sugar beet, fruits, and tobacco are cultivated; -ii. Central zone specialized in the production of grapes and -iii. South zone specialized in the production of grapes, sunflowers, fruits, and corn. According to the National Bureau of Statistics of the Republic of Moldova in the period 2014-2017, the production of fruits increased from 497 thousand tonnes to 666 thousand tones and of grapes from 594 to 675 thousand tones. In 2019 Moldova produced 840 thousand tons of fruits and 657 thousand tons of grapes [335].

Besides the grapes, the apples “Golden Delicious” and the plums “Record” were collected in August- September 2017 in the village Danceni, district Ialoveni (the Central zone of the country). At each site, samples of leaves and fruits were collected from three to five trees. The soils were collected from the corresponding roots of plum and apple trees at the depths varying from 10 to 20 cm In the studied areas chernozem soil of brownish grayish color with the following pH values: 6.26±0.02 for apples and 6.05±0.02 for plums predominates [329].

The interest in the determination of fruits' elemental composition is determined by their nutritional importance. Fruits are a significant source of carbohydrates, organic acids, also, they are rich in vitamins and minerals [336]. According to Markowski et al. [337]. the consumption of fruits and vegetables may help to reduce the risk of cardiovascular diseases and even prevent the cancer. For vegetarians and vegans. the intakes of minerals and trace elements from fruits become particularly vital [338]. In addition to macro and essential trace elements, fruit also can contain toxic or potentially toxic elements. Thus, it is important to trace their content and to prevent consumers from the possible effect of toxic elements on human health.

4.3.1 Determination of apples and plums fruits and leaves elemental content

Using NAA a total of 25 major and trace elements were determined in apple samples and 23 elements in plum samples as well as 26 elements in leaves samples. In the leaves besides the elements determined in fruits was determined also Ba. The elemental composition of studied leaves and fruits, values for the RP [263] as well as data reported by the other researchers are presented in Table 4.9. Obtained data shows that concentrations of some macro- and microelements in apples were higher than in plums. Thus, the content of Na in apples was 3.6 times higher than in plums, of Cl – 12 times, of Ni -2 times, of Sc-2.5 times, of Mo - 10 times, and of La – 4 times. Content of K, Ca, Mn, As, U was almost on the same level. The plums were characterized by the higher content of Fe, Co, Zn, Br, Sr, Rb, Sm, and Th. Chromium and V were detected only in the apple samples, while in plum samples it was below the detection limits [329].

Determination of cations, such as K, Ca, Mg, and Na, in fruits is important due to their dietary significance [339]. Among the macroelements analyzed, K was the most abundant element and its content in both the types of fruits was almost similar: 23700 mg/kg in apples and 23500 mg/kg in plums. The content of Ca, Na, and Mg was also higher in the apple fruits. Calcium and Mg are macro elements necessary for the development of bone and structural tissue formation. They are also involved in the regulation and dilation of blood vessels and a regular heartbeat [340]. As it was previously mentioned, the high content of Ca in leaves and fruits can be linked to the abundant limestone soil in Moldova.

Among the trace elements, the highest content in fruits was determined for Fe: 25±1.0 mg/kg in apples and 120±6 mg/kg in plums. Iron is a vital component of heme proteins, hemoglobin, and myoglobin required for oxygen transportation, glucose metabolism, and vascular functions [340]. Zinc was the second most abundant microelement in fruits. Its function in organisms includes the formation of metalloenzymes, protein metabolism, RNA conformation, and membrane stabilization [341]. The manganese content in apples was 2.6±0.1 mg/kg and 1.7±0.08 mg/kg in plums. The content of Co, Br, and Mo in both types of fruits was significantly lower in comparison with the above-mentioned microelements. Cs, La, Sm, Ta, Th, and U content in fruits was on the level of several ppm and their main source in fruits can be considered soil. Nickel was more abundant in apple fruits than Cr and V. Nickel is moderately required for proper absorption of Fe in the body [340]. Toxic elements, Sb and As were detected in both types of fruits [329]. Their source in fruits can be soil as well as the application of pesticides and fertilizers.

In the leaves, the concentration of the main part of determining elements was higher than in fruits. The accumulation of heavy metals by leaves depends on the binding and solubility of particles deposited on leaf surfaces, as well as on concentrations and bioavailability of elements in the soil. In the case of leaves, it is difficult to distinguish whether the accumulated elements originate from the soil or the air [342]. The presence of Sr and Fe in leaves in high concentrations can be explained by aerial deposition [116]. It should be mentioned that all the leaves were washed before analysis.

Barium was determined only in the leaves samples. As it was mentioned previously the high Mg content can be explained by its participation in photosynthesis [116]. A higher concentration of Sb, As in leaves can be associated with their accumulation during the process of trees spraying.

The data obtained in the present study were in good accordance with the values given in the literature (Table A1.6, Annex). The content of elements in leaves in fruits was compared with the corresponding *RP* values. The content of Mg in the leaves was significantly higher than in RP. In leaves, the content of the main part of the elements was higher in comparison with RP. In apple fruits, the content of almost all elements, except for Na, Mg, Sc, Ca, Ni, As, Th, and U was lower than the corresponding values for RP. In the plum fruits, the content of Na, Sc, Co, As, and Rb was significantly higher than RP values. The content of other elements was similar to or lower than RP [329].

4.3.2 Elemental composition of soil collected at the root of apple and plum trees

Determination of the soil elemental composition is extremely important to assess its anthropogenic pollution and to calculate metal bioaccumulation. A total of 29 major and trace elements were determined in soils taken at the root of apples and 30 elements at the roots of plum (Table 4.10). The table also contains data for the UCC (Rudnick and Gao 2013) and AS [301].

Table 4.9 Elemental content of studied soils (average ± SD) as well as the corresponding values of the UCC and the AS

Element	**Apple**	**Plum**	**UCC** [300]	**AS** [301]	**RM** [330]
Major elements, %					
Na_2O	0.72	0.71	3.27	0.85	
MgO	3.02	2.35	2.5	1.0	
Al_2O_3	9.70	6.20	15.4	13.5	
SiO_2	54.50	32.00	66.6	70.6	
CaO	4.14	1.97	3.6	1.9	
TiO_2	0.67	0.38	0.64	0.77	
K_2O	1.74	1.88	2.8	1.5	
MnO	0.1	0.06	0.1	0.11	0.07
FeO	3.9	3.9	5.6	5.4	
Microelements, mg/kg					
Cl	<60	167	370	100	
Sc	10.9	10.3	14	7	
Cr	83	81	92	200	91
V	90	60	97	100	91
Ni	39	36	47	40	39
Co	12	12	17	8	
Zn	76	88	67	50	76 – 91
As	8.2	8.6	5	5	
Br	8.3	8.9	2.1	5	
Sr	110	130	320	300	240
Rb	93	96	84	60	
Mo	1.1	1.2	1.1	1.1	0.9-4.8
Sb	0.9	0.9	0.4	0.2	
Ba	520	480	624	500	460
Cs	5.4	5.5	4.9	0.2-26	
La	30	28	31	40	44
Sm	5.2	5.0	4.7	-	
Ta	1.0	0.9	0.9	1.5	
W	1.5	1.5	1.9	-	
Th	10.4	9.41	11	6	
U	2.2	2.1	2.8	1	

The content of major elements, except of Si, Ca, Ti, Cl, and Al, in the studied soil samples was very similar. The content of the above-mentioned elements was higher in the soils taken at the roots of apple trees. The content of Na, Al, Si, Fe, and Sr in the studied soil samples was lower with respect to UCC and AS, while the content of As, Sb, and Br was significantly higher. The content of other elements does not differ too much from the UCC/AS values [329]. The content of microelements and trace elements (Mn, Ni, Cr, V, Ba, Zn, and La) in the soil taken at the root of the horticultural plants corresponds to their content in common chernozems, characteristic for the RM [330] (see Table 4.10). Regarding the concentration of elements which can be considered as potential anthropogenic pollutants their content in analyzed soil did not exceed the corresponding limits defined by the national authorities (see Table 4.1), which indicates insignificant anthropogenic soil pollution.

Pollution indices are a powerful tool for soil quality assessment. For nine elements *CF*, I_{geo}, and *PLI* values were calculated (Table. 4.11). For soil collected at the roots of apples CF for all elements, except of Zn, As, Br and Sb were lower than 1.0 indicating on the soil uncontaminated by these elements. The values for Zn, As and Sb lied between 1.0 and 3.0 and pointed at the slight soil pollution by these elements.

Table 4.10 The experimental values of CF, I_{geo} and PLI calculated for analyzed soil

	CF			*Igeo*		
Element	apple	plum	grape	apple	plum	grape
Cr	0.92	0.88	1.03	-0.71	-0.77	-0.54
V	0.93	0.58	1.11	-0.69	-1.37	-0.43
Mn	0.80	0.60	0.87	-0.90	-1.33	-0.79
Ni	0.83	0.77	0.86	-0.85	-0.96	-0.80
Fe	0.71	0.70	0.81	-1.07	-1.10	-0.89
Co	0.72	0.71	0.78	-1.05	-1.08	-0.94
Zn	1.14	1.32	1.36	-0.40	-0.19	-0.14
As	1.71	1.78	2.10	0.19	0.25	0.49
Br	5.21	5.59	5.83	1.80	1.90	1.96
Sb	2.35	2.38	2.88	0.65	0.66	0.94
			PLI	1.24	1.15	1.43

The highest value of *CF* was obtained for Br showing the moderate soil pollution with this element. The same pattern was observed for soil collected at the root of plum trees. In the case of grapes, except for the mentioned elements, the *CF* values of Cr and V were higher than 1.0. According to I_{geo} the moderate soil pollution with Br was observed, with regards to other elements it can be characterized as uncontaminated to moderately contaminated soil. PLI values were higher than 1.0 for all the analyzed soil samples, which means that the slight contamination exists [329].

Bromine makes a significant contribution to calculated values. Sources of the high content of Br in the soil can be natural (volcanic exhalation and sea evaporation) and anthropogenic (automobile exhaust and pesticides). Considerable amounts of Br (as methyl bromide and ethylene dibromide) are used in agriculture as pesticides (fungicides, herbicides, and insecticides). Bromine is also a component of K fertilizers [116,345].

4.3.3 Metal bioaccumulation in the soil-leaf-fruit system

The values of *TF* were calculated to reveal accumulation of elements the following systems: $TF_{L/S}=C_{leaves}/C_{soil}$, $TF_{F/S}=C_{fruits}/C_{soil}$, $TF_{F/L}=C_{fruits}/C_{leaves}$, $TF_{F/St}=C_{fruits}/C_{stone}$, and $TF_{St/S}=C_{stone}/C_{soil}$. It was found that the apple leaves mostly accumulate from soils K ($TF_{L/S}$=3.1), Zn ($TF_{L/S}$=2.1), Sr ($TF_{L/S}$ =1.9), and Mo ($TF_{L/S}$ =1.1) and Ca ($TF_{L/S}$=0.96). To a smaller extent Ca ($TF_{L/S}$=0.96) and Br ($TF_{L/S}$=0.73) were accumulated. For other elements, $TF_{L/S}$ values were found to be lower than 1.0, for most of them in the order of 0.1 (Fig. 4.10). Concerning the elements transfer from the soil in apple fruits the highest *TF* values were obtained for K ($TF_{F/S}$= 1.6) and U ($TF_{F/S}$ = 2.1). In the system apple leaf- fruit) *TF*> 1.0 was obtained for Na, Cl, and Cr [329].

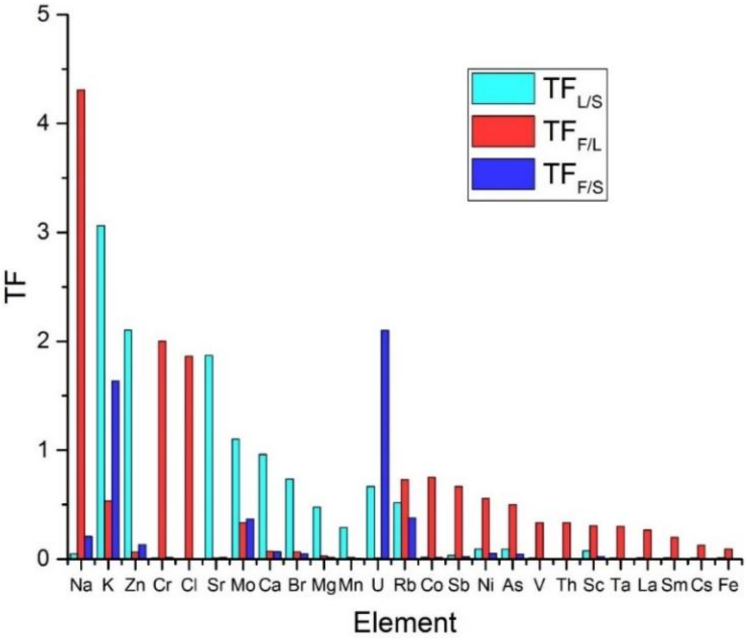


Fig. 4.10 Transfer factors of system soil-leaf-apple

The plum leaves have accumulated from soil preferentially Ca ($TF_{L/S}$=1.5), Br ($TF_{L/S}$ =1.5), Sr ($TF_{L/S}$ =1.2), and K ($TF_{L/S}$ =1.1) (Fig. 4.11). In a lesser degree Rb and Cl were accumulated, for both elements $TF_{L/S}$ was0.9. The plum fruits accumulated mainly K ($TF_{F/S}$=

1.5) and Rb ($TF_{F/S}$ = 1.9) from soil. The degree of other elements accumulation was very low ($TF_{F/S}$<1).

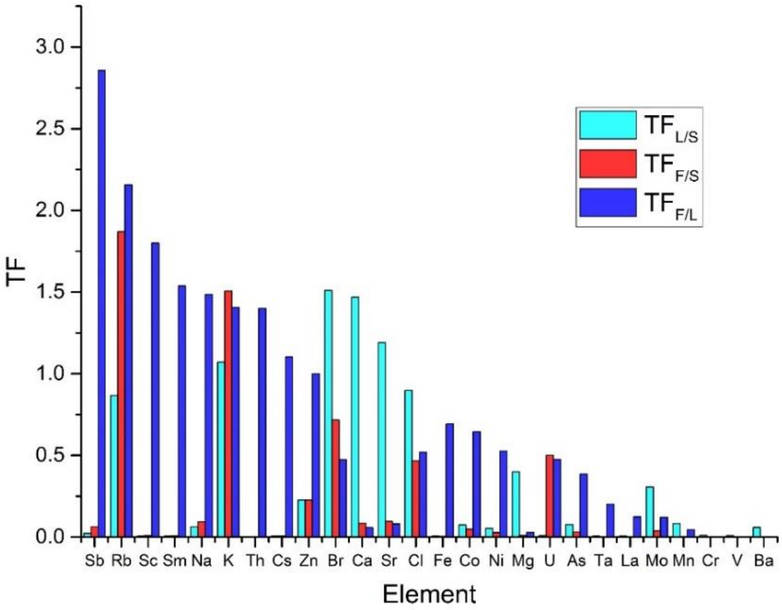


Fig. 4.11 Transfer factors for system soil-leaf-plum

It is interesting to mention that *TF* for Na was lower than 1.0, indicating that Na is not accumulated in the plum fruits from the soil. The main part of elements plum fruits accumulates from leaves. $TF_{F/L}$ values for Na, K, Sc, Zn, Rb, Sb, Sc, Sm, and Th were greater than 1.0 [329].

Plums are stone fruits, thus, the chemical composition of stones was also determined (Table 4.9). For the main part of elements in the system fruit-stone, the *TF* values were higher than 1.0 (Fig. 4.12). Content of Mg, Cl, Ca. Cr, Mn, Ni, Sr, and Mo in stones was higher than in leaves. The concentrations of other elements were the same or lower. As in the case of fruits in stones from the soil are mainly accumulated K ($TF_{St/S}$ =1.4) and Rb ($TF_{St/S}$ =1.1) [329].

For all the studied systems, the accumulation of rare earth elements and elements, which can be considered as environmental pollutants was very low. As it was priviosly mentioned plants act as a barrier to the accumulation of pollutants in the food chain.

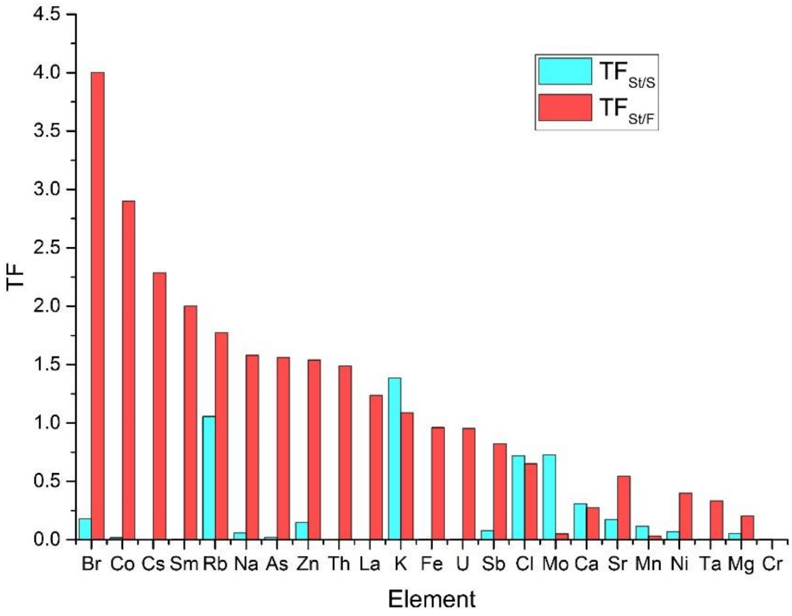


Fig. 4.12 Transfer factors for system soil-stone-plum

4.3.4 Health risk assessment

The risk assessment indices were calculated according to formula presented in Chapter 1 (Section 1.4.5) for nine elements (Cr, Co, Fe, Mn, Ni, V, Zn, As, and Sb), considered as environmental pollutants. The data obtained are presented in Table 4.12.

Table 4.11 Estimated daily intake of metal and potential health hazards of analyzed fruit

	Apple			**Plum**			**Grape**			
Element	C	DIM,	HQ,	C	DIM,	HQ,	C	DIM,	*HQ,*	R_fD,
Cr	3.9	1.2	0.01	0	0	0	0.48	0.1	0.004	105
Co	0.5	0.1	0.05	0.7	0.2	0.07	0.6	0.2	0.06	3
Fe	78	23	0.4	151	45	0.75	8.8	2.6	0.04	10-60
Mn	8.1	2.4	0.5	2.2	0.6	0.1	1.7	0.5	0.1	0.5-5.0
Ni	6.7	2.0	1.4	1.4	0.4	0.3	1.8	0.2	0.2	1.4
V	0.6	0.2	0.1	0	0	0	1.3	0.4	0.3	1.8
Zn	33	9.9	0.7	25	7.5	0.5	6.4	1.9	0.1	15
As	1.1	0.35	0.23	0.32	0.09	0.06	0.22	0.07	0.05	1.5
Sb	0.06	0.018	6	0.08	0.02	6.7	0.006	0.002	0.7	0.003

*C - mg/kg f.w; DIM - mg/day; HQ, mg/kg/day

To calculate DIM and HQ values the concentration of the abovementioned elements given in mg/kg dry weight were recalculated in mg/kg fresh weight. The drying coefficient constituted 0.32 for apple, 0.79 for plum, and 0.63 for grape. DIM of metals from fruits varies greatly between the three fruits type. The highest intake of Cr, Mn, Ni, and Zn was from the apple fruits, while of Fe from the plum fruits. The intake of toxic metals, As and Sb, from fruits, was very low. The HQ values for the main part of the elements were below 1.0, which is considered safe. It should be noted that values for As were significantly lower than 1.0. In the case of Sb HQ value for apples and plums significantly higher than 1.0, so there is a concern for potential health effect

4.4 Determination of major and minor elements in fruits from different zones of RM by NAA and assessment of their provenance

As it was previously mentioned the RM is an important exporter of fruits in Europe. The quality of the fruits, especially their elemental composition is strongly dependent on soil chemical composition. The soil often contains chemical elements considered as fingerprints of the origin of the studied soil and fruits. The most explored fingerprinting techniques combine the chemical analysis and multivariate statistical analysis of chemical data to classify fruits according to their geographical origin. To evidence any correlation between the different types of fruits and the place where were cultivated, fruits were collected in September 2018 in four zones in the Republic of Moldova: South-East (Purcari), South (Cahul), Center (Ialoveni) and Codru (Criuleni) (Fig. 4.13). The following type and sorts of fruits were collected: in Purcari - the grapes Merlot (P_m), Feteasca Neagra (P_f) and Saperav (P_s); in Cahul – grapes ”Muscat de Hamburg” (C_{mh}), ”Moldova” (C_{md}), apples (C_a) and plums (C_p); in Ialoveni – grapes ”Alb de Suruceni” (I_{as}), apples ”Golden” (I_{ag}) and plums “Vengherca” (I_p); in Criuleni - grapes „Moldova” (C_{rmd}), apples (C_{ra}) and plums (C_{rp}). Collected fruits were washed several times with distilled water and dried at 105 ºC (convection drying) until the constant weight. Then, samples were burnt inside the muffle furnace at 400 ºC [346].

The soils samples were collected and prepared for analysis according to the procedure described in Section 4.3.2.

Fig. 4.13 The geographical location of sampling points (green stars)

Determination of the elemental composition of fruits collected in different zones of the RM using NAA

The NAA allowed determining 22 elements (Na, Mg, Cl, K, Ca, Sc, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Rb, Sr, Sb, Cs, Ba, La, Th, and U) in the analyzed grape, apple, and plum samples. The average concentrations determined for each element are summarized in Table 4.13, while the content of each type of fruit is presented in Table A1.7 (Annex). The determined elements can be divided into three main groups: major elements present in all fruits, Na, Cl, K, Ca, and Mg; microelements, playing an important role in biological processes, Co, Zn, Fe, etc.; and elements, which do not have biological functions Sb, As, Th, U, etc [346]. All these elements enter into the human body by daily consumption of vegetal food such as vegetables and fruits as well as animal sub-products.

For the human body, Ca, Mg, Na, and K are critical structural elements, which control the action of nerves and muscles, help maintain the body's water balance and buffer the pH of the cell and extracellular fluids [347]. As in the case of fruits collected in 2017 in Danceni district, K was the most abundant element in all analyzed fruits with an average content of 38770 ± 5590 mg/kg, with a maximum of 46200 mg/kg for Criuleni apples and a minimum of 27270 mg/kg for

“Feteasca Neagra" from Purcari. The content of K in the fruits decreased in the following order plum>apple>grape. Calcium, the second major element has a different behavior with an average content of 4370 ± 2800 mg/kg, reaching a maximum value of 8450 mg/kg for the "Feteasca Neagra" grape collected from Purcari vineyard and a minimum of 1110 mg/kg in the case of Cahul apples. Fruits from Criuleni were characterized by a high content of Mg, while its lowest concentration was determined in plums from Cahul. The content of Mg varied between 590 mg/kg and 3000 mg/kg with an average value of 1360 ± 790 mg/kg. Sodium levels in the analyzed fruits were in the range of 211-1820 µg/g and its mean values increased in the following order plum>grape>apple. Chlorine was determined in all fruits, except grape "Feteasca Neagra" from Purcari. The range of its content varied between 120 and 1720 mg/kg, with an average value of 630 ± 500 mg/kg, plums presenting the highest content, and the apple the lowest [346]. As a general remark, the content of all mentioned above elements is distributed onto a great interval of values, as the corresponding Coefficient of Variation [348] varied between 130 for Na and 690 for K.

The second group of elements includes Fe, Mn, Co, Cu, Zn, Ni, and Br known to be essential and playing an important biological role in the human body [127] or enzymatic role in plant metabolism [349]. According to the data presented in Table 4.13, Fe had the highest concentration in all the analyzed fruits followed by Cu and Zn. The lowest Fe concentration was determined in plums from Criuleni -32 µg/g, while the highest in apples from Cahul- 156 µg/g. The highest mean Fe content was determined in grapes followed by apples and plums. Copper levels in the samples ranged from 10 to 41 µg/g with the highest concentration in grape „Merlot” from Purcari. Zinc concentration in the analyzed samples ranged between 3.3 µg/g (apple Cahul) and 43 µg/g (grape "Feteasca Neagra" from Purcari). The average concentrations of Cu and Zn were the highest in grapes [346].

Table 4.12 The mean content of investigated elements in correlation with existing literature data

Element	Grapes		Apples		Plums	
	Present work	Literature data	Present work	Literature data	Present work	Literature
Na	575 ± 580	90† - 329#	455 ± 185	15 §	606 ± 380	490†
Mg	1140 ± 460	150† – 1214#	1190 ± 200	233 ‡ – 297§	1675 ± 285	160†
Cl	550 ± 600	226# – 730†	380 ± 330	940 ‡ – 1060§	1020 ± 150	78 †
K	31640 ± 3280	14500† – 36133#	41040 ± 1930	7242 § – 23700†	42810 ± 5710	23500†
Ca	5900 ± 1090	47800† – 57800#	1390 ± 190	290 § -1960†	1900 ± 460	1190†
Sc	0.14 ± 0.1	0.06† – 0.28#	0.19 ± 0.17	0.25†	0.1 ± 0.14	0.1†
Mn	5.7 ± 1.9	1.1† – 8.6#	2.2 ± 1.5	2.6† – 4.3§	2.4 ± 0.3	1.7†
Fe	86 ± 54	5.6† – 96#	83 ± 17	9.3 § – 225†	41 ± 8	120†
Co	0.05 ± 0.01	0.05# – 0.5 ‡	0.08 ± 0.01	0.15† – 0.4 ‡	0.04 ± 0.01	0.58†
Ni	0.8 ± 0.3	0.5† – 0.6 ‡	0.6 ± 0.1	<0.2 § – 2†	1.3 ± 0.6	1†
Cu	26 ± 11	2.13 ‡ – 35#	15 ± 2	1.25 ¤ – 1.5 ‡	15 ± 6	--
Zn	16 ± 5	1.33 ‡ – 17#	1.4 ± 0.1	0.16 § – 9.9†	16 ± 4	20†
As	0.08 ± 0.04	0.14† - .17#	0.14 ± 0.1	0.37† – 1.4 §	0.05 ± 0.01	0.25†
Br	0.9 ± 0.3	0.56# – 2.7†	1.1 ± 0.4	04†	0.9 ± 0.7	6.4†
Rb	46 ± 16	88# – 5.1†	44 ± 4	15†	25 ± 6	179†
Sr	51 ± 108	26† – 56#	8 ± 9	0.85 § – 1.5†	11 ± 14	13†
Sb	0.01 ± 0.01	0.01#	0.01 ± 0.01	002†	0.01 ± 0.01	0.046†
Cs	0.07 ± 0.02	0.01† – 0.17#	0.07 ± 0.01	0.01†	0.03 ± 0.01	0.06†
Ba	5.6 ± 1.3	5.9#	5.2 ± 0.4	--	2.8 ± 2.3	--
La	0.15 ± 0.05	0.03† -0.12#	0.11 ± 0.02	0.02†	0.05 ± 0.01	0.021†
Th	0.02 ± 0.01	0.02# – 0.06†	0.01 ± 0.01	--	0.01 ± 0.01	0.007†
U	0.01 ± 0.01	0.01† – 0.17#	0.01 ± 0.01	0.02†	0.01 ± 0.01	0.01†

†Zinicovscaia et al. [350], ‡Elbgami et al. [351] , #Mitic et al. [127], §Michenaud-Rague et al. [338],and ¤Radwan et al. [352]

The high content of Cu in grapes can be also explained by the use of copper sulfate as pesticides [127]. Generally, the plants contain the amount of Cu, which is inadequate for normal growth of plants. However, the application of micronutrient fertilizers and copper-based fungicides may sometimes increase it to the alarming levels [352].

Manganese is an essential element for both animals and plants, being a cofactor of several classes of enzymes [347]. Manganese's lowest content was determined in the apples from Criuleni (1.6 μg/g) while the highest in grape "Muscat de Hamburg" from Cahul (8.6 μg/g). The mean content of Br in all types of analyzed fruits was on the same level. Nickel plays the numerous roles in hormonal activity, lipid metabolism, activation of some enzymes, and stabilization of DNA and RNA [347]. The highest Ni content (2.03 μg/g) was determined in plums from Criuleni and the lowest in plums from Cahul (0.6 μg/g). The content of Co in analyzed fruits was significantly lower in comparison with other essential elements and lied in the range of 0.025-0.09 μg/g [346].

The third group of elements constituted Sc, As, Rb, Sr, Rb, Cs, Ba, La, Th, and U. The sources of these elements in fruits can be considered soil (Sc, Rb, Sr, Rb, Cs, Ba, La, Th, and U) as well as anthropogenic activity (As, and U). Among the selected fruits, the highest concentrations of As were determined in apples from Cahul (0.2 μg/g) and the lowest in grape "Feteasca Neagra" from Purcari (0.036 μg/g). In the grapes „Merlot" from Purcari and all their fruits collected in Criuleni As values were lower than the detection limit of the NAA technique [346].

According to data provided in Table 4.13, our results were in good concordance with the similar ones reported in literature Zinicovscaia et al. [350], Elbgami et al. [351], Mitic et al. [127], Michenaud-Rague et al. [338], and Radwan [352].

4.4.1 Determination of the elemental composition of soils collected in the different zones of the RM using NAA

The multi-elemental capability of NAA was employed for the determination of 39 major and trace elements in 13 soil samples collected in four zones Criuleni, Ialoveni, Cahul, and Purcari. The final results concerning the mean content of the eight major, rock-forming elements: Na, Mg, Al, Ca, Si, K, Mn, and Fe as well as of the other 31 trace elements: Sc, Ti, V, Cr, Co, Ni, Zn, As, Br, Rb, Sr, Zr, Mo, Cd, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Dy, Tm, Yb, Hf, Ta, W, Th, and U are presented in Table A1.8 (Annex). Table 6 also contains the data for the Upper Continental Crust [303] and Pedological Limits (PL) established for Moldavian soil [353].

The matrix of Spearman's correlation coefficients, as well as more statistic tests such as Tukey's Q, Mann-Whitney U or Kruskal-Wallis test (Table 4.14) for equal medians, showed that at $p < 0.05$, the distribution of major elements which compose investigated soils are similar and very close to the UCC [303] (Table 4.14). This finding is well explained by the fact that all considered locations are within a radius of less than 200 km, and belongs to the same geological formation, the Moldavian Platform. The highest content of Si and Mn was determined in soil collected in the Cahul region. The content of Na in soils collected in the Ialoveni and Criuleni regions was lower than in soils from Cahul and Purcari zones.

A relatively similar situation we have found in the case of trace elements, which content falls within the pedological limits established for the RM [353] as well in those previously presented for Ialoveni (Danceni district) [350]. However, the content of As, Zr, Sb, Cd, Sb, Dy, Tm, Yb, and Hf were in some cases higher than UCC one, the highest difference was achieved for Br by a factor of 5.7-8.2 [346].

Table 4.12 The matrix of Spearman's r correlation factor with Bonferroni correction at p < 0.01

	Cahul	**Criuleni**	**Ialoveni**	**Purcari**	**UCC**
Cahul	------------	0.999	1.000	1.000	1.000
Criuleni	0.933	------------	1.000	1.000	0.995
Ialoveni	0.938	0.950	------------	1.000	0.999
Purcari	0.950	0.950	0.967	------------	0.997
UCC	0.933	0.800	0.833	0.833	------------

*locations (lower diagonal) and of the Tukey's Q test (p the same) (upper diagonal)

Since the presence of As, Br, and Cd in the soil in high concentrations could be related to the human activity, to quantify their presence we have calculated for each of them the corresponding *EF*, C_F, I_{geo} as well as the more general *PLI*. For a more complete analysis, we have taken into account besides As, Br, and Cd the other nine potential pollutant elements, i.e. V, Cr, Mn, Fe, Ni, Co, Zn, Sb, and U (Table 4.15).

The *EF* values for Mn, Fe, Co were less than unit, for V, Cr, Ni, Zn, As, Cd, and U they lied between 1.0 and 3.0 and for Br they were higher than 5.0. According to Puckett and Finnegan [354] the values of *EF* less than unit attests to an uncontaminated environment, between one and three characterize a moderately polluted to highly polluted environment, and higher than five indicate a severely polluted environment. The values of C_F for Mn, Fe, Zn (Purcari), U (Ialoveni and Criulieni) were lower than 1.0, indicating that soils were practically uncontaminated by these elements. The values for V, Cr, Zn, As, Cd, Sb, and U lie between 1.0 and 3.0 and point at slight soil pollution. The values obtained for Br indicate moderate pollution by this element. In the case of I_{geo}, the negative value obtained for V, Cr, Mn, Fe, Co, Ni, Zn, and U are indicators of uncontaminated environment, values between 0 and 1.0 (Cd, Sb) are attributed to the uncontaminated to moderately contaminated environment [346], while the values between 2.0 and 3.0 (Br) characterize moderately to strongly polluted environment [248]. The PLI values were very similar for analyzed zones and indicate moderately to the unpolluted environment [249].

Table 4.13 The experimental values of *EF*, *CF*, I_{geo}, and *PLI*

	EF				***CF***				***Igeo***			
Element	Purcari	Cahul	Ialoveni	Criuleni	Purcari	Cahul	Ialoveni	Criuleni	Purcari	Cahul	Ialoveni	Criuleni
V	1.36	1.44	1.24	1.40	1.16	1.13	1.19	1.14	-0.37	-0.41	-0.33	-0.39
Cr	1.38	1.48	1.31	1.39	1.18	1.16	1.11	1.14	-0.34	-0.37	-0.44	-0.40
Mn	0.91	1.20	0.97	0.87	0.78	0.94	0.82	0.71	-0.94	-0.67	-0.87	-1.08
Fe	0.81	0.87	0.86	0.84	0.69	0.68	0.73	0.68	-1.11	-1.14	-1.04	-1.13
Co	0.86	0.84	0.84	0.84	0.73	0.66	0.71	0.69	-1.03	-1.18	-1.07	-1.12
Ni	1.06	1.13	1.18	1.15	0.90	0.89	1.00	0.94	-0.73	-0.76	-0.58	-0.67
Zn	1.07	1.62	1.50	1.50	0.92	1.27	1.27	1.22	-0.71	-0.24	-0.24	-0.29
As	2.45	2.50	2.30	2.48	2.10	1.96	1.95	2.03	0.48	0.38	0.38	0.43
Br	9.55	7.89	7.25	7.04	8.19	6.19	6.15	5.75	2.45	2.04	2.03	1.94
Cd	2.44	2.35	2.29	2.81	2.09	1.84	1.94	2.30	0.48	0.30	0.37	0.61
Sb	3.09	3.53	3.30	3.58	2.65	2.77	2.80	2.93	0.82	0.88	0.90	0.96
U	1.40	1.41	1.13	1.22	1.20	1.10	0.96	0.99	-0.32	-0.44	-0.65	-0.59
PLI	**1.39**	**1.38**	**1.37**	**1.36**								

The concentrations of twelve elements were also compared with the maximum admissible concentrations (MAC) defined by the National Authorities of Moldova [273] and neighboring counties: Romania [307] and Russian Federation [308]. The concentrations of V, Mn, Cd, Sb, Ni, and Zn in analyzed soils were lower than MAC. The concentrations of Co and Cr were difficult to compare with MAC since in the present work the bulk amount of the element in the samples was determined, while the National Authorities of both counties give the MAC values of the mobile metal forms. The concentration of As was almost 5 times higher than MAC established for both countries. The main anthropogenic sources of As are coal combustion and application of arsenical sprays particularly in orchards, which most often produced apples, but also peaches, cherries, pears, and other tree crops [116,355].

For Br and U there no established MAC values, however, their content in analyzed soil fall within the limits reported in Kabata-Pendias [116] and overpassed the UCC value by a factor of 5.7-8.2. The Sources of the high content were mentioned previously. The concentration of U in analyzed soil was similar to the UCC value [346].

4.4.2 Assessment of elements accumulation in soil-fruits systems

To assess the metal accumulation from the soil in fruits *TF* values were calculated for all the systems. In all the analyzed fruits the highest values of *TF* were obtained for K, which varied from 1.79 in grape "Feteasca Neagra" from Purcari to 2.9 in plums from Criuleni (Fig. 4.14). Potassium is usually accumulated by the plants from the soil, its accumulation in the plant depends on the physicochemical properties of the soil, and differs greatly with soil type. Significant reserves of K can be maintained in the fertilized soils and released into the soil solution over the years following manuring, significantly increasing the plant-available K [356]. In the grapes ”Muscat de Hamburg” and ”Moldova” the *TF* values calculated for Rb were higher or near 1.0. Rubidium, as the other monovalent cations can be easily taken up by plants, it can partly substitute for K sites in plants, as their properties are similar, but cannot substitute K metabolic roles [116]. Relatively high values of *TF* were obtained for Ca (0.63), Sr (0.7), and Zn (0.76) in grapes "Feteasca Neagra" from Purcari. Calcium is an essential plant nutrient, playing the structural roles in the cell wall and membranes, as a counter-cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol [346]. Calcium is taken up by the roots from the soil solution and delivered to the shoot via the xylem. It may traverse the root either through the cytoplasm of cells linked by plasmodesmata (the symplast) or through the spaces between cells (the apoplast) [357]. Zinc is one of the eight trace elements (manganese, copper, boron, iron, zinc, chlorine, molybdenum, and nickel) that are essential for the normal, healthy growth and reproduction of plants [346].

The availability of Zn to plants and fruits depends on several soil factors such as the concentration of Zn in solution, ion speciation, and the interaction of Zn with other macronutrients and micronutrients [358]. In the higher plants, primary translocation of Sr takes place in three steps each influencing the other: (1) radial transport within the root from the outer medium (soil solution resp. apparent free space) to the xylem; (2) long-distance transport within the root or from the root to the shoot within the xylem; (3) radial transport from the xylem vessels to the xylem-surrounding tissues (parenchymas) of roots, stems, leaves, and flowers or fruits [359]. For other elements, including elements considered as environmental pollutants, *TF* values were lower than 1.0. It should be mentioned that even *TF* for Sr was lower than 1.0, its highest values were determined in grapes ranging from 0.32 to 0.57 [346].

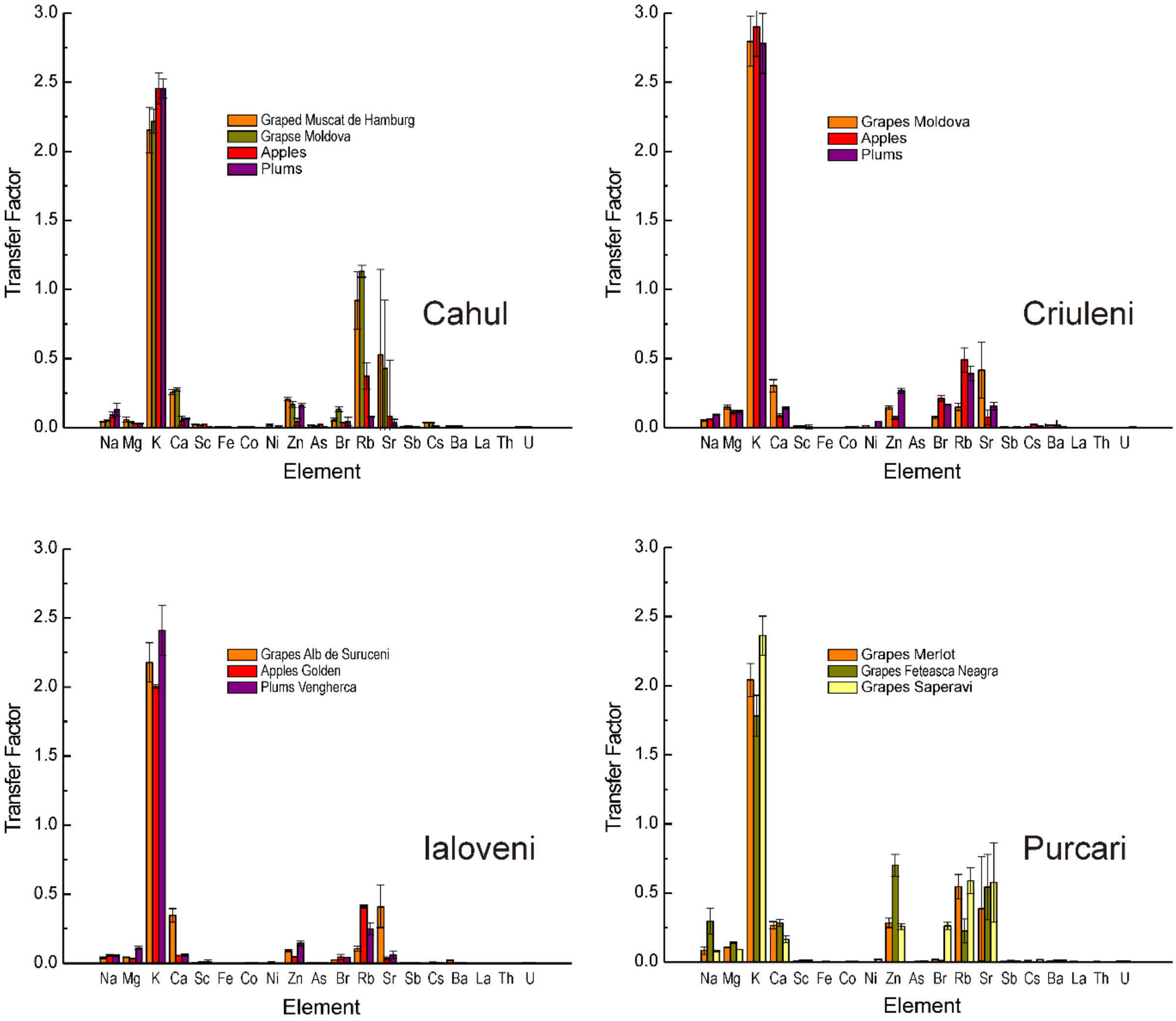


Fig. 4.14 Transfer factor values in the system soil-fruit for fruits collected in four regions of the Republic of Moldova

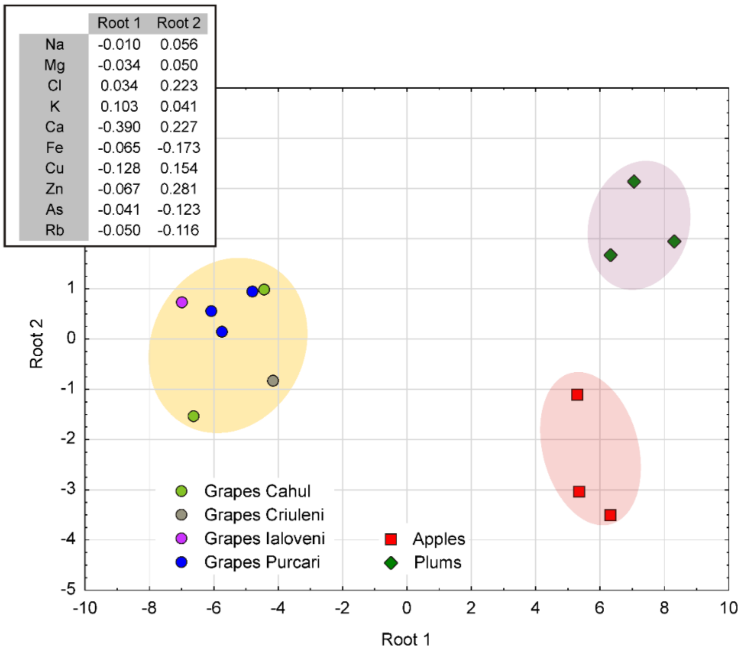
4.4.3 Evaluation of the risk assessment associated with the consumption of analyzed fruits and Discriminant analysis

Although the microelements such as Cr, Zn, Mo, Co, etc. play an important biological role their excess can be harmful to human health. For example, Cr and Ni are established the human carcinogens while an excessively high intake of Co may damage the heart muscles and may cause an overproduction of red blood cells or damage to the thyroid gland [347]. Thus, DIM and potential HQ values were calculated for seven elements (Co, Fe, Mn, Ni, Zn, As, and Sb), considered as environmental pollutants. The data obtained are given in Table A1.9 (Annex). According to the calculated values, the daily intake of metals from fruits varies greatly between the analyzed fruits. The uptake of Co from analyzed fruits was very low. The lowest uptake of Fe was from fruits collected in the Criuleni region, followed by Ialoveni, Purcari, and Cahul regions. The lowest accumulation of Fe was from plums. Accumulation of Mn changed in the following order grapes>apples>plums, while of zinc in order grapes>plum>apple. Bioaccumulation of toxic elements, As and Sb, from the analyzed fruits, was very low. The HQ values for all elements, except Sb in fruits collected in Criuleni and Cahul regions, were below 1.0, suggesting that analyzed fruits are safe for consumption [346].

To get more information concerning the similarities as well as the dissimilarities between the investigated fruits, the DA as one of the most appropriate statistic methods of analysis was used. Following this method, it was possible not only to discriminate between grapes, apples, and plums but also to evidence the differences between grapes according to the vineyards from where they have been collected. The results of this analysis are better illustrated by the Root 2 vs, Root 1 bi-plot reproduced in Fig. 4.15, as well as by the corresponding structure of Roo1 and Root 2.

Given the reduced number of samples (seven varieties of grapes distributed over four vineyards, and three varieties of apples and plums) the main contribution to DA was restrained to 10 elements (Na, Mg, Cl, K, Fe, Cu, Zn, As and Rb) which showed the greatest variability, able to assure the maximum discernibility between cases. As can be observed in Fig. 4.15, Root 1 showed a net separation between the apples and plums on one hand and grapes on the other while Root 2 proved the better discrimination between the apples and plums, and a partial overlap between the grapes and apples. From this point of view, Root 1 and Root 2 showed a net difference between plums and apples, and grapes [346].

Further, within the grape cluster, the Purcari vineyard presented the best homogeneity, while the Cahul, Ialoveni, and Purcari showed to be quite different. By analyzing the structure of Root 1 and Root 2, it can be remarked that while in the case of Root 1, only K, Ca and Cu have a relatively significant contribution, in the case of Root 2, the contribution comes from more elements, i.e. Cl, Ca, Fe, Cu, Zn, As and Rb. Because of this, it should be noted that, according to Markert et al. [349], Fe, Cu, Zn belong to the group of enzymatic elements while K and C represent one of the major constituents of vegetal tissue [346].



	Root 1	Root 2
Na	-0.010	0.056
Mg	-0.034	0.050
Cl	0.034	0.223
K	0.103	0.041
Ca	-0.390	0.227
Fe	-0.065	-0.173
Cu	-0.128	0.154
Zn	-0.067	0.281
As	-0.041	-0.123
Rb	-0.050	-0.116

Fig. 4.15 The result of Discriminant Analysis illustrating the existence of three clusters, each of them consisting of a single type of fruits

4.5 Determination of the elemental composition of medicinal plants of *Lamiaceae* family using NAA

Fertile soil and climatic condition in the RM are favorable for the cultivation of medicinal plants. Currently, the demand for medicinal and aromatic plants is growing, and Moldova is an important player in this sector. Among 2,000 vascular species in the flora of the Republic of Moldova and about 130 species of medicinal plants are present [360]. Aromatic and medicinal plants are used as high-quality pharmaceutical, perfumery, and food raw material with a much more enhanced content of active principles in comparison with the types collected in wild flora. Given the fact that about 3,500 tons of essential oil, 10,000 tons of food additives, 13,000 tons of perfumery additives are produced in the world, the development and cultivation of medicinal and aromatic plants occupy an important place in agriculture [361]. Besides being an important source of essential oil medicinal plants are widely used today in traditional medicine. The use of herbal medicinal products and supplements has increased tremendously over the past decades with about 80% of the world population relying on them for primary healthcare [362].

The elemental content of medicinal plants is affected by the geochemical characteristics of soil, application of natural and artificial fertilizers, climatic conditions, vicinity of industry and extensive agricultural activity, and by the ability of herb species to accumulate elements

[30,363]. Often medical herbs and their mixtures can present a health risk due to the presence of toxic elements such as Pb, Cd, Al, Hg, or Cr [363]. Thus, the plants used in therapeutics should be collected in the environmentally clean areas and their chemical content needs to be carefully controlled.

Concentrations of macro and trace elements in 45 species of herbs of *Lamiaceae* family collected in the RM were determined by the mean of NAA. The *Lamiaceae* is one of the most important plant families with the extensive economic importance and a wide spectrum of applications in medicine, due to the production of essential oils, tannins, saponins, and organic acids that possess antimicrobial, antifungal, antibacterial, anti-inflammatory, and antioxidant activities [364,365]. The majority of *Lamiaceae*, species are used in the perfumery, cosmetics, food, and pharmaceutical industries as active ingredients or as flavor and fragrance [366,367].

In the flora of the RM, the *Lamiaceae* family is represented by 28 genera and 82 species [368]. In the Collection of Medicinal Plants from National Botanical Garden (Republic of Moldova), the *Lamiaceae* family is represented by 99 taxa belonging to 30 genera [369]. Aerial parts of the plants were collected at the full flowering stage during 2017 from the Experimental subdivision of the Collection of Medicinal Plants (CMP) of National Botanical Garden (Institute), RM. The plants were cultivated in ecological conditions and without use of fertilizer. The aerial parts were dried separately at room temperature (20-23 °C) in dark. Voucher specimens of each species/cultivar were deposited at the Herbarium of the National Botanical Garden (Republic of Moldova) [370]. Samples preparation and the irradiation procedure are described in Chapter 2.

Using NAA, it was possible to determine 26 major and trace elements in analyzed herbs. The results of major elements (Na, Mg, K, Ca, and Cl) determinations in the analyzed plant samples as well as *RP* values [263] are summarized in Table A1.9 (Annex) and graphically presented in Fig. 4.16a. The content of major elements in analyzed plants changed in the following order K>Ca>Mg>Cl>Na, and were on the level of mg/kg, except Na (μg/g). The concentration of K in plants ranged from 10700 μg/g in *Melissa officinalis* to 44800 μg/g in the *Lamium album*. The primary role of K consists in the maintenance of water balance in plant cells and its content in plants varied from 1.58% to 3.75%. It also plays the essential role in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, and stress resistance [371,372].

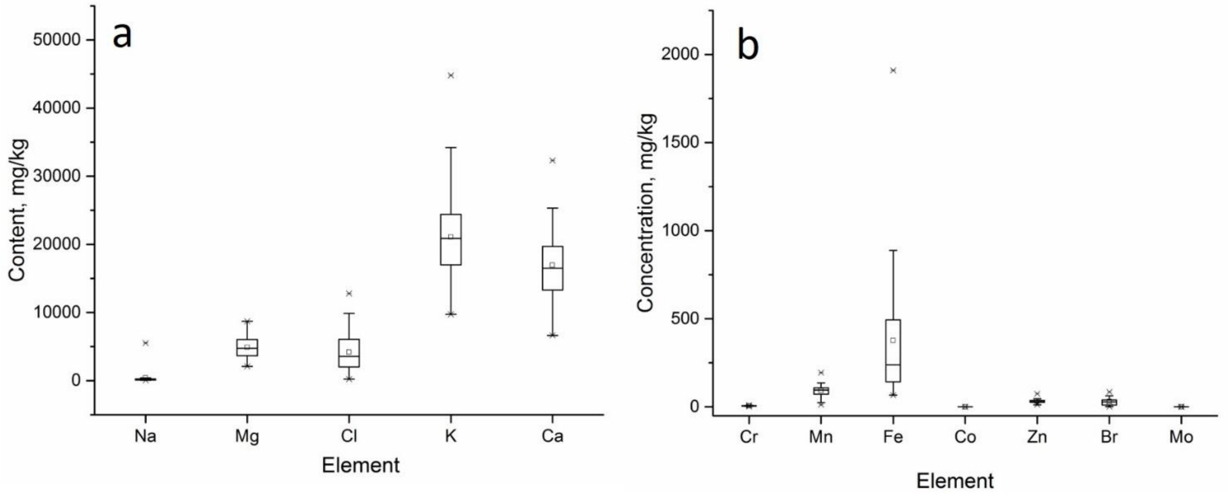


Fig. 4.16 The graphical presentation of (a) macroelements and (b) microelements content in medicinal plant raw materials determined by NAA

As it was shown in previous sections K in plants is mainly accumulated from the soil. Calcium content in analyzed plants varied from 6650 μg/g in *Lavandula angustifolia* to 32300 μg/g in *Nepeta parviflora*. High Ca content in studied plants may be linked to the abundant limestone soil in Moldova [370]. Calcium is an essential plant nutrient, required for various structural roles in the cell wall and membranes, it is a counter-cation for inorganic and organic anions in the vacuole, and the cytosolic Ca^{2+} concentration ($[Ca^{2+}]$cyt) is an obligatory intracellular messenger coordinating the responses to numerous developmental cues and environmental challenges (White and Broadley 2003). Magnesium content in analyzed plants varied from 2100 μg/g (*Mentha verticillata*) to 8710 μg/g (*Salvia tesquicola*). Magnesium is one of the core macronutrients in plant growth and development, playing a central role in plant chlorophyll biosynthesis and carbon fixation as a cofactor of a series of enzymes involved in carbon metabolism [374]. Chlorine is an essential nutrient of higher plants and participates in several physiological metabolism processes. Its functions in plant growth and development include osmotic and stomatal regulation, the evolution of oxygen in photosynthesis, and disease resistance and tolerance [375]. Chlorine content in analyzed plants varied considerably from 208 μg/g in *Salvia sclarea* to 12800 μg/g in *Mentha x gracilis'Variegata'* [370].

Among the major elements, Na concentrations in the plants were the lowest: 44.5 μg/g (*Origanum virens*) - 5530 μg/g (*Mentha x piperita citrata)*. Only in several plants (*Menthax piperita citrata, Mentha x gracilis'Variegata'*, *Mentha verticillata,* and *Rosmarinus officinalis),* Na content was on the level of mg/g. Due to Na^+ similarity to K^+, K^+ transporters often are not discriminated sufficiently between these cations, thus, the excess of external Na^+ can not only impair K^+ acquisition but also lead to accumulation of Na^+ in the plant cells [376]. For almost all terrestrial plants, Na is not an essential element for either growth and development or reproduction [377]. The K/Na ratio is usually used to characterize the diuretic activity of medicinal plants. The values obtained for the analyzed plants varied between 1.8:1 and 548:1. The mean values of major elements determined in analyzed plants were higher than the corresponding *RP* values [370].

The concentration of essential elements Cr, Mn, Fe, Co, Zn, Mo, and Br determined in the analyzed plants varied significantly (Fig.4.16b, Table A1.11, Annex). The comparison with RP values revealed that the mean concentration of Mo and Co were on the level of data presented by Markert for *RP* [263], values for Zn and Mn were lower, while those for Cr, Fe, and Br higher. Iron was the most abundant microelement in plants, its content varying greatly from 1910 µg/g in *Thymus citriodorus 'Aureus'* plant to 67 µg/g in *Nepeta parviflora* [370]. The redox properties of Fe make it an essential element for practically all life forms, playing a significant role in various physiological and biochemical pathways in plants as well. It serves as a component of many vital enzymes such as cytochromes of the electron transport chain, and it is thus required for a wide range of biological functions. Plants mainly acquire Fe from the rhizosphere. Although Fe is one of the most abundant metals in the Earth's crust, its availability to plant roots is very low [378–380].

The zinc level in the analyzed herbs ranged from 13 µg/g (*Scutellaria baicalensis*) to 75 µg/g (*Agastache urticifolia*). Zinc is an essential component of thousands of proteins in plants, although it is toxic in excess. As a component of proteins, zinc acts as a functional, structural, or regulatory cofactor of a large number of enzymes. It was already proved that Zn plays a key role in stabilizing of RNA and DNA structure, maintaining the activity of DNA synthesizing enzymes, and controlling the activity of RNA degrading enzymes. Its deficiency is associated with the disruption of normal enzyme activity, and consequently inhibition of photosynthesis [381]. Cobalt, an essential component of several enzymes and co-enzymes, can affect the growth and metabolism of plants, in different degrees, depending on its concentration and form in the rhizosphere and soil [382]. Minimal Co concentration was determined in *Nepeta parviflora* (0.03 µg/g) and maximum in *Thymus citriodorus 'Aureus'* (0.88 µg/g). Chromium was detected only in *Ajuga genevensis*, *Origanum vulgare*, *Thymus commosus*, *Thymus vulgare*, and *Mentha x gracilis 'Variegata'* samples [370].

In plants, Mn is one of the essential elements, intervening in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor. It is only needed in small quantities for plants but is ultimate as critical to growth as are the other nutrients [383]. The manganese content in samples ranged from 12.7 µg/g (*Lavandula angustifolia*) to 194 µg/g, with the highest concentration in *Salvia verticillate* [370]. Despite many plant species could accumulate large amounts of Br its role in various biochemical processes is still unknown. The main source of bromine in plants is considered to be soil, in case if there no oblivious anthropogenic sources [384]. At the same time, according to Scott McCall and co-authors [385], Br is an essential trace element for all animals, and its deficiency may be relevant to BM alterations observed in the nutritional and smoking-related disease. Bromine content in samples varied between 2.0 µg/g (*Thymus calcareus*) and 85 µg/g in *Ocimum basilicum* and was lower than the maximum limit established by USP for bromine (< 125 µg/g) [386]. Molybdenum in plants is utilized by selected enzymes to carry out redox reactions. Enzymes that require molybdenum for activity include nitrate reductase, xanthine dehydrogenase, aldehyde oxidase, and sulfite oxidase [387]. In the analyzed plants, its content varied between 0.21 µg/g (*Ajuga reptans*) and 1.7 µg/g in *Ajuga genevensis* [370]. The requirements of plants for Mo are between 0.2–5 mg/kg [116].

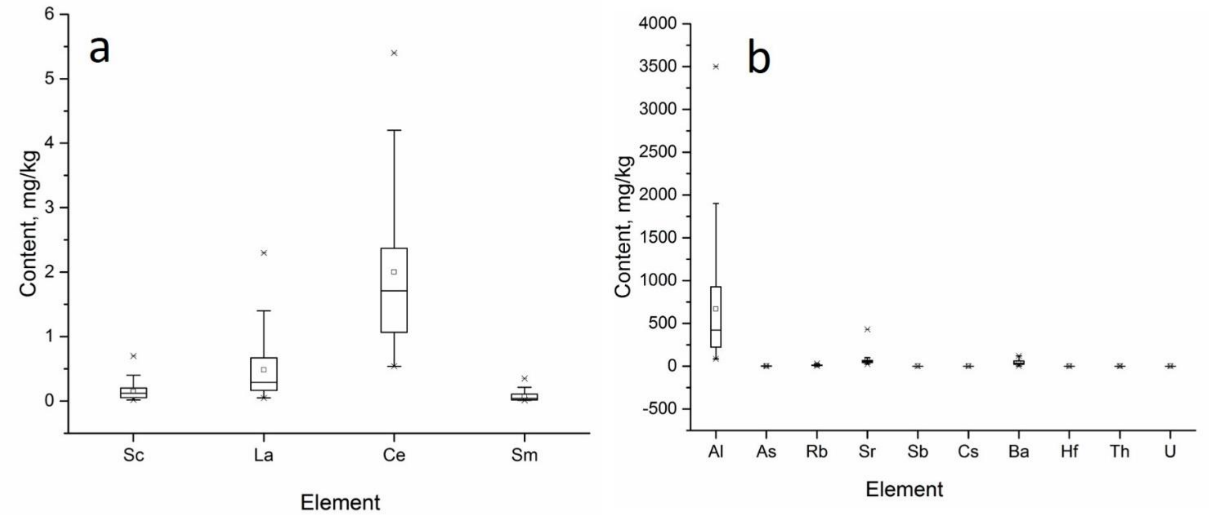


Fig. 4.17 The graphical presentation of (a) REE and (b) non-essential elements content in medicinal plant raw materials determined by NAA

The concentration of 14 elements, including four rare earth elements are given in Fig. 4.17 and Table A1.12, Annex. Aluminum is an amphoteric element without any established biological function. The availability of Al and, in consequence, the possibility of Al to interact with plants is mostly restricted to an acid environment [388]. Aluminum was determined in analyzed plants in a wide range of concentrations: from 86 µg/g in *Origanum virens* to 3500 µg/g in *Thymus citriodorus 'Aureus'* [370]. The concentration of As in analyzed plants exceed the limits of 1 mg/kg recommended for medicinal plants [389] and some species reached a value higher than 2.0 µg/g. The active biomonitoring studies performed in Chisinau and described in

Chapter 3, Section 3.2 showed that the level of As in moss exposed in BG for five months was on the level of control. Thus, high As content in plants can be associated with its accumulation from the soil. Plants acquired Ba from the soil in considerable amounts and its accumulation in vascular plants varies among the species. It is suggested that high plant uptake of Ca enhances Ba uptake [390]. Its concentration in the analyzed plants ranged from 1.3 μg/g (*Origanum vulgare*) to 119 μg/g in *Thymus calcareus*. Cesium common range in various plants can be presented as < 0.1–3 mg/kg [116]. In analyzed herbs, Cs content was significantly lower, within 0.007 (*Origanum virens*), and 0.3 μg/g (*Thymus citriodorus 'Aureus'*). The Main Rb source in plants is soil and its intake by plants increases with the lower soil pH. The accumulation of Rb is dependent on K concentration in soil. Thus, high K content inhibits Rb uptake by plants [391]. Rubidium values in plants changed from 2.84 μg/g (*Ajuga reptans*) to 31 μg/g (*Salvia sclarea*) [370].

High Sr accumulation capacity was observed for *Thymus calcareus* (429 μg/g), *Thymus marschallianus* (390 μg/g), *Thymus moldavicus* (391 μg/g), *Melissa officinalis* (112 μg/g), *Melissa officinalis* ssp. *altissima* (100 μg/g), and *Mentha x gracilis'Variegata'* (105 μg/g). For the other plants, it was lower than 100 μg/g. Although Sb is considered a nonessential metal, it is relatively easily taken up by plants if present in soluble forms [116]. Antimony has no known biological function and it can be toxic, in some cases carcinogenic, at elevated concentrations. Information about Sb uptake by crop plants is very limited. It is suggested that Sb is translocated within plants primarily along the apoplastic pathway through the xylem [392]. In medicinal plants from the BG, the content of Sb lied in the range 0.01-0.2 μg/g. The content of the Hf (0.01 - 0.5 μg/g) felt within the range characteristic for plants given as 0.01–0.4 mg/kg [116]. In the present study, its content ranged from 0.01 to 0.5 μg/g. As reported in the literature values of concentrations of U and Th in various plant species differ significantly. Thorium was accumulated by analyzed plants in the range of 0.008-0.75 μg/g and U - 0.005-0.16 μg/g [370].

Information about the rare earth elements (REE) determinations in medicinal plants is rather scarce in the literature. The concentration of REEs in plants growing in the uncontaminated environment is around 10^{-2}-10^{-4} μg/g dry mass [393]. It was shown [393] that REEs can regulate the chlorophyll activities and photosynthetic rate in plants. They can promote the protective function of the cell membrane and enhance the plant resistance capability to the stress produced by environmental factors., in variable amounts. Their mean concentration of four REE elements determined in the analyzed medicinal plants changed in the following order: Ce>La>Sc>Sm (Fig.4.17a). However, from 45 analyzed species Ce was determined only in 16 samples (in other it was below the detection limit).

Table 4.145 Comparison of results obtained in the present study (in mg/kg) with literature data [370]

Element	Present work (mean value)	*Mentha spicata L.* [394]	*Salvia officinalis* [395]	*Rosmarinus officinalis*/ *Rosmarinus tournefortii* [396]	*Salvia officinalis* [397]	*Lavandula angustifolia* [398]
Na	211	1020	567	190/ 4400	280	-
Mg	4908	-	4250	1740/ 1710	-	-
Al	648	-	1830	-	-	-
Cl	4035	-	2430	-	-	-
K	21411	22940	16400	13950/ 11190	23000	-
Ca	16910	14040	14500	2950 /19120	-	-
Sc	0.14	0.18	-	-	-	-
Cr	4.88	4.85	7	2656/5602	-	90-540
Mn	90.8	-	56	22.1/ 18.4	716	41-163
Fe	364	463	1050	268/342	1176	66-477
Co	0.25	0.35	-	-	-	-
Zn	31.6	38.5	123	33.1/ 29.3	55.6	-
As	0.67	0.819	2.1	--	--	-
Br	30.42	13.3	-			-
Rb	10.3	9.7	-	--	--	-
Sr	76.3	42.1	-	-	-	--
Mo	0.66	-	2.8	-	-	-
Sb	0.033	0.04	0.6	-	-	-
Cs	0.066	0.11		-	-	-
Ba	40.8	22.57	84	-	-	-
La	0.47	0.42	-	0.38/0.5	-	-
Ce	1.98	2.82	-	-	-	-
Sm	0.07	0.08	-	-	-	-
Hf	0.12	0.07	-	-	-	-
Th	0.14	-	-	-	-	-
U	0.04	-	-	-	-	-

In the plants, metal ions can be accumulated from the soil, groundwater, irrigation water or plants can absorb metals from atmospheric particles, deposited on them. The active biomonitoring study performed in the BG showed that for Ni, Fe, Sc, Mg, Na, Rb, La, Hf, Th, U, and Sb mean RAF values were higher than 1.0. The main source of these elements can be considered re-suspension of soil particles on leaves as well as transport. Thus, even when fertilizers were not applied for analyzed plants growth they can accumulate metals from soil and atmosphere. The mean concentrations of elements were compared with the data presented in the literature (see Table 4.16). The number of elements determined for plants from the RM was significantly higher than the data reported for other plants of the *Lamiaceae* family.

The values obtained values were comparable with data obtained by Begaa et al. [394] for all elements, except Na, Br, Sr, Ba, and Ce. The differences in the elemental composition of medical plants can be explained by geological particularities of the soil in the zones where they were grown, applied agricultural practices, anthropogenic loading as well as the composition of the water used for irrigation.

4.5.1 Estimation of the Dietary Intake

Since the medicinal plants are often used as a tee and chemical elements are transferred from plants in tea infusions and further in the human body it is very important to determine the tolerable daily intake (TDI) of potentially toxic elements. The elements in tea infusions can be classified into three groups poorly (Ca, Co, Fe, La, Sb, Sm, Sc, Sr, and Th), moderately (Al, As, Cr, Mg, Mn, Zn), and highly extractable (K, Cs, Na, Rb) [399,400]. The TDI values for some elements considered as toxins were calculated assuming an intake of 10 g (dry weight) of plant leaves ration per person according to [394] and 8 g according to the State Pharmacopoeia of the Russian Federation. The values obtained together with the data prescribed by the World Health Organization (WHO) [389] are given in Table 4.17.

Table 4.15 Tolerable Daily Intake for elements, considered as potentially toxic

	Element							
	As	Sb	Co	U	Br	Cr	Zn	Mn
	µg/day/person				mg/day/person			
[1]TDI, adult with 70 kg b.w.	6.7	0.3	2.5	0.4	0.3	0.05	0.3	0.9
[2]TDI, adult with 70 kg b.w.	5.3	0.26	2.0	0.36	0.24	0.04	0.25	0.7
TDI value according to WHO	2.1	6	11	0.6	70	0.2	1	3

The calculated TDI values did not exceed the daily intake set by the WHO. The only element, which mean TDI values exceeded permissible level was As. The results obtained revealed that medicinal plants should be carefully used keeping in mind the possible negative impact on human health.

Principal component analysis

To identify the relations between the determined elements in studied plant materials the principal component analysis was applied. As a result of the performed calculations, four components were extracted (eigenvalues higher than one), which explains about 75% of the total variance with the contribution of each factor being 45%, 11.4%, 9.6%, and 8.8%. The extracted factors were used for further analysis. The matrix of rotated factor loadings is given in Table 4.18.

Factor 1 (Al, Sc, Fe, Co, Cs, La, Sm, Hf, Th, and U) is the strongest factor, which explains 45% of the total variance. It includes the elements, which are naturally distributed (geogenic association of elements). The second factor accounts for 11% of the total variance and includes K and Rb (positive correlation), Sr, and Ba (negative correlation). Factor 3, loaded by Mg, Ca, and Mn, explains 9% of the total variance. The second and third factors are most likely of vegetation origin. The fourth factor has high loading for Na and Sb and can be considered as natural/anthropogenic associations of elements.

Table 4.167. Matrix of varimax rotated loadings

Element	F1	F2	F3	F4	Element	F1	F2	F3	F4
Na	0.12	-0.08	-0.07	**0.90**	As	0.43	-0.06	-0.15	-0.40
Mg	-0.056	0.04	**0.91**	-0.16	Br	0.38	0.37	-0.01	-0.04
Al	**0.96**	0.03	0.08	0.06	Rb	-0.09	**0.59**	0.35	-0.16
Cl	0.24	0.33	0.49	0.31	Sr	-0.12	**-0.85**	0.13	-0.13
K	0.08	**0.58**	0.22	-0.38	Sb	0.46	0.001	0.06	**0.79**
Ca	-0.13	-0.44	**0.68**	0.06	Cs	**0.95**	0.09	0.04	0.077
Sc	**0.97**	0.01	0.03	0.08	Ba	0.42	**-0.74**	0.21	-0.17
Mn	0.53	0.22	**0.55**	0.09	La	**0.98**	-0.01	-0.01	0.033
Fe	**0.97**	0.02	0.06	0.09	Sm	**0.96**	-0.04	0.02	0.13
Co	**0.92**	0.16	0.02	0.05	Hf	**0.91**	0.15	0.03	0.12
Zn	0.35	-0.14	0.18	0.09	Th	**0.98**	0.04	-0.01	0.031
Expl. Var	10.3	2.63	2.20	2.03	U	**0.92**	-0.24	-0.08	0.027
Prp. Totl	0.45	0.11	0.09	0.088					

The 3-D plot of the principal analysis components is shown in Fig 4.18. The relation between elements rewords the results of FA.

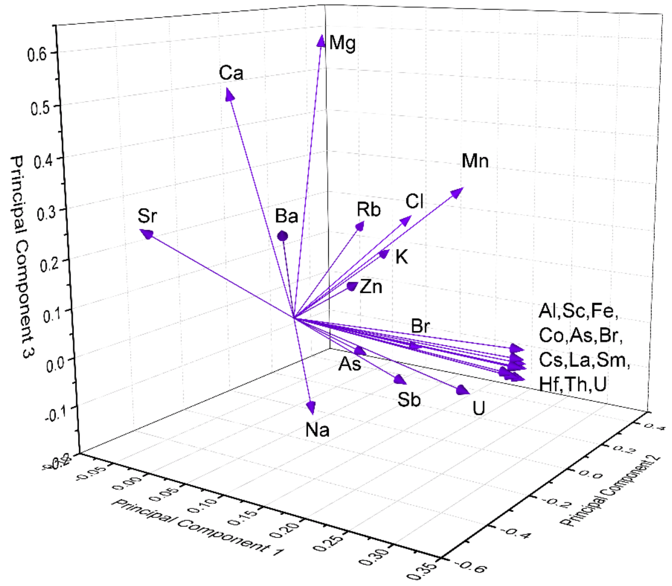


Fig. 4.18 Principal component analysis results in the three-dimensional space: plot of loadings of the first three principal components.

4.6. Conclusions to Chapter 4

1. Neutron activation analysis proved to be a suitable technique for the determination of a wide range of chemical elements (up to 40) in soil, wine, fruits and medicinal plants samples collected in the Republic of Moldova.
2. For the first time, the elemental composition of the soils of two renowned Moldavian vineyards: Romanesti and Cricova was determined. The elemental content of the studied soils showed to be very close to Average soil values and felt within the limits established for Moldavian soils. The analysis of Rb, Sr, and Zr distribution evidenced a significant degree of soil chemical weathering. The high content of As (approximately 1.5 higher than MPL) and Br (two times higher than UCC) in the soil can be associated with agricultural activity.
3. Neutron activation analysis enables evidence of the presence of 18 elements in the analyzed wines samples. Potassium was the dominant element in wine, which transfer factor values varies between 21 and 36 mg/L for Romanesti wines and between 27 and 32 mg/L for Cricova wines. The content of other elements, including the toxic ones, did not exceed the limits established by the International Organisation of Vine and Wine.
4. For the first time, the neutron activation analysis was applied to determine the content of elements in soils and fruits of different sorts collected in the different zones of the Republic of Moldova. According to data obtained, the soils can be characterized as slightly polluted

with Zn, As, Cr, V, and Sb and moderately polluted with Br, which sources can be pesticides and transport. The concentration of As in the soil exceeded the maximum admissible concentration established by the national authorities approximately 1.5 times, thus, it may present a risk for human health.

5. In all types of fruits as well as the leaves, high content of K, Mg and Ca was found, the highest values were obtained for K. The concentration of essential and trace elements varied in the analyzed fruits within a wide range, but they were corresponding to the data reported in the literature.
6. The daily intake values calculated for a selected number of elements varied greatly depending on the fruits sort and place of provenance. The potential health hazard index values showed that Sb is the element that presents a danger for human health, especially in Criuleni, Ialoveni, and Cahul regions.
7. For the first time, the elemental composition of 45 plant species of plants of the Lamiaceae family growing in the Republic of Moldova was determined by NAA. The plants analyzed were particularly rich in major elements (K, Cl, Mg, and Ca). Iron was the most abundant in plant material, followed by Mn, Zn, Br, Cr, Mo, and Co. Arsenic concentration in analyzed plants changed in a wide range, but just in several species, it exceeds the value established by the World health organization.
8. The tolerable daily intake values calculated for the selected elements, except for As were lower than the values established by the World Health Organization.
9. Statistical instruments, factor analysis and discriminant analysis were powerful tools allowing the identification of elements sources and samples differentiation.
10. Based on the analysis performed, it can be concluded that As, Sb, and Br are elements, which can pose a risk to human health in the Republic of Moldova.

5. ELABORATION OF ENVIRONMENTALLY FRIENDLY SORBENTS FOR WASTEWATER TREATMENT

Water quality monitoring is a major issue toward maintaining the safety and security for many countries, including RM. The most important water resources in RM are Danube, Nistru, and Prut rivers, and Raut, Bac, and Botna small rivers. According to the national authorities, the hydrobiological monitoring of the Prut, Danube, and Dniester rivers showed the water quality class III (“moderately polluted”) [22]. The content of heavy metals is higher in the river Nistru, downstream of the towns of Soroca, Tighina, and Tiraspol, as well as after the inflow of the tributaries Raut and Bac. Between 30% and 76% of the total heavy metals (Cu, Zn, Pb and Cd) content in water is bound to the suspended particles [401]. The pollution of small internal watercourses remained severe, with water quality classes III–V („moderately polluted” - „intensely polluted”). For example, river Bac, flowing through Chisinau is badly affected due to the inflow of poorly treated wastewater and runoff water as well as its low dilution capacity [401].

Today, water pollution with heavy metals has become one of the most serious environmental and social problems for Moldova [216]. Water contamination can occur in different ways, such as discharge of both treated and untreated wastewater, accidental contamination, runoff from residential and industrial areas, agricultural practice, soil erosion, etc. [179]. Effluents from metal plating facilities, paper industries, power generation facilities, electronic device manufacturing units, tanneries, iron-sheet cleaning, metal processing, automobile parts manufacturing, dyeing, textile, fertilizer, and petroleum industries release heavy metals into the environment in concentrations exceeding the permissible levels [239].

Due to the global water scarcity, many countries use wastewater obtained after different industrial processes as well as domestic ones for irrigation. At least 20 million ha in 50 countries are irrigated using wastewater. Wastewater usage for irrigation has some benefits as the reduction of groundwater and fertilizers utilization and solution of effluent disposal problem. However, since wastewater may contain toxic components, especially heavy metals and often are inappropriately cleaned or used without any pretreatment it can result in the contamination of soil and agricultural crops, and finally have a negative impact on human health. As it was shown in Chapter 1, release of wastewater containing metal ions in natural water and soil may result in the death of organisms or disruption of metabolic processes [402].

Today, in accordance with the European Water Framework Directive (EU-WFD, 2000/60/EC), the ecological status of water bodies is assessed based on five biological indicators such as phytoplankton, macrophytes, phytobenthos, benthic invertebrates, and fish [403]. Toxicity test performed on different aquatic organisms has shown that responses to metal ions is very different and depend on the experimental conditions such as pH, temperature, salinity and other water quality parameters. Thus, according to PAN Pesticides Database 50%-lethal concentrations (LC_{50}) of metal ions for fish are the following: for Ni 1.3-10.4 mg/L, for Zn 0.35-30 mg/L, for Cr 14.3-93 mg/L, for Pb 0.44-2mg/L. The values for *Daphnia magna* are significantly lower [404]. In Gheorghe et al. study [403] the LC_{50} for Zn, Pb, and Ni and Cr in fish from Danube Delta were found to be 12.2, 30.10, 65.8, and 120 mg/L, respectively. The LC_{50} values for Cr varied from 7.3 mg/L in Sanyal et al. [405] study to 164 mg/L in Shaukat and Javed [406] research. Ionic Ni is lethal to sensitive species of aquatic organisms at 11-113 μg/L, while fish *Anabas testudineus* were resistant to Ni concentration of 143 mg/L [407]. The 96-h LC_{50} for $AgNO_3$ lies in the range of 5 to 70 μgAg/L, however, in saline water it increases to 0.33-2.7 mg/L [408].

There is a lack of information in the literature about the toxic effect of complex metal-containing systems on water organisms. The experimental studies of the effect of copper-containing effluents with different chemical compositions and metal ion concentrations on *Spirulina platensis* biochemical composition showed that such effluents are toxic for spirulina even at low copper concentrations. Effluents (Cu/Fe, Cu/Fe/Ni, Cu/Fe/Zn and Cu/Fe/Ni/Zn) with the following metal concentrations: copper ions 10 mg/L, iron – 5 mg/L, and zinc and nickel – 2 mg/L lead the cyanobacteria to grow only for one vital cycle, leading to a significant reduction of the biomass (Fig. 5.1). In the Cu/Fe system amount of biomass was reduced by 32.8%, in Cu/Fe/Zn and Cu/Fe/Ni systems approximately by 40%. The most pronounced decrease of biomass, by 52.6% in comparison with control biomass, occurred in the Cu/Fe/Ni/Zn system. It is seen that with the increase in the number of metal ions in solution the decrease in biomass amount was more pronounced. Thus, at given concentration and composition, the effluents constitute a serious stress factor for cyanobacteria. Copper toxicity for cyanobacteria can be explained by the production of reactive oxygen species through the Fenton and Haber–Weiss reactions, which lead to severe damage of lipids, proteins, DNA, and other cytoplasmic molecules and substitution of Mg in chlorophyll as well as by the ability of copper ions to chelate the sulfhydryl groups, thereby interfering with the cell proteins or enzymes [52,409].

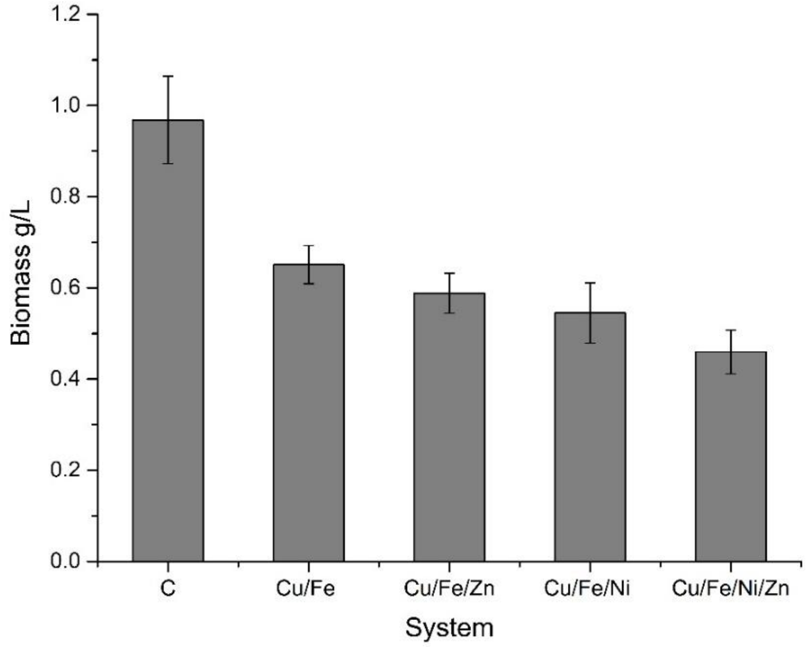


Fig. 5.1 *S. platensis* biomass in four analyzed systems: I) Cu/Fe, II) Cu/Fe/Zn, III) Cu/Fe/Ni and IV) Cu/Fe/Zn/Ni (C_{iCu} 10 mg/L, C_{iFe} 5 mg/L, C_{iNi} 2 mg/L and C_{iZn} 2 mg/L)

The protein content was affected in the same manner as biomass amount. From Fig. 5.2 can be seen that in Cu/Fe system protein content was reduced by 50% and constituted 27.75% of absolutely dry biomass. In Cu/Fe/Ni, Cu/Fe/Zn, and Cu/Fe/Ni/Zn systems proteins content in biomass constituted 22.2, 22.3 %, and 20.25%, respectively, indicating their content reduction by 66-69%. The decrease of the proteins levels in cyanobacteria cultivated in media supplemented with metal ions was reported in several studies [409,410]. This decrease of the protein content can be explained by the competition of metal ions present in the systems their binding sites in proteins, resulting in a perturbation of protein function and further degradation [52]. Copper, zinc, iron ions in spirulina are mainly accumulated in protein and carbohydrate fractions, while nickel ions mainly affect proteins related to photosynthesis and carbon metabolism [179].

Carbohydrates were less affected by the presence of metal ions in the cultivation medium, their content in biomass was reduced by 19.7 - 29.2% with respect to control biomass. It should be mentioned that the variation of carbohydrates content in the systems was within the limits of standard deviation.

The changes in the lipids content were also statistically insignificant. Lipids are the only macromolecules, which maintained their content in biomass, affected by metal ions. A decrease in carbohydrates content can be attributed to their use by spirulina biomass as the source of carbon for lipids biosynthesis. Thus, the lipids peroxidation process resulting from the presence of metal ions in the cultivation medium is partly offset, which explains the survival of certain number of cells [411].

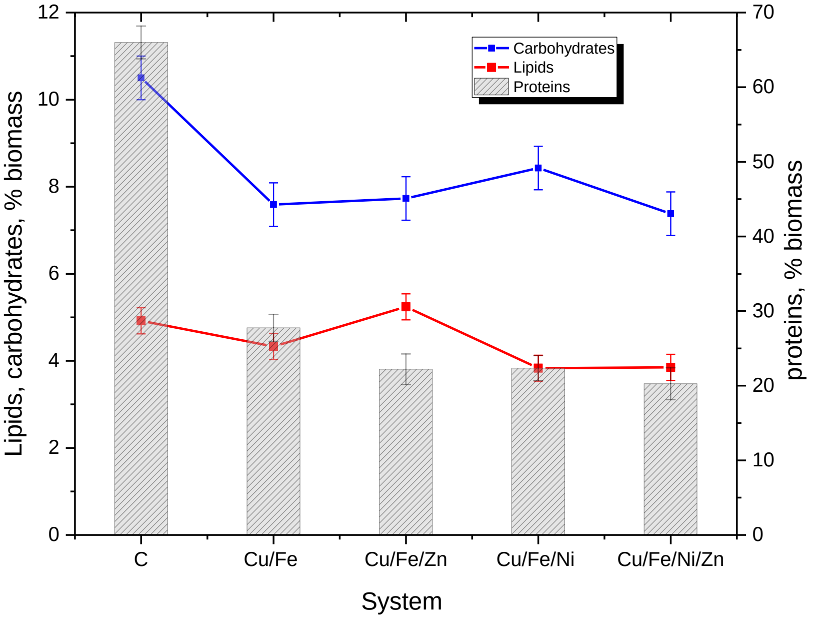


Fig. 5.2 The proteins, lipids and carbohydrates content in *S. platensis* biomass cultivated in in four analyzed systems: I) Cu/Fe, II) Cu/Fe/Zn, III) Cu/Fe/Ni and IV) Cu/Fe/Zn/Ni (C_{iCu} 10 mg/L, C_{iFe} 5 mg/L, C_{iNi} 2 mg/L and C_{iZn} 2 mg/L)

Even, *S. platensis* was significantly affected by metal ions it showed very good metal bioaccumulation capacity. According to NAA data (Fig. 5.3), in Cu/Fe system spirulina biomass accumulated 200 times more copper and 1.6 times more iron - 5990 µg/g and 7200 µg/g, respectively than their content in control biomass. In the Cu/Fe/Zn system biomass accumulation capacity for copper and iron remain on the level of Cu/Fe system 6000 µg/g for copper and 6740 µg/g for iron, and the amount of zinc accumulated by biomass was 26 times 1160 µg/g higher than in control biomass. In the Cu/Fe/Ni system amount of accumulated copper increased in comparison with two previous systems by approximately 1000 µg/g, while iron uptake was significantly reduced, it was approximately two times lower than in control biomass - 2460 µg/g. The amount of accumulated nickel ions constituted 162 µg/g. Comparing Cu/Fe/Zn and Cu/Fe/Ni systems, spirulina preference for zinc ions was observed. Although nickel and zinc were added to the solution in the same concentrations *S. platensis* accumulated approximately 7 times more zinc. One of the peculiarities of *S. platensis* is the zinc accumulation in the biomass in amounts that exceed the optimal amount determined for its growth and development [179]. At the same time nickel ions even in concentration 0.1 µg/g caused a net reduction in *Anacystis nidulans* and *Spirulina platensis* biomass. Nickel toxicity for cyanobacteria is associated with its binding to sulfhydryl-like groups and consequent reduction in Chl-A content [412]. In the quaternary system, the highest accumulation for all elements was observed: 9720 µg/g for copper, 7760 µg/g for iron, 1500 µg/g for zinc, and 593 µg/g for nickel. Since spirulina biomass was able to grow at given metal concentrations only for one cycle it can be considered as biosorbent [411].

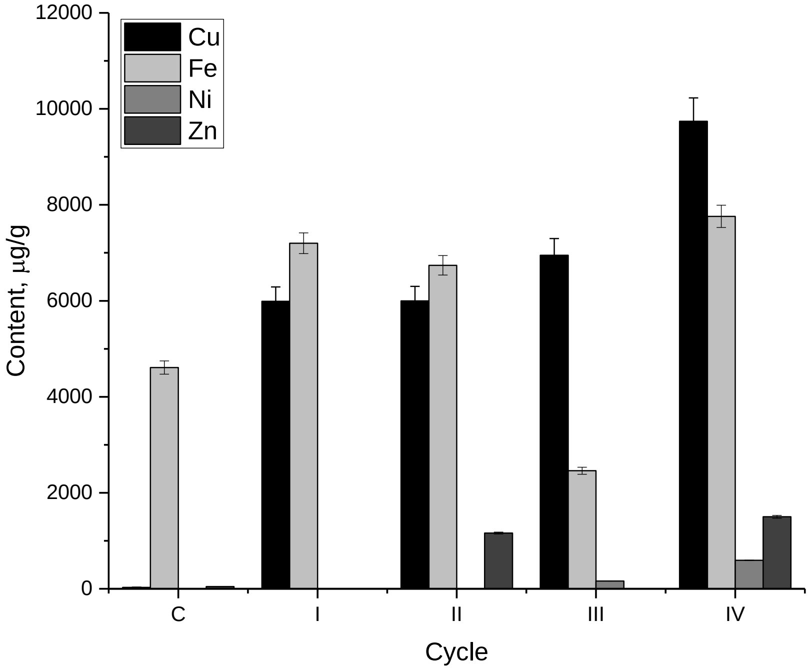


Fig. 5.3 Metal uptake by *S. platensis* biomass in four analyzed systems: I) Cu/Fe, II) Cu/Fe/Zn, III) Cu/Fe/Ni and IV) Cu/Fe/Zn/Ni (C_{iCu} 10 mg/L, C_{iFe} 5 mg/L, C_{iNi} 2 mg/L and C_{iZn} 2 mg/L)

Thus, *Spirulina platensis* in line with other biological objects can be considered as a suitable material for metal removal from wastewater before their discharge in the natural water bodies. Several remediation techniques to remove metal ions from aqueous solutions are available ranging from the traditional physicochemical methods to emerging biotechnological techniques such as biosorption and bioaccumulation [225]. Biosorption is the metabolism-independent uptake of metals by non-living biomass, while bioaccumulation is a slow and nutrient-dependent process. The main scope of these processes is to reduce the contanimants concentrations to the levels, which will not present danger for aquatic organisms and human health. In the present Chapter examples of biosorbents application for metal removal from synthetic and real wastewater will be discussed.

5.1 Metal removal from the synthetic effluents using *Spirulina platensis*.

Cyanobacteria and microalgae are of particular interest in the development of new materials with biosorption properties. These organisms accumulate metals from the environment by passive adsorption of metals on the cell surface, such as the cell wall, membrane, and capsular polysaccharides, or by the formation of complexes with intracellular or exocellular molecule [413]. Numerous investigations have shown that *Spirulina platensis* (*S. platensis*) can be efficiently applied as bioaccumulator and sorbent of different metals and radioactive ions [38,52,168,179,195,202]. As metals of interest Ag, Pb, Ni, Zn, Cr(III), Cr(VI), and Re(VII) were chosen. Lead is a common environmental pollutant, its widespread use has discontinued in many countries of the world, but it is still used in many industries like car repair, battery manufacturing, and recycling, refining, smelting, *etc.* Lead is highly poisonous metal affecting almost every organ in the body. Of all the organs, the nervous system is the most affected target in lead toxicity, both in children and adults [414]. Wide applications of metals such as zinc, nickel, chromium in different industrial processes resulted in the significant increase in their environmental levels relative to normal background. The abovementioned metals are often found in effluents discharged from the industries involved in galvanization, electroplating, and the manufacture of alloys. Re(VII) is a rare and valuable metal, which due to its special properties is extensively used in petrochemical and chemical industries, metallurgy, medicine, defense, aviation, and alloy production [415,416]. Silver is widely used in chemical industry, photography, electroplating, and medicine. Removal of silver and rhenium from industrial effluents is mainly determined by their price, at the same time silver ions are toxic for living organisms, thus its recovery presents ecological interest as well.

Metal removal by sorbents depends on several parameters, the most important are: pH, initial metal concentrations, contact time, temperature, and the concentration of biosorbent. The influence of the abovementioned parameters on Pb, Zn, Ag, Cr(III), Cr(VI), Ni, and Re removal was investigated.

Biosorption experiments were conducted in 100 mL Erlenmeyer flasks containing 50 mL of metal synthetic solution. The effect of pH on metal ions removal was determined by adding 0.5 g of cyanobacterial biomass in the medium, containing metal ions with a pH ranging from 1.0 to 6.0 (depending on the studied metal). The initial solution was adjusted to the desired pH with diluted or concentrated HNO_3 and NaOH solutions. The change in the working volume due to the addition of HNO_3 and NaOH was negligible in this experiment. In all experiments, interaction time was 1 hour and temperature 22-23 ^{0}C.

The effect of biosorbent dosage on batch experiments was examined by varying its concentration from 2.0 to 20 g/L. In all the experiments the working volume was 50 mL, metal ions concentration 10 mg metal/L, pH was chosen optimally for each metal and temperature 22 - 23^0C.

Initial metal concentration varied within the range of 5 to 100 mg/L. Experiments were carried out under the ambient conditions at 22 ˚C, for 1 hour. In all experiments, the working volume of the solution was 50 mL. The effect of temperature on metal ions biosorption was studied in the temperature range 20-50 ^{0}C. The kinetics experiments were performed at optimal initial pH for each metal, metal concentrations in solution 10 mg Me/L, and biosorbent dosage of 0.5 g. In all the experiments, the working volume was 50 mL. To determine the contact time required for the sorption equilibrium, the samples were withdrawn at predetermined time intervals (3, 5, 7, 10, 15, 30, 45, 60, 120 min) [413,417–422].

All the experiments were performed in triplicate and the average value of the obtained experimental values was used. After the experiments, the biosorbent was separated from the solution by filtration and the concentration of a metal ion in the solution was determined by AAS. NAA was used to assess metal sorption by biomass. Information related to the measurement of metal concentrations in the obtained solutions is presented in Chapter 2, Section 2.3.

5.1.1 Effect of pH on metal biosorption by S. platensis biomass

pH is one of the most important parameters affecting the biosorption of metal ions. It not only influences the properties of a sorbent surface but also affects the metal speciation in solution. The effect of pH on metal removal efficiency is presented in Fig. 5.4.

Lead form in solution depends on the pH. Thus, at pH 2.0–6.0 predominant forms of lead in solution are Pb^{2+} and $PbOH^+$, while above pH 6, it is hydrolyzed to $PbOH^+$ and $Pb(OH)_2$ [423]. The lowest efficiency of lead removal by *S. platensis* was obtained at pH 2 (52%). With the increase of the pH, its removal was increased up to 88-89% and remained almost constant at pH range 3.0-5.0. At low pH values due to the high protons concentration, the competition between protons and positively charged metal cations takes place, thus less lead ions could be adsorbed [421]. Aneja *et al*. [424] and Al-Homaidan *et al*. [425] in their studies have shown that at pH 2.0 lead removal by *Spirulina* sp. and *Spirulina platensis* was less than 5% and 16.5%, respectively.

At pH values higher than 5.0, a decrease of biosorption parameters was observed, due to the formation of soluble hydroxocomplexes of the lead ions. Therefore, pH 5.0 was considered as the optimum pH and used in further experiments [421].. In other studies, related to cyanobacteria use for lead removal it was shown that optimal pH for lead biosorption by *Spirulina* sp. was 4.0 [424] and by *Spirulina platensis* – 3.0 [425].

The maximum silver biosorption (4.3 mg/g) by *S. platensis* was achieved at pH 3.0, which corresponds to the removal of 49% of metal ions initially present in the supernatant. At the pH values lower or higher than 3.0 the decrease of sorption capacity was noticed [426]. According to literature data, the optimal pH values for the biosorption of silver onto various biosorbent vary between 4.0 and 6.0 [427,428].

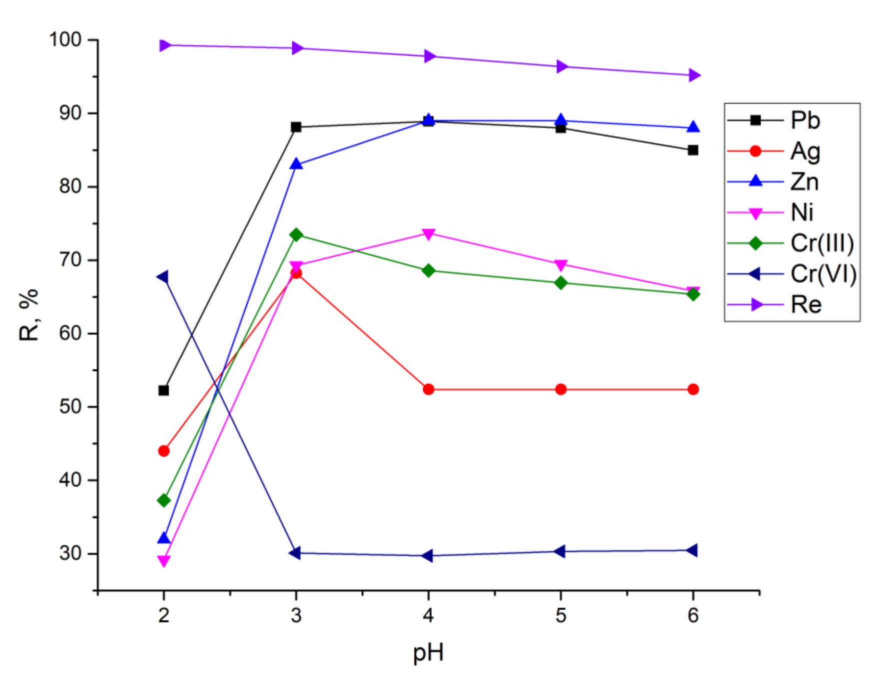


Fig. 5.4 Removal of metal ions at different initial pH (T 20 ^{0}C; C$_i$ 10 mg/L; sorbent dosage 10 g/L; adsorption time 1 h)

According to the data presented in Fig. 5.1 the lowest zinc removal by *S. platensis* was obtained at pH 2.0 (32%). At pH increase from 2.0 to 4.0 its removal efficiency increased from 32 to 89% and remain on the same level (0.85 mg/g) at pH range 4.0 to 6.0 [418]. The data obtained for other cyanobacteria have shown that maximum zinc sorption on *Aphanothece halophytica* took place at pH within 6.5-7.0 and on *Oscillatoria* sp. at pH 5.0 [429,430].

Nickel sorption by *S. platensis* biomass reached the maximum (760 µg/g) at pH 4.0. Low efficiency (29%) of nickel removal at pH 2 can be explained by competition of nickel ions with protons for binding sites [431]. At pH values higher than 4.0 a decrease of biomass removal efficiency was observed, which can be connected with the formation of anionic hydroxide [419]. For cyanobacteria *Chlorella vulgaris* and *Nostoc linkia*, optimal pH values for maximum nickel removal were 4.5 [36] and 5.5 [432], respectively. In Seker et al. [433] study, in contrast, optimal pH for nickel ions biosorption by *A. platensis* biomass was found to be 6.0.

The results obtained showed that Cr(III) sorption by *S. platensis* reached the maximum at pH 3.0 and then the decrease of biomass adsorption capacity was noticed. At pH 3.0 74% of chromium ions were removed from the solution [420]. Cr(III) being present in solution in cationic forms ($Cr(OH)^{2+}$, $Cr(OH)_2^+$ and Cr(III)) is better adsorbed at higher pH values when cyanobacteria surface is charged negatively. Under the alkaline conditions, weakly soluble $Cr(OH)_3$ and $Cr(OH)_4^-$ forms are formed [170, 389]. The data obtained are in agreement with [215], who reported maximum Cr(III) removal by *Spirulina* sp. at pH 3.0. In Gagrai et al. study [390] maximum Cr(III) sorption (74%) was achieved at pH 4.0.

The maximum removal of Cr(VI) 68% was achieved at pH 2.0 [420]. Cr(VI) ions are presented in solution in anionic forms $HCrO_4^-$ and $Cr_2O_7^{2-}$ and are better adsorbed at the low pH values due to the protonation of functional groups on the cyanobacteria surface [435]. The data obtained by [436] and [435] for cyanobacteria: *Chlorella vulgaris, Scenedesmus obliquus, Synechocystis sp.,* and *S. platensis* support this fact.

Rhenium is also present in solution in anionic form, thus the maximum Re(VII) biosorption was obtained at pH 2.0 (0.98 mg/g), 99% of rhenium ions were removed from the solution. An increase of pH from 2.0 to 6.0 leads to the insignificant reduction of its removal (by 6%) [437]. The data obtained were supported by [416] study, where it was shown that the maximum Re(VII) biosorption by *Bacillus* sp. GT-83-23 was obtained at pH 2.0 (117.9 mg/g dry wt). Thus, further experiments were conducted at pH values under which maximum removal of metal ions was achieved.

According to the data obtained, it is seen that metal ions present in solution in anionic form (chromium(VI) and rhenium) are more efficiently removed at low pH values. At the lower pH values, the biosorbents surface is surrounded by hydronium ions, which enhance the anions interaction with binding sites of the biosorbents by greater attractive forces [438]. The drop in the anions removal with the increase of pH is explained by the decrease of the amount of positively charged functional groups. The increase of pH facilitates cations removal since the sorbent surface becomes negatively charged.

5.1.2 Effect of sorbent concentration on metal biosorption by S. platensis biomass

The biosorbent adsorption efficiency is highly dependent on the biomass dosage in the solution. With the increase of *S. platensis* concentration from 2 to 20 g/L the percentage of lead adsorbed increased from 54% to 88%, but the equilibrium uptake was decreased from 2.5 to 0.4 mg/g (Fig.5.5) [421]. Al-Homaidan *et al*. [425] have shown that maximum adsorption of the lead ions by spirulina biomass was attained at the sorbent dosage 2.0 g/L. Kőnig-Péter and coworkers (2015) studying lead biosorption by cyanobacteria have shown the increase of biomass uptake capacity with the increase of biosorbent dosage from 0.25 to 1.0 g.

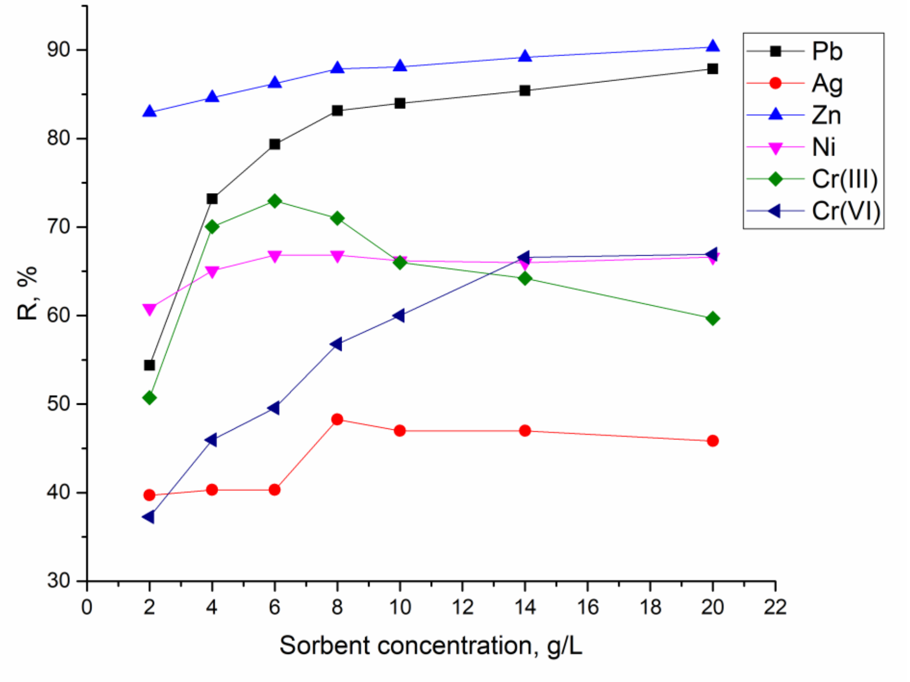


Fig. 5.5 Effect of biosorbent concentration on efficiency of metal ions removal by *S. platensis* biomass (T 22 ^{0}C; C$_0$ 10 mg/L; adsorption time 1h)

Maximum sorption of silver ions by *S. platensis* of 14 mg/g was attained at the lowest biosorbent concentration, and the amount of silver bound by the unit weight of biomass decreased with increment in biomass concentration. At the same time, maximum silver removal efficiency (48.3%) was observed at a dosage of 8 g/L, and it was slightly reduced at the sorbent dosage of 20 g/L. It is well known that at the increase of biosorbents amount, more binding sites are available for metal capture and thus the removal efficiency goes up. At the sorbent dose of 4, 6, and 10 g/L, the silver removal efficiency was 40, 43, and 47% respectively [426].

Zinc ions were efficiently removed at all studied sorbent concentrations. At *S. platensis* concentration 2 g/L zinc removal efficiency was 83%. With the increase of biosorbent concentrations from 2 to 20 g/L, biosorption efficiency increased only by 7 %, from 83% to 90% [418]. The amount of zinc adsorbed by the *Aphanothece halophytica* reached a maximum at cell concentration of 0.2 g/L and *Oscillatoria* sp. at sorbent concentration 0.04 mg/mL [429,430]. The results presented in Fig. 5.2 showed that nickel removal efficiency increased from 61% to 67% with the rise of *S. platensis* concentration from 2 to 20 g/L. It should be mentioned that nickel removal efficiency was the same at the sorbent dosage in the range of 6-20 g/L [419].

Cr(VI) removal efficiency varied from 37% to 67% for the increase of biosorbent concentration from 2 to 14 g/L. Further increase of sorbent concentration up to 20 g/L did not influence the efficiency of its removal. In case of Cr(III), maximum metal removal was achieved at sorbent concentration of 6 g/L, then its removal efficiency decreased to 60% at sorbent concentration 20 g/L [420].

The increase of spirulina concentration has a positive effect on the efficiency of metal removal. This is possible since more binding sites are available for metal capture and thus the removal efficiency ramps up. Despite the increase in efficiency of metal removal, the sorption capacity of *S. platensis* biomass was decreased, which can be explained by the sorbent aggregation and reduction of biosorbent surface area. In the presence of high biomass concentration, there is a very fast superficial adsorption onto the biomass that produces a lower metal concentration in solution, than in case of lower biomass concentration [436].

5.1.3 Effect of initial metal concentrations on S. platensis sorption capacity and biosorption equilibrium modeling

The initial metal ion concentration is a very important factor to be investigated in adsorption studies since wastewaters usually present different concentrations of metal ions. Thus, determination of its effect is necessary for the adsorption study [439]. The equilibrium experiments were performed at optimal initial pH and sorbent concentrations for each metal and initial metal concentrations in solution in the range of 5–100 mg/L.

With the increase of initial lead concentration in solution from 5 to 100 mg/L the *S. platensis* removal capacity increased from 0.23 to 7.24 mg/g [421] (Fig. 5.6). The increase of silver concentration in solution from 5 to 30 mg/L leads to the growth of biomass sorption capacity from 0.8 to 3.7 mg/g [426]. The same pattern was observed for other metal ions as well. Thus, the increase of zinc concentration in solution from 5 to 100 mg/L leads to an increase in biomass sorption capacity from 0.8 to 4.9 mg/g [418]. The amount of nickel adsorbed by spirulina biomass was proportional to the initial nickel concentration in the solution, the maximum adsorption capacity being 4.2 mg/g [419]. The increase of chromium concentration in solution from 10 to 200 mg/L led to the increase of the chromium amount adsorbed by biomass from 1.2 to 11.2 mg/g for Cr(III) and from 0.6 to 9.5 mg/g for Cr(VI). The increase of rhenium concentration in solution leads to an increase in its content in biomass [420]. At rhenium concentration in solution 100 mg/L, its content in biomass reached the value of 4.6 mg/g [437]. The increase in equilibrium uptake with the increase in the initial metal concentration can be explained by the increase in the number of collisions between cyanobacteria cells and metal ions [396].

The experimentally obtained equilibrium data were described using Langmuir, Freundlich, and Temkin equilibrium models. The description of the models is given in Chapter 1. The Freundlich and Temkin constants were calculated from nonlinear regression (Fig. 5.6), while Langmuir model constants were calculated from the approximation of linearized experimental plot data (Fig. 5.7). The values of the parameters calculated on the base of the models used are shown in Table 5.1.

The coefficient of determination (R^2) values indicated that Langmuir and Freundlich models were suitable to describe the biosorption equilibrium of lead ions. Thus, lead sorption by spirulina biomass was homogeneous and multilayer in the nature process. The maximum uptake capacity (q_{max}) estimated by the Langmuir model normally could not be reached at experimental conditions, while *b* is the equilibrium constant representing the standard Gibbs adsorption energy. According to the Langmuir model, the maximum uptake capacity of spirulina biomass was determined as 11.1 mg/g. Calculated q_{max} was higher than experimentally obtained one (7.42 mg/g), suggesting that lead biosorption by spirulina biomass would proceed at higher lead concentrations. The constant *b* value of 0.01 indicates a significant binding strength affinity of metal with biomass [421]. In the Freundlich model, the *n* value was less than 1.0 showing that with the increase of metal concentration in solution the relative adsorption will decrease. Applicability of the Freundlich model for the description of lead biosorption by spirulina species was shown in [425] and [397] works.

In the case of silver, the R^2 values for Freundlich and Temkin models were significantly lower than those of the Langmuir model. The predicted q_{max} was 31.6 mg/g and the correlation coefficient 0.98 [426]. Silver biosorption by *Cladosporium cladosporioides* fits well with the Freundlich and Langmuir models, however, the correlation coefficient obtained by the Langmuir model was higher in comparison with Freundlich one [398]. Satisfactory fitting of the Langmuir model to the silver adsorption on biosorbents and extracellular polymeric substances has been reported by various researchers [399, 400].

The R^2 value obtained for zinc showed that experimental data fits well Langmuir model with its value of 0.99. Calculated q_{max} according to the Langmuir model 7.1 mg/g, is higher than the experimentally obtained value of 4.9 mg/g, indicating that zinc biosorption by spirulina biomass would proceed at higher zinc concentrations. The constant *b* value of 0.014 indicates that spirulina possesses a high capacity for metal removal [418].

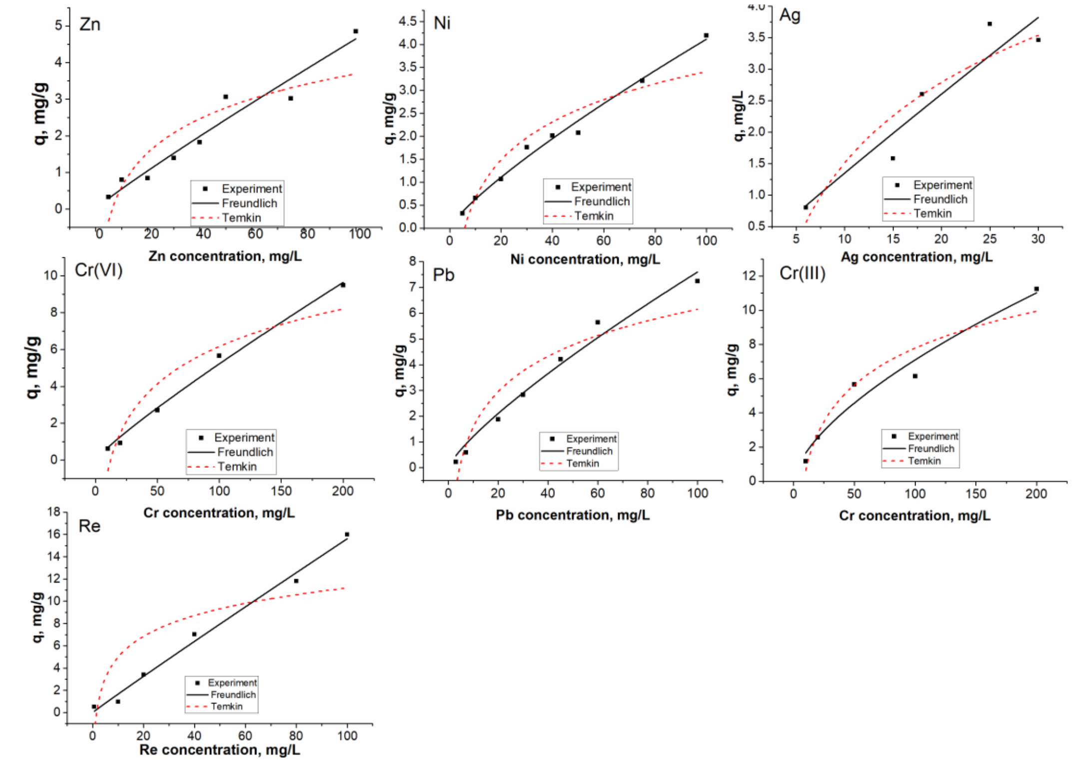


Fig. 5.6 Freundlich and Temkin isotherm models of studied elements

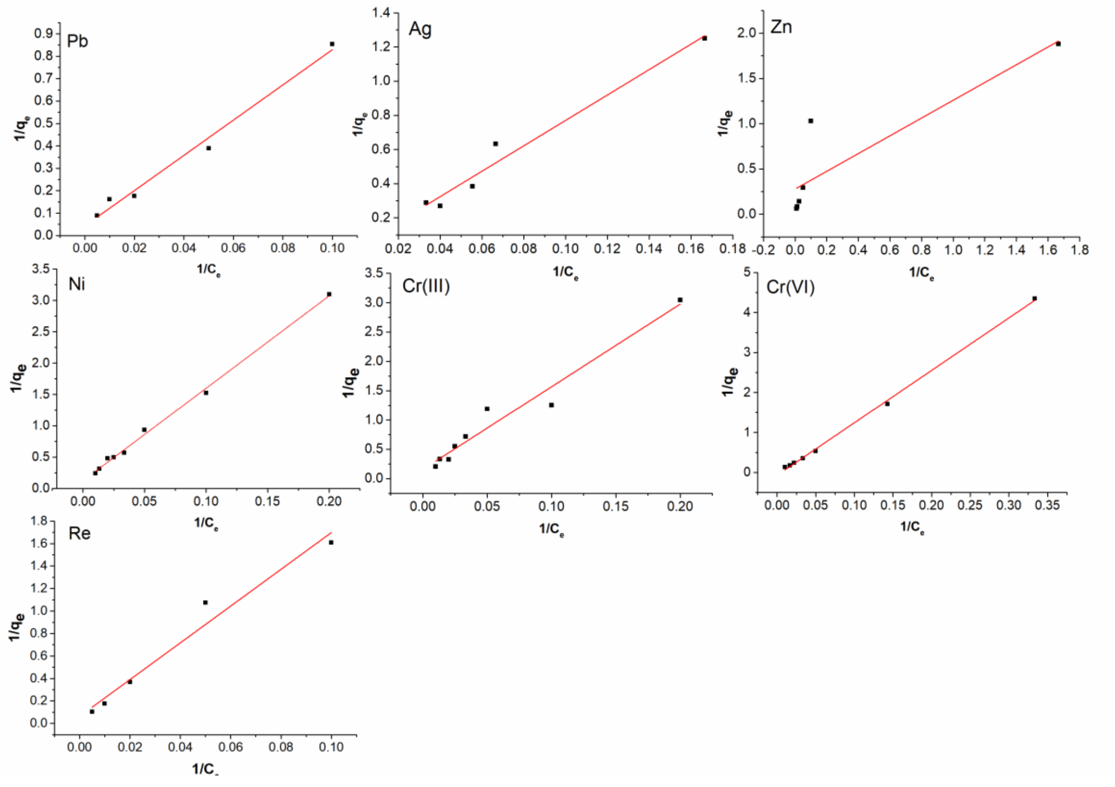


Fig. 5.7 Langmuir isotherm models of studied elements

The q_{max} value obtained in the present study fall into the range of data obtained for the other microalgae: *Scenedesmus obliquus* and *Scenedesmus quadricauda* [401], but was considerably lower than values reported by [33] and [429] for *Scenedesmus obliquus* and *Aphanothece halophytica*. Freundlich isotherm is suitable to fit almost all the experimental adsorption data and is especially good for fitting the data from highly heterogeneous sorbent like for example spirulina biomass. The *n* value higher than 1.0 indicates that zinc ions are favorably adsorbed by spirulina biomass [418].

High R^2 obtained for Langmuir and Freundlich models point to the fact that the models applied are suitable for describing the nickel biosorption by *S. platensis* biomass in the studied concentration range. Applicability of the two models suggests that the nickel biosorption by spirulina biomass involves several mechanisms. Seker *et al.* [433] showed the applicability of the Freundlich model to describe nickel biosorption by *Chlorella vulgaris.* The q_{max} obtained in the present work (13.4 mg/g) was higher than values reported by [161] for *Chlorella miniata and Chlorella vulgaris:* 2.98 mg/g and 1.28 mg/g, respectively; and by Micheletti et al. [402] for Nostoc PCC 7936 - 1.9 mg/g and lower than the data presented by Aksu [36] for *Chlorella vulgaris* - 48.1- 60.2 mg/g. Relatively low nickel biosorption by microbial biomass can be also explained by generation of stable nickel aqueous complexes, that probably reduce their rate of exchange with the protons bound to the active sites of the biosorbent [402].

Values of R^2 obtained for Cr(III)/Cr(VI) show that the Langmuir model described better the experimental data for Cr(III), while the data obtained for Cr(VI) fit both models. The determined q_{max} value for Cr(III) was found to be 25 mg/g and for Cr(VI)-16.8 mg/g [420]. It should be mentioned that in the case of Cr(III) ions R^2 calculated for Freundlich and Temkin models were also higher than 0.9. Both Freundlich and Langmuir adsorption models were applicable for Cr(VI) removal by three cyanobacteria species [392].

Lodi and co-authors [447] showed that the Langmuir model described better the Cr(III) biosorption process by *S. platensis.* Freundlich and Langmuir isotherm models were applicable for the description of the rhenium equilibrium model. The constant *b* value of 0.001 indicates a significant binding strength affinity of rhenium towards biomass [393]. Ghorbanzadeh Mashkani et al. [416] showed that Freundlich isotherm was suitable to describe experimental data obtained for *Bacillus* sp. Strain GT-83-2. A good correlation ($R^2 > 0.93$) reported that the *Bacillus* sp. strain GT-83-23 possesses high sorption capacity and high affinity for Re(VII). For the main part of elements, except of Cr(III), for the Temkin model the low R^2 values were obtained, indicating its low applicability for the description of experimentally obtained data.

Table 5.1 Isotherm parameters for the biosorption of metal ions on *S. platensis* biomass

Element	Isotherm model					
	Freundlich		Temkin		Langmuir	
Pb	R^2	**0.99**	R^2	0.86	R^2	**0.99**
	K_f, mg/g	0.19	a_T, L/g	0.22	q_m, mg/g	11.1
	n	1.25	b_T	1.23	b, L/mg	0.01
Ag	R^2	0.87	R^2	0.82	R^2	**0.98**
	K_f, mg/g	0.15	a_T, L/g	0.23	q_m, mg/g	31.6
	n	1.05	b_T	1.34	b, L/mg	0.004
Zn	R^2	0.94	R^2	0.76	R^2	**0.99**
	K_f, mg/g	0.06	a_T, L/g	0.15	q_m, mg/g	7.1
	n	1.1	b_T	1.81	b, L/mg	0.014
Ni	R^2	**0.99**	R^2	0.86	R^2	**0.99**
	K_f, mg/g	0.1	a_T, L/g	0.17	q_m, mg/g	13.4
	n	1.22	b_T	2.1	b, L/mg	0.006
Cr(III)	R^2	0.95	R^2	0.9	R^2	**0.99**
	K_f, mg/g	0.38	a_T, L/g	0.12	q_m, mg/g	25.0
	n	1.57	b_T	0.78	b, L/mg	0.005
Cr(VI)	R^2	**0.99**	R^2	0.86	R^2	**0.985**
	K_f, mg/g	0.09	a_T, L/g	0.08	q_m, mg/g	16.8
	n	1.13	b_T	0.83	b, L/mg	0.004
Re	R^2	**0.99**	R^2	0.58	R^2	**0.99**
	K_f, mg/g	0.17	a_T, L/g	0.63	q_m, mg/g	142.9
	n	1.02	b_T	0.90	b, L/mg	0.001

5.1.4 Effect of contact time on metal biosorption by S. platensis and kinetics modeling

The kinetic experiments were performed at optimal initial pH and sorbent concentration for each metal with metal concentration in solution 10 mg Me/L. The time required for the maximum removal of metals from solution is an important parameter. The effect of time on studied metal biosorption is shown in Fig. 5.8. Maximum lead biosorption was reached after 45 min of sorbent-sorbate interaction and no further increase in the level of bound lead occurred after this time [421]. The optimal time for silver biosorption can be considered 5 min when its content increased up to 2.54 mg/g (removal efficiency 40%), and it did not change significantly during the next period, which can be explained by the saturation of binding sites on the cell surface [426]. Singleton and Simmons [428] in their study have shown that silver removal by *Saccharomyces cerevisiae* biomass reached maximum levels within few minutes and no further marked increase in metal uptake occurred. The maximum zinc removal efficiency (90%) was achieved after 30 min of biomass-solution interaction [418]. *S. platensis* biosorption capacity toward nickel ions was increased with increasing of contact time and almost 68% of nickel was removed by biomass in the first 30 min of sorbent-sorbate interaction. Equilibrium was reached after 60 min of interaction. It should be mentioned that 52% of nickel was sorbed during the first

3 min of interaction [419]. The results obtained are supported by the literature data. For example, [161] demonstrated that nearly 50% of total nickel amount was adsorbed by *Chlorella vulgaris* after 1-min biosorption. The results presented in Fig. 5.5 show the rapid Cr(III) sorption in the first 30 min of sorbent- sorbate interaction after equilibrium was reached. In 30 min 70% of chromium ions were removed from the solution. The maximum amount of Cr(VI), 62%, was removed in 45 min of interaction and it did not change further [420]. The highest speed of sorption was attained for rhenium, during 1 min- sorption 94% of rhenium was removed from the solution. Then it increased up to 97% in 15 min and the equilibrium was reached [437]. Similar trend was observed for rhenium removal by *Bacillus* sp. GT-83-23 [416]. This rapid sorption is considered advantageous at full scale, where the short retention times are required for maximal recovery efficiency.

Kinetic models are generally used to understand the sorbent-sorbate interactions. Four models, namely pseudo-first-order (PFO), pseudo-second-order (PSO), Elovich model (EM), and the intra-particle Weber and Morris diffusion model (IPM), were applied in the present study to describe experimental data (Fig.5.8).

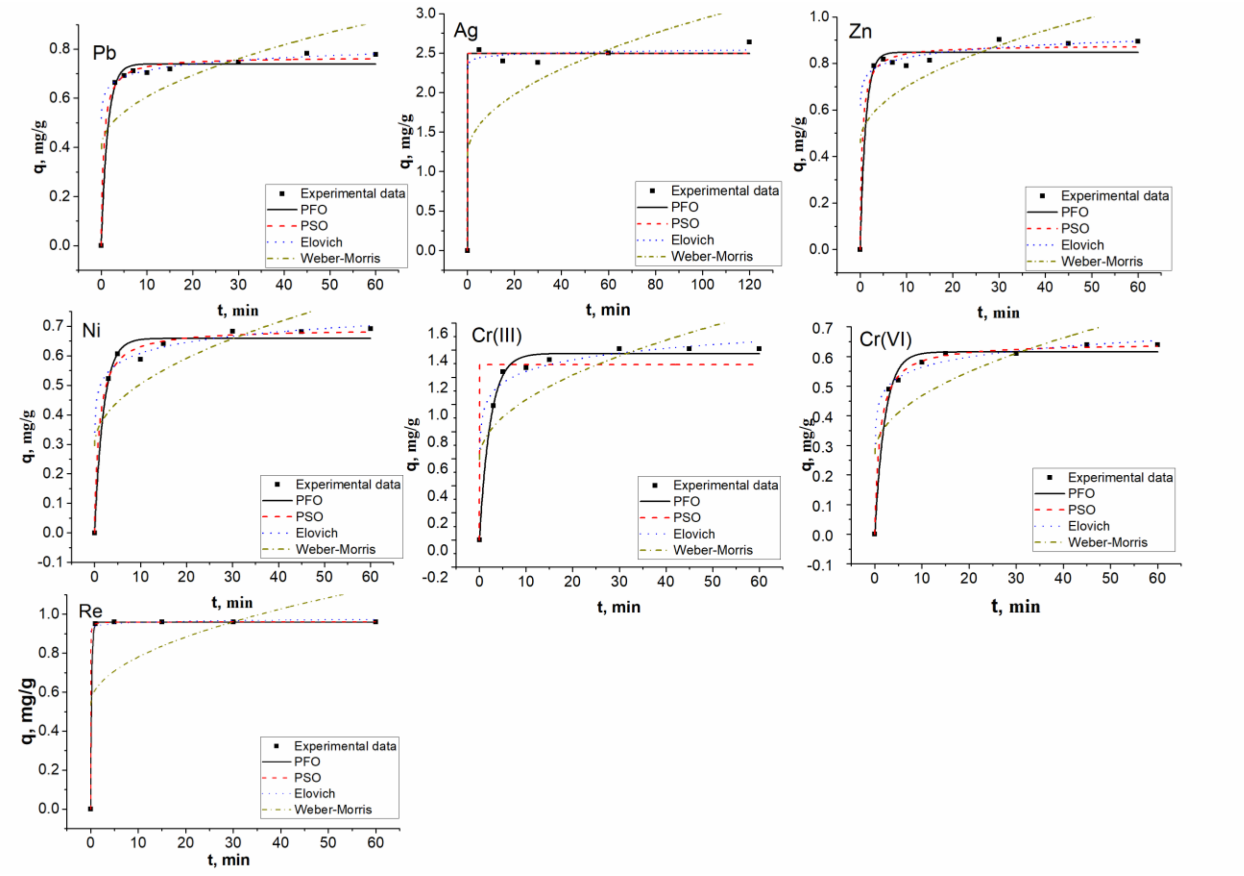


Fig. 5.8 Kinetics of the metal adsorption using *S. platensis* biomass

The corresponding coefficients of values determination values, as well as the kinetic parameters of metal sorption on *S. platensis* biomass, are given in Table 5.2.

Table 5.2 Kinetic parameters for the biosorption of metal ions on *S. platensis* biomass

Element	Kinetic model							
	PFO		PSO		EM		IPM	
Pb	R^2	0.98	R^2	**0.99**	R^2	**-**	R^2	0.39
	q_e, mg/g	0.74	qe, mg/g	0.77	α, mg/g·min	-	k_{diff}	0.07
	k_1, min^{-1}	0.70	k_2, g/mg·min	2.42	β, g/min	-	C_i	0.39
	q_{exp}, mg/g	0.78						
Ag	R^2	**0.99**	R^2	**0.99**	R^2	**-**	R^2	0.31
	q_e, mg/g	2.49	qe, mg/g	2.49	α, mg/g·min	-	k_{diff}	0.17
	k_1, min^{-1}	138	k_2, g/mg·min	4.15	b, L/mg	-	C_i	1.19
	q_{exp}, mg/g	2.5						
Zn	R^2	0.98	R^2	**0.99**	R^2	**-**	R^2	**0.38**
	q_e, mg/g	0.85	qe, mg/g	0.88	α, mg/g·min	-	k_{diff}	0.08
	k_1, min^{-1}	0.85	k_2, g/mg·min	2.59	b, L/mg	-	C_i	0.46
	q_{exp}, mg/g	0.89						
Ni	R^2	0.98	R^2	**0.99**	R^2	**0.99**	R^2	0.5
	q_e, mg/g	0.66	qe, mg/g	0.69	α, mg/g·min	615	k_{diff}	0.07
	k_1, min^{-1}	0.5	k_2, g/mg·min	1.5	b, L/mg	19	C_i	0.23
	q_{exp}, mg/g	0.68						
Cr(III)	R^2	**0.99**	R^2	0.90	R^2	0.98	R^2	0.51
	q_e, mg/g	1.37	qe, mg/g	1.29	α, mg/g·min	315	k_{diff}	0.14
	k_1, min^{-1}	0.43	k_2, g/mg·min	9.73	b, L/mg	8.2	C_i	0.6
	q_{exp}, mg/g	1.41						
Cr(VI)	R^2	0.98	R^2	**0.99**	R^2	**0.99**	R^2	0.5
	q_e, mg/g	0.62	qe, mg/g	0.64	α, mg/g·min	373	k_{diff}	0.06
	k_1, min^{-1}	0.47	k_2, g/mg·min	1.49	b, L/mg	19.9	C_i	0.27
	q_{exp}, mg/g	0.64						
Re	R^2	**1.00**	R^2	**1.00**	R^2	**-**	R^2	0.17
	q_e, mg/g	0.96	qe, mg/g	0.96	α, mg/g·min	-	k_{diff}	0.08
	k_1, min^{-1}	4.6	k_2, g/mg·min	9.39	b, L/mg	-	C_i	0.53
	q_{exp}, mg/g	0.96						

The high values of coefficient of determination (0.99) obtained for the pseudo-second-order and small differences between theoretical and experimental adsorption capacity show this model applicability to describe the experimental for lead, zinc, nickel, and chromium(VI). For chromium(III) the pseudo-first order-model fits better the experimental data, while for silver and rhenium both models were applicable, the differences between $q_{e,exp}$ and $q_{e,cal}$ were insignificant.

Applicability of the second-order model is indication that the rate-limiting step in biosorption is chemisorption involving the electrons exchange between the adsorbent and adsorbate due to the covalent forces and ion exchange [256]. This model also assumes that the biosorption process occurs in two reactions, the first one is fast and reaches equilibrium quickly and the second is slow and keeps going for a long period. Elovich model gives a good correlation for adsorption on highly heterogeneous surfaces and it also shows that along with surface adsorption chemisorption is also a dominant phenomenon taking place [404]. Elovich model was applicable to describe nickel and chromium (VI) biosorption. For the intra-particle diffusion model, the correlation coefficients were relatively low lying between 0.17 and 0.51.

5.1.5 Effect of temperature on metal removal by S. platensis biomass and thermodynamic study

Industrial effluents are often discharged at relatively high temperatures; thus, the temperature is an important parameter affecting the sorbent biosorption capacity. With the increase of temperature biosorption capacity of biomass toward lead ions was increased from 0.76 to 0.96 mg/g (Fig. 5.9). A maximum lead removal efficiency corresponding to 93% was obtained at the temperature of 30 ºC. In case of silver, increase in temperature lead to the decrease of adsorption capacity from 1.74 to 1.20 mg/g. Maximum biosorption of 960 µg Zn/g was obtained at 50 ºC. The increase of temperature leads to pores enlargement thereby causing the rise of sorption [405].

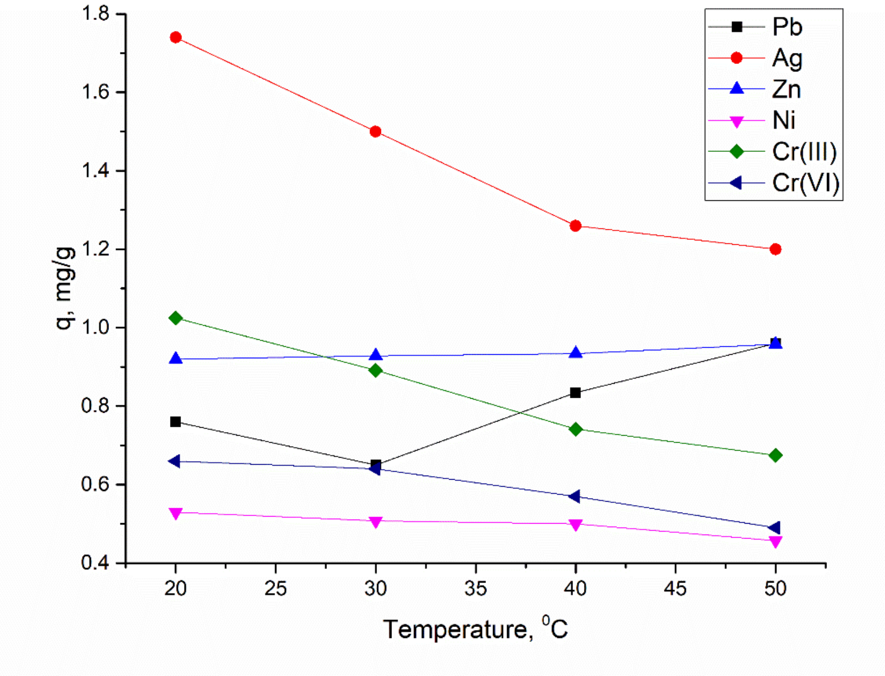


Fig. 5.9 Effect of temperature on removal efficiency metal ions by *S. platensis* biomass (C_0 10 mg/L; adsorption time 1h)

This suggests that zinc biosorption by spirulina biomass could involve a combination of chemical interaction and physical adsorption. Increase of temperature had an opposite effect on the biosorption of nickel, Cr(III), and Cr(VI), leading to a decrease of nickel biosorption from decrease Temperature increase from 20 to 50 ˚C led to the decrease of biomass biosorption capacity from 883 μg/g to 762 μg/g. Increase of temperature from 20 to 50 ºC leads to the decline of biomass biosorption capacity from 1025 to 675 μg/g for Cr(III) and from 660 to 490 μg/g for Cr(VI) [420].The thermodynamic constants namely, free energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°) were calculated to evaluate the thermodynamic feasibility of the process and to confirm the nature of the adsorption process according to equations presented in Chapter 1. The values of enthalpy (ΔH°) and entropy (ΔS°) were evaluated from the slope and intercept of ln K_d vs. 1/T plots and obtained values are presented in Table 5.3.

Table 5.3 Thermodynamic parameters for lead biosorption on *S. platensis*

Temperature,	**ΔG°, kJ/mol**	**ΔH°, kJ/mol**	**ΔS°, J/mol·K**
Pb			
293	-1.53	-0.83	34.8
303	-1.87		
313	-2.2		
323	-2.5		
Ag			
293	-8.35	-19.24	-37.15
303	-7.98		
313	-7.61		
323	-7.24		
Zn			
293	-10.9	1.33	41.8
303	-11.3		
313	-11.7		
323	-12.1		
Ni			
293	-3.83	3.9	26.4
303	-4.09		
313	-4.35		
323	-4.62		
Cr(III)			
293	-11.24	-11.1	0.35
303	-11.25		
313	-11.25		
323	-11.25		

Negative ΔG° values obtained for all the elements showed the spontaneity of the adsorption process. At ΔG° values between −20 and 0 kJ/mol, adsorption is classified as physical adsorption, while in chemical adsorption ΔG° values change range from −80 to −400 kJ/mol [406]. Since the values of ΔG° were less than 0 kJ/mol it can be suggested that biosorption was mostly physical in nature. Positive standard enthalpy change suggests that the interaction of metal ions with spirulina is an endothermic process, while the negative standard enthalpy indicates the exothermic process. The negative ΔH° value obtained for lead, silver, chromium(III), and chromium(VI) revealed that the adsorption process was exothermic, and for zinc and nickel – endothermic. Negative ΔS^0 values show that metal ions are stable on the biosorbent surface and that no significant change occurs in the internal structure of spirulina during the biosorption process [407]. At the same time, positive value of ΔS° reflects the increased randomness at the solid-solution interface during the biosorption and it also indicates that ion replacement reactions occur [408]. Silver biosorption by spirulina biomass was predominantly physical, while for the other elements it can be considered as a physicochemical adsorption process [418,420,421,426].

5.1.6 Regeneration of biosorbent obtained after biosorption experiments

Reutilization of biosorbent used for the industrial wastewater treatment is very important economically, since it allows reducing the price of treatment process. As examples can serve data presented on desorption analysis of *S. platensis* biomass loaded with lead, zinc, and nickel ions. Four different eluents, namely 0.1 M HNO_3, 0.1 M HCl, 0.1 M CH_3COOH, and 0.1 M NaOH were tested as desorbing agents. *S. platensis* biomass was first exposed to 10 mg Me/L solution after that it was separated from the solution by filtration and then brought in contact with 50 mL of effluent for 1 h on an orbital shaker at 200 rpm. The procedure was repeated three times. The data obtained are presented in Table 5.4.

The mineral acids were efficient as desorbing agents (cycle I) for lead recovery: 65 % is removed by HNO_3 and 66.7 % by HCl. However, in the next cycle, the efficiency of lead desorption was decreased, especially when HNO_3 was used as an eluent. The decrease in the biomass sorption/desorption capacity can be explained by structural damage of biosorbent and blockage of binding sites by metal complex. Application of 1.0 M HNO_3 for desorption of Cr(III), Cd(II), and Cu(II) adsorbed by cyanobacteria *Spirulina* sp. reduced metals desorption by 50% [409]. CH_3COOH and NaOH showed the low elution efficiency. At the same time, biosorption efficiency remained almost constant when CH_3COOH or NaOH were used. That can be explained by the concentration increase of carboxyl and hydroxyl groups under the biomass interaction with the abovementioned chemicals [421].

In case of zinc, the mineral acids and CH_3COOH were efficient as desorbent agents in the first cycle. However, in the next two cycles, the elution efficiency of mineral acids was significantly decreased due to the possible biomass damage. The lowest elution efficiency was obtained for NaOH. However, the efficiency of zinc removal by *S. platensis* biomass treated with NaOH was increased from 89% (I cycle) to 97.5% (III cycle).

Table 5.4 Elution efficiency of different type of desorbing agents

Cycle	**Eluent**			
	CH_3COOH	HNO_3	HCl	NaOH
	Pb			
I	19.2%	65%	66.7%	20%
II	24%	31%	28.8%	6.0%
III	21%	8.9%	29%	7.8%
	Zn			
I	48.5%	49%	55%	12.8%
II	22%	3.8%	5.8%	5.0%
III	12.4%	2.5%	2.9%	15.0%
	Ni			
I	71%	70%	78%	25%
II	81%	8.6%	19%	6.1%
III	20%	4.1%	14%	1.9%

High removal efficiency can be explained by the increase of the hydroxyl groups concentration under the biomass interaction with NaOH. When acids were used as desorbing agents the decrease of adsorption efficiency was observed: approximately by 60% for HNO_3 and HCl, and by 30% for CH_3COOH. A decrease of adsorption efficiency can be connected with the competition of protons and the dissolved metal ions for binding sites [430] or the structural damage of biosorbent and blockage of binding sites by the metal complex. Babu and co-authors [391], studying zinc biosorption by *S. platensis* biomass have used 0.1 M HCl as eluent for column regeneration during four cycles. Both, removal efficiency and uptake capacity of biosorbent were found to progressively decrease with the increase in the number of cycles [419].

Mineral acids were also efficient as desorbing agents for nickel recovery in the first cycle: 70 % by HNO_3 and 78% by HCl. However, once the biomass was reused in the next two biosorption cycles the elution efficiency was significantly decreased. CH_3COOH showed good desorption capacity during two sorption-desorption cycles. The lowest elution efficiency was obtained for NaOH (Table 5.4) [419].

5.1.7 Mechanisms of metal ions biosorption by S. platensis biomass

Metal biosorption includes several processes: metal binding to functional groups, ion-exchange, and microprecipitation. The Fourier-transform infrared spectroscopy (FTIR) allows identifying functional groups on the *S. platensis* surface, which may be involved in the metal ions binding. FTIR spectra were recorded 4000−600 cm^{-1} region using a Perkin Elmer Spectrum 100 FT-IR spectrometer.

In the control spirulina FT-IR spectrum (Figs. 5.10-5.12) some intense characteristic bands corresponding to –OH (1055, 1397.9 cm^{-1}), $–NH_2$, $S-CH_2$, $NHC(O)_{amid}$ (1235.0), C=O (1535.0), P=O (1625.0), $N-CH_2$ (2925.0), and NH_2 (3277.0) groups were observed. As examples, the spectra of control and metal-loaded biomass for selected elements are presented.

After lead biosorption the bands 1055, 1397.9, 1537.5, and 1625.0 cm^{-1} were shifted to 1070.5, 1401.0, 1538.5, 1627.5 cm^{-1}, respectively (Fig. 5.10). The change in the wavenumbers of these bands suggests that OH, C=O, and P=O groups can be involved in the lead ions biosorption onto the spirulina biomass [421]. Involvement of amino, hydroxyl, and carboxyl groups in lead biosorption by cyanobacteria *Spirulina maxima, Chroococcus multicoloratus,* and *Oscillatoria trichoides* was shown in [440,441].

FTIR analysis of Ag-loaded biomass showed the shift of adsorption bands of OH, C=O, NH_2 groups by ~ 3÷12 cm^{-1} indicating their involvement in the adsorption process. The same results were obtained by the other researchers, who had studied silver biosorption by other types of sorbents. For example, [427] have shown that the ionized carboxyl of amino acid residues and amide of peptide chains in the biomass were involved in the silver biosorption by *Aeromonas* strain SH10. The results provided by the FTIR suggest that C=O groups represent the main reactive site for silver uptake by *the Rhodotorula mucilaginosa* (strain UFMG-Y27) [444].

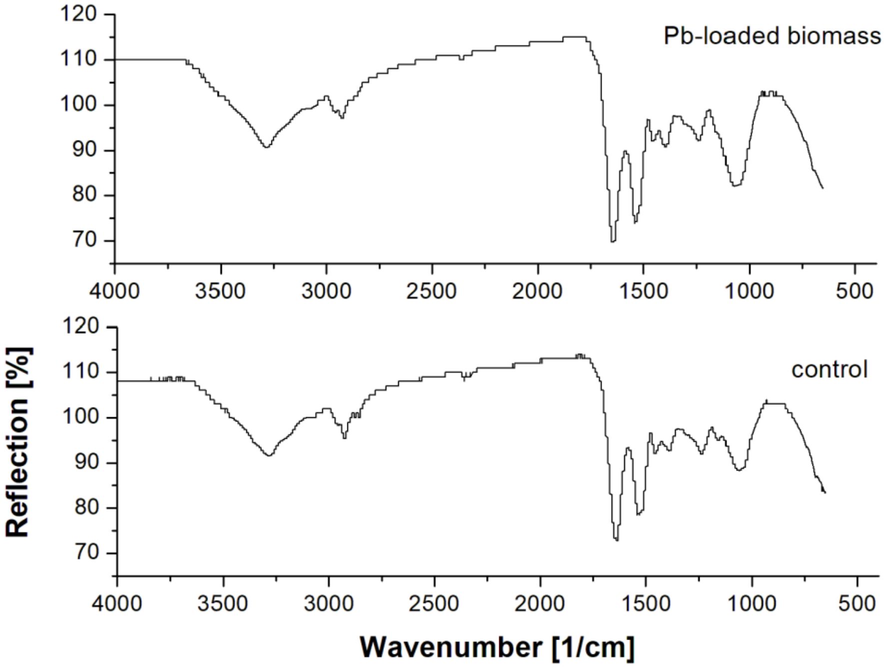


Fig. 5.10 FT-IR spectra of *S. platensis* biomass: control and Pb-loaded biomass

In the FTIR spectrum (Fig.5.11) of Ni-loaded *S. platensis* biomass, the position of the peaks of amine (3282 cm^{-1}), carboxyl (1750–1350, 1250–1000 cm^{-1}), hydroxyl (3282, 1250–

1000 cm^{-1}), phosphate, and sulfate (900–500 cm^{-1}) groups were slightly shifted indicating on their involvement in nickel ions binding [419].

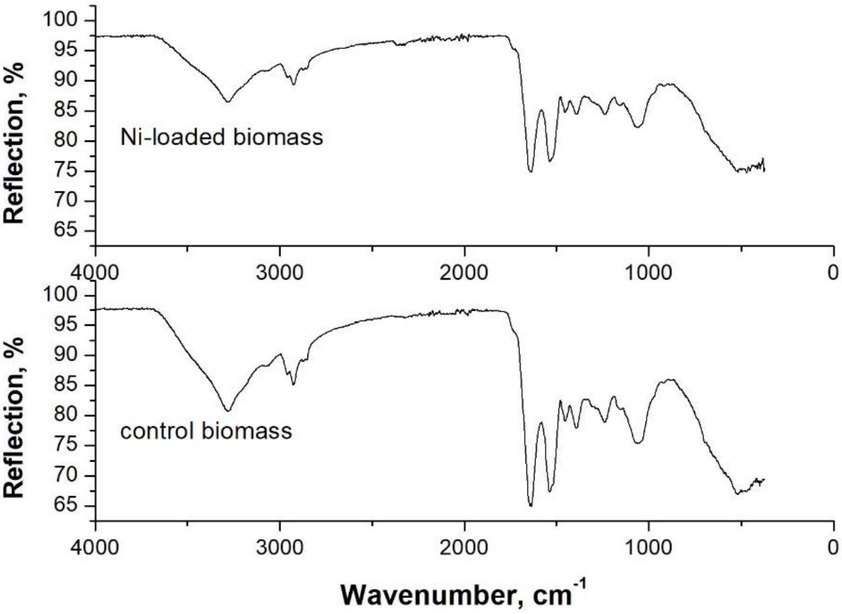


Fig. 5.11 FT-IR spectra of *S. platensis* biomass: control and Ni-loaded biomass

The spectrum of rhenium- loaded biomass (Fig. 5.12) showed the appearance of a Re-O band of perrhenate group in spectrum in the region 900 cm^{-1}. The shift of the Re-O band from 900 cm^{-1} to the high-frequency region and its splitting in two parts 909 - 911 cm^{-1} and 871 cm^{-1} was observed. This is indicative of the decrease of perrhenate ions symmetry due to their interaction with the organic ligands.

After one-hour experiment, the biomass was washed with the distilled water. The spectrum obtained for the barren solution showed a Re-O peak, indicating rhenium ion binding to water-soluble complexes. The Re-O peak was also present in the washed biomass, indicating that besides the ionic interaction rhenium ions form the stable complexes with amide and carboxyl groups [393].

The data obtained by NAA (Table 5.5) show that besides the metal binding to functional groups on the sorbent surface, the ion-exchange is another mechanism of metal uptake by *S. platensis* biomass. For example, as shown in Table 5.5, nickel ions sorption by spirulina biomass led to the decrease of Na, K, Fe, As, Br, and Sr content in biomass, indicating their possible involvement in the exchange process.

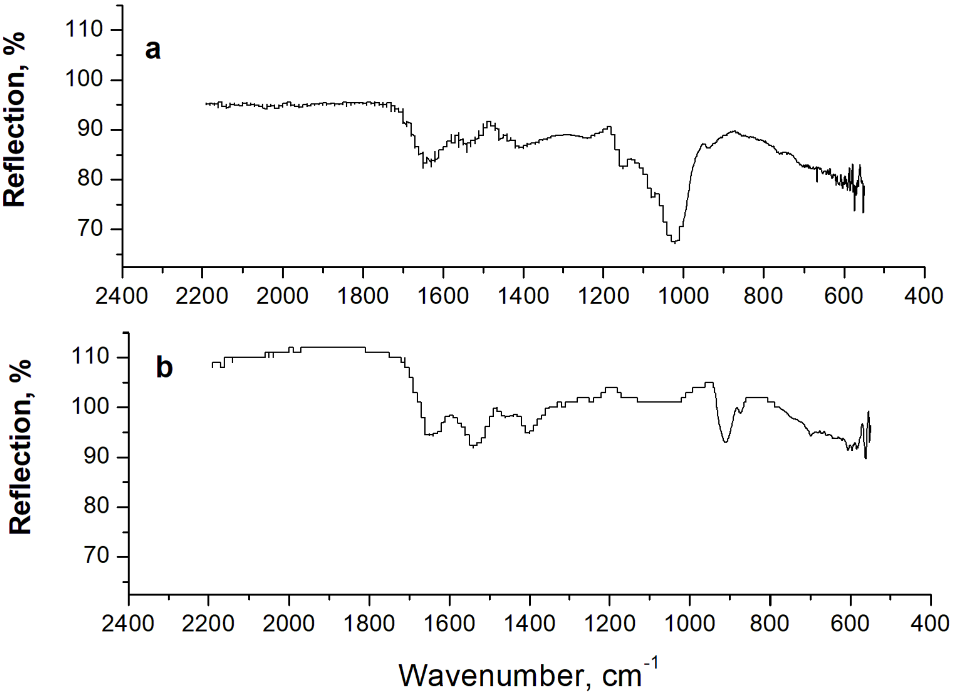


Fig. 5.12 FTIR spectra of (a) *S. platensis* biomass control; (b) Re-loaded biomass

Table 5.5 Change of element content in *S. platensis* biomass during nickel biosorption

Ni concentration, mg/L	Element content in biomass, µg/g					
	Na	K	Fe	As	Br	Sr
0	5830±300	14700±730	4900±340	1.93±0.08	2.26±0.09	107±11
10	1380±70	10800±540	1420±100	1.22±0.05	1.31±0.05	38.6±3.9
25	1080±50	9150±450	970±65	1.22±0.05	1.37±0.05	33.5±3.3
50	920±45	9840±490	925±65	1.19±0.05	1.32±0.04	30.5±3.0
75	841±40	8600±430	899±65	0.98±0.04	1.14±0.04	48.5±4.9
100	866±40	8520±430	927±65	0.96±0.04	1.13±0.04	59.8±6.0

5.2 Metal removal from the synthetic effluents using *Saccharomyces cerevisiae*.

Four complex effluents with the following composition: Cr(VI)-Fe(III), Cr(VI)-Fe(III)-Ni(II), Cr(VI)-Fe(III)-Ni(II)-Zn(II), and Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) were modeled. The concentration of chemical elements in the analyzed system were as follows: chromium - 10 mg/L, iron 5 mg/, nickel, zinc and copper - 2 mg/L). The effect of pH (2.0-6.0), time (5-120 min), temperature (20 -50 °C), and chromium concentration (10 -100 mg/L) on metal biosorption was studied. In all experiments, the working volume was 50 mL and the sorbent dosage 0.5 g. Experiments were conducted in triplicate and the averages of the measurements for each treatment were used. Copper concentration in solution was determined by applying AAS, the content of Cr, Fe, Ni, and Zn in biosorbent samples was determined by NAA [237].

Biosorbent was characterized using several techniques: FTIR, scanning electron microscopy, and zeta potential was measured. The Scanning Electron Microscopy (SEM) characterization was performed using the S3400N (Hitachi) microscope. Fig. A1.1 (Annex) presents the SEM images of *S. cerevisiae* before and after interaction with metal ions. The surface morphology of the biomass did not change significantly after its interaction with metal ions, thus, it can be concluded that metal ions biosorption occurs mainly on the surface of biosorbent [237].

FTIR was applied to confirm the presence of functional groups in the samples of *S. cerevisiae* and to reveal the chemical modification induced by metal biosorption. Infrared spectra were recorded in the range of 4,000–400 cm^{-1} using Nicolet 6700 spectrometer (Thermo Scientific). FTIR spectrum of native *S. cerevisiae* biomass (Fig. 5.13) indicates the strong absorption bands in the regions 1036 and 1518 cm^{-1}, which correspond to OH-groups.

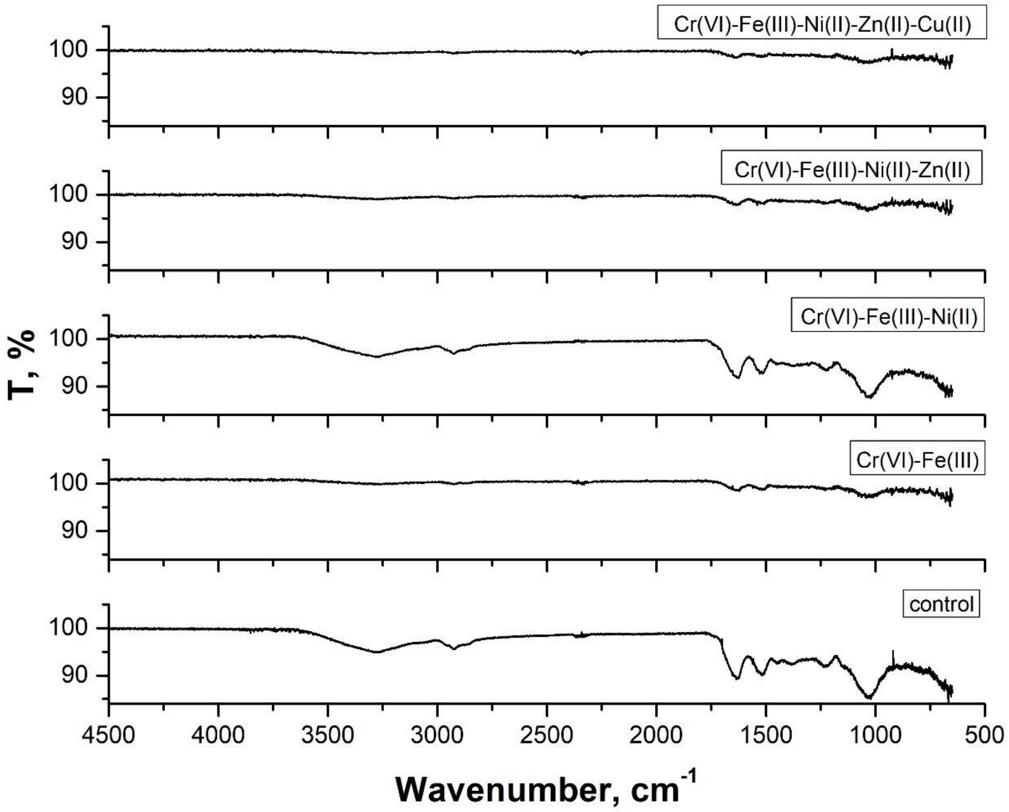


Fig. 5.13 FTIR spectrum of *S. cerevisiae* biomass before and after metal biosorption

The peaks at 1214 cm^{-1} and 1740 cm^{-1} are related to the stretching vibration of carboxyl (C=O) groups, whereas the peaks at 1393 and 2950 cm^{-1} represent the stretching vibration of alkyl groups (-CH_3 or CH_2). The peak at 1518 cm^{-1} is related to the vibration of aromatic groups, and the absorption band at 1626 cm^{-1} corresponds to CH=CH groups. The peak of symmetrical stretching vibration of the phosphodiester group $[-PO_2^-]$ was entered at wavenumber 1092 cm^{-1} and the vibration absorption bands of polysaccharide skeleton at wavenumber 1043 cm^{-1} were observed. The strong boarded peak at wavenumber area 3600-3200 cm^{-1} could be attributed to hydroxyl (–OH) and amine (–NH) functional groups. The presence of methyl (–CH) stretching vibrations could be confirmed by the adsorption peak at the wavenumber's region 2950-2800 cm^{-1}. The band at 3288 cm^{-1} is relevant to the standard absorption band of the amido group (HN=O).

Besides, –C–O, –C–C, and –C–OH stretching vibrations could be found at the adsorption peaks in the 1650–1200 cm^{-1} region or the strong bands in this area could also correspond to the amide I–III bands of polypeptide/proteins. After biomass interaction with the solution containing Cr(VI) and Fe(III), the IR spectra revealed a slight shift of bands 1036, 1518, and 3288 cm^{-1} indicating the involvement of OH, CH=CH, C=O и N=O groups in metal binding. In case of Cr(VI)-Fe(III)-Ni(II), Cr(VI)-Fe(III)-Ni(II)-Zn(II) and (VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) no significant changes in metal loaded biomass spectra were observed. However, the transmittance of the peaks in the metal loaded biomass was lower than in the control sample. Zeta potential of *S. cerevisiae* measured at the pH range 2.0-9.0 was negative at all pH values [237].

5.2.1 The influence of pH on metals adsorption

Effect of pH, parameter, which affects the speciation of metal and charge of biomass, was studied in the pH range 2.0-6.0 (Fig.5.14) since at pH > 7.0 precipitation of metal cations take place. The sorption of Cr(VI) ions was very high at low pH values. Cr(VI) in solution regardless of its form is present as an anion, while the functional groups on the yeast surface at low pH values are protonated, which facilitates the sorption of anionic species. Anionic species of Cr(VI) can be linked to the microbial surface through the electrostatic interaction with amines, which are positively charged, or bound to carbonyl, amide, and phosphate groups [390, 410]. In Cr(VI)-Fe(III) system, the maximum removal of Cr(VI) 97.7 % was achieved at pH 2.0, while with the pH increase it was significantly reduced at pH > 4.0 when the yeast removal capacity for Cr(VI) was only 30 %. Removal of iron was also favorable at low pH values: 98.1 % of iron was removed at pH 2.0. Fe(III) exists in the ionic form in a very narrow range of pH. According to the literature data, OH groups play the main role in iron ions binding [411]. The decrease of

Fe(III) ions removal with the pH increase can be explained by the increase of the concentration of OH- ions and their precipitation in the form of iron hydroxide. The pH 2.0 was found as optimal for chromium and iron ions removal in other studies as well [193,206].

In Cr(VI)-Fe(III)-Ni(II) and Cr(VI)-Fe(III)-Ni(II)-Zn(II) systems for chromium and iron removal the same pattern was maintained as in the previous system with maximum removal at pH 2.0. The pH 3.0 was found to be optimal for maximum nickel ions removal in both systems, while zinc maximum removal was achieved at pH 4.0. Addition of zinc ions in the system Cr(VI)-Fe(III)-Ni(II)-Zn(II) leads to the reduction of nickel and iron ions removal. Carboxyl, phosphate, sulfhydryl, hydroxyl, and nitrogen-containing groups of yeast biomass surface participated in the removal of Zn(II) ion [219]. Thus, the decrease of nickel and iron removal can be explained by their competition with zinc ions for the same binding sites on the yeast surface or formation of heterogeneous hydroxocomplexes containing two or more metal ions, that reduce metal ions biosorption [412]. Decrease of Ni(II) removal at pH≥ 4.0 in the systems, in which it was present can be associated with the beginning of the nickel hydroxide formation or low biomass affinity for nickel ions. [413] suggested that mannoproteins and glucans play the main role in nickel binding on the yeast surface.

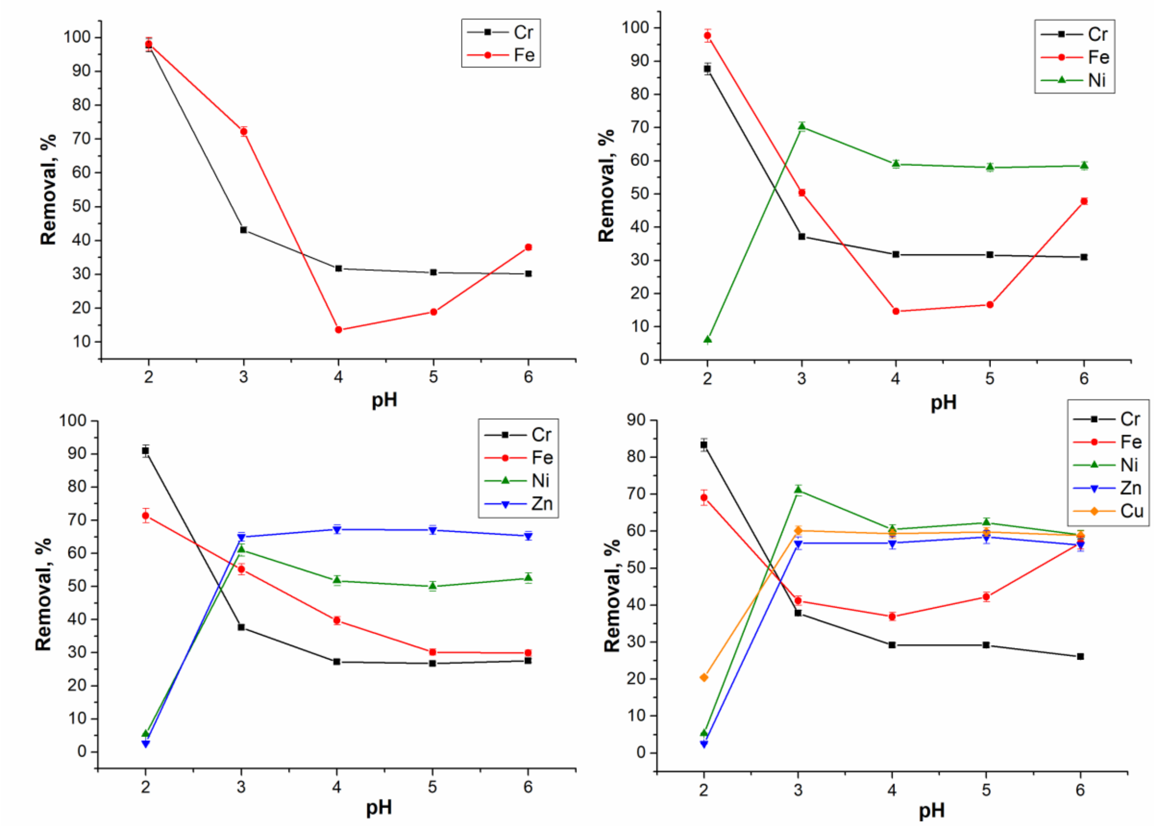


Fig. 5.14 Removal of metal ions at different initial pH (at T 20 0C; sorbent dosage 0.5 g/L; adsorption time 1h)

In Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system at pH 2.0 83 % of chromium and 69 % of iron ions were removed from the solution. The optimum pH for nickel (71 %) and copper (60%) ions removal was 3.0, while at pH 5.0 maximum removal of zinc ions (58 %) was achieved. Addition of copper ions affected mainly iron and zinc metal removal, while the removal of nickel ions was maintained on the level of Cr(VI)-Fe(III)-Ni(II) system [237]. This is in agreement with [414] study. Machado et al. [218] showed the involvement of carboxyl, amino, hydroxyl, and amide groups of protein and carbohydrate fractions of the cell wall in the yeast *S. cerevisiae* in Cu(II), Ni(II), and Zn(II) uptake. Guler et al. [415] reported that in *S. cerevisiae* hydroxyl and carbonyl groups are involved in Cu(II) binding, while Ni(II) biosorption occurs in hydroxyl, amine, and carboxylate groups of polysaccharides on peptidoglycan layer.

The experiment performed with the use of a brewing strain of *S. cerevisiae* for metal removal from electroplating effluent showed that 98% of Cr(VI) ions were removed at pH 2.0, while Ni(II) (52%) and Cu(II) (78%) ions were better removed at higher pH values [218]. Due to the acidic nature of chromium-containing industrial effluents, further experiments were performed at pH 2.0 [237].

5.2.2 The effect of contact time on metal biosorption by S. cerevisiae and kinetics of sorption

The effect of contact time on the adsorption of metal ions onto *S. cerevisiae* was studied at 20 ^{0}C and pH 2.0 and is shown in Fig. 5.15.

In Cr(VI)-Fe(III) system 91 % of chromium and 94% of iron ions were adsorbed by biomass in the first 45 min of biomass interaction with the solution and then the equilibrium was attained. At the addition of nickel ions in the system, chromium ions were almost completely removed from the solution (99.8%), while iron removal was reduced by 28 %, indicating the competition of iron and nickel ions for the binding sites on the yeast surface. In Cr(VI)-Fe(III)-Ni(II)-Zn(II) system the metal maximum removal was changed in the following order: Cr(VI) (95 %)>Fe(III) (79 %)>Ni(II) (9.9 %)>Zn(II) (6 %). In Cr(VI)-Fe(III)-Ni(II) and Cr(VI)-Fe(III)-Ni(II)-Zn(II) systems equilibrium for all elements was achieved in 30 min of sorbate-sorbent interaction. The addition of copper ions in the system mainly affected Cr(VI) biosorption, the sorption of other elements was on the level of two, three, and four-component systems. Equilibrium for Cr(VI) and Fe(III) ions was attained in 60 min, while for Zn(II) and Cu(II) in 30 min of sorbate-sorbent interaction. Since the experiments were performed at pH 2.0, the removal of metal ions present in the solution in cationic form was significantly lower and their presence in solution did not affect chromium ions removal [389].

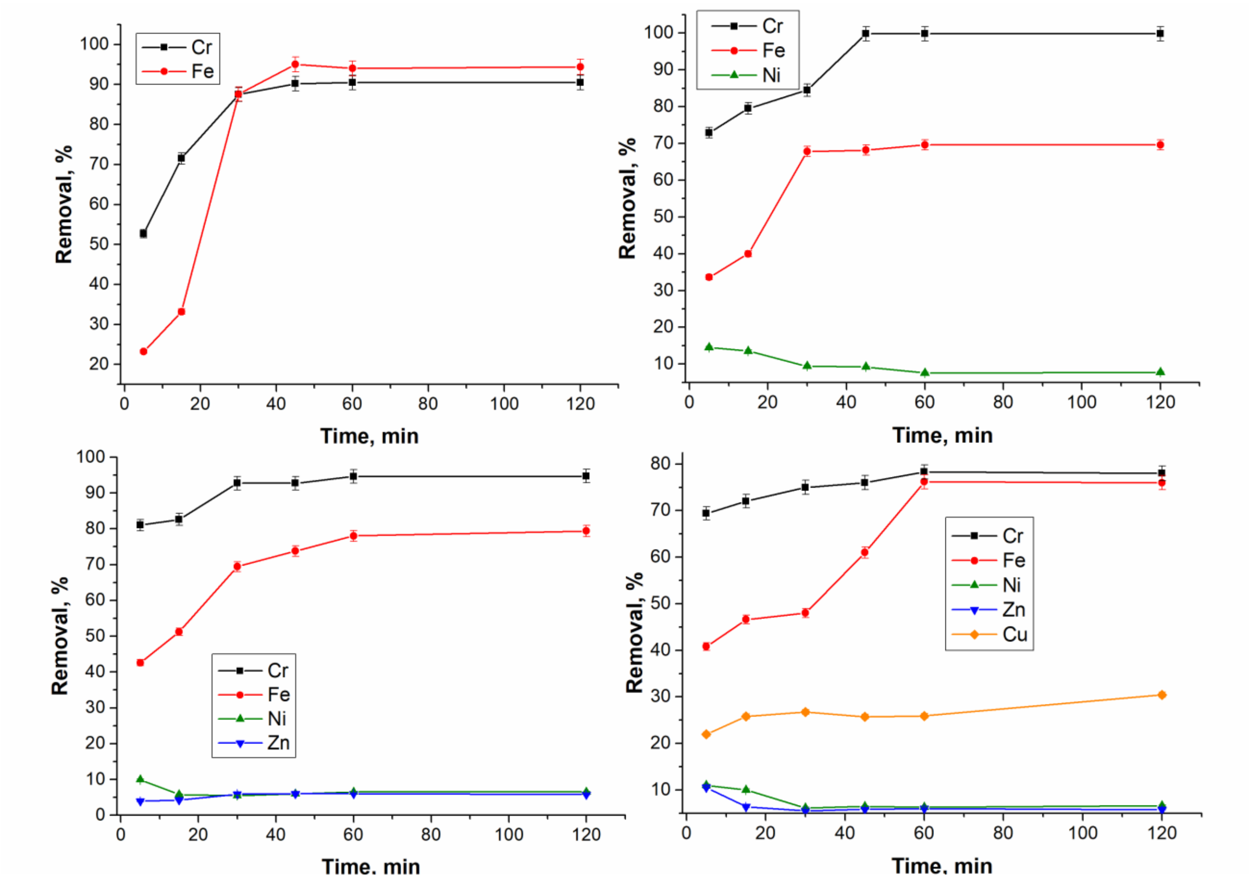


Fig. 5.15 Adsorption of metal ions on *S. cerevisiae* as the function of time (at T 20 ^{0}C; sorbent dosage 0.5 g/L)

Four kinetic models, PFO, PSO, EM and IPM, were applied to describe the experimental data. The description of models is given in Chapter 1. Graphical presentation of the data is given in Figs. A1.2-A1.5 in the Annex and the coefficients of correlation as well as the kinetic parameters are given in Table 5.6. Since Ni(II) in all systems and Zn(II) (in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system) adsorption was decreased with the increase of time of contact it was not possible to describe the obtained experimental data by kinetic models.

In Cr(VI)-Fe(III) and Cr(VI)-Fe(III)-Ni(II) systems, the experimental values obtained for chromium obey the PSO model, while PFO and PSO models fit well data obtained for iron ions. PSO model is based on the assumption that in the sorption the rate-limiting step can be chemical sorption or chemisorption involving the valency forces through electrons sharing or exchange between sorbent and sorbate [256]. The pseudo-first-order Lagergren model is obtained under the ideal assumption of a totally homogenous adsorption surface [416]. In Cr(VI)-Fe(III)-Ni(II)-Zn(II) system according to the coefficient of determination (R^2) values, the PSO model was suitable to describe the data obtained for chromium ions, while data obtained for iron and zinc were better described by PSO and EM models.

Table 5.6 The constants and determination coefficients (R^2) of the kinetics models

				Model											
				PFO			**PSO**			**EM**			**IPM**		
Systems		**Metal**	q_{exp}, mg/g	q_e, mg/g	k_1, min^{-1}	R^2	q_e, mg/g	k_2,	R^2	α, mg/g·min	β, g/min	R^2	k_{diff}	C_i	R^2
	Cr(VI)-Fe(III)	Cr	0.91	0.88	0.15	0.89	0.96	0.2	0.96	2.2	7.7	0.83	0.04	0.5	0.59
		Fe	0.47	0.5	0.05	0.88	0.6	0.08	0.82	0.06	7.3	0.77	0.04	0.08	0.6
	Cr(VI)-Fe(III)-Ni(II)	Cr	0.84	0.83	0.4	0.8	0.86	1.32	0.95	2.7	26.3	0.77	0.01	0.7	0.5
		Fe	0.34	0.35	0.08	0.81	0.39	0.03	0.83	0.17.	14.8	0.77	0.02	0.15	0.5
	Cr(VI)-Fe(III)-Ni(II)-Zn(II)	Cr	0.95	0.91	0.42	0.35	0.94	1.1	0.7	6.9	19.5	0.81	0.01	0.79	0.67
		Fe	0.39	0.38	0.11	0.74	0.4	0.39	0.9	0.33	15.2	0.91	0.02	0.19	0.77
		Zn	0.012	0.01	0.17	0.58	0.012	2.3	0.77	0.06	670	0.71	0.0004	0.008	0.49
	Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II)	Cr	0.78	0.76	0.48	0.47	0.77	1.97	0.78	5.35	32.9	0.93	0.01	0.7	0.8
		Fe	0.38	0.33	0.11	0.29	0.39	0.3	0.58	0.22	16.1	0.76	0.02	0.15	0.81
		Cu	0.034	0.04	0.33	0.46	0.037	1.85	0.6	1.921	354	0.72	0.001	0.03	0.74

The Elovich equation is used to describe the chemical adsorption processes and is suitable for the systems with heterogeneous adsorbing surfaces [416]. In Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system R^2 value 0.9 indicates that the adsorption of Cr(VI) on *S. cerevisiae* follows the EM, while IPM was more applicable for the description of Fe(III) and Cu(II) biosorption. Determination coefficients determined for the intra-particle diffusion model were, in general, lower in comparison with other models and lied within 0.49-0.81. It should be mentioned that in Cr(VI)-Fe(III) and Cr(VI)-Fe(III)-Ni(II) systems Cr(VI) and Fe(III) ions sorption was described by the same models. At the addition of Zn(II) ions in the system the data obtained for Cr(VI) and Fe(III) ions were better described by EM, while at the addition of Cu(II) ions by EM and IPM. Applicability of different models to describe the metal biosorption in multi-metal systems indicates the complex mechanism of metal ions interaction with biosorbent. Since PSO and EM described better the sorption of ions present in the studied systems it can be suggested that chemical sorption plays an important role in the metal ions binding onto *S. cerevisiae* [237].

5.2.3 Effect of temperature on metal biosorption by S. cerevisiae and thermodynamic studies

The effect of temperature on metal removal by *S. cerevisiae* was studied in the temperature range of 20 – 50 ºC. Experimentally obtained data showed that the increase in temperature did not affect significantly chromium ions removal in analyzed systems (Fig. 5.16). The temperatures 25-35 ºC were found to be optimal for Cr(VI) removal by *S. cerevisiae* [417].

The temperature effect on iron removal by *S. cerevisiae* was very different. The maximum removal of iron in Cr(VI)-Fe(III) and Cr(VI)-Fe(III)-Ni(II) systems was 92 % and 96 %, respectively and was achieved at 40 ^{0}C. In the Cr(VI)-Fe(III)-Ni(II)-Zn(II) system the highest iron ions sorption occurred at 50 ^{0}C, while in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system at 20 ^{0}C. The temperature increase leads to the decrease in nickel ions removal. In Cr(VI)-Fe(III)-Ni(II)-Zn(II) system maximum Zn(II) ions adsorption was achieved at 40 ºC, while in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system at 20 ºC. Temperature increase from 20 to 50 ºC resulted in the decrease of copper removal from 37 to 16 %, which is in agreement with Brady and Duncan [418], who reported the maximum uptake of Cu(II) ions by *S. cerevisiae* at 25°-30°C.

Thermodynamics of metal ions removal by *S. cerevisiae* was evaluated using the standard thermodynamic variables: Gibbs free energy change ($\Delta G°$), enthalpy change ($\Delta H°$), and entropy change ($\Delta S°$). The values were calculated according to formulas (26-27) presented in Chapter 1, Section 1.5.1.2. A linear van't Hoff plot (Fig. A1.6, Annex) of $\ln K_d$ versus 1/T gives slope and intercept to determine the value of ΔH° and ΔS°, respectively. The results obtained are listed in Table 5.7 [237].

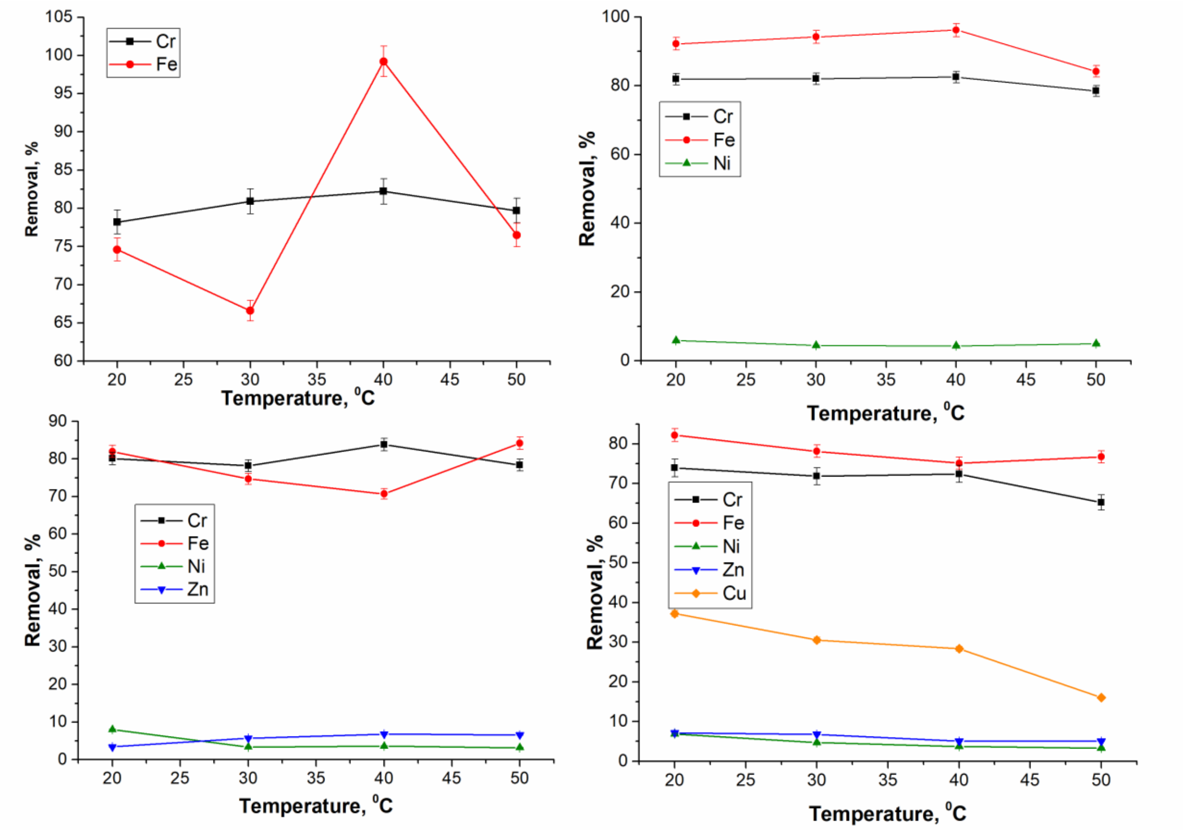


Fig. 5.16 Effect of temperature on the sorption of metal ions by *S. cerevisiae* biomass (at T 20 ^{0}C; sorbent dosage 0.5 g/L; adsorption time 1h)

The negative values of ΔG° obtained for all elements present in the analyzed systems indicate on feasible and spontaneous biosorption process. The decrease of the ΔG° values with the increase of temperature, except for Ni(II) and Zn(II) (in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system), shows that temperature rise positively affects the adsorption process [419].

The energy ΔG° values between 0 and 20 kJ/mol indicate that the adsorption process is physisorption, while the values between 80 and 400 kJ/mol correspond to chemisorption [463]. The ΔH° value gives information about the nature of the adsorption process if ΔH° is positive metal ions sorption is considered endothermic, while at the negative ΔH° value the process is exothermic. In Cr(VI)-Fe(III) system positive ΔH° values were obtained for both elements, while in Cr(VI)-Fe(III)-Ni(II) system the character of metal biosorption was exothermic. The sorption of chromium and zinc in Cr(VI)-Fe(III)-Ni(II)-Zn(II) system was endothermic and of iron and nickel ions exothermic process. In Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system ΔH° values were negative for all elements, indicating the exothermic character of biosorption. The sorption process is generally considered as physical if ΔH°<25 kJ/mol and as chemical when ΔH° >40 kJ/mol [464]. According to the calculated ΔH° and ΔG° values, the metal sorption present in the analyzed system can be described as physical sorption. The positive ΔS° value suggests an increasing randomness due to metal ions sorption on *S. cerevisiae*. Negative ΔS° values were obtained only for Ni(II) and Zn(II) (in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system) and indicate the decrease in the degree of freedom of the adsorbed species [237].

Table 5.7 Thermodynamics parameters for metal biosorption on *S. cerevisiae*

		Metal	ΔG°, kJ/mol				ΔH°, kJ/mol	ΔS°, J/mol·K
			293 K	303 K	313 K	323 K		
System	Cr(VI)-Fe(III)	Cr	-10.5	-10.9	-11.3	-11.7	0.6	38.2
		Fe	-10.3	-10.8	-11.3	-11.8	4.1	49
	Cr(VI)-Fe(III)-Ni(II)	Cr	-17.8	-18.4	-18.9	-19.5	-1.2	56.5
		Fe	-11.1	-11.4	-11.7	-12	-2.1	30.7
		Ni	-4.09	-4.09	-4.08	-4.08	-4.2	-0.5
	Cr(VI)-Fe(III)-Ni(II)-Zn(II)	Cr	-10.6	-11	-11.3	-11.7	-0.8	36.5
		Fe	-10.4	-10.7	-11.1	-11.4	-0.8	35.7
		Ni	-4.7	-4.1	-3.5	-2.9	-22.3	-60.3
		Zn	-3.2	-4	-4.7	-5.4	18.2	73.2
	Cr(VI)-Fe(III)-Ni(II)-Zn(II) – Cu(II)	Cr	-10.5	-10.8	-11	-11.3	-2.9	25.9
		Fe	-10.6	-10.9	-11.2	-11.5	-2.1	29.1
		Ni	-4.6	-4.1	-3.6	-3	-20.4	-54
		Zn	-5.1	-4.9	-4.7	-4.5	-10.9	-19.9
		Cu	-9.9	-10	-10.1	-10.2	-6.9	9.9

5.2.4 Effect of chromium ions concentration on metals adsorption by S. cerevisiae and equilibrium study

The effect of initial Cr(VI) concentration on metal biosorption was studied by maintaining the constant concentration of interfering metals (Fe, Zn, Ni, and Cu) and varying Cr(VI) concentration from 10 and 100 mg/L. The data presented in Fig. 5.17 show that an increase of chromium concentration in solution leads to an increase in its content in biomass. The maximum Cr(VI) adsorption was found to be 8.3 mg/g in Cr(VI)-Fe(III) system, 7.9 mg/g in Cr(VI)-Fe(III)-Ni(II) system, 7.9 mg/g in Cr(VI)-Fe(III)-Ni(II)-Zn(II) system and 6.9 mg/g in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system. It is evident that with the increase of the number of metal ions in solution chromium uptake by biomass was reduced due to competition of metal ions for binding sites [237]. Brady et al [465] showed that the yeast biomass was effective for simultaneous uptake of Zn(II), Cr(VI), and Cu(II) ions from electroplating wastewater, showing the highest removal capacity for Cu(II) ions. At the same time at the simultaneous presence of lead and copper ions in solution, the biosorption capacity of waste beer yeast biomass was decreased with the increasing of the competing metal ion concentration [185].

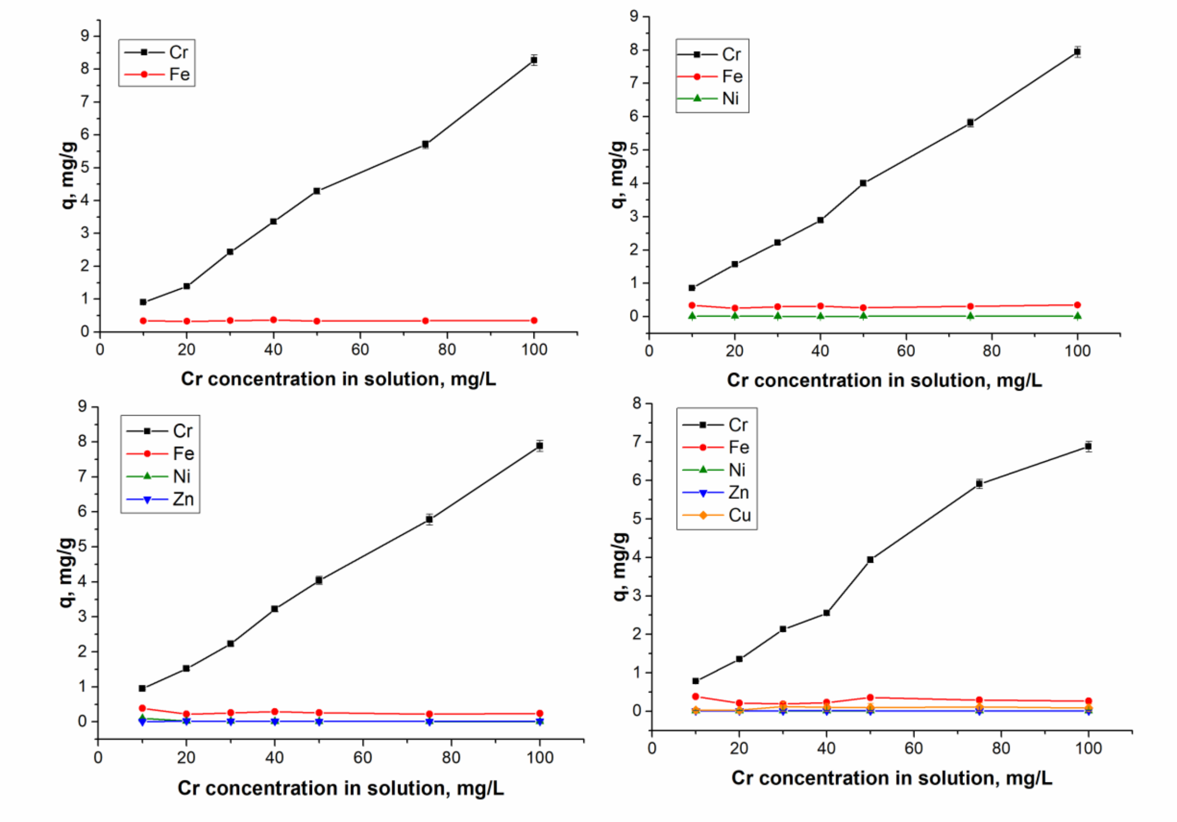


Fig. 5.17 Removal of metal ions at different chromium concentration in solution (at T 20 0C; sorbent dosage 0.5 g/L; adsorption time 1h)

The increase of Cr(VI) concentration did not affect iron sorption in Cr(VI)-Fe(III) and Cr(VI)-Fe(III)-Ni(II) systems, but it was slightly reduced at the addition of zinc and copper ions in the systems. Since the experiments were performed at pH 2.0, the sorption of zinc and nickel by *S. cerevisiae* was very low and it was not affected by an increase of Cr(VI) concentration in solution. At the same, the rise of chromium concentration in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system resulted in the increase of copper sorption from 15 to 50% and the reduction of chromium ions removal. Thus, copper can be considered the main chromium competitor for binding sites (amine groups).

The equilibrium data obtained for Cr(VI) removal by *S. cerevisiae* were described using Langmuir, Freundlich, Temkin, and Dubinin–Radushkevich equilibrium models. The Freundlich, Temkin, and Dubinin-Radushkevich' constants were calculated from nonlinear regression (Fig. A1.7, Annex), while Langmuir model constants were calculated from the approximation of linearized experimental plot data (Fig. A1.8, Annex). The values of the parameters calculated using these models are shown in Table 5.8.

Table 5.8 The parameters and coefficients of determination (R^2) of the adsorption isotherms

Model	Parameters	System			
		Cr(VI)-Fe(III)	Cr(VI)-Fe(III)-Ni(II)	Cr(VI)-Fe(III)-Ni(II)-	Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II)
Langmuir	q_m, mg/g	26.8	27.3	17.40	31.8
	b, L/mg	0.003	0.003	0.006	0.002
	R^2	0.97	0.99	0.98	0.99
Freundlich	K_F, mg/g	0.08	0.07	0.08	0.08
	1/n	0.99	0.97	1.0	1.0
	R^2	0.99	0.99	0.99	0.98
Temkin	a_T, L/g	0.09	0.09	0.09	0.09
	B, J/mol	3045	3045	3045	3045
	R^2	0.86	0.84	0.85	0.86
Dubinin-Radushkevich	q_{DR}, mg/g	8.2	8.3	8.0	7.5
	β, mol^2/kJ^2	225	256	233	240
	R^2	0.87	0.86	0.87	0.9
	E, kJ/mol	0.05	0.04	0.05	0.05

The maximum adsorption capacity of *S. cerevisiae* toward chromium ions was calculated from the Langmuir adsorption isotherm and constituted: 26.8 mg/g for Cr(VI)-Fe(III) system, 17.4 mg/g for Cr(VI)-Fe(III)-Ni(II)-Zn(II) system, 27.3 mg/g for Cr(VI)-Fe(III)-Ni(II) system and 31.7 mg/g for Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system. Cr(VI) biosorption onto *S. cerevisiae* in all analyzed systems was better described by the Freundlich isotherm model. The *1/n* values calculated from the Freundlich model for all elements were near 1.0, which shows that the biosorption of chromium ions onto *S. cerevisiae* biomass is favorable. The R^2 values 0.99 for Cr(VI)-Fe(III)-Ni(II) and Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) indicate the applicability of Langmuir isotherm for the description of experimentally obtained data. The R^2 values calculated from Dubinin–Radushkevich and Temkin isotherm models were lower than those obtained for Langmuir and Freundlich models [237]. A sorption process is considered as physical if free energy (*E*) is less than <8 kJ/mol and as chemical when the *E* value is between 8 and 16 kJ/mol [456]. Calculated *E* values from the Dubinin–Radushkevich model indicate the predominance of physical sorption of metal ions present in studied systems. In all the studied systems the constant b_T, related to the heat of adsorption, has positive values, indicating the endothermic character of the sorption process [466].

Applicability of Langmuir and Freundlich models to describe biosorption of metal ions present in analyzed systems was shown in other studies as well. The process was mainly described for single element systems [206,456,467].

5.3 Metal removal from the synthetic effluents using *Shewanella xiamenensis*

Besides the yeast and cyanobacteria, bacteria can be also used for metal removal from wastewater. A wide variety of pure and mixed bacterial cultures have been reported to be capable of removing metal ions under aerobic and/or anaerobic conditions [468]. Bacteria can remove metals through adsorption, intracellular uptake, methylation, oxidation, reduction, and precipitation. Since bacteria are often present in the solution in free suspended form, the separation of microbial biomass from the treated effluent becomes a difficult task. Immobilization of bacteria onto the different cheap supports of natural provenance (zeolites, clinoptilolite, activated carbon, etc.) is considered a suitable approach to face challenge [469].

In recent years, bacteria of Shewanella species have attracted the great interest concerning environmental bioremediation. Shewanella species, gram-negative facultative anaerobe bacteria, able to form a stable biofilm, are widely distributed in various environments such as marine and freshwater, spoiled food, and oil field wastes and can reduce iron, manganese, selenium, chromium, and other multivalent elements to insoluble forms [470–472]. Accumulation and bioreduction capacity of Shewanella species was widely discussed in the literature, while information of their use as biosorbent is very scanty. Mamba et al. [473] investigated the effect of different parameters on the biosorption of copper and cobalt by *Shewanella* spp.

5.3.1 Biosorbent characterization

A confocal laser scanning microscope Leica SP5 (Leica, Germany) was used to evaluate the formation of biofilms on the zeolite surface (Fig. 5.18). The results obtained were analyzed using the software package Imaris 7.0.0. (Bitplane, Switzerland, https://www.bitplane.com/biofilms.aspx). The software allows calculation of the number of signals of each laser (color) and assess the area covered by the biofilm. The surface area of zeolite was covered by biofilm by 98%, which mainly consisted of polysaccharides (49.4%) and bacterial cells (46.6%).

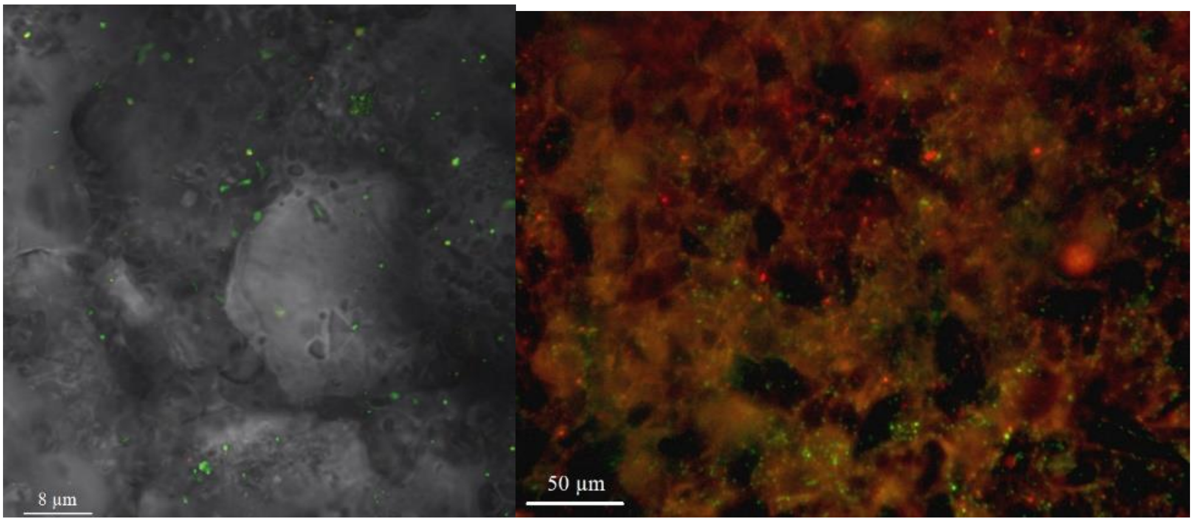


Fig. 5.18. Zeolite surface before (left) and after (right) biofilm formation

To characterize the porous texture of the biosorbent by physical adsorption of nitrogen at 77 K (purity 99.9%), the automatic volumetric adsorption apparatus AUTOSORB 1MP from Quantachrome instruments was used. The sample was degassed overnight at 573 K before the adsorption measurement. The N_2 physisorption measurements made at liquid nitrogen temperature (77K) for bio-zeolite are shown in Fig. A1.8 Annex. The hysteresis loops were identical to typical mesoporous materials with flat slits between two crystalline planes [474]. The convergence of desorption and adsorption curves to form a closed-loop was found at the point of hysteresis. Thus, the isotherm has shown two distinct features such as rapid adsorption at near-zero relative pressure itself and the initiation of adsorption/desorption hysteresis at a relative pressure of 0.3. According to the IUPAC classification, the isotherm obtained corresponds to type IV, which is characteristic of mesoporous materials. The specific surface areas calculated using the BET equation were 34 m^2/g. The total volume of pores was calculated by converting the gaseous nitrogen adsorbed at $P/P_0 = 0.99$ in liquid nitrogen. The value obtained was 0.154 cm^3/g. According to IUPAC, pores are classified into three groups: micropores (<2nm), mesopores (2–50 nm), and macropores (>50 nm) [474]. Fig. A1.8, Annex shows the distribution curve of pore volumes as a function of radius, obtained by the density function theory method. In the analyzed sorbent the pore size was in the mesoporous range. The mesopore size distribution ranged between 2.5 and 40 nm, with the dominant pore sizes of 3.0, 5.5, and 7.5 nm and in a smaller amount – pores with a radius of 10 and 25 nm [241].

5.3.2 Metal removal from chromium-containing synthetic effluents by Shewanella xiamenensis biofilm supported on zeolite

The concentrations of metal ions in the analyzed systems and the composition of systems were similar to the systems described in Section 5.1.2.

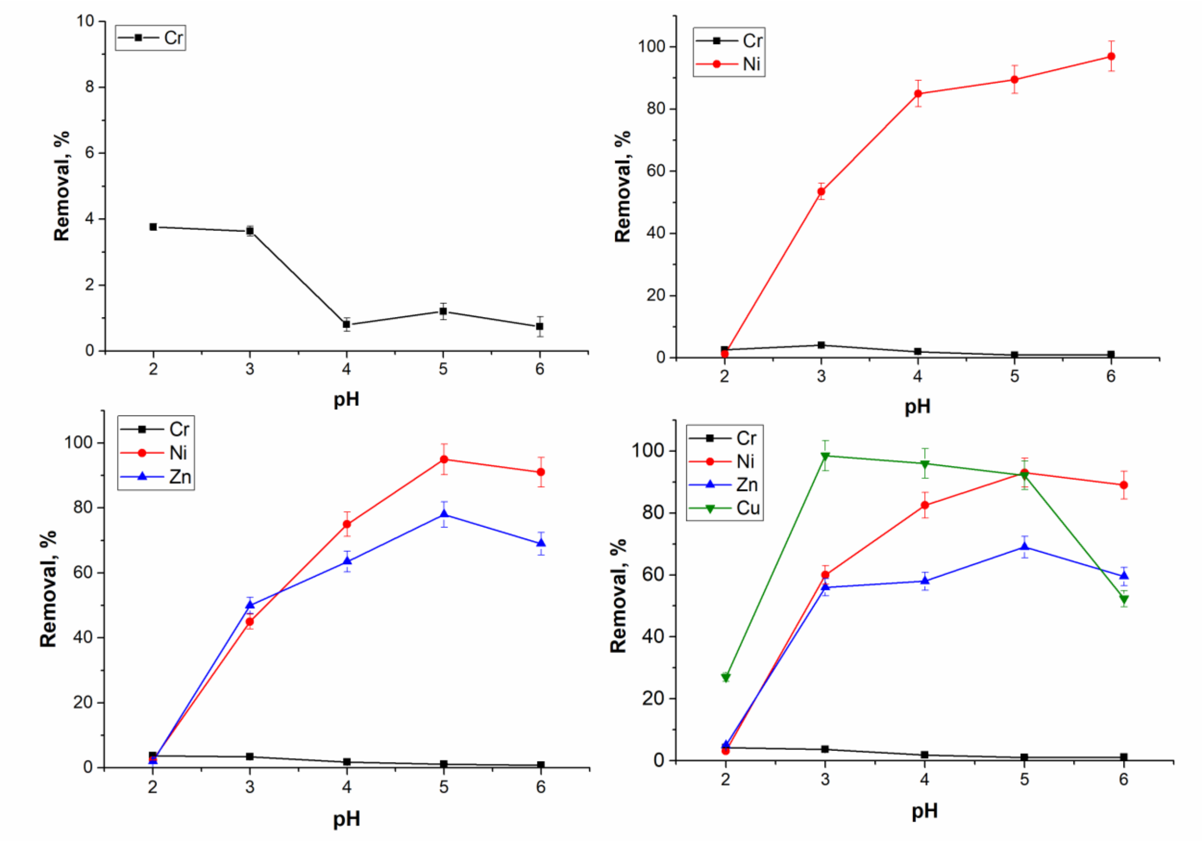


Fig. 5.19 Removal of metal ions on biosorbent at different initial pH values (at T 20° C; sorbent dosage 0.5 g/L; adsorption time 2h)

Sorption experiments were carried out in 100-mL flacks in which 50 mL of solution and 0.5 g of the sorbent were added. Experimental solutions were continuously stirred for one hour. All the experiments were conducted in triplicate and the average values were used for further calculation. The adsorption capacity of biosorbent was studied in the pH range of 2.0-6.0. The metal uptake by biomass was traced using NAA and the procedure of samples irradiation is given in Chapter 2. In biosorption, studies pH is the parameter, which markedly affects biosorbent removal capacity. Usually, it is one of the first parameters, which influence the materials biosorption capacity. The effect of other parameters (time, concentration) is studied at optimal pH value.

According to the data obtained (Fig. 5.19), the removal efficiency of biosorbent toward chromium ions was very low in all analyzed systems and did not exceed 5 %, it constituted 3.8% in the Cr(VI)-Fe(III) system, 4.2% in Cr(VI)-Fe(III)-Ni(II) and Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) systems, and 3.7% in the Cr(VI)-Fe(III)-Ni(II)-Zn(II) system. These values were obtained at pH 2.0 at higher pH values chromium removal efficiency was near 0%.

The low sorption of chromium ions can be explained by the structure of the natural zeolite, which contains the interconnected voids that are filled with cations that can be easily exchanged with other metal ions present in cationic form. Since at pH range 2.0-6.0 chromium exists in solution in anionic forms, $HCrO_4^-$ and $Cr_2O_7^{2-}$ species, its removal by bio-zeolite was low. In the preliminary experiments performed on natural zeolite within 24 hours only 0.01% of chromium ions were removed from the solution. Immobilization of biomass on the zeolite surface resulted in the increase of its removal due to the participation of functional groups of biofilms in the metal ions binding [241].

The low removal of the chromium from the solution by natural and modified zeolites was shown in the literature. Thus, Kučić et al. [475] showed that natural and modified zeolite removed 5% and 13% of Cr(VI) from the solution, respectively. Silva et al. [476] reported that maximum removal of chromium by a biofilm of *Actinomyces viscosus* supported on zeolites constituted 20%. The removal of chromium in the present study was even lower that can be explained by the multi-metal composition of studied systems.

The efficiency of nickel, zinc, and copper ions removal present in solution in cationic form was markedly higher and was attained at higher pH values. This is in agreement with [477] study, which showed that *Escherichia coli* biofilm supported on zeolite NaY was more efficient in the removal of Ni(II) than of Cr(VI) and Cd(II) ions. In the present study, the maximum removal of nickel ions was achieved at pH 5.0-6.0, and removal efficiency was higher than 90%: 97% in the Cr(VI)-Fe(III)-Ni(II) system, 95% in Cr(VI)-Fe(III)-Ni(II)-Zn(II) system, and 93% in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system. The maximum removal of zinc ions was reached at pH 5.0 (78% in the Cr(VI)-Fe(III)-Ni(II)-Zn(II) system and 69% in the Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system). Zinc removal in analyzed systems was lower that of nickel. The maximum removal of copper ions (98%) was achieved at pH 3.0 [241]. The low removal of metals present in solution at acidic pH is associated with the competition between hydrogen and cations for sorption sites, which limits the approaching of metal cations due to repulsive forces, and the removal of cations is inhibited [477].

Since zeolite was almost completely covered by biofilm, it is assumed that the main role in the removal of metals from solutions belong to the functional groups. Ha and co-authors [478] showed that in bacterium *S. oneidensis* strain MR-1, Zn(II) ions mainly interacted with phosphoryl groups and in a less extend with carboxyl and amide groups. Carboxylate, amine, and hydroxyl groups play an important role in the binding of Cu(II) ions, while the biosorption of Ni(II) occurs on hydroxyl, amine, and carboxylate groups [458]. Machado et al. [218] showed the participation of carboxyl, amino, hydroxyl, and amide groups of the cell wall in Cu(II), Ni(II), and Zn(II) uptake by yeast biomass. Decrease in the removal of Zn(II) in the Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system can be explained by its competition for the same binding sites with other metal ions.

The high sorption capacity of biofilms was presented in other studies. Almost 99% of Zn(II) and Cu(II) ions were removed by *Escherichia coli* ATCC29522 and *Staphylococcus epidermidis* RP62A biofilms at pH 5.0 removed [479]. The removal of zinc and chromium from the aqueous solution using *Escherichia coli* ATCC 25922 and *Staphylococcus epidermidis* biofilms supported on kaolin was investigated at pH 5.0. *Escherichia coli* biofilm showed a higher biosorption capacity compared to *Staphylococcus epidermidis* biofilm due to the highly negative surface of the cell wall structure and high bacterial activity of bacteria. Chromium was preferably adsorbed over zinc by both biofilms [217]. The maximum removal of cobalt and copper by *Shewanella* spp. was achieved at pH 6.5. With the simultaneous presence of metal ions in the solution, the decrease in the removal efficiency from 44% to 31% for copper and from 33% to 15% for cobalt ions was observed [473].

The situation with iron, even if it was present in solution in cationic form, was different from other metal ions. Although iron was present in the analyzed systems, its content in the metal-loaded biosorbent was lower or at the level of the control sample (Fig. A1.9, Annex). The low removal of iron can be explained by the formation of iron hydroxide $Fe(OH)_3$. Iron removal occurred only in the Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system, where 40-60% of its ions were removed from the solution. The sorption of iron, in this case, can be explained by its co-deposition on the metal ions already sorbed on the surface of *S. oneidensis* [241].

The results obtained showed that *S. xiamenensis* biofilm possesses the high removal capacity for nickel, copper, and zinc ions and can be applied for selective removal of these metal ions from complex solutions. At the same time, the problem of removing of chromium and iron ions remained unsolved. Since it is necessary to attain the complete metal removal from wastewater, the process of metal removal needs to be improved. Chromium was the element of main interest in the analyzed system and since its removal in the biosorption experiment was very low the effect of other parameters such as chromium concentration, time of contact, and temperature on biosorbent removal capacity was not investigated. Our further research showed that chromium and iron can be removed from the solution through bioaccumulation process [241].

5.3.3 Metal removal from nickel-containing synthetic effluents by S. xiamenensis biofilm supported on zeolite

Since the analyzed biosorbent showed the high affinity for metal cations four systems containing nickel ions with the following composition: Ni(II), Ni(II)-Sr(II)-Cu(II)-Zn(II), Ni(II)-Cr(VI)-Fe(III), Ni(II)-Zn(II)-Mo(VI)-Cu(II) were modeled. The interest in nickel removal from wastewater is explained by the low affinity of used sorbents for nickel ions. The concentration of Ni(II) in all systems was 10 mg/L, of Cr(VI), Sr(II) and Fe(III) – 5 mg/L, of Mo(VI) – 0.5 mg/L, and of Zn(II) – 2 mg/L. Cu(II) was 1 mg/L in Ni(II)-Cu(II)-Sr(II)-Zn(II) system and 5 mg/L in Ni(II)-Zn(II)-Mo(VI)-Cu(II) system [480]. The experimental conditions were similar to those for chromium-containing systems.

5.3.3.1 Effect of pH on metal ion removal

The pH is one of the most important parameters, which affect metal removal from the solution. According to data presented in Fig. 5.20 the highest removal of nickel, 94%, was achieved in Ni(II) and Ni(II)-Cr(VI)-Fe(III) systems. As it was previously showed the biosorbent obtained showed a low affinity for metal anions and their removal was very low. Low affinity for anions can be explained by the negative values of zeta potential values at the studied pH range. At the pH range 2-8.2, nickel is present in solution in the dissolved form, while at pH >8.2, low-soluble $Ni(OH)_2$ is formed.

In Ni(II)-Sr(II)-Cu(II)-Zn(II) and Ni(II)-Zn(II)-Mo(VI)-Cu(II) systems 68% of nickel ions were removed. In the two mentioned systems removal of other metal cations was also very high. Maximum strontium removal was attained at pH 6.0 and constituted 95 %. The optimal pH for copper removal in Ni(II)-Sr(II)-Cu(II)-Zn(II) system was 6.0 when 70% of copper ions were sorbed from the solution, and in Ni(II)-Zn(II)-Mo(VI)-Cu(II), pH in the range 3.0-6.0 was favorable for copper ions removal. More than 90% of zinc was removed from the systems containing this element. The decrease of nickel removal can be explained by the competition of metal cations for the binding sites. In Ni(II)-Cu(II)-Sr(II)-Zn(II) system, metal ions are present in solution in the dissolved form at the studied pH range. The preference of biosorbent for the analysed elements changed in the following order Zn>Sr>Cu>Ni. In the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system, Zn(II) and Ni(II) were present in soluble forms. Copper is present both in the dissolved form and in precipitated form as molybdate, $CuMoO_4$ [480]. Since molybdenum was present in solution in anionic for or in the form of copper molybdate it is removal from the solution did not occur.

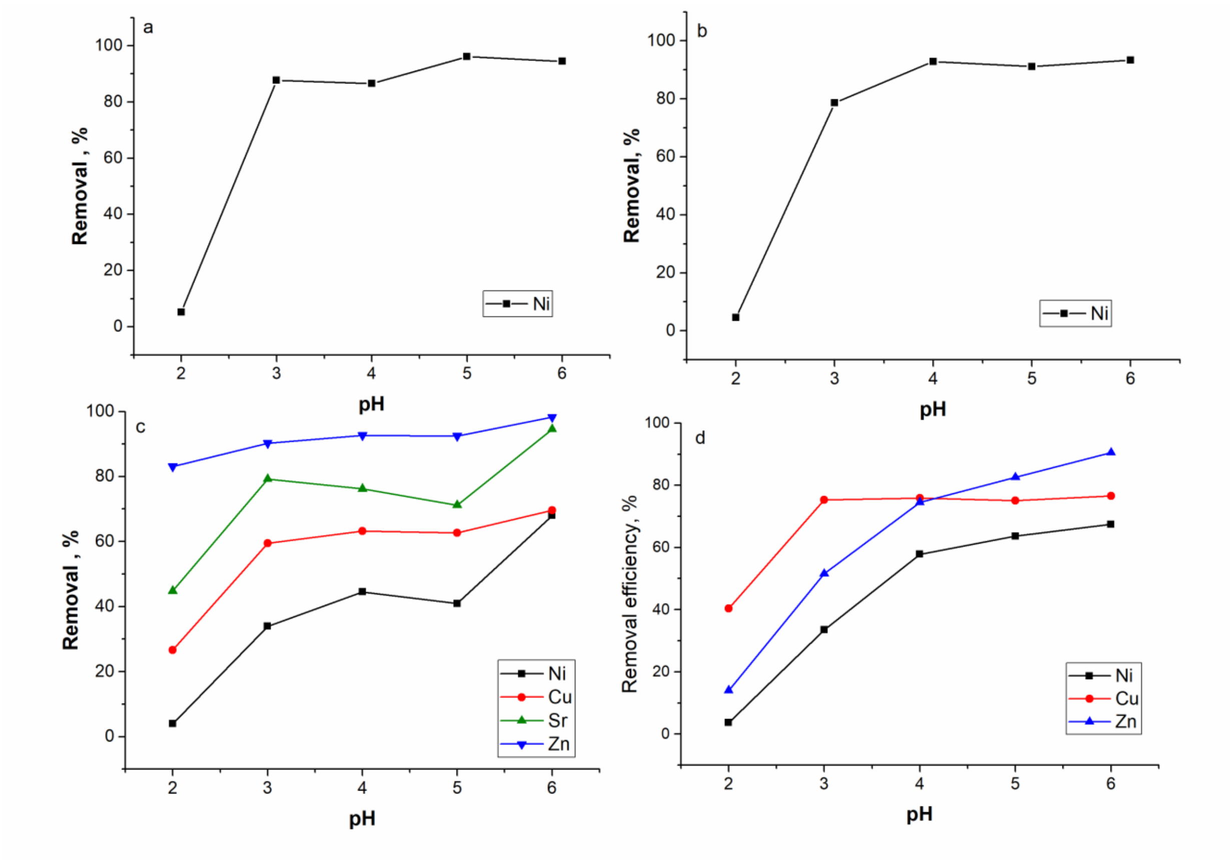


Fig. 5.20 Effect of pH on metal removal by hybrid adsorbent: a) Ni(II), b) Ni(II)-Cr(VI)-Fe(III), c) Ni(II)-Sr(II)-Cu(II)-Zn(II) and d) Ni(II)-Zn(II)-Mo(VI)-Cu(II)

Several mechanisms can be involved in metal removal from the analyzed systems. Metal ions can interact with functional groups of *Shewanella xiamenensis*, as well as with zeolite. Hydroxyl, carboxyl, carbonyl, and amino groups may play an important role in metal ions binding by microorganisms [237,418,419]. However, FTIR analysis showed that biofilm functional groups were not involved in metal ion trapping. Thus, it can be suggested that the main role in metal immobilization belongs to zeolites, in which process of chemical immobilization of heavy metals is based on the exchange of the alkali and alkaline earth metal cation with heavy metals [241]. The data obtained for Na and K using neutron activation analysis confirmed this fact since their content in the metal-loaded adsorbent decreased by 2-18% in respect to the control adsorbent. Besides, the ion-exchange, metal precipitation can be expected as another mechanism of metal removal [480].

Considering the role of biofilm in metal removal Handley-Sidhu et al. [481] showed that the biofilm was responsible for less than 30% of Sr^{2+} sorption by biogenic hydroxyapatite. The main role in Ni(II) removal by *Arthrobacter viscosus* supported on zeolite 13 X, 94.1%, belongs to zeolite, while the bacteria accelerated the process [482]. Therefore, it can be suggested that the main role in metal removal from the analyzed systems belonged to zeolite and bacterial biofilm, which accelerated the metal sorption process.

The maximum removal of copper (54.98 %) and zinc (57.32%) by *Escherichia coli* biofilm placed on zeolite was attained at pH values of 4.8–5.7 and 4.5–5.5, respectively [483]. Since Ni(II) was the element of main interest, further experiments were performed at pH 6.0.

5.3.3.2 Effect of time on metal removal and kinetic study

The biosorption of metal ions by *Shewanella xiamenensis* biofilm placed on zeolite was a quick process. The optimal time for maximum removal of metal ions varied from 60 to 150 min In the Ni(II) system, in two hours, nickel was completely removed from the solution (Fig. 5.21), and in the Ni(II)-Cr(VI)-Fe(III) system, its maximum removal, 95%, was attained in 150 min, then the equilibrium was attained. Optimum nickel uptake by natural zeolite was achieved at a contact time of 56–68 min and a pH of 4.8–6 [484].

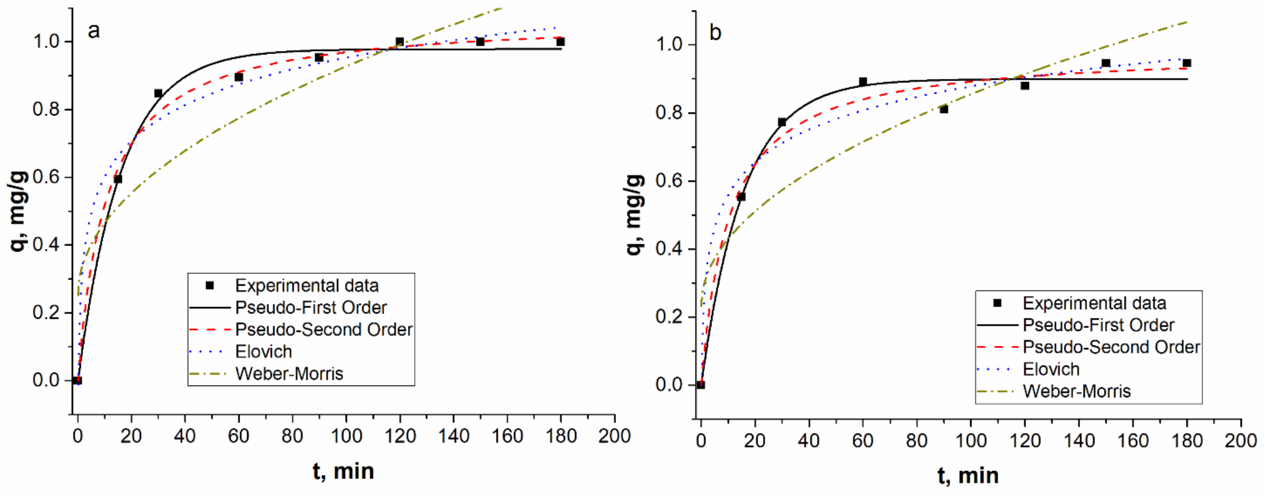


Fig. 5.21 Ni(II) adsorption on hybrid adsorbent in the (a) Ni(II) and (b) Ni(II)-Cr(VI)-Fe(III) systems

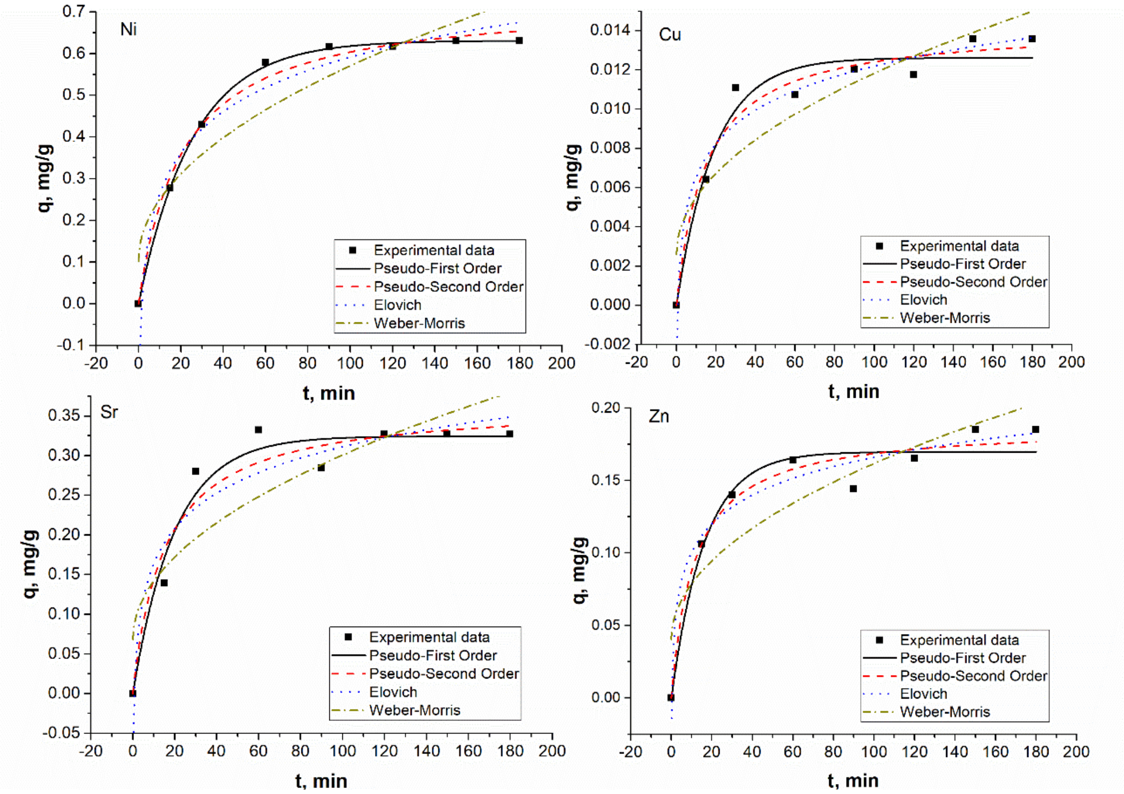


Fig. 5.22 Metal adsorption on hybrid adsorbent in Ni(II)-Sr(II)-Cu(II)-Zn(II) system

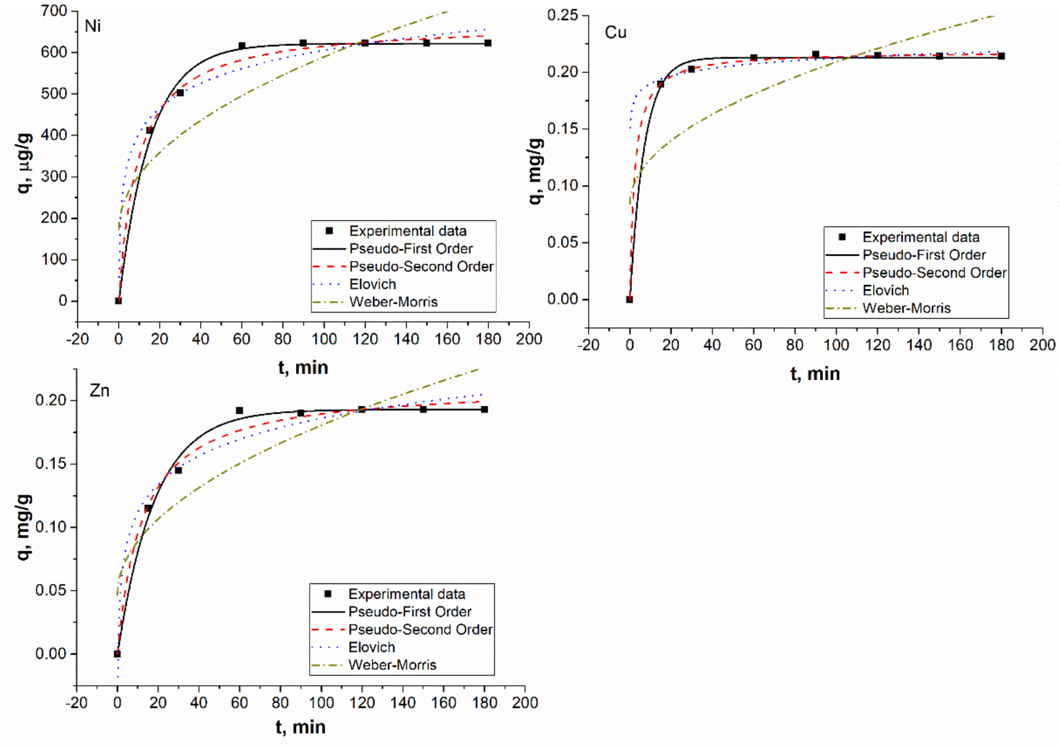


Fig. 5.23 Metal adsorption on hybrid adsorbent in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system

In the Ni(II)-Sr(II)-Cu(II)-Zn(II) and Ni(II)-Zn(II)-Mo(VI)-Cu(II) systems, 62% of nickel was removed in 90 min and then its removal stabilized at the same level. In the Ni(II)-Sr(II)-

Cu(II)-Zn(II) system, maximum zinc and copper removal (98.5% and 74%, respectively) was achieved in 150 min, and of strontium in 120 min (65%) (Fig. 5.22). In the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system (Figure 4), 60 min was enough for equilibrium to be attained for copper and zinc. The maximum removal of copper was 79% and of Zn(II) 96.5% (Fig. 5.23). Mo(VI), which was present in the solution in anionic form, was not adsorbed onto the biosorbent [480]. The equilibrium time for adsorption of copper and zinc on *Escherichia coli* biofilm placed on zeolite was achieved after 5 and 4 days, respectively [483].

Experimentally obtained data were described using four models, namely PFO, PSO, EM, IPM, which are presented in Chapter 1 and calculated coefficients are listed in Table 5.9. The *q* values, both calculated and experimental ones, for the PFO and PSO models were in good agreement. In Ni(II) and Ni(II)-Cr(VI)-Fe(III) systems, PFO and PSO models were applicable for the description of experimental data. The R^2 values showed that the adsorption of cations present in the Ni(II)-Sr(II)-Cu(II)-Zn(II) system obeyed the different models: PSO for Ni(II) and Cu(II); EM for Zn(II); and PFO model for Sr(II).

Table 5.9. Parameters calculated from the applied models

		Ni(II)	**Ni(II)-Cr(VI)-Fe(III)**	**Ni(II)-Sr(II)-Cu(II)-Zn(II)**				**Ni(II)-Zn(II)-Mo(VI)-Cu(II)**		
	Metal	**Ni**	**Ni**	**Ni**	**Zn**	**Cu**	**Sr**	**Ni**	**Cu**	**Zn**
	q_{exp}, mg/g	1	0.63	0.95	0.18	0.014	0.33	0.62	0.21	0.19
PFO	q_e, mg/g	0.98	0.63	0.9	0.16	0.01	0.32	0.62	0.21	0.19
	k_1, min^{-1}	0.06	0.04	0.06	0.06	0.05	0.05	0.06	0.14	0.05
	R^2	**0.99**	**0.99**	0.98	0.95	0.95	**0.96**	**0.99**	**0.99**	**0.99**
PSO	q_e, mg/g	1.07	0.73	0.98	0.19	0.014	0.36	0.67	0.22	0.21
	k_2, g/mg·min	0.09	0.06	0.1	0.5	4.7	0.2	0.002	2	0.4
	R^2	**0.99**	**0.99**	**0.99**	0.96	**0.96**	0.93	**0.99**	**0.99**	**0.99**
EM	α, mg/g·min	0.7	0.8	2.7	0.1	0.003	0.08	0.9	n.a	0.1
	β, g/min	6.6	7.2	5.8	35	407	15.6	0.01	n.a	31
	R^2	0.98	0.97	0.97	**0.98**	0.93	0.9	0.98	n.a	0.97
IPM	k_{diff}	0.07	0.06	0.06	0.01	0.001	0.02	0.4	0.01	0.01
	C_i	0.25	0.1	0.2	0.04	0.003	0.07	1.7	0.08	0.05
	R^2	0.76	0.85	0.75	0.8	0.81	0.74	0.72	0.52	0.77

n.a. -not applicable

In the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system, PFO and PSO models were found to be suitable for describing the experimentally obtained data for Ni(II), Zn(II), and Cu(II). The R^2 values for EM in all systems were higher than 0.9, while those for IPM were significantly lower. The applicability of PSO and EM showed that chemical absorption and ion exchange are the main mechanisms of metal sorption by the hybrid adsorbent [485]. For all elements except for Ni(II) in Ni(II)-Zn(II)-Mo(VI)-Cu(II), the adsorption rate values calculated for the PSO model were higher than for the PFO model, pointing to a higher rate of adsorption.

5.3.3.3 Effect of nickel concentration on metal removal and equilibrium study

The increase in Ni(II) concentration in the analyzed solutions up to 100 mg/L led to the continuous increase of biosorbent adsorption capacity from 0.9 to 2.9 mg/g in the Ni(II) system, from 0.8 to 2.8 mg/g in the Ni(II)-Cr(VI)-Fe(III) system, from 0.9 to 2.8 mg/g in the Ni(II)-Sr(II)-Cu(II)-Zn(II) system, and from 0.6 to 1.7 mg/g in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system. In the Ni(II)-Cr(VI)-Fe(III) system, adsorption of chromium and iron did not occur and the same was observed for molybdenum in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system. In the Ni(II)-Sr(II)-Cu(II)-Zn(II) system, nickel sorption was similar to that of the Ni(II) system, and removal of other metal ions present in the system was not affected negatively by the increase of nickel concentration in solution. Thus, removal of zinc ions was almost the same at all nickel concentrations, while the removal of copper and strontium was increased by 12% and 10%, respectively. In the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system, copper removal was increased by 10 % with the increase in nickel concentration from 10 to 100 mg/L, while zinc removal was continuously decreased. Thus, at a nickel concentration in the solution of 10 mg/L, 99% of zinc was removed from the solution but a 10-fold increase in nickel concentration led to a decrease in its removal up to 39%. The decrease in Na and K content in the metal-loaded adsorbent did not exceed 10%. The lowest adsorption of Ni(II) was in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system. It is important to mention that in this system, a significant reduction of Na (by 20%) and K (by 30%) took place, which may indicate their involvement in ion exchange.

The Langmuir, Freundlich, and Temkin isotherm constants were calculated by nonlinear regression and are presented in Figure 5.24 along with the experimental data.

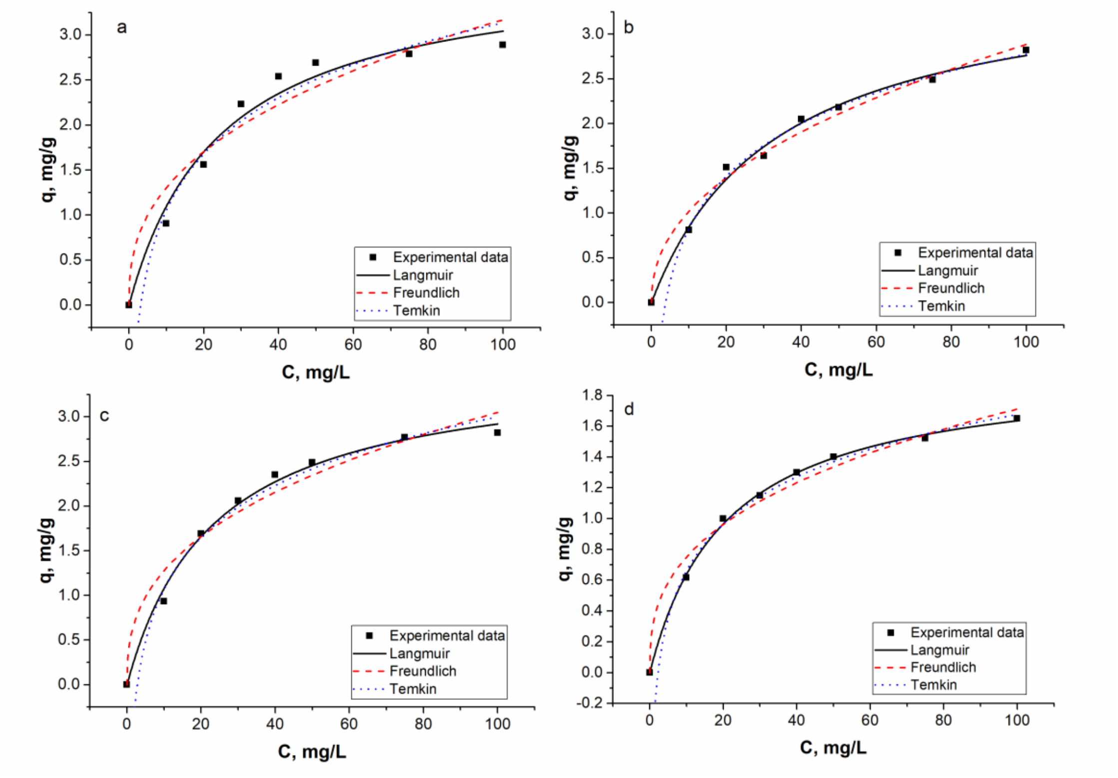


Fig. 5.24 The adsorption isotherms for Ni(II) ion removal on hybrid adsorbent: (a) Ni(II), (b) Ni(II)-Cr(VI)-Fe(III), (c) Ni(II)-Sr(II)-Cu(II)-Zn(II), and (d) Ni(II)-Zn(II)-Mo(VI)-Cu(II)

The parameters obtained are listed in Table 5.10. The Langmuir model, which assumes the monolayer sorption, fits well the data obtained for nickel. The maximum sorption capacity in three of four systems was on the level of 3.6-3.9 mg/g, while in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system, it was significantly lower (1.98 mg/g). The separation factors values lower than 1.0 indicate favorable nickel biosorption.

The R^2 values for the Freundlich model were lower. The coefficient of determination obtained for the Freundlich model was lower; however, the Freundlich constant 1/n was less than 1.0 in all analyzed systems, confirming favorable adsorption. For the Temkin isotherm, R^2 values were on the level of the Langmuir model and the constant (B) values related to the heat of adsorption were in the range 0.4-0.9 kJ/mol. Adsorption is considered to be a physical process if the bonding energy is in the range of 5–40 kJ/mol and the chemical one in the energy range 40-800 kJ/mol. The low values of energy obtained in the Temkin model in the present study suggested the weak ionic interaction between the sorbate and the sorbent [486].

Table 5.10 The parameters of the applied adsorption isotherm models

Model	Parameters	System			
		Ni(II)	Ni(II)-Cr(VI)-Fe(III)	Ni(II)-Sr(II)-Cu(II)-Zn(II)	Ni(II)-Zn(II)-Mo(VI)-Cu(II)
Langmuir	q_m, mg/g	3.9	3.7	3.6	1.98
	b, L/mg	0.04	0.03	0.04	0.05
	R_L	0.2–0.7	0.2–0.8	0.19–0.7	0.17–0.68
	R^2	0.97	0.99	0.99	0.99
Freundlich	K_F, mg/g	0.53	0.4	0.53	0.33
	1/n	0.39	0.45	0.38	0.36
	R^2	0.92	0.98	0.96	0.98
Temkin	a_T, L/g	0.3	0.3	0.4	0.4
	B, kJ/mol	0.9	0.85	0.8	0.4
	R^2	0.96	0.99	0.99	0.99

5.3.3.4 Effect of temperature on metal removal and thermodynamic studies

An increase in the temperature in all analyzed systems was favorable for nickel removal (Fig.5.25). A temperature rises up to 50 °C resulted in the removal of 99% of nickel from Ni(II)-Sr(II)-Cu(II)-Zn(II) and Ni(II)-Cr(III)-Fe(III) systems and of 90% in the other two systems. Copper removal was not affected by the temperature. In the Ni(II)-Sr(II)-Cu(II)-Zn(II) system, the removal of zinc was not affected by the temperature, while strontium removal was increased by 20% with temperature increase from 20 to 50 °C. Zinc removal in the Ni(II)-Zn(II)-Mo(VI)-Cu(II) system increased by 35% at a temperature of 50 °C in comparison to removal at 20 °C.

The thermodynamic parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$ were calculated according to Equations presented in Chapter 1.

From the plot of lnKd versus 1/T (data are not shown), enthalpy and entropy values were calculated and their values are given in Table 5.11. The Gibbs free energy was negative for all the metal ions present in analyzed systems in cationic form, indicating the spontaneous character of biosorption process. The enthalpy and entropy values were positive for cations. Positive values of $\Delta H°$ indicated the endothermic character of biosorption. Positive $\Delta S°$ values acquired for cations in the analyzed systems suggested the high affinity and presence of low energy barrier of metal adsorption processes using Shewanella xiamenensis biofilm placed on zeolite [487].

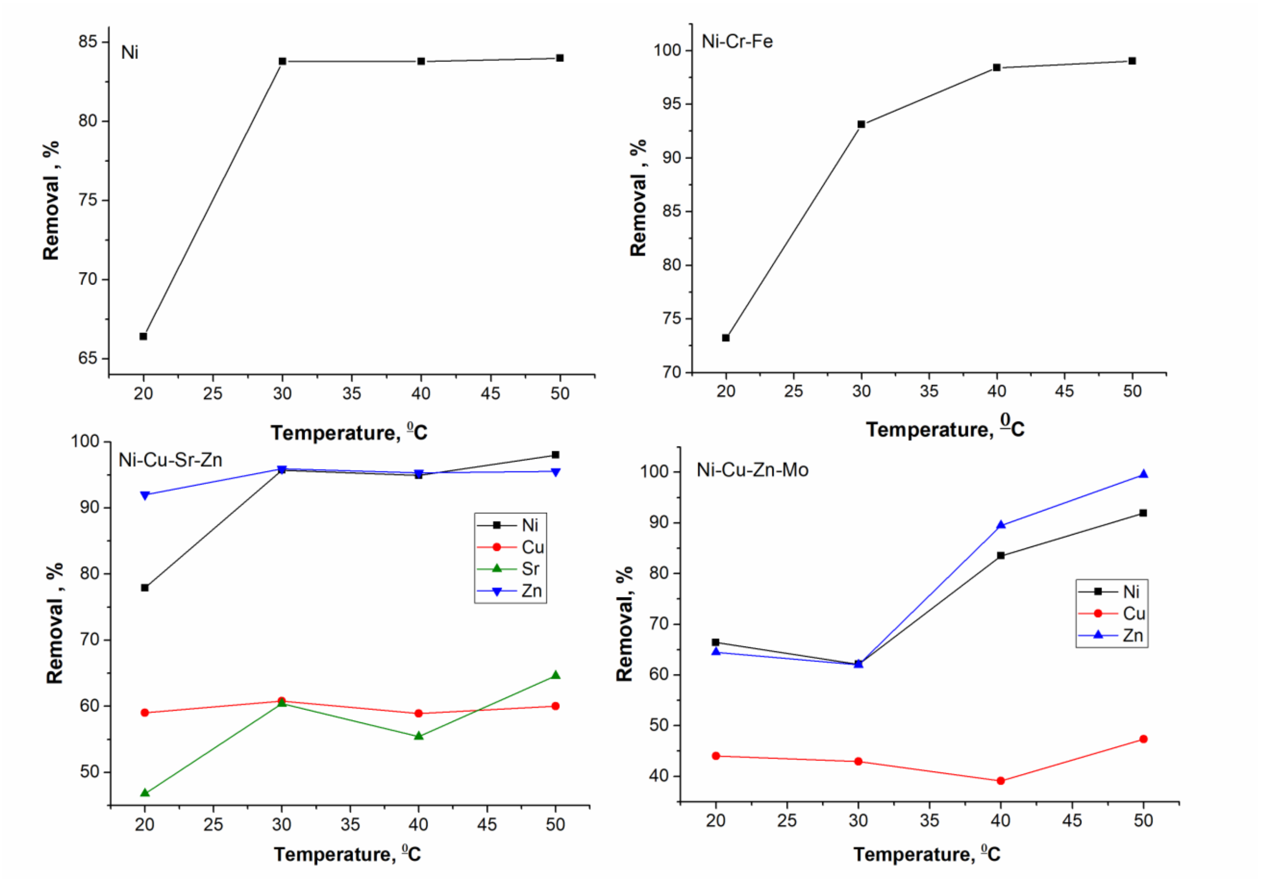


Fig. 5.25 Effect of temperature on metal removal by hybrid adsorbent: (a) Ni(II), (b) Ni(II)-Cr(VI)-Fe(III), (c) Ni(II)-Sr(II)-Cu(II)-Zn(II), and (d) Ni(II)-Zn(II)-Mo(VI)-Cu(II)

Table 5.11 Thermodynamic parameters for metal sorption on the hybrid adsorbent

System	**Metal**	**ΔG°, kJ/mol**				**ΔH°, kJ/mol**	**ΔS°, J/mol·K**	**R^2**
		293 K	**303 K**	**313 K**	**323 K**			
Ni(II)	Ni	−10.4	−11.0	−11.5	−12.1	5.7	54.9	0.79
Ni(II)-Cr(VI)-Fe(III)	Ni	−10.6	−11.2	−11.8	−12.5	6.4	58	0.88
Nin(II)-Sr(II)-Cu(II)-Zn(II)	Ni	−10.6	−11.2	−11.7	−12.3	5.9	54.9	0.98
	Zn	−10.8	−11.3	−11.9	−12.4	5.0	54.0	0.99
	Sr	−9.6	−10.2	−10.7	−11.3	7.0	56.5	0.99
	Cu	−8.5	-9.2	−9.9	−10.5	11.4	68.2	0.98
Ni(II)-Cu(II)-Zn(II)-Mo(VI)	Ni	−11.1	−11.5	−12.0	−12.4	0.8	40.7	0.81
	Cu	−15.1	−15.8	−16.6	−17.4	7.8	60.6	0.92
	Zn	−9.9	−10.7	−11.4	−12.2	13	78.1	0.91

5.4 Metal removal from industrial effluents using biological sorbents

It is well known that industrial effluents represent complex systems that contain metal ions in cationic as well as anionic forms in a wide range of concentrations. Thus, the sorption capacity of biosorbent can significantly differ from batch systems. The potential of biological and combined sorbents for metal removal from real wastewater was investigated and examples are presented below.

5.4.1. Metal removal from zinc-containing effluents using biological sorbents

Spirulina platensis

The industrial effluent, containing zinc in concentration 45 mg/L (pH 6.5) was taken from the electroplating unit of the "Atom" company, producer of a large volume of construction steel parts, dedicated tanks, and vessels, including those operated under pressure and in aggressive environments. Industrial effluent was collected directly after the electroplating process. Zinc concentration in the effluent was determined using AAS. The effect of two parameters: time and biosorbent dosage on zinc removal from effluent was examined. The experiments were performed at measured pH since as it was shown in batch experiments that zinc biosorption by *S. platensis* was almost the same in the pH range 4.0-7.0.

To determine the contact time required for the sorption equilibrium experiment, the samples were withdrawn at the predetermined time intervals (5, 10, 15, 30, and 60 min). The sorbent concentration was 10 g/L, the working volume was 50 mL, and the temperature of 23 ºC. All the experiments were conducted in triplicate and the averages of the measurements for each treatment were used. From the data presented in Fig. 5.26 it can be seen that the maximum amount of zinc 2.74 mg/g, which corresponds to 61% was adsorbed from effluent in 30 min of sorbent sorbate interaction [418].

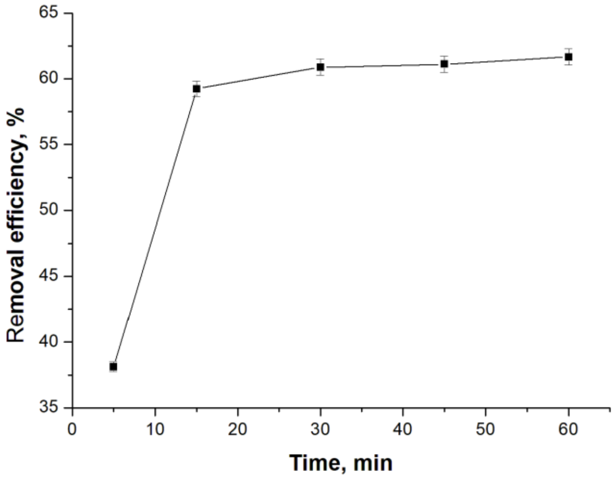


Fig. 5.26 Effect of contact time on the sorption of zinc ions from wastewater by *S. platensis* biomass (T 20 ^{0}C; C$_{i}$ 45 mg/L; pH 6.5; sorbent concentration 10 g/L)

The time required for maximum zinc biosorption coincide with the batch experiment, described in Section 5.1.1, however, zinc removal efficiency from the effluent was lower. The lower removal efficiency can be explained by higher zinc concentration in effluents (4.5 times) and/or the presence of other ions, besides zinc, in effluents and consequently their competition for binding sites. For example, in Rodrigues et al. study [488] it was shown that the adsorption capacities of *S. platensis* for zinc were significantly decreased in binary and ternary systems in comparison with single metal one due to the competition of nickel, lead, and zinc ions for binding sites. In the present study, the concentrations of other metal ions present in the wastewater were negligible and did not affect the removal of zinc ions. Spirulina was able to remove 50% of zinc from wastewater, containing zinc iron, tungsten, and antimony ions [38]. The native biomass of *Oscillatoria anguistissima* was able to remove from 66 to 95% of zinc ions from different types of industrial effluent [430].

To increase the efficiency of zinc removal from effluent the process was studied at biosorbent concentration of 10-60 g/L. The increase of biomass concentration resulted in the increase of zinc removal from 61 to 83% (Fig. 5.27).

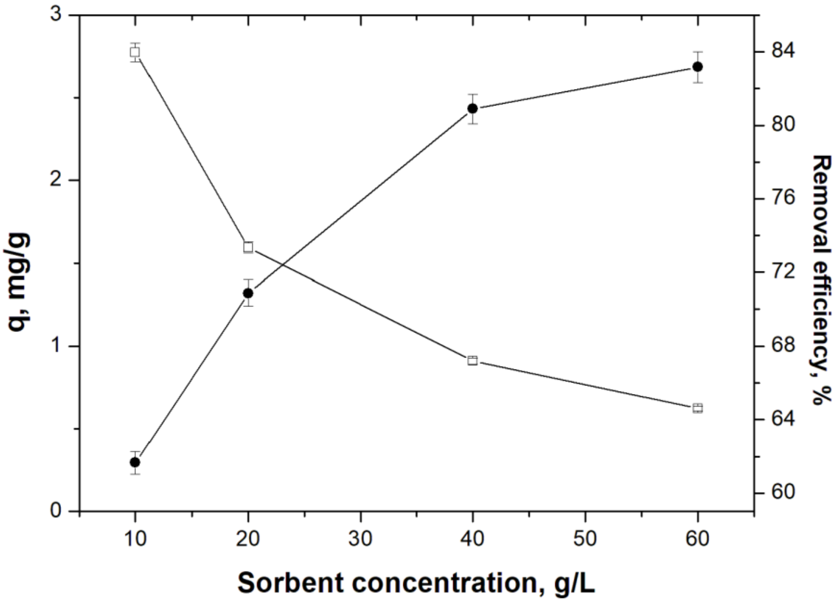


Fig. 5.27 Effect of biosorbent concentrations on biosorption capacity and removal efficiency of zinc ions from wastewater by *S. platensis* biomass (T 20 ^{0}C; C$_i$ 45 mg/L; pH 6.5; adsorption time 1 h)

Maximum zinc removal (83%) was achieved at the adsorbent dosage of 60 g/L. However, the difference in biomass removal capacity at sorbent dosage 40 and 60 g/L was just 3%. Therefore, the use of a 40 g/L biosorbent dose is justified for economical purposes [418].

Saccharomyces cerevisiae

In the experiments with *S. cerevisiae* the effluent containing zinc was obtained from the same "Atom" company. The effluent chemical composition was determined by ICP-MS and is presented in Table 2.1. In the effluent, beside zinc ions, the other metal ions were present and the effect of pH on metal removal was studied. For this purpose, 10 g/L g of dry biomass was added to 50 mL of effluent in a 100-mL flask and continuously stirred for 60 min. The pH of the effluent varied from 2.0 to 6.0. According to data presented in Fig. 5.28 the removal of zinc ions was more favorable at high pH values, it was increased from 1.2% at pH 2.0 to 51% at pH 6.0 [238].

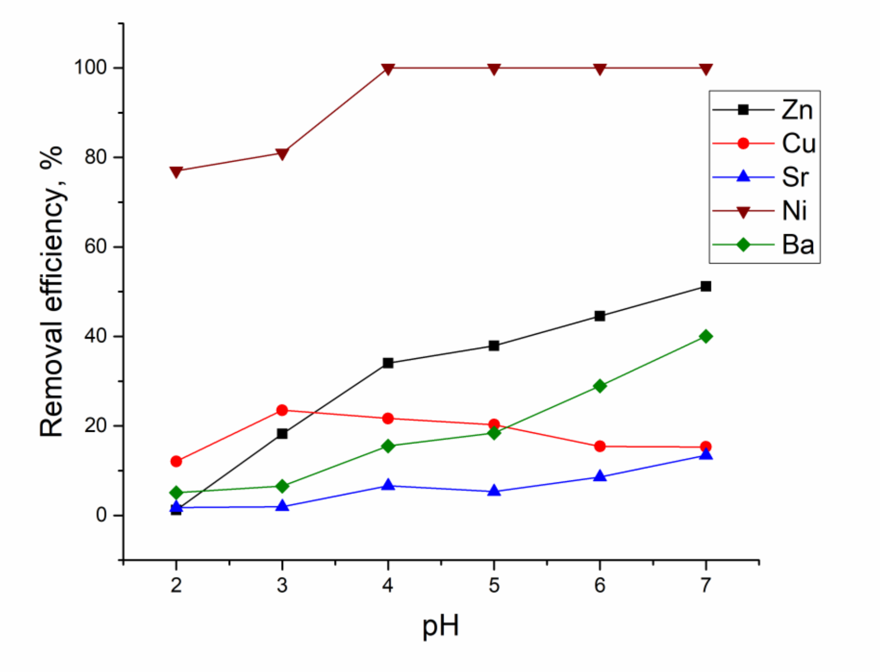


Fig. 5.28. Efficiency of metal ions removal from the industrial effluent at different initial pH (at T 20 ºC; sorbent dosage 10 g/L; adsorption time 1h)

Zinc removal was lower than in the batch system that can be explained by its higher concentration in the effluent and competition with other metal ions for binding sites. Removal of the other metal ions was also dependent on pH. Thus, for barium and strontium ions maximum removal was achieved at pH 60, for nickel at pH 5.0, and for copper at pH 5.0. Variation of the pH values at sorbent concentrations of 10 g/L allowed reducing concentrations of all elements, except of zinc in the effluent, under the values of MPC [238].

Since zinc was the element of the main interest on the next step the effect of sorbent on the zinc removal was investigated. The experiment was performed in the subsequent scheme with the addition of new sorbent to the effluent. In the first stage sorbent in the dosages ranging from 20 to 40 g/L was added to 100 mL of effluent at initial effluent pH (6.0). The suspension was shaken at 200 rpm for 60 min, then sorbent was removed by filtration. The supernatant obtained for each sorbent dosage was divided into three parts. One part was used for ICP-MS analysis, while two others were used for the second stage of the experiment. On the second stage, 1.0 g/L or 10 g/L of new biomass were added to the effluent, obtained after the first stage, and shacked for 60 min. Then, biomass was again separated from the supernatant by filtration. Metal concentration in obtained solutions was determined using ICP-MS.

The increase of sorbent concentration on the first stage from 10 to 40 g/L led to a rise of yeast removal capacity from 44 to 72% (Fig. 5.29a). To increase zinc removal, to the effluent obtained after the first stage the new sorbent was added in concentration 1.0 or 10 g/L (Fig.5.29b). The addition of 1.0 g/L of new biosorbent to the treated effluent resulted in the removal of 17% of zinc ions in all experimental variants, while the addition of 10 g/L of biosorbent lead to sorption of 47%-52% of zinc onto yeast cells. Therefore, during two cycles it was possible to remove 72-85% of zinc ions from the effluent. Thus, zinc concentration in the effluent was reduced to 1.5MPC [238].

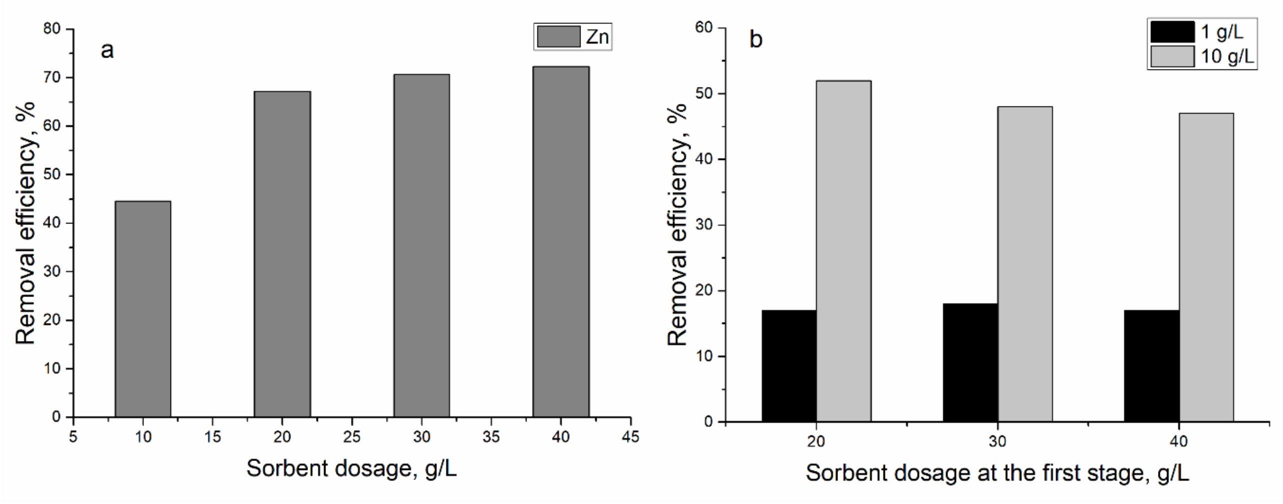


Fig. 5.29 Removal of zinc ions from industrial effluent at different sorbent dosage (at T 20 ºC; adsorption time 1h)

The experiments performed showed that the optimal scheme to achieve the maximal zinc removal is as follows: adding of 20 g/L of yeast biomass on the first stage and 10 g/L on the second stage [238]. In Hernández Mata et al. study [489] after 40 days of the experiment, the reduction of 81.5% zinc, 76.5% copper, manganese 95.5%, and 99.8% of the iron in the subsequent system by yeast biomass was attained.

5.4.2 Metal removal from nickel-containing effluents by biological sorbents

Spirulina platensis

The applicability of *S. platensis* biomass for nickel removal from two types of industrial effluents containing nickel in concentration 14.1 mg/L and 117 mg/L, respectively was investigated. The pH of both effluents was 6.0. Effluents were obtained from electroplating units of the Scientific Production Association "Atom" (Dubna, Russia).

To determine the contact time required to attain the equilibrium sorption, samples were withdrawn at predetermined time intervals (5, 10, 15, 30, and 60 min). The sorbent concentration was 10 g/L, the working volume of wastewater - 50 mL, and temperature 23 ºC. All the experiments were conducted in triplicate and the averages of the measurements for each treatment were used.

The efficiency of nickel removal from the first effluent was studied using the AAS technique, while its accumulation in biomass was traced by NAA. The experimental conditions were as follows: sorbent concentration 10 g/L, interaction time – 1 hour, the volume of the experimental solution- 50 mL. The AAS data showed that during 60 min of reaction 66% of nickel was removed from the industrial effluents. Nickel content in biomass increased from 5 µg/g to 625 µg/g (Fig. 5.30).

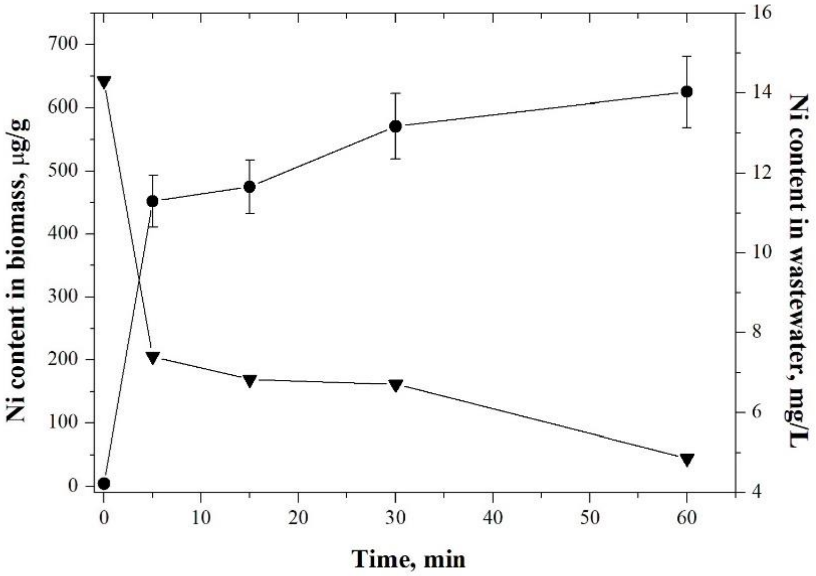


Fig. 5.30 Nickel content in the *S. platensis* biomass and in the industrial effluents versus the (contact time T 20 ^{0}C; C_i 14.1 mg/L; pH 6; interaction time 60 min)

NAA data showed that besides nickel *S. platensis* biomass removed from wastewater iron, zinc, and barium. Thus, the amount of iron and barium in biomass increased twice in comparison with control, while of zinc – 1.5 times, indicating the possibility of spirulina use for complex wastewater treatment. As in the case of the batch system increase of sorbent, dosage did not influence significantly the nickel removal efficiency. FTIR analysis revealed the participation of OH and $NHC(O)_{amid}$ groups in metal ions binding [490].

In the case of the second effluent due to higher nickel concentration, the process of its removal was studied at the dosage range of 6–40 g/L. The time of biomass interaction with effluent was 60 min, working volume of the solution - 50 mL and temperature 23 ºC. All the experiments were conducted in triplicate and the averages of the measurements for each treatment were used.

The data presented in Fig. 5.31 shows that with the increase of sorbent dosage from 6 to 40 g/L nickel removal efficiency increased from 42 to 68%, however, the increase of sorbent concentration from 20 to 40 g/L resulted in the increase of nickel removal only by 2% [419]. Relatively low nickel biosorption by microbial biomass can be explained by (i) its electronic configurations, when the number of electrons lying in very diffused and distant orbitals (d and f), which are notoriously more available than those closer to the nucleus, are lower and (ii) formation of stable aqueous complexes in solution, that decreases its exchange capacity [446,491].

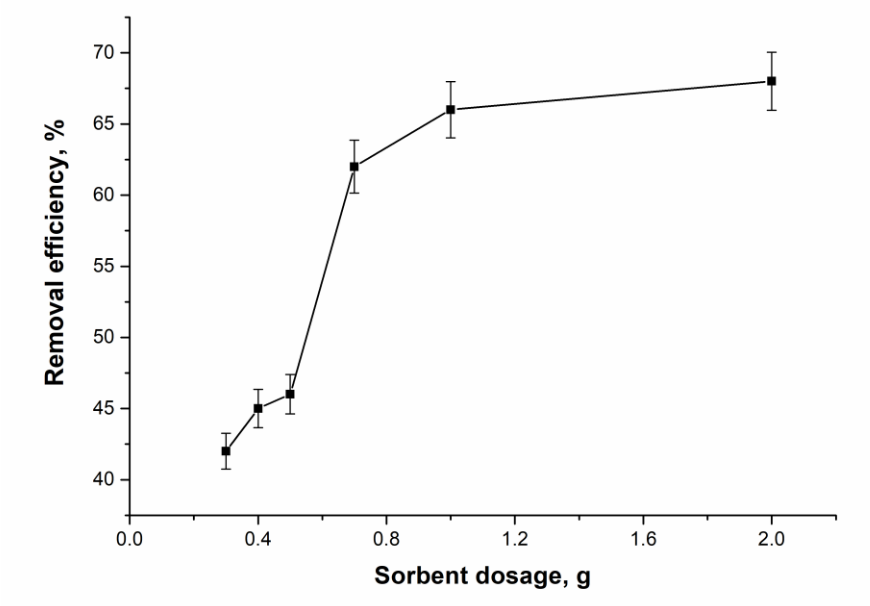


Fig. 5.31 Effect of biosorbent dose on biosorption capacity and removal efficiency of nickel ions from industrial effluent by *S. platensis* biomass (T 20 ^{0}C; C_i 117 mg/L; pH 6; interaction time 1 h)

At the same time, the removal efficiency of *S. platensis* biomass toward nickel ions was higher in comparison with literature data. In Corder and Reeves study [492] it was shown, that from the eight studied cyanobacteria only *Anabaena flos-aquae* demonstrated the high binding capacity for nickel. However, only 28 % of nickel was removed from wastewater contained 7.1 mg/L of nickel ions. The rate of nickel biosorption by *Chlorella pyrenoidosa* and Chlorella HKBC-C3 was less than 20 % in 72 hours of cyanobacteria contact with [493].

As the nickel concentration in the effluent was relatively high, it is reasonable to treat it firstly in a chemical way to reduce the nickel concentration and after that to apply spirulina biomass for the effluent post-treatment or to use the scheme of subsequent water treatment in several stages with the addition of the new sorbent at each stage.

Saccharomyces cerevisiae

Working with *S. cerevisiae* the nickel-containing effluent was obtained from the same company (“Atom”). The chemical composition of the effluent and the initial pH are given in Table 2.1. The effect of pH and sorbent dosage on the metal removal was investigated according to the scheme presented in Section 5.2.1 for *S. cerevisiae. S. cerevisiae* showed to possess the good sorption capacity for cations as well as for anions present in the effluent. The metal removal was dependent on the effluent pH. Thus, maximum removal of metals presented in anionic forms molybdenum and chromium was achieved at pH 3.0 55 and 75%, respectively. Removal of iron and cobalt was also maximal at pH 3.0. Nickel and strontium were better removed at pH 6.0: 42% and 10%, respectively, and zinc at pH 7.0 (52%). Thus, by varying the pH of the solution it is possible to achieve selective metal removal as well as the complex metal removal. According to FTIR analysis (data not shown), the main groups, which participated in metal binding at the studied pH range were OH, C=O and HN-C=O [239].

As it was shown in the case of *S. platensis* the increase of the sorbent dosage from 6 to 40 g/L leads to the rise of nickel removal from 42 to 68%. In order to reduce the nickel content in the effluent the subsequent scheme with the addition of new sorbent was applied. At the first stage the increase of biomass concentration from 10 to 40 g/L lead to the rise of nickel removal from 42 to 52%. At the addition of 1 g/L of new yeast biomass at the second stage nickel removal constituted 5.7% (first stage 20 g/L), 15% (first stage 30 g/L) and 18% (first stage 40 g/L), thus, the total cumulative removals constituted 54.5%, 66% and 71%, respectively. Addition of 10 g/L of new biomass resulted in the removal of 16% (first stage 20 g/L), 31% (first stage 30 g/L) and 26% (first stage 40 g/L) of nickel ions. Thus, cumulative removal constituted 65%, 82%, and 78%, respectively (Fig. 5.32) [239].

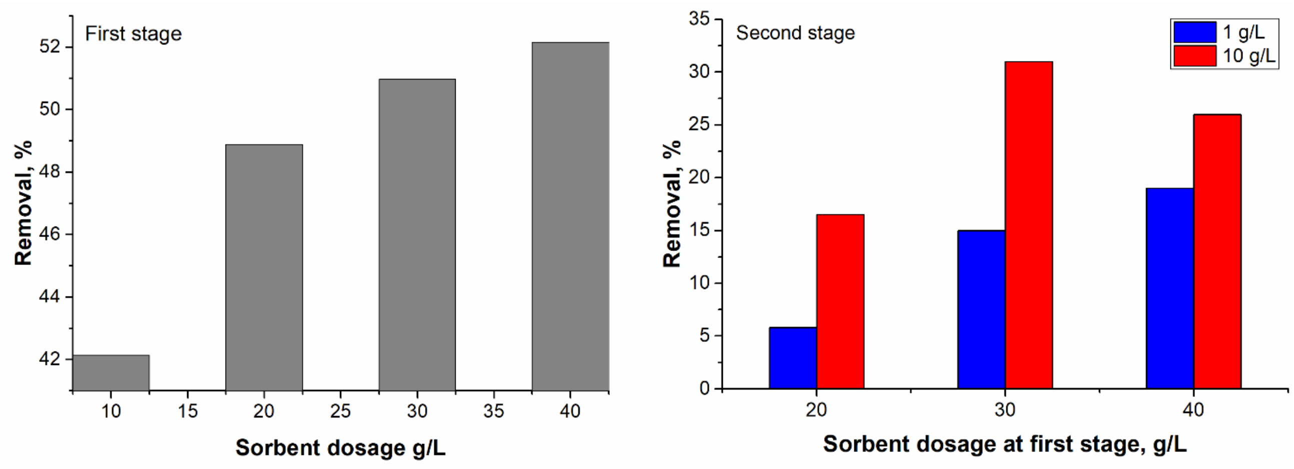


Fig. 5.32 Removal of metal ions from nickel containing effluent at different sorbent dosage (at T 20 ^{0}C; adsorption time 1h)

Mahado et al. [494] used 12 g/L of yeast cells to remove nickel from the effluents in three steps that resulted in the removal of 89% of nickel ions. It should be mentioned that the initial nickel concentration in the effluent in the presented study was 17.8 mg/L, significantly lower than in the present experiment.

Shewanella xiamenensis biofilm placed on zeolite

For the treatment of the same effluent *Shewanella xiamenensis* biofilm placed on zeolite was used as well. The effect of the pH, sorbent dosage, and effluent dilution on the nickel removal efficiency was assessed. Studying the effect of pH on nickel ions removal it was observed that in comparison with the batch experiments, removal of nickel from real effluent was very low, 17% at pH 6.0. Since the concentrations of other metal ions were considerably lower, and they were excluded from further discussion.

In order to improve the nickel removal, the amount of biomass was increased from 0.5 to 2.0 g (Figure 5.33). The increase in the biosorbent mass resulted in increased Ni(II) removal, from 17% (sorbent dosage 0.5 g) to 27% (at sorbent dosage 2.0 g). The increase in sorbent dosage four times resulted in a rise in nickel removal only by 10%. The percent of Ni(II) adsorbed by coir pith from the effluent containing 145 mg/L of Ni(II) was 88% at 5% (w/v) adsorbent dosage and a further increase in sorbent dosage did not increase nickel removal [495].

In the batch experiments described in Section 5.1.1, it was shown that the increase in nickel concentration in solution lead to the significant decrease of nickel removal, from 90% to 28%. Since it was shown that applied sorbent is more efficient at low nickel concentrations in solution in the next experiment, the effluent was diluted two and twelve times and 0.5 or 1.0 g of sorbent was added to it. At sorbent dosage of 1.0 g, 26% of Ni(II) was removed from the solution; however, Ni(II) removal after 12-fold dilution amounted to 66% at a sorbent dosage of 0.5 g and 72% at a dosage of 1.0 g (Fig. 5.33). It can be concluded that *Shewanella xiamenensis* biofilm placed on zeolite can be better used for wastewater post-treatment [495].

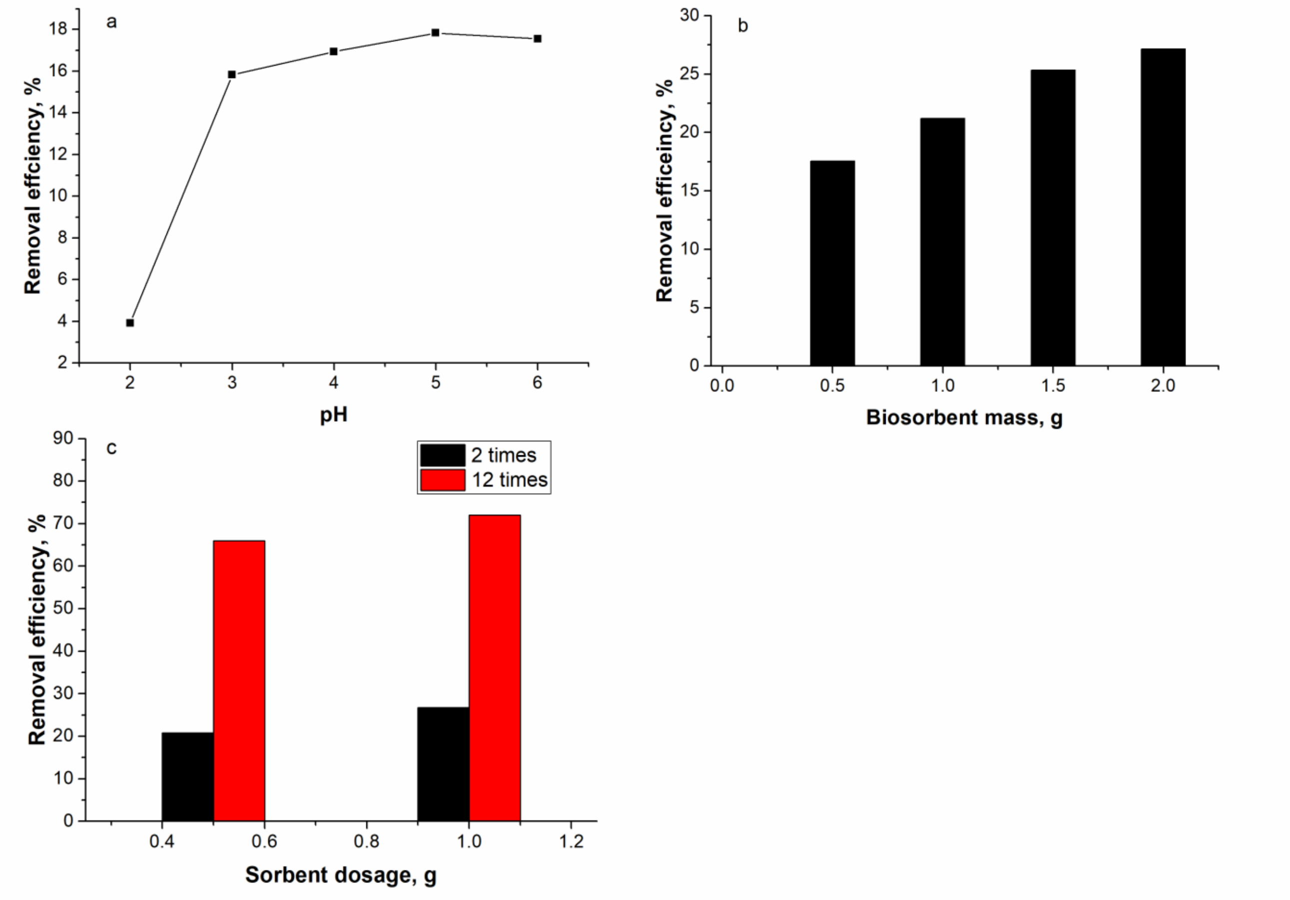


Fig. 5.33 Effect of different parameters on Ni(II) removal from industrial effluent: (a) pH, (b) sorbent dosage (initial effluent), (c) sorbent dosage (diluted effluent)

E. coli biofilm placed on the zeolite showed to be more efficient for zinc and copper removal from output wastewater treated previously by traditional techniques [496].

5.4.3 Metal removal from chromium-containing effluents using biological sorbents

Two types of industrial effluents: the first one containing chromium in concentration 9.4 mg/L, along with other metals such as barium, cobalt, copper, iron, scandium, strontium, and zinc, and the second with chromium concentration of 27 mg/L were obtained from electroplating units of the Scientific Production Association "Atom". Industrial effluents were collected directly after the electroplating process. The pH of both effluents was 4.0.

To study the efficiency of chromium removal from the first effluent 10g/L of *S. platensis* biomass were added to 100 mL of industrial effluents in Erlenmeyer 250-mL capacity flasks placed on a rotary shaker set at 100 rpm. The dynamics of the adsorption processes was studied for 45 h. The samples were removed at different time intervals (5, 15, 30, and 45 min), filtered, and dried. All the experiments were conducted in triplicate and the averages of the measurements for each treatment were used [490].

According to AAS data (Fig. 5.34), the time required for maximal uptake of chromium by biomass was found to be 15 min. During 45 min of interaction, chromium was completely removed from industrial effluents, its concentration in solution was lower than the detection limit. The NAA showed that in 45 min the content of chromium in biomass increased 60 times.

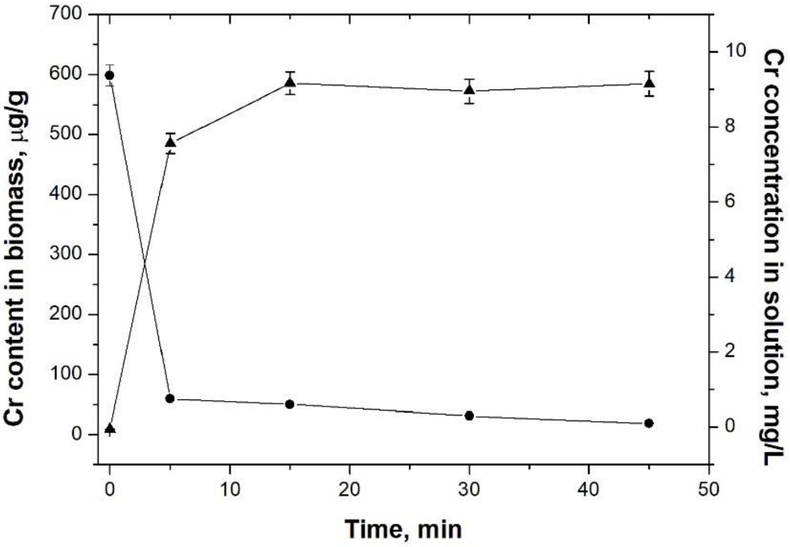


Fig. 5.34 Chromium content in the *S. platensis* biomass and in the industrial effluents versus the contact time biomass (T 20°C; C_0 9.4 mg/L; sorbent concentration 10 g/L, pH 4)

Besides chromium, NAA revealed the uptake of iron, copper, and nickel ions by biomass. In the Cr-loaded biomass, the concentration of iron increased twice and of nickel in 45 times in comparison with the native biomass. The copper content in the native biomass was below the detection limit of its determination, but its concentration achieved the value of 250 µg/g after 45 min of interaction [490].

The experiments with model solution proved that metal biosorption is affected by different parameters, among which pH is one of the most important. At pH 4.0, the predominant species of Cr(VI) in solution are $Cr_2O_7^{2-}$, $HCrO_4^{-}$, and $Cr_2O_4^{2-}$, which can be easily attracted to positively charged functional groups on the cyanobacteria surface. Iron exists in the ionic form in a very narrow range of pH. At pH > 4.0, it starts to precipitate. Thus, its uptake by biomass can be run through two mechanisms: binding to OH groups and microprecipitation on the spirulina. Uptake of copper and nickel ions can be associated with their binding to carboxyl, amino, hydroxyl, and amide groups of protein and carbohydrate fractions of the cell wall [237]. FTIR data indicated the involvement of NH_2, P=O, and C=O groups in metal ions binding.

The decrease of sodium content from 10.6 to 4.4 mg/g, chlorine from 6.4 to 0.5 mg/g, and potassium from 18.6 to 12.0 mg/g indicates their replacement with metal ions presented in the effluent. Thus, the ion-exchange can be considered as one of the mechanisms of metals biosorption [490].

To compare the removal efficiency of biological sorbent with one of the most well-known sorbents, activated carbon, the experiments on its testing the removal capacity for chromium removal from the same effluent were performed. As a competitor for spirulina biomass, G-5 activated carbon produced from fine dust (a mixture of coals) and a binding agent (wood-tar) with further thermal treatment in a water-steam medium at a temperature of 850-950 °C was used. As in the case, of experiments with spirulina biomass, the effect of sorbent dosage and contact time on the removal capacity was investigated.

To determine the time required for maximum chromium removal, the samples were removed from the solution at different time intervals: 1, 3, and 24 hours. Sorbent concentration in solution was 4 g/L (chosen according to preliminary batch experiments), temperature 23 ºC, and solution volume 100 mg/L. All the experiments were conducted in triplicate and the averages of the measurements for each treatment were used. The data presented in Fig. 5.35 show that even after 24 hours of sorbent-sorbate interaction chromium removal from solution was very low, around 2.0 %.

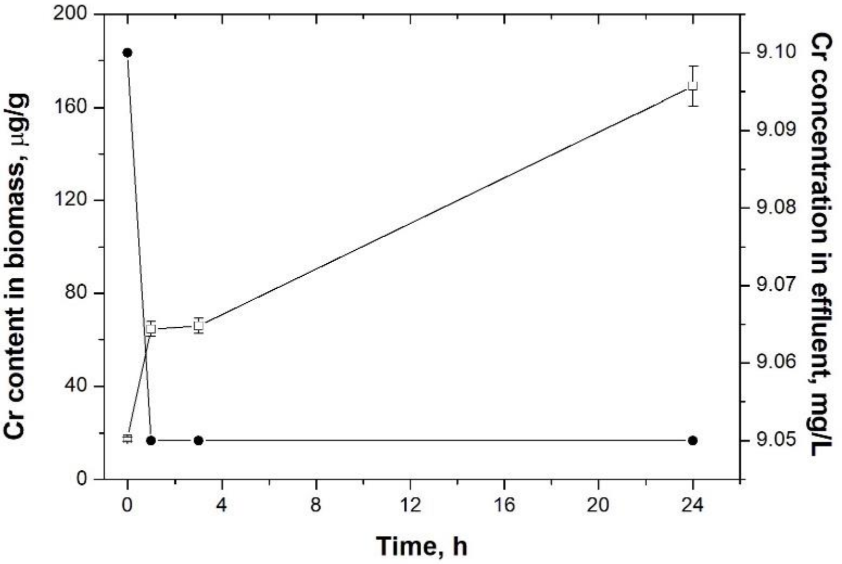


Fig. 5.35 Chromium content in the AG-5 and in the wastewater versus the contact time (T 20 ^{0}C; C_0 9.4 mg/L; pH 4, sorbent concentration 4 g/L)

Low chromium sorption can be explained by the competition of elements present in the effluent for binding sites. According to the NAA data concentration of Fe, Ni and Cu determined in sorbent after interaction with wastewater was increased in comparison with their content in the control sample. Thus, iron content increased from 3.1 to 4.7 mg/g, of Ni from 14 to 57 μg/g. Copper was not detected in the control activated carbon sample, but its concentration in carbon after 24 hours increased up to 256 μg/g [497]. According to [498] the ions with the same valence are adsorbed better if their effective radius is larger. Ions of larger radius hydrate to a lesser degree, consequently, their sorption increases on a polar sorbent surface.

The NAA data show that after the interaction of activated carbon with wastewater the concentration of some elements like Al, Na, Mg, Ti, K, Ba was decreased, whereas those of Sr, V, Sc, Co, Zn, As, Br, Rb did not change significantly. The decrease of Na content in carbon from 11.6 to 0.4 mg/g, of Al from 5.4 to 2.4 mg/g and of Mg from 1.7 to 0.7 mg/g indicate their participation in the ions-exchange process. The FT-IR did not show significant changes in control and metal-loaded sorbent, confirming that physical absorption and ion-exchange were the main mechanisms of metal ions interaction with activated carbon [497].

The comparison of two sorbent types showed that *S. platensis* biomass is more efficient sorbent for chromium removal from industrial effluents. Thus, instead of producing industrial quantities of sorbents, which would be prohibitively expensive, is more profitable to use as biosorbents waste materials from large-scale biotechnological processes (can be used for free).

For the second effluent (chromium concentration 27 mg/L) the experiments were conducted in 100 mL Erlenmeyer flasks containing 50 mL of effluent and 10 g/L of biosorbent. The flasks were shaken on a shaker incubator at a constant rate of 120 rpm. The effect of interaction time and sorbent concentration was studied. After the experiment, the biomass was removed by filtration and the obtained solution was used for further analysis. All experiments were carried out in triplicate and the average value of obtained experimental values was used for calculations. To study the effect of contact time on chromium absorption experiment was performed at sorbent 14 g/L, determined as a more efficient dosage in batch experiments (see Item 5.1.5).

The data presented in Fig 5.36. show that equilibrium was reached after 45 min of interaction. However, the efficiency of Cr(VI) removal was lower than in batch experiments. During 60 min of sorbent-sorbate interaction, 51% of Cr(VI) ion was removed from the effluent. Lower removal efficiency in comparison with batch experiments can be explained by its higher concentration in effluent and/or presence of co-ions in the effluent.

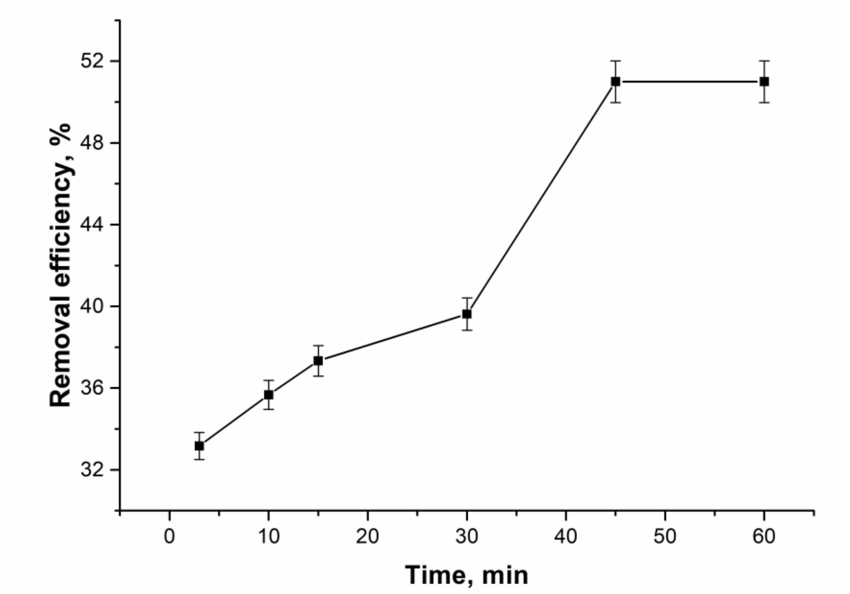


Fig. 5.36 Effect of contact time on the sorption of Cr(VI) ions by *S. platensis* biomass (T 20°C; C_0 27 mg/L; sorbent concentration 14 g/L, pH 4)

Thus, to increase the efficiency of chromium ions removal the sorbent dosage was increased from 10 to 40 g/L. With the increase of sorbent dosage, chromium removal increased almost twice from 35 to 72% (Fig. 5.19) [420]. Machado et al. [467] showed 98% removal of chromium at pH 2.3 by yeast from real electroplating effluent containing Cu, Ni, and Cr. *Spirulina fusiformis* was very effective in the removal of chromium (93–99%) besides removing other toxicants from retained chrome liquor [220].

5.4.4 Metal removal from rhenium-containing effluents using biological sorbents

Re(VII) is a rare and valuable metal, which removal is important not just because the of ecological, but economic reasons as well. Two industrial effluents, containing rhenium were taken from different stages of leaching from polymetallic ore plant (Russia). The chemical composition as well as pH values of effluents are given in Table 2.1. Current treatment schemes of studied industrial effluents include a complex of chemical methods. The experiment on rhenium removal from effluents was conducted for 60 min at the biosorbent dosage 0.05 g. The working volume of the experimental solution was 50 mL. Is the first study on the application of biological sorbents for rhenium removal from real industrial effluents, another data there is not available in the literature.

The data presented in Table 5.12 show that for both effluents, *S. platensis* removal rhenium removal efficiency was almost the same - 51-55%. In the case, of the first effluent the low rhenium sorption, in comparison with the batch system (Section 5.1) can be explained by the neutral pH of effluent and the presence of co-ions in the effluent. As it was shown in batch experiments optimal pH value for rhenium biosorption is 2.0. Usually, pH within the range 4.0–7.0 is considered optimal for metals represent in solution in cationic form removal, as the competition of hydrogen ions and metal cations for binding sites is minimized, due to the negative charge of the cell surface. Thus, it can be suggested that in the first effluent the biosorption of metal cationic species was more favorable.

Table 5.12 The efficiency of rhenium removal from industrial effluents

	C_i, mg/L	C_f, mg/L	Q, mg/g	Efficiency, %
Effluent I	0.7	0.34	0.069	51
Effluent II	20	8.9	2.23	55

In the second effluent, pH was favorable for maximum rhenium removal, however, the efficiency of its removal was approximately two times lower than in batch solution: 97% for the batch solution and 55% for industrial effluent. The lower values obtained for the effluent can be explained by competition of anionic species presented in effluents for binding sites and higher rhenium concentration in comparison with batch solutions [437].

5.4.5 Metal removal from complex effluent

The chemically complex wastewater containing the following metals: Al, Ba, Cr, Fe, Sr, and Zn in different concentrations (pH=6.0) was obtained from electroplating units of the Tactical Missiles Corporation (Dubna, Russia). Chemical composition of wastewater, was determined by AAS and the maximum permissible concentrations (MPC) of metals established by Russia and the World Health Organization (Table 5.13) [499,500]. The concentrations of Al, Ba, Sr, and Zn in the effluent were lower than MPC, while concentrations of Cr and Fe exceed MPC values.

Table 5.13 Industrial effluent chemical composition and MPC values

Metal	Concentration in wastewater, mg/L	MPC for Russia, mg/L	MPC established by WHO, mg/L
Al	0.2	0.2	0.2
Cr	0.1	0.5 (Cr^{3+}) 0.05 (Cr^{6+})	0.05
Fe	3.0	0.3	0.2
Zn	0.2	1.0	3.0
Ba	0.1	0.7	0.7
Sr	0.5	7.0	-

To determine the contact time required for equilibrium sorption, 10 g/L of *S. platensis* biomass was added to 100 mL of industrial effluents in a 250-mL capacity Erlenmeyer flask placed on a rotary shaker set at 200 rpm. The dynamics of the adsorption processes was studied for one hour. The samples were collected at different time intervals (5, 15, 30, 45, and 60 min), and then filtered and dried. All the experiments were conducted in triplicate, and the averages of the measurements for each treatment were used for the final result. Metal uptake by spirulina biomass was traced using NAA and change of concentration in a solution by AAS. The experiments were performed at room temperature (22 ^{0}C).

The data obtained by NAA (Fig. 5.37) show that the maximum amount of metals was adsorbed by biomass at the first 5-30 min of spirulina biomass interaction with effluent then the equilibrium was achieved. Iron ions are typical for almost all types of industrial effluents. At pH value 6.0 iron is present in solution in the form of the suspended solid phase, $Fe(OH)_3$. Despite this fact, 94% of iron was removed from the effluent, with the increase of its content in biomass from 5.2 to 8.0 mg/g (Table 5.14, Fig. 5.37).

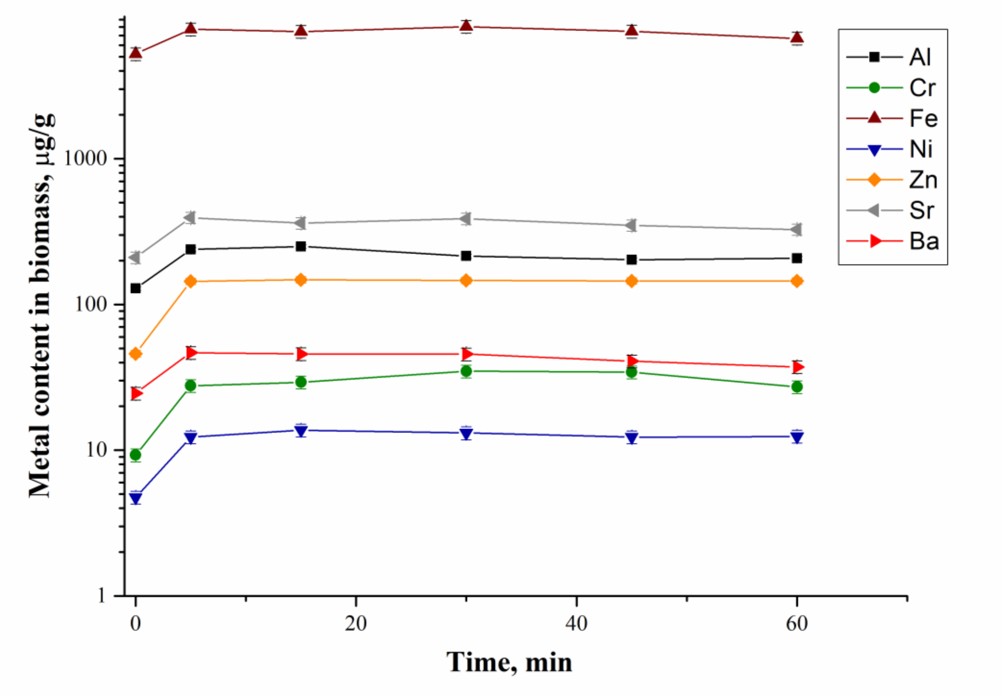


Fig. 5.37 Metal uptake from industrial effluent by *S. platensis* biomass determined by NAA (pH 6, T 22^{0}C, time 60 min, sorbent concentration 10 g/L)

The main mechanism of iron biosorption on spirulina can be considered its precipitation induced by siderophores. According to AAS data barium was completely removed from wastewater and its content in biomass increased from 210 to 394 μg/g. The efficiency of strontium and aluminum removal from effluent was lower 68% and 60%, respectively.

Table 5.14 Amount of metal adsorbed from wastewater at equilibrium time and removal efficiency values

Metal	Concentration of metal remaining in water (C_f), mg/L	q_e, mg/g	R, %
Al	0.08	0.12±0.002	60
Ba	n.d.	0.02±0.001	100
Cr	0.063	0.026±0.001	37
Fe	0.018	2.81±0.08	94
Sr	0.16	0.18±0.005	68
Zn	0.1	0.1±0.002	50

According to thermodynamic examination barium and strontium were both presented in wastewater in solid state [501]. Thus, precipitation can be considered as the main mechanism of their biosorption. 57% of aluminum in the solution was in soluble form and the rest in the form of precipitate [422]. Thus, the removal efficiency of 60% indicates that only the soluble form of aluminum was removed from the solution. It can take place through the mechanism of ion-exchange and/or interaction with the functional groups on the cell surface.

The data obtained by NAA (Table 5.15) showed that during the interaction of biomass with effluent, the release of sodium, potassium, calcium, chlorine, and magnesium ions from cells was observed. For example, magnesium content in biomass decreased three-fold in comparison with control biomass. Aluminum ions can replace magnesium ions as they tend to bind to the magnesium uptake system directly [502]. The release of calcium from spirulina cells can be also connected with its replacement by aluminum. Replacement of cell wall calcium by aluminum has been reported for algae *Chara corallina* [503].

Table 5.15 Change of Na, Cl, K, and Ca concentration in *S. platensis* biomass as a function of the contact time with the wastewater

Time, min	Element, µg/g				
	Na	Cl	K	Ca	Mg
control	15300±1071	7480±598	18400±1472	19800±1188	4680±140
5	11800±826	230±18	13400±1072	16800±1008	401±12
15	13300±931	364±29	14400±1152	16200±972	453±13
30	13400±938	427±34	13900±1112	16100±966	403±12
45	13500±945	514±41	12900±1032	15200±912	382±11
60	12800±896	355±28	13400±1072	15700±942	406±12

Zinc and chromium ions at pH 6.0 were present in wastewater in soluble form. Their concentration in wastewater decreased by 50% and in biomass increased from 46 to 146 μg/g. Zinc ions can be removed from wastewater by the mechanism of ion-exchange and/or zinc ions interaction with functional groups on the cell surface. Zinc, divalent metal cations, being structurally similar to calcium and manganese ions can replace them in the spirulina cell. The release of sodium, potassium, and chlorine can be also explained by the alteration of cell wall permeability for small-sized ions. The FT-IR data showed the involvement of OH, C=O, and P=O - groups in metal binding [422].

Although chromium was present in solution in soluble form *S. platensis* showed the lowest removal capacity for this element. The amount of chromium in biomass after one hour of interaction increased approximately fourfold (from 9 to 35 μg/g), however, its removal efficiency was not so high – 37%. At pH 6, the main part of functional groups on the spirulina cell wall is charged negatively, while chromium is present in solution in anionic form, predominantly as $HCrO_4^-$. Gagrai et al. [434] have shown that amine groups may play an important role in Cr(VI) ions binding. However, the position of amine groups in the obtained IR spectrum of metal-loaded biomass did not suffer critical changes during the interaction process. Thus, cations biosorption was more preferable in the case of studied effluent, and consequently, for complex effluent treatment, it is necessary to vary the pH of the effluent as well [422].

S. platensis showed to be an efficient biosorbent for metal removal not only from the batch system but also from the real industrial effluents. Application of spirulina biomass in absorption experiments revealed several shortcomings: biomass degradation during desorption studies and its high cost, if biomass is cultivated in laboratory conditions and not waste of biotechnological processes are used. At the same time, *S. platensis* contains a unique physiologically balanced combination of substances, vital for human: 60–70% protein, 10–20% carbohydrates, 5% fat, 0.9% chlorophyll, and also vitamins, minerals, carotenoids, and important microelements, which make its extracts to be extensively employed in food, in pharmaceutical as well as perfume and cosmetics industries, and medicine. But despite everything, *S. platensis* biomass can be considered an ideal object for biosorption experiments, since data obtained using this biosorbent in batch mode can be extrapolated to another type of sorbent, which are cheaper for large scale application.

Applied biological sorbents allowed to reduce the concentrations of toxic metals to levels, which according to literature data presented at the beginning of the Chapter can be considered low toxic for aquatic organisms-bioindicators.

The price of the sorbent is a critical parameter, which determines its application in industrial processe. The yeast *Saccharomyces cerevisiae* used in the experiments was a waste obtained after beer production and it was free of charge. The price of zeolite in Russia varies from 78 to 916 USD per ton, depending on the supplier. The price of zeolite used in the present work was 380 USD per ton. Bacteria *Shewanella xiamenensis* do not require special growth conditions and the cost of mineral salt used for growth constituted 0.2 USD per liter of cultivation medium. The final price of the hybrid adsorbent would constitute 530 USD per ton of sorbent [432]. The price of mineral salt used for growth of 1 g of *S. platensis* biomass used for biomedical application constituted 0.3 USD. As biosorbent is recommended to use spirulina biomass, which remain as a waste after biotechnological process.

5.3 Conclusions to Chapter 5

1. The possibility of biological and mineral-organic sorbents application for metal removal from batch system and real wastewater was tested.
2. Using dry *Spirulina platensis* biomass it is possible to achieve maximum metal removal at the following optimal parameters:
    for lead: 11.1 mg/g at pH 5 and sorbent dosage 0.5 g;
    for silver: 31.6 mg/g at pH = 5 and sorbent dosage 0.4 g;
    for nickel: 13.4 mg/g at the pH 4 and sorbent dosage 0.3 g;
    for zinc: 7.1 mg/g at the pH e 5.6 and sorbent dosage 0.5 g;
    for chromium (III): 25 mg/g at pH 3 and sorbent dosage 0.3;
    for chromium (VI): 16.8 mg/g at pH 2 and sorbent dosage 0.7;
    for rhenium: 142.9 mg/g at pH 2 and sorbent dosage 0.05.
3. Biosorption of metal ions on *Spirulina platensis* fitted well the Langmuir and Freundlich models and obeyed the pseudo-second-order equation with good correlation. Biosorption of lead, silver, Cr(III)-Cr(VI) was an exothermic process and that of nickel and zinc - endothermic spontaneous process.
4. Application of several desorbing agents showed that CH_3COOH can be considered as the most efficient one, leading to multiple biomass use as biosorbent.
5. *S. cerevisiae* showed to be a suitable biosorbent for chromium-containing effluents treatment. The optimal pH values for maximum metal removal varied from 2.0 to 5.0 depending on the element.
6. The experimental data of Cr(VI) sorption were better described by Langmuir and Freundlich models, as compared to other tested models. The maximum adsorptive

capacity of S. cerevisiae for Cr(VI) was 26.8 mg/g in Cr(VI)-Fe(III) system, 27.3 mg/g in Cr(VI)-Fe(III)-Ni(II) system, 17.4 mg/g in Cr(VI)-Fe(III)-Ni(II)-Zn(II) system and 31.8 mg/g in Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) system.

7. Bacteria *S. oneidensis* biofilm placed on zeolite used to remove metal ions from complex chromium-containing effluents, showed high affinity for nickel, zinc and copper removal at pH 5.0-6.0, while chromium and iron removal with analyzed biosorbent did not occur. Removal of both elements from the solution was only possible when living biomass was used.
8. *S. xiamenensis* biofilm placed on zeolite showed to be a more efficient sorbent for metal removal from synthetic nickel-containing effluents. The process of cation sorption showed to be pH-dependent and the optimal pH for maximum metal ion removal (> 70%) was 6.0. The experimental values at equilibrium were better adjusted to the Langmuir and Temkin isotherm models. The good fit of the pseudo-second-order and Elovich models suggested that the chemisorption and ion exchange were the main mechanisms of metal sorption. The decreased content of Na and K in adsorbent was determined by NAA and supported this finding.
9. *S. platensis* biomass showed the high sorption capacity for metal ions present in wastewater and resulted in the removal of 83% of zinc, 66-68% of nickel, 72-100% of chromium(VI), and 51-55% of rhenium ions. In the case of complex industrial effluent, the preference of spirulina biomass for metal ions was Ba>Fe>Sr>Al>Zn>Cr. The main mechanisms of metal removal were: (i) precipitation for iron, barium, and strontium; (ii) ion-exchange and interaction with functional groups for zinc and aluminum, (iii) interaction with functional groups for zinc.
10. The highest efficiency of removal of zinc (85%) and nickel (72%) ions by S. cerevisiae from complex real effluents was possible to achieve in a two-stage subsequent system. In case of nickel maximum removal was obtained after 12-fold dilution of the effluent.

Spirulina platensis can be applied in water bioremediation studies, however, due to its high nutritional value it is better to use it for medical and pharmaceutical studies. In bioremediation studies spirulina biomass as the waste of biotechnological processes or other types of microbial biomass can be thus used. The yeast *Saccharomyces cerevisiae* is considered a cheap, safe biosorbent with high metal removal capacity, which can be obtained as a result of fermentation processes. *Shewanella xiamenensis* biofilm placed on zeolite showed the lowest efficiency in metal removal from wastewater and can be proposed mainly for the wastewater post-treatment.

CONCLUSIONS AND RECOMMENDATIONS

The results outlined in this thesis support the proposed scientific hypothesis, claiming that the current level of heavy metals in the Republic of Moldova has a negative effect on the quality of the environment and human health, compliment the research in the field of ecological chemistry, and are summarized as follows:

1. Neutron activation analysis proved to be an efficient and highly sensitive technique for the simultaneous determination of considerable number of chemical elements in the environmental samples collected in the Republic of Moldova.
2. Moss biomonitoring techniques (passive and active) applied, for the first time, to assess the quality of air in the Republic of Moldova showed that the main air pollution sources in the country are thermoelectric power plants (V, U, Sb, As), transport (Pb, Zn, Sb, Cu), industrial activity (Fe, Cr, Zn, As, Sb, Br, Ni) and agricultural practice (Cu, As, Sb, Br). The level of air pollution in Moldova can be classified as moderate to severe contaminated environment with the most polluted places being municipalities of Chisinau and Balti [262, 286].
3. The values of the Hazard Quotient and Hazard Index showed that the emissions of vanadium could negatively affect the human health, especially of children, in the entire country and in Chisinau in particular, and requires special attention of the national authorities [281].
4. In comparison with the European countries, participating in the moss surveys under the ICP Vegetation programme content of As, Cd, Cr, and Sb in the Republic of Moldova was among the highest, while the concentration of V was the highest [281].
5. According to neutron activation analysis, the data soils in Moldova can be classified as slightly to moderately polluted with heavy metals. The main contribution to soil pollution with As, Br, and Sb is primarily associated with human activity, namely the intensive use of fertilizers and pesticides [299, 329].
6. Potassium, followed by Ca and Mg was the dominant element in the analyzed fruits and vine samples. According to the *TF* values, the agricultural crops mainly accumulate these elements from soil. The daily intake values varied greatly depending on the fruit type and place of provenance, while the Hazard index values for Sb in almost all fruit samples were higher than 1.0, indicating that their consumption may present risk for the consumers' health [297, 298, 346, 350].
7. For the first time, the elemental composition of 45 plant species of the *Lamiaceae* family growing in the Botanical garden in the Republic of Moldova was determined. The analyzed plants were particularly rich in K, Cl, Mg, and Ca. Among the essential elements, Fe was

most the abundant, followed by Mn, Zn, Br, Cr, Mo, and Co. In general, metal concentrations in plants were in line with the literature data, except of As, which concentration in several species exceeded the value established by WHO. Since the tolerable daily intake values for As overpassed the values stated by WHO, this element can constitute threat to human health [370].

8. Natural water pollution with toxic metals in Moldova as well as the other countries can be reduced through the rigorous industrial and domestic wastewater treatment. The biological sorbents can be considered as excellent alternative to the conventional techniques of metal removal from the wastewater due to their ecological safety, low price, and high efficiency.
9. Three types of biological sorbents (*S. platensis; S. cerevisiae* and bacteria *S. oneidensis* placed on zeolite) were tested for their ability to remove metal ions from synthetic and real, mono- and multi-element effluents. To achieve the maximum metal removal, the effect of different parameters on sorption efficiency of process was investigated. Among the tested biosorbents the yeast *S. cerevisiae* can be regarded as more preferred biosorbent due to its safety, low cost, and possibility to treat complex effluents. *S. platensis* due to its high nutritional value, can be applied as biosorbent only as waste from the different biotechnological processes. Bacteria *S. oneidensis* placed on zeolite is more suitable for the selective metal removal from complex effluents [237-293, 411, 413, 417-422, 426, 431, 432].
10. The results obtained in the thesis are in line with the main objectives of ecological chemistry, allow to expand the area of its application, and contribute for the development of long-lasting approaches of monitoring and prevention of environmental pollution.

Personal contribution. The results obtained and presented in the thesis are entirely based on the author’s contributions and correspond to the thesis specific objectives.

The obtained result, which contributes to the creation of the new scientific direction: Application of neutron activation analysis in estimation of the biological value of the habitat for life based on the determination of the elemental composition of the different environmental samples.

In theoretical aspect, a new theoretical and practical approach in the field of ecological chemistry has been proposed, which allow based on the chemical composition evaluation of the non-carcinogenic risk, calculation of hazard coefficient for air, soil, agricultural crops polluted with metals and the values of transfer factor in soil-agricultural crops systems. The optimal physico-chemical conditions for metals maximum removal using different types of biological sorbents have been established..

In application aspect, the inclusion of the results of the biomonitoring study of air pollution in the Republic of Moldova in the „European Atlas of Heavy Metal Atmospheric Deposition” edited by „ICP Vegetation Programme Coordination Center” indicate the value of ang high appreciation of the performed research. Determination of the elemental composition of fruits, wines, and medicinal plants is extremely important for the assessment of their quality and effect on the consumers' health as well as identification of their origin. The results obtained for different sorbents can be used for the elaboration of the methods of metals removal with the purpose to reduce their concentration to maximum admissible levels..

Scientific results stated in the thesis were presented at the following national and international conferences:

13th Conference of the Society of Physicists of Macedonia (CSPM 2020), September 16-19, Macedonia (**2021**), International Conference on Radiation Applications (RAP 2021), September 6-8, Thessaloniki, Greece (**2021**), XI Всероссийская Научная конференция и школа «Аналитика Сибири и Дальнего Востока» посвящена 100-летию со дня рождения И.Г. Юделевича, 16 - 20 августа, Новосибирск, Россия (**2021**), 13th European Exhibition of Creativity and Innovation Iasi, Romania, 20-21 May, (**2021**), National Scientific Symposium with International Participation: Modern Biotechnologies – Solutions to the Challenges of the Contemporary World. Chișinau, 20-21 Mai, (**2021**), 5th Balkan Scientific Conference on Biology, Plovdiv, Bulgaria, April 15-16, (**2021**), Central European Conference ECOpole'20, October 7-10, Opole, Poland (**2020**); Vth International Conference Actual Scientific & Technical Issues of Chemical Safety, Kazan, Russia (**2020**); International Chemical Engineering and Material Symposium, Bucharest, Romania (**2020**); Fourteenth International Conference on Management Science and Engineering Management, Chisinau, Moldova (**2020**); International Conference “Achievements and perspectives of modern chemistry” dedicated to the 60th anniversary from the foundation of the Institute of Chemistry, Chisinau, Moldova (**2019**); 19th International Balkan Workshop on Applied Physics, Constanţa, Romania (**2019**); 7th International Conference on Radiation in Various Fields of Research, Herceg Novi, Montenegro (**2019**); 23rd International Scientific Conference of Young Scientists and Specialists, Dubna, Russia (**2019**); TFM-2019, Targoviste, Romania (**2019**); 5th Symposium «Advances and Challenges in Physics by JINR and South Africa», Somerset West, Republic of South Africa (**2018**); International Conference on Microbial Biotechnology 4th Edition, Chisinau, Moldova

(**2018**); 8th International Workshop on Biomonitoring of Atmospheric Pollution, Dubna (**2018**); The XXII International Scientific Conference of Young Scientists and Specialists, Dubna, Russia (**2018**); VI Сабининские чтения, Москва, Россия (**2017**, plenary talk); 8th International Student Summer School "Nuclear Physics – Science and Applications", Brasov, Romania (**2017**); International Seminar on Interaction of Neutrons with Nuclei, Dubna, Russia (2017; plenary; 2014); Ecological & Environmental chemistry, Chisinau, Moldova (**2017**); Radioanalytical and Nuclear Chemistry, Budapest, Hungary (**2016**); The XX International Scientific Conference of Young Scientists and Specialists Dubna, Russia (**2016)**; 29th Task Force Meeting, Dubna, Russian Federation (**2016**); 7th International Student Summer School on "Nuclear Physics – Science and Applications", Poznan, Poland (**2015**); 4-я Школа-конференция молодых ученых и специалистов ОИЯИ, Алушта, Крым (**2015**); Вторая Всероссийская молодежная научно-техническая конференция с международным участием "ИННОВАЦИИ В МАТЕРИАЛОВЕДЕНИИ", Москва, Россия (**2015**); The International Conference dedicated to 55th anniversary from the foundation of the Institute of Chemistry of the Academy of Science of Moldova, Chisina, Moldova (**2014**); XVIII International Conference of Young Scientists and Specialists, Dubna, Russia (**2014**); 1st EuChemMS Congress on Green and Sustainable Chemistry, Budapest, Hungary (**2013**).

The scientific results presented in the thesis were published in **50** papers, including **24** articles with impact factor, **2** books, **4** book chapters and **1** conference paper.

The obtained results were implemented at Public Institution "Central laboratory for testing alcoholic/non-alcoholic beverages and canned goods", Moldovan Association of Aromatic and Medicinal Plant Producers "AROMEDA", Institute of Chemistry, and Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova.

Recommendations

1. It is recommended to participate in every five-year moss survey in order to accumulate the data on air pollution with heavy metals in Moldova and to elaborate the legislation which will help to improve the air quality in the country, following the other European countries, for example, Norway.
2. It is recommended to assess the quality of fruits and beverages widely consumed by the population using the modern analytical techniques to determine the concentration of toxic elements. This information will enable to assess the risk of agricultural products consumption on human health and prevent their excessive consumption.

3. It is recommended to apply the biological sorbents for metal removal from wastewater generated by industrial enterprises before their discharge into the natural water bodies. Treatment of wastewater using biological sorbents allows the repeated use of purified water in the technological process thus, reducing clean water consumption and the impact on the environment.

Suggestions for future research

1. Passive and especially active moss biomonitoring studies in the Republic Moldova need to be continued in order to detect specific sources of chemical elements emission in the country
2. It is necessary to continue the experiments on the development of the techniques of wastewater treatment using biological sorbent and to move from laboratory experiments beyond implementation of the technique on the industrial enterprises

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Annex 1

Experimental data

Table A1.1 Pearson correlation coefficient between element content in mosses in Moldova

Na	Mg	Al	Cl	Sc	Ti	V	Cr	Fe	Ni	Co	As	Rb	Sb	Cs	La	Ce	Sm	Eu	Gd	Tb	Yb	Hf	Ta	W	Th	U
1	0.72	**0.71**	0.10	**0.87**	0.68	0.63	**0.93**	**0.88**	**0.90**	**0.95**	**0.95**	**0.87**	**0.81**	**0.84**	**0.93**	**0.96**	**0.83**	**0.94**	**0.84**	**0.93**	**0.92**	**0.96**	**0.94**	**0.96**	**0.95**	**0.98**
	1.00	**0.96**	-0.05	**0.88**	**0.93**	**0.94**	**0.87**	**0.89**	**0.83**	**0.86**	**0.80**	**0.89**	0.61	**0.91**	**0.82**	**0.78**	**0.86**	**0.82**	**0.73**	**0.83**	**0.78**	**0.72**	**0.84**	**0.74**	**0.83**	**0.77**
		1.00	-0.18	**0.91**	**0.98**	**0.98**	**0.89**	**0.91**	**0.85**	**0.85**	**0.83**	**0.92**	0.56	**0.94**	**0.84**	**0.80**	**0.90**	**0.83**	**0.75**	**0.85**	**0.81**	**0.73**	**0.86**	**0.70**	**0.85**	**0.78**
			1.00	-0.03	-0.17	-0.21	-0.01	-0.05	-0.08	0.04	0.02	-0.05	-0.07	-0.06	0.02	0.08	-0.03	0.07	0.07	0.03	0.05	0.09	0.02	0.15	0.02	0.06
				0.45	0.51	0.49	0.43	0.45	0.36	0.41	0.29	0.45	0.09	0.48	0.39	0.30	0.46	0.33	0.29	0.41	0.38	0.32	0.40	0.25	0.38	0.32
				0.61	0.44	0.42	0.69	0.62	0.73	**0.76**	0.71	0.63	0.78	0.61	0.65	0.72	0.55	0.69	0.67	0.66	0.67	0.70	0.68	**0.86**	**0.70**	**0.82**
				1.00	**0.92**	0.87	**0.98**	**1.00**	**0.93**	**0.96**	**0.96**	**0.99**	0.63	**0.99**	**0.98**	**0.95**	**0.98**	**0.96**	**0.89**	**0.98**	**0.96**	**0.92**	**0.98**	**0.85**	**0.98**	**0.93**
					1.00	**0.97**	**0.89**	**0.93**	**0.83**	**0.84**	**0.82**	**0.92**	0.53	**0.94**	**0.86**	**0.79**	**0.92**	**0.83**	**0.76**	**0.87**	**0.83**	0.74	**0.87**	0.67	**0.85**	**0.76**
						1.00	**0.84**	**0.88**	**0.79**	**0.79**	**0.75**	**0.87**	0.49	**0.91**	**0.79**	0.72	**0.85**	**0.76**	0.67	**0.80**	**0.75**	0.65	**0.80**	0.62	**0.79**	0.71
							1.00	0.99	**0.95**	**0.98**	**0.97**	**0.98**	**0.74**	**0.97**	**0.98**	**0.96**	**0.96**	**0.96**	**0.88**	**0.99**	**0.96**	**0.94**	**0.99**	0.91	**0.99**	**0.96**
								0.40	0.42	0.41	0.40	0.39	0.32	0.41	0.37	0.36	0.41	0.37	0.22	0.39	0.34	0.32	0.39	0.28	0.38	0.38
								1.00	**0.94**	**0.96**	**0.96**	**0.99**	0.65	**0.99**	**0.98**	**0.95**	**0.98**	**0.96**	**0.88**	**0.98**	**0.96**	**0.92**	**0.98**	**0.86**	**0.98**	**0.93**
									1.00	**0.94**	**0.94**	**0.94**	**0.78**	**0.92**	**0.93**	**0.92**	**0.91**	**0.91**	**0.85**	**0.93**	**0.90**	**0.89**	**0.95**	**0.89**	**0.95**	**0.93**
										1.00	**0.97**	**0.96**	**0.75**	**0.95**	**0.97**	**0.97**	**0.93**	**0.96**	**0.87**	**0.97**	**0.95**	**0.94**	**0.98**	**0.93**	**0.98**	**0.97**
											0.53	0.49	0.64	0.45	0.48	0.51	0.44	0.47	0.52	0.50	0.51	0.50	0.50	0.61	0.51	0.55
											1.00	**0.95**	0.72	**0.93**	**0.98**	**0.99**	**0.94**	**0.98**	**0.89**	**0.98**	**0.97**	**0.96**	**0.98**	**0.92**	**0.99**	**0.97**
												-0.14	-0.17	-0.16	-0.21	-0.21	-0.19	-0.25	-0.13	-0.20	-0.21	-0.23	-0.20	-0.26	-0.22	-0.23
												0.26	0.08	0.18	0.22	0.25	0.16	0.21	0.17	0.24	0.26	0.25	0.23	0.30	0.23	0.23
												1.00	0.66	**0.98**	**0.97**	**0.94**	**0.96**	**0.95**	**0.87**	**0.97**	**0.95**	0.91	**0.98**	**0.84**	**0.97**	**0.92**
													0.32	0.26	0.28	0.31	0.18	0.28	0.39	0.28	0.31	0.30	0.29	0.51	0.31	0.36
													1.00	0.62	0.68	0.70	0.60	0.67	0.63	0.69	0.65	0.71	0.71	**0.81**	0.71	**0.77**
														0.68	0.72	0.69	0.70	0.69	0.61	0.72	**0.70**	0.70	0.71	0.63	0.71	0.68
														1.00	**0.96**	**0.92**	**0.96**	**0.93**	**0.87**	**0.96**	**0.93**	**0.87**	**0.96**	**0.83**	**0.96**	**0.91**
															1.00	**0.98**	**0.96**	**0.98**	**0.92**	**1.00**	**0.98**	**0.97**	**1.00**	**0.90**	**0.99**	**0.96**
																1.00	**0.92**	**0.98**	**0.92**	**0.98**	**0.98**	**0.98**	**0.98**	**0.93**	**0.99**	**0.98**
																	1.00	**0.94**	**0.85**	**0.97**	**0.94**	**0.90**	**0.96**	**0.81**	**0.95**	**0.88**
																		1.00	**0.90**	**0.98**	**0.98**	**0.96**	**0.98**	**0.90**	**0.98**	**0.96**
																			1.00	**0.90**	**0.93**	**0.89**	**0.90**	**0.85**	**0.91**	**0.89**
																				1.00	**0.98**	**0.97**	**1.00**	**0.90**	**0.99**	**0.95**
																					1.00	**0.97**	**0.98**	**0.88**	**0.98**	**0.95**
																						1.00	**0.97**	**0.92**	**0.97**	**0.96**
																							1.00	**0.91**	**1.00**	**0.97**
																								1.00	**0.92**	**0.95**
																									1.00	**0.97**
																										1.00
Na	Mg	Al	Cl	Sc	Ti	V	Cr	Fe	Ni	Co	As	Rb	Sb	Cs	La	Ce	Sm	Eu	Gd	Tb	Yb	Hf	Ta	W	Th	U

Table A1.2 Elemental content [mg/kg d.w.] of the moss exposed for five months (d.w. = dry weight)

Site	**Control**	**TPP**					**BG**				
Month	I	I	II	III	IV	V	I	II	III	IV	V
Na	134±8	269 ±8	544 ±16	608 ±32	1370 ±82	2720 ±163	348 ±21	341 ±20	258 ±15	673 ±40	431 ±25
Mg	84±5	n.d.	n.d.	n.d.	n.d.	1040 ±62	1030 ±61	945 ±57	1120 ±68	924 ±55	887 ±53
Cl	579±50	394 ±35	529 ±47	568 ±51	2430 ±218	1720 ±155	157 ±14	413 ±37	376 ±33	353 ±31	559 ±50
K	10800±860	5020 ±400	6890 ±550	2440 ±190	5020 ±400	4440 ±350	4400 ±350	3730 ±300	3280 ±260	3470 ±280	2750 ±220
Sc	0.02±0.001	0.057 ±0.003	0.079 ±0.004	0.091 ±0.005	0.125 ±0.006	0.174 ±0.009	0.048 ±0.002	0.081 ±0.004	0.059 ±0.003	0.077 ±0.004	0.077 ±0.004
Ca	2680±400	2980 ±450	4360 ±650	8150 ± 1220	9680 ±1450	6500 ±975	5560 ±830	4260 ±640	5880 ±880	4180 ±630	4910 ±735
Ti	78±11	31 ±4	72 ±10	75 ±10	110 ±16	53 ±8	39 ±6	n.d.	n.d.	n.d.	n.d.
Cr	0.74±0.1	0.7 ±0.1	2.0 ±0.3	1.5 ±0.5	2.0 ±0.3	2.3 ±0.3	2.1 ±0.3	1.0 ±0.2	1.1 ±0.2	1.0 ±0.2	1.5 ±0.2
V	0.54±	2.0 ±0.1	2.7 ±0.1	5.4 ±0.3	10.8 ±0.5	3.9 ±0.2	0.93 ±0.04	0.78 ±0.03	0.75 ±0.03	0.77 ±0.03	0.88 ±0.04
Mn	243±12	239 ±12	295 ±14	390 ±20	327 ±16	282 ±17	357 ±17	303 ±15	332 ±16	232 ±12	251 ±12
Ni	0.8±0.1	1.5 ±0.2	4.7 ±0.7	3.6 ±0.5	2.1 ±0.3	2.7 ±0.4	3.6 ±0.5	1.3 ±0.2	1.6 ±0.2	1.2 ±0.2	1.8 ±0.3
Fe	134±9	276 ±12	332 ±23	378 ±26	514 ±35	646 ±45	253 ±17	338 ±23	276 ±19	329 ±23	327 ±23
Co	0.14±0.01	0.25 ±0.02	0.29 ±0.02	0.33 ±0.03	0.41 ±0.04	0.47 ±0.04	0.19 ±0.01	0.25 ±0.02	0.27 ±0.02	0.24 ±0.02	0.26 ±0.02
Zn	28±1.0	43 ±1	46 ±1	88 ±3	54 ±1	55 ±1	44 ±1	53 ±1	49 ±1	50 ±1	55 ±1
As	0.71±0.05	0.74 ±0.05	0.76 ±0.05	1.04 ±0.07	1.20 ±0.08	1.24 ±0.09	1.20 ±0.08	0.74 ±0.05	0.61 ±0.04	0.62 ±0.04	0.65 ±0.04
Br	3.4±0.1	5.9 ±0.2	4.9 ±0.1	3.5 ±0.1	6.4 ±0.2	7.1 ±0.2	3.2 ±0.1	4.4 ±0.1	3.4 ±0.1	3.9 ±0.1	4.4 ±0.2
Sr	6±0.6	10 ±1	19 ±2	24 ±2	31 ±3	35 ±3	11 ±1	22 ±1	15 ±1	17 ±1	26 ±2
Rb	58±10	26 ±4	31 ±5	11 ±2	27 ±5	19 ±3	21 ±4	16 ±3	19 ±3	12 ±2	15 ±2
Mo	0.20±0.006	0.15 ±0.04	0.17 ±0.05	0.11 ±0.03	0.22 ±0.07	0.21 ±0.06	0.12 ±0.03	0.13 ±0.04	0.11 ±0.03	0.09 ±0.03	0.13 ±0.04
Sb	0.03±0.003	0.25 ±0.02	0.42 ±0.03	0.34 ±0.03	0.59 ±0.05	1.00 ±0.09	0.099 ±0.009	0.30 ±0.02	0.28 ±0.02	0.28 ±0.02	0.26 ±0.02
Ba	10±1	25 ±2	18 ±2	28 ±3	29 ±3	29 ±3	23 ±2	23 ±2	27 ±2	15 ±1	27 ±2
La	0.02±0.001	0.21 ±0.01	0.28 ±0.01	0.28 ±0.01	0.48 ±0.02	0.57 ±0.02	0.18 ±0.01	0.27 ±0.01	0.20 ±0.01	0.34 ±0.01	0.29 ±0.01
Ce	0.30±0.03	0.27 ±0.03	0.95 ±0.09	0.56 ±0.06	0.73 ±0.07	1.0 ±0.1	0.26 ±0.03	0.56 ±0.05	0.26 ±0.03	0.45 ±0.05	0.53 ±0.05
Hf	0.01±0.004	0.029 ±0.009	0.05 ±0.01	0.04 ±0.01	0.08 ±0.02	0.11 ±0.02	0.07 ±0.02	0.05 ±0.01	0.03 ±0.01	0.03 ±0.01	0.04 ±0.01
Cs	0.02±0.001	0.140 ±0.007	0.152 ±0.007	0.086 ±0.004	0.143 ±0.007	0.154 ±0.007	0.122 ±0.006	0.096 ±0.006	0.120 ±0.006	0.108 ±0.006	0.121 ±0.006
Th	0.01±0.001	0.043 ±0.003	0.067 ±0.004	0.059 ±0.004	0.109 ±0.007	0.163 ±0.01	0.037 ±0.002	0.064 ±0.004	0.043 ±0.002	0.092 ±0.006	0.061 ±0.004
U	3.2±0.1	0.023 ±0.001	n.d.	0.032 ±0.001	0.047 ±0.002	0.064 ±0.002	0.014 ±0.001	0.028 ±0.001	0.015 ±0.001	0.026 ±0.002	0.027 ±0.002
Cu	0.5±0.02	2.47 ±0.01	3.15 ±0.01	4.18 ±0.01	4.53 ±0.01	6.92 ±0.02	3.55 ±0.01	3.37 ±0.01	3.85 ±0.01	3.58 ±0.01	3.58 ±0.01
Pb	0.11±0.005	1.30 ±0.04	1.08 ±0.03	1.34 ±0.04	1.28 ±0.04	1.64 ±0.04	0.97 ±0.03	0.53 ±0.01	1.36 ±0.03	0.84 ±0.01	1.55 ±0.03
Cd	134±8	0.193 ±0.008	0.153 ±0.008	0.334 ±0.009	0.169 ±0.008	0.173 ±0.008	0.214 ±0.008	0.176 ±0.008	0.167 ±0.008	0.196 ±0.008	0.257 ±0.009

Table A1.2 Elemental content [mg/kg d.w.] of the moss exposed for five months (d.w. = dry weight) (continuation)

Site	**AS**				
Month	I	II	III	IV	V
Na	393 ±25	217 ±13	786 ±45	1530 ±90	1050 ±63
Mg	725 ±43	639 ±38	980 ±59	1170 ±70	949 ±57
Cl	171 ±15	264 ±24	740 ±67	1360 ±122	987 ±88
K	3530 ±280	816 ±65	2100 ±170	2330 ±180	3880 ±310
Sc	0.08 ±0.004	0.15 ±0.008	0.26 ±0.01	0.18 ±0.09	0.20 ±0.01
Ca	10700 ±1600	11000 ±1650	7970 ±1120	11000 ±1650	6310 ±940
Ti	82 ±12	32 ±5	96 ±14	69 ±10	36 ±5
Cr	2.4 ±0.4	1.9 ±0.3	4.0 ±0.6	2.9 ±0.4	3.2 ±0.5
V	0.96 ±0.05	0.79 ±0.04	1.94 ±0.06	1.22 ±0.06	2.20 ±0.07
Mn	230 ±11	192 ±10	264 ±13	359 ±18	334 ±17
Ni	0.7 ±0.1	0.7 ±0.1	2.2 ±0.3	1.5 ±0.2	3.1 ±0.5
Fe	324 ±23	446 ±31	942 ±65	707 ±50	778 ±55
Co	0.33 ±0.03	0.30 ±0.03	0.57 ±0.05	0.54 ±0.05	0.53 ±0.05
Zn	47 ±1	46 ±1	67 ±2	61 ±2	64 ±2
As	0.21 ±0.01	0.23 ±0.01	0.37 ±0.03	0.38 ±0.03	0.42 ±0.03
Br	6.5 ±0.2	5.0 ±0.1	7.9 ±0.2	9.5 ±0.2	6.9 ±0.1
Sr	24 ±2	52 ±5	45 ±4	35 ±3	41 ±4
Rb	16 ±3	3 ±1	10 ±2	12 ±2	11 ±2
Mo	0.10 ±0.03	0.13 ±0.04	0.30 ±0.09	0.26 ±0.08	0.21 ±0.06
Sb	0.47 ±0.04	0.91 ±0.08	1.3 ±0.1	0.95 ±0.08	0.61 ±0.05
Ba	19 ±2	16 ±1	27 ±3	27 ±3	31 ±3
La	0.28 ±0.01	0.32 ±0.01	0.64 ±0.03	0.64 ±0.03	0.66 ±0.03
Ce	0.26 ±0.03	0.50 ±0.05	1.3 ±0.1	1.5 ±0.1	1.0 ±0.1
Hf	0.04 ±0.01	0.09 ±0.02	0.14 ±0.04	0.09 ±0.02	0.08 ±0.02
Cs	0.095 ±0.002	0.072 ±0.001	0.120 ±0.005	0.107 ±0.005	0.108 ±0.005
Th	0.066 ±0.004	0.081 ±0.005	0.17 ±0.01	0.17 ±0.01	0.17 ±0.01
U	0.047 ±0.004	0.15 ±0.01	0.14 ±0.01	0.077 ±0.006	0.079 ±0.006
Cu	5.36 ±0.02	4.93 ±0.01	8.59 ±0.03	8.30 ±0.03	5.33 ±0.02
Pb	0.71 ±0.02	1.90 ±0.06	1.69 ±0.06	1.42 ±0.04	1.41 ±0.04
Cd	0.148 ±0.007	0.157 ±0.008	0.139 ±0.007	0.187 ±0.009	0.195 ±0.009

Table A1.3 The numerical values (average ± standard deviation) of the content of seven major and 29 trace elements in soils collected from Romanesti and Cricova vineyeards as well as the corresponding vales of the UCC and of the AS. All values are expressed in mg/kg

	Romanesti – red wine vineyard			Romanesti – white wine vineyard			Cricova vineyard			UCC	AS
Element	Mean		S.D.	Mean	±	S.D.	Mean		S.D.		
Na	4178	±	396	4071	±	154	4100	±	396	25400	6300
Mg	19528	±	3034	19050	±	2028	21440	±	3034	11600	5000
Al	60214	±	4600	60183	±	5708	59500	±	4600	78300	67000
K	18671	±	976	18450	±	2198	16860	±	976	15000	
Ca	11991	±	2821	13661	±	10925	10062	±	2821	31500	13700
Sc	10	±	1	10	±	1	10	±	1	14	7
Ti	4158	±	744	4048	±	368	4438	±	744	3300	4600
V	89	±	6	85	±	11	82	±	6	140	100
Cr	91	±	8	90	±	9	88	±	8	69	200
Mn	671	±	62	676	±	73	715	±	62	770	
Fe	30085	±	1178	30033	±	4618	26540	±	1178	41700	38000
Co	13	±	1	13	±	1	12	±	1	17	8
Ni	33	±	2	37	±	5	35	±	2	55	40
Zn	72	±	4	70	±	10	58	±	4	67	50
As	8.9	±	1.2	8.4	±	1.1	8.8	±	0.3	1.6	
Br	8	±	0	7	±	1	9	±	0	2.1	5
Rb	105	±	3	103	±	16	93	±	3	110	100
Sr	85	±	21	89	±	25	127	±	21	350	300
Zr	667	±	271	651	±	63	492	±	271	193	300
Sb	0.9	±	0.1	0.9	±	0.1	0.8	±	0.1	0.2	
Cs	5.3	±	0.7	5.3	±	1.2	4.4	±	0.2	3.7	5
Ba	400	±	59	397	±	16	359	±	59	570	500
La	34	±	1	33	±	1	33	±	1	30	40
Ce	62	±	5	61	±	0	70	±	5	58	50
Nd	32	±	7	31	±	4	39	±	7	27	
Sm	5.4	±	1.1	4.6	±	0.3	5.6	±	1.2	4.59	
Eu	1.1	±	0.1	1.1	±	0	1.3	±	0.2	1	
Gd	4.3	±	0	4.4	±	0.1	4	±	0.2	4	
Tb	0.8	±	0	0.8	±	0	0.8	±	0	0.6	
Tm	0.3	±	0	0.3	±	0.1	0.4	±	0.1	0.3	
Yb	2.7	±	0.3	2.6	±	0.3	2.7	±	0.7	2	
Hf	8.5	±	1.3	8.4	±	1.1	9.4	±	0.8	4	6
Ta	1	±	0	1	±	0	1	±	0	1.5	
Th	11.4	±	1.6	12	±	1.1	10.9	±	1.3	11	6
U	2.3	±	0.5	2.1	±	0	2.7	±	0.6	2.8	1

Table A1.4 Content of 18 elements determined by NAA in wines from Romanesti vineyard

	Wine sort											
	Cabernet, *red*	Regent, *red*	Pinot Noir, *red*	Nero, *red*	Syzar, *red*	Merlot, *red*	Malbec, *red*	Sauvignon, *white*	Riesling, *white*	Sauvignon, *white*	Pinot Gris, *white*	Muscat, *white*
Element	**Concentration, mg/L**											
Na	23±0.9	32±1.1	7.5±0.1	29±0.9	12±0.3	16±0.5	14±0.3	15±0.3	19±0.3	18±0.2	18±0.3	17±0.3
Mg	118±3.5	105±3.0	117±3.2	110±2.8	89±1.9	90±1.5	104±2.0	74±1.8	91±1.9	82±1.9	98±1.4	74±1.2
Al	1.6±0.06	0.8±0.03	2.1±0.08	0.8±0.03	1.1±0.08	2.3±0.08	2.4±0.09	1.3±0.04	2.2±0.09	2.4±0.09	1.5±0.08	1.4±0.05
K	666±60	695±70	698±70	563±56	820±80	723±72	844±84	290±29	299±29	253±25	329±32	285±28
Ca	47±4.7	70±7	54±5.4	73±7	35±3	51±5	50±5	69±6	121±12	80±8	64±6	70±7
Mn	1.3±0.05	1.4±0.04	1.6±0.05	1.2±0.03	1.4±0.04	1.2±0.03	1.6±0.05	1.0±0.03	1.3±0.04	1.0±0.03	1.4±0.03	1.1±0.03
Fe	6.1±0.3	0.4±0.02	8.8±0.4	0.9±0.4	1.4±0.02	3.6±0.8	5.9±0.07	1.3±0.06	1.3±0.06	1.2±0.05	1.2±0.06	1.1±0.05
Zn	1.3±0.09	0.2±0.01	0.6±0.02	0.3±0.02	0.8±0.05	0.4±0.03	0.6±0.04	0.5±0.03	0.5±0.03	0.5±0.03	0.6±0.03	0.5±0.03
Br	0.15±0.01	0.27±0.02	0.32±0.03	0.32±0.03	0.40±0.04	0.21±0.02	0.25±0.02	0.11±0.03	n.d	0.02±0.03	0.13±0.04	0.04±0.03
Rb	1.9±0.1	1.8±0.1	1.8±0.1	1.4±0.1	1.8±0.08	1.6±0.09	2.0±0.1	0.8±0.06	1.0±0.01	0.8±0.05	1.2±0.1	0.7±0.06
Sr	1.4±0.03	1.1±0.02	1.0±0.02	1.0±0.02	0.7±0.012	0.9±0.03	0.9±0.02	0.5±0.01	0.8±0.01	0.5±0.01	0.6±0.01	0.5±0.05
	Concentration. µg/L											
Ni	34±3	40±4	57±5	33±3	34±3	n.d	15±1.5	40±5	46±4.5	28±3	41±3	28±
Co	7.0±0.6	4.6±0.4	13.2±1.1	4.8±0.5	5.2±0.4	3.1±0.2	8.2±0.7	9.3±0.8	10.9±1	9.1±0.8	12.0±1.1	9.4±0.8
Sb	n.d	0.22±0.01	0.23±0.05	0.19±0.01	0.26±0.02	0.14±0.02	0.23±0.01	0.37±0.01	0.62±0.03	0.48±0.01	0.41±0.01	0.37±0.02
Ba	129±6	323±15	180±9	217±610	100±5	92±64	141±66	76±3	154±7	96±5	103±6	93±5
Cs	17.1±0.03	3.1±0.01	3.6±0.01	3.3±0.01	3.9±0.01	3.9±0.01	7.6±0.01	1.5±0.01	4.3±0.02	1.6±0.01	2.6±0.01	1.2±0.01
U	0.03±0.00	0.15±0.00	0.17±0.00	0.10±0.00	0.18±0.00	0.12±0.00	0.13±0.00	0.25±0.00	0.15±0.00	0.24±0.001	0.14±0.001	0.24±0.001

Table A1.5 Content of 18 elements determined by NAA in wines from Cricova vineyard

	Wine sort										
	Pinot. C-18. red	Pinot. C-42. red	Pinot. C-191. red	Pinot. C-197. red	Chardonnay. C-13. *white*	Chardonnay. C-179. *white*	Chardonnay. C-163. white	Chardonnay. C-173. *white*	Chardonnay. white	Pinot frank. *white*	Cabernet. *red*
Element	**Concentration. mg/L**										
Na	13±0.4	11±0.3	8±0.4	15±0.7	10±0.2	8±0.4	16±0.8	10±0.2	16±0.7	11±0.04	10±0.03
Mg	69±2	81±4	76±3	83±4	94±5	65±3	95±5	89±6	132±6	83±4	78±4
Al	3.2±0.12	1.0±0.05	0.7±0.02	1.4±0.03	2.7±0.01	1.8±0.01	1.5±0.01	1.0±0.03	1.1±0.02	0.6±0.02	0.7±0.03
K	600±60	433±40	441±44	458±45	594±59	561±56	636±63	644±64	459±45	383±38	381±38
Ca	72±7	72±7	75±7	72±7	88±8	72±7	79±8	83±8	60±6	29±2	43±4
Mn	0.8±0.02	1.0±0.03	1.1±0.03	1.0±0.02	1.1±9.03	0.8±0.02	1.1±0.02	1.1±0.02	1.2±0.04	0.7±0.02	0.8±0.02
Fe	1.07±0.06	0.72±0.02	1.20±0.05	2.24±0.08	0.66±0.02	0.48±0.01	1.29±0.06	0.61±0.02	0.83±0.03	0.15±0.001	0.45±0.02
Zn	0.7±00.3	1.3±0.05	1.1±0.05	1.2±0.05	0.5±0.02	0.5±0.02	0.5±0.02	0.6±0.02	0.4±0.01	0.5±0.02	0.3±0.01
Br	0.06±0.003	0.08±0.004	0.05±0.002	0.11±0.005	0.09±0.004	0.05±0.002	0.15±0.007	0.16±0.008	0.12±0.006	0.07±0.003	0.06±0.003
Rb	2.0±0.2.	1.5±0.15	1.7±0.17	1.6±0.16	1.5±0.15	1.8±0.18	2.0±0.2	1.3±0.13	1.6±0.16	1.2±0.12	1.1±0.11
Sr	0.6±0.003	1.1±0.005	1.2±0.006	0.9±0.003	0.8±0.004	0.6±0.004	0.9±0.003	0.9±0.003	0.7±0.02	0.5±0.01	0.5±0.01
	Concentration. µg/L										
Ni	7.2±0.7	28±2.7	16±1.5	28±3	21±2	18±1.7	29±2.5	16±1.5	26±2	16±1	12±1
Co	4.3±0.1	5.3±0.2	5.3±0.2	4.4±0.2	3.4±1.1	2.4±0.1	3.6±0.1	3.8±0.1	3.6±0.1	2.0±0.1	1.5±0.1
As	0.2±0.006	0.2±0.006	0.2±0.006	0.6±0.009	0.2±0.006	n.d	0.38±0.003	0.40±0.003	n.d	n.d	n.d
Sb	0.72±0.04	0.31±0.02	0.65±0.03	1.11±0.05	0.63±0.03	0.22±0.01	0.57±0.02	0.81±0.04	0.72±0.03	0.20±0.01	0.16±0.009
Ba	106±5	87±4	107±5	13±0.5	107±5	71±3	105±5	123±6	61±3	57±3	41±2
Cs	11±0.5	8.0±0.4	8.3±0.4	6.5±0.3	6.3±0.3	5.5±0.2	7.4±0.3	4.8±0.2	7.2±0.3	4.9±0.3	3.6±0.1
U	0.17±0.008	0.09±0.004	0.06±0.003	0.14±0.001	0.13±0.001	0.11±0.005	0.15±0.007	0.12±0.006	n.d	0.13±0.006	0.16±0.008

Table A.1.6 Elemental content of analyzed fruits determined by NAA (in mg/kg)

Element	**Apple**							**RP**
	leaves			***Fruits***				
	Present	[119]	[343]	**Present work**	[343]	[338]	[119]	
Na	260±10		50.0–250	1120±50	30.0–90.0	15	-	150
Mg	8660±420		3590–7440	233±10	360-610	297	-	200
Cl	505±190			940±440		1060	-	2000
K	44400±2200		6600–16500	23700±1180	6,000–14,700	7242	-	19000
Sc	0.82±0.04		0.1–0.2	0.25±0.01	0.1–0.4		-	0.02
Ca	28400±1400		11400–25700	1960±90	300–600	290	-	10000
Cr	0.6±0.03	1.17	0.4–4.9	1.2±0.06	1.5–2.6	0.43	0.24	1.5
V	0.6±0.03			0.2±0.01	-	0.007	-	0.5
Mn	180±10		32.0–380	2.6±0.1	2.0–9.0	4.3	-	200
Ni	3.6±0.2		2.3–16.0	2.0±0.1	0.05–0.7	<0.2	-	1.5
Fe	270±10		110–690	25±1.0	10.0–150	9.3	-	150
Co	0.2±0.01		0.14–0.64	0.15±0.07	0.01–0.1	0.16	-	0.2
Zn	16±0.8	82.2	8.7–33.6	9.9±0.5	1.1–10.3	1.4	34.2	50
As	0.74±0.03		0.1–1.5	0.37±0.02	0.1–0.4		-	0.1
Br	6.1±0.3			0.4±0.02			-	4
Sr	200±10		17.0–69.8	1.5±0.07	0.5–1.7	0.85	-	50
Rb	48±2.4			35±1.5			-	50
Mo	1.2±0.06		0.07–1.72	0.4±0.02	0.02–0.16		-	0.5
Sb	0.03±0.001		0.03–0.09	0.02±0.001	0.01–0.04		-	0.1
Ba	17±0.8		5.8–73.5	n.d			-	40
Cs	0.04±0.002			0.05±0.003			-	0.2
La	0.3±0.01			0.08±0.004			-	0.2
Sm	0.05±0.002			0.01±0.007			-	0.04
Ta	0.008±0.0004			0.0024±0.0001			-	0.001
Th	0.009±0.004			0.003±0.001			-	0.005
U	0.03±0.001			0.02±0.001			-	0.01

Table A1.6 Elemental content of analyzed fruits determined by NAA (in mg/kg), (continuation)

Element	**Plum**							**RP**
	leaves			***stone***	***fruits***			
	present	[336]	[344]		***present***	[336]	[344]	
Na	330±15			310±15	490±20			150
Mg	5580±270			760±40	160±8			200
Cl	150±15			120±30	78±8.5			2000
K	16700±830			21600±1080	23500±1170			19000
Sc	0.055±0.02			<0.02	0.099±0.005			0.02
Ca	20700±1030			4350±210	1190±60			10000
Cr	0.9±0.04			0.5±0.03	<3.6			1.5
V	0.5±0.82			<0.3	<0.4			0.5
Mn	38±2		20–62	5.4±2	1.7±0.08		2.8-6.5	200
Ni	1.9±0.1			2.5±0.1	1.0±0.05			1.5
Fe	173±8.5		58–173	125±6	120±6		12-79	150
Co	0.9±0.04			0.2±0.01	0.58±0.03			0.2
Zn	20±9	20-80	15–35	13±6.5	20±1.0	5-20	0.35-0.79	50
As	0.65±0.03			0.16±0.01	0.25±0.01			0.1
Br	13.5±0.7			1.6±0.08	6.4±0.3			4
Sr	150±7			22±1	13±0.6			50
Rb	83±4.1			101±5	179±8.9			50
Mo	0.38±0.02			0.9±0.05	0.046±0.002			0.5
Sb	0.021±0.001			0.073±0.004	0.06±0.003			0.1
Ba	28.5±1			n.d	n.d			40
Cs	0.029±0.001			0.014±0.0007	0.032±0.001			0.2
La	0.17±0.008			0.017±0.0009	0.021±0.001			0.2
Sm	0.026±00.01			0.0028±0.0001	0.04±0.0002			0.04
Ta	0.005±0.0003			0.0003±0.00001	0.001±0.0001			0.001
Th	0.005±0.003			0.005±0.002	0.007±0.003			0.005
U	0.02±0.001			0.02±0.001	0.01±0.0004			0.01

Table A1.7 Elemental content of fruits collected in different regions in the Republic of Moldova determined by NAA, µg/g

	1	2	3	4	5	6	7	8	9	10	11	12	13
	P_m	**P_f**	**P_s**	**C_{mh}**	**C_{md}**	**C_a**	**C_p**	**I_{as}**	**I_{ag}**	**I_p**	**C_{rmd}**	**C_{ra}**	**C_{rp}**
Element	**Purcari**			**Cahul**			**Cahul**	**Ialoveni**			**Criuleni**		
Na	524	1820	500	329	400	737	1036	211	310	299	265	313	485
Mg	980	1300	847	1214	869	675	593	823	634	2097	3003	2263	2337
Cl	157	-	1713	226	571	116	950	432	276	1186	200	755	918
K	31250	27267	36150	36233	37333	41300	41300	38700	35633	42833	44533	46200	44300
Ca	7957	8495	4980	5780	6215	1110	1440	7047	1167	1227	6457	1903	3027
Sc	0.09	0.16	0.17	0.28	0.20	0.30	0.08	0.08	0.14	0.16	0.11	0.13	0.06
Mn	5.6	6.1	6.6	8.6	6.2	2.3	3.4	3.3	2.6	1.9	3.2	1.6	1.8
Fe	54.1	145.0	115.8	129.1	137.0	156.5	44.4	45.9	61.3	45.8	42.2	32.6	32.3
Co	0.03	0.07	0.06	0.06	0.07	0.09	0.03	0.03	0.08	0.05	0.04	0.08	0.06
Ni	-	-	0.845	-	0.935	-	0.605	-	0.64	-	0.63	-	2.035
Cu	41	37	24	35	21	10	11	17	12	23	11	-	12
Zn	17.4	42.7	15.7	17.0	13.8	3.3	13.2	7.8	4.0	12.4	11.9	5.9	21.9
As	-	0.036	0.078	0.174	0.096	0.215	0.043	0.048	0.058	0.059	-	-	-
Br	0.26	0.16	3.42	0.56	1.34	0.35	0.46	0.47	0.88	0.80	0.68	1.91	1.50
Rb	55	23	59	88	109	36	8	12	47	28	15	49	39
Sr	45	63	66	56	46	9	4	53	5	8	54	10	20
Sb	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.004	0.004	0.005	0.009	0.005	0.007
Cs	0.06	0.02	0.09	0.17	0.16	0.05	0.01	0.01	0.05	0.03	0.03	0.11	0.06
Ba	3.5	6.4	6.8	5.9	5.3	4.2	1.7	8.6	2.9	2.5	7.8	8.4	4.0
La	0.22	-	0.08	0.12	0.13	0.14	0.05		0.07	0.05	-	-	-
Th	0.075	-	0.011	0.017	0.027	0.021	0.006	0.005	0.010	0.013	0.005	0.006	0.008
U	0.03	0.03	0.01	0.01	0.02	0.02	0.01	0.001	0.01	0.02	0.01	0.01	0.01

Table A1.8 Elemental content of analyzed soils collected investigated zones in the Republic of Moldova as well as UCC values and Pedological Limits established for the Republic of Moldova (contents expressed in mg/kg)

Element	Purcari	Cahul	Ialoveni	Criuleni	UCC	PL
Na	6133±429	7885±550	5353±370	5196±360	24259	
Mg	19233±1150	20825±1250	18833±1130	20133±1200	14957	
Al	47300±1980	47175±1880	56366±2255	45000±1800	81505	
Si	252666±25270	330250±33025	282333±28230	248000±28400	313315	
K	15300±920	16825±1010	17766±1070	15933±960	23244	
Ca	29933±2590	22475±1690	20300±2440	21333±2560	25568	
Sc	12±0.36	10.9±0.33	11.8±0.36	11.4±0.34	14	
Ti	6510±520	6517±520	6480±520	5436±435	383	4000-49000*
V	113±6.8	109±6.5	115±7	111±6.7	97	15 – 165
Cr	108±6	106±6	102±6	105±6	92	91
Mn	606±42	730±50	634±45	550±40	774	150 – 2250
Fe	27133±1260	26575±1330	28500±1425	26800±1340	39176	
Co	12.5±0.9	11.2±0.8	12.1±0.8	11.7±0.8	17	1.3-10
Ni	42.5±3.4	41.7±3.3	48±3.8	44±3.5	47	5 – 75
Zn	61±3	82±4	85±4	82±4	67	76 – 91
As	10±0.6	9.4±0.6	9.3±0.6	9.7±0.5	4.8	
Br	13.1±0.5	9.9±0.4	9.8±0.4	9.2±0.4	1.6	
Rb	101±16	96±16	114±18	100±17	84	
Sr	115±9	107±8.6	130±10	130±10	320	50 – 400
Zr	462±70	401±60	263±40	380±58	193	331-445
Mo	0.9±0.3	0.94±0.3	0.93±0.3	0.82±0.3	1.1	0.9 – 4.8
Cd	0.19±0.02	0.16±0.02	0.17±0.02	0.21±0.02	0.09	0.2-0,84
Sb	1.06±0.06	1.1±0.07	1.12±0.07	1.17±0.07	0.4	1-5
Cs	5.2±0.2	4.5±0.2	6.1±0.2	4.9±0.2	4.9	1-14
Ba	449±49	440±48	400±44	435±48	628	140 – 640
La	39±2	35±1.7	30±1.5	35±1.7	31	30 – 60
Ce	74±5±4.6	66.5	56±3.9	65±4.5	63	70-130
Nd	33.9±3.3	31.2±3.1	26.7±2.7	34.5±3.6	27	30-50
Sm	7.4±0.6	6.6±0.5	5.7±0.4	6.3±0.5	4.7	
Eu	1.27±0.11	1.15±0.1	1.2±0.1	1.1±0.1	1	1.6-2.5
Tb	1.03±0.04	0.98±0.04	0.84±0.03	0.91±0.04	0.7	
Dy	7.8±1.6	8.9±1.8	8.2±1.6	7.7±1.5	3.9	
Tm	0.54±0.09	0.45±0.07	0.39±0.06	0.48±0.08	0.3	
Yb	3.7±0.3	3.2±0.3	2.6±0.2	2.9±0.2	2	
Hf	11.6±1.7	9.9±1.5	6.7±1	9.2±1.4	5.3	
Ta	1.26±0.04	1.2±0.03	1.03±0.03	1.08±0.03	0.9	
W	1.64±0.3	1.63±0.3	1.61±0.3	1.5±0.2	1.9	
Th	15.2±0.6	12.15±0.5	11.0±0.4	13.2±0.5	10.5	
U	3.2±0.2	2.9±0.2	2.5±0.2	2.6±0.2	2.7	

Table A1.9 Estimated daily intake of metal (DIM) and potential health hazards (HQ) from fruit

		Element	Co	Fe	Mn	Ni	Zn	As	Sb
Sort		**RfD, mg/day**	**3**	**10 or 60**	**0.5-5.0**	**1.4**	**15**	**1.5**	**0.003**
$\mathrm{P_m}$	Purcari	C mg/kg f.w.	0.04	65.18	6.75	n.d	20.96	n.d	0.01
		DIM, mg/day	0.01	19.55	2.02	n.d	6.29	n.d	0.004
		HQ, mg/kg/day	0.004	0.33	0.40	n.d	0.42	n.d	1.20
$\mathrm{P_f}$		C mg/kg f.w.	0.08	166.67	7.01	n.d	49.08	0.04	0.01
		DIM, mg/day	0.02	50.00	2.10	n.d	14.72	0.01	0.003
		HQ, mg/kg/day	0.01	0.83	0.42	n.d	0.98	0.01	1.15
$\mathrm{P_s}$		C mg/kg f.w.	0.07	136.90	7.86	1.01	18.69	0.09	0.01
		DIM, mg/day	0.02	41.07	2.36	0.30	5.61	0.03	0.004
		HQ, mg/kg/day	0.01	0.68	0.47	0.22	0.37	0.02	1.19
$\mathrm{C_{mh}}$	Cahul	C mg/kg f.w.	0.07	146.59	9.77	n.d	19.32	0.20	0.01
		DIM, mg/day	0.02	43.98	2.93	n.d	5.80	0.06	0.003
		HQ, mg/kg/day	0.01	0.73	0.59	n.d	0.39	0.04	1.14
$\mathrm{C_{md}}$		C mg/kg f.w.	0.09	167.07	7.56	1.14	16.83	0.12	0.01
		DIM, mg/day	0.03	50.12	2.27	0.34	5.05	0.04	0.004
		HQ, mg/kg/day	0.01	0.84	0.45	0.24	0.34	0.02	1.22
$\mathrm{C_a}$		C mg/kg f.w.	0.11	196.25	2.88	n.d	4.13	0.27	0.01
		DIM, mg/day	0.03	58.88	0.86	n.d	1.24	0.08	0.004
		HQ, mg/kg/day	0.01	0.98	0.17	n.d	0.08	0.05	1.25
$\mathrm{C_p}$		C mg/kg f.w.	0.04	57.89	4.47	0.80	17.50	0.06	0.01
		DIM, mg/day	0.01	17.37	1.34	0.24	5.25	0.02	0.004
		HQ, mg/kg/day	0.004	0.29	0.27	0.17	0.35	0.01	1.32
$\mathrm{I_{as}}$	Ialoveni	C mg/kg f.w.	0.04	68.66	4.93	n.d	11.64	0.07	0.01
		DIM, mg/day	0.01	20.60	1.48	n.d	3.49	0.02	0.002
		HQ, mg/kg/day	0.004	0.34	0.30	n.d	0.23	0.01	0.60
$\mathrm{I_{ag}}$		C mg/kg f.w.	0.09	70.11	2.99	0.74	4.60	0.07	0.00
		DIM, mg/day	0.03	21.03	0.90	0.22	1.38	0.02	0.001
		HQ, mg/kg/day	0.01	0.35	0.18	0.16	0.09	0.01	0.46
$\mathrm{I_p}$		C mg/kg f.w.	0.07	63.01	2.60	n.d	16.99	0.08	0.01
		DIM, mg/day	0.02	18.90	0.78	n.d	5.10	0.02	0.002
		HQ, mg/kg/day	0.01	0.32	0.16	n.d	0.34	0.02	0.68
$\mathrm{Cr_{md}}$	Criuleni	C mg/kg f.w.	0.05	51.22	3.90	0.77	14.51	n.d	0.01
		DIM, mg/day	0.01	15.37	1.17	0.23	4.35	n.d	0.003
		HQ, mg/kg/day	0.005	0.26	0.23	0.16	0.29	n.d	1.10
$\mathrm{Cr_a}$		C mg/kg f.w.	0.09	37.93	1.84	n.d	6.78	n.d	0.01
		DIM, mg/day	0.03	11.38	0.55	n.d	2.03	n.d	0.002
		HQ, mg/kg/day	0.01	0.19	0.11	n.d	0.14	n.d	0.57
$\mathrm{Cr_p}$		C mg/kg f.w.	0.07	39.51	2.20	2.87	27.04	n.d	0.01
		DIM, mg/day	0.02	11.85	0.66	0.86	8.11	n.d	0.003
		HQ, mg/kg/day	0.01	0.20	0.13	0.61	0.54	n.d	0.86

Table A1.10 Elemental content of major elements in analysed samples determined by NAA as well as Reference plant data (in µg/g)

Plant	Concentration, µg/g														
	Na			Mg			Cl			K			Ca		
Agastache rugosa	133	±	11	4660	±	280	4550	±	364	16200	±	1296	18600	±	2046
Agastache urticifolia	140	±	11	4490	±	269	6730	±	538	18100	±	1448	18900	±	2079
Ajuga genevensis	104	±	8	3860	±	232	3780	±	302	18000	±	1440	20600	±	2266
Ajuga reptans	122	±	10	2180	±	131	2840	±	227	25600	±	2048	12000	±	1320
Betonica officinalis	70	±	6	2720	±	163	3080	±	246	17600	±	1408	7980	±	877.8
Hyssopus officinalis (f. alba)	55	±	4	4440	±	266	6070	±	486	21100	±	1688	21400	±	2354
Hyssopus officinalis	52	±	4	5480	±	329	4770	±	382	19000	±	1520	21200	±	2332
Hyssopus officinalis	58	±	5	4650	±	279	2420	±	194	16700	±	1336	19400	±	2134
Lamium album	110	±	9	6640	±	398	6590	±	527	44800	±	3584	14700	±	1617
Lavandula angustifolia	139	±	11	3420	±	205	2200	±	176	22500	±	1800	6650	±	731.5
Melissa officinalis ssp. *altissima*	886	±	71	7020	±	421	4520	±	362	16000	±	1280	31000	±	3410
Melissa officinalis	799	±	64	4860	±	292	4990	±	399	10700	±	856	19800	±	2178
Melissa officinalis	260	±	21	4760	±	286	3520	±	282	19300	±	1544	19700	±	2167
Mentha verticillata	1060	±	85	2100	±	126	4320	±	346	16300	±	1304	13600	±	1496
Nepeta pannonica	51	±	4	3590	±	215	1290	±	103	18100	±	1448	12400	±	1364
Nepeta parviflora	98	±	8	7920	±	475	6530	±	522	19100	±	1528	32300	±	3553
Nepeta transcaucasica	113	±	9	4330	±	260	4930	±	394	21700	±	1736	16600	±	1826
Ocimum basilicum	176	±	14	6160	±	370	8150	±	652	27000	±	2160	19000	±	2090
Origanum laevigatum	255	±	20	3620	±	217	3210	±	257	20900	±	1672	17800	±	1958
Origanum tyttanthum	316	±	25	4290	±	257	6800	±	544	28500	±	2280	12500	±	1375
Origanum virens	44.5	±	4	3990	±	239	1200	±	96	24400	±	1952	14800	±	1628
Origanum vulgare	201	±	16	3020	±	181	4770	±	382	23800	±	1904	10400	±	1144
Origanum vulgare	346	±	28	2940	±	176	4490	±	359	22800	±	1824	9630	±	1059.3
Origanum vulgare	60	±	5	4820	±	289	2140	±	171	12100	±	968	13200	±	1452
Origanum vulgare	45	±	4	4850	±	291	813	±	65	23000	±	1840	17800	±	1958
Origanum vulgare	54	±	4	4880	±	293	1050	±	84	24800	±	1984	13600	±	1496
Rosmarinus officinalis	1020	±	82	2260	±	136	5480	±	438	21800	±	1744	7820	±	860.2
Salvia kopetdaghensis	231	±	18	6880	±	413	9780	±	782	30400	±	2432	9860	±	1084.6

Table A1.10 Elemental content of major elements in analysed samples determined by NAA as well as Reference plant data (in µg/g), continuation

Plant	Concentration, µg/g														
	Na			Mg			Cl			K			Ca		
Salvia nemorosa	158	±	13	8200	±	492	12400	±	992	25100	±	2008	15600	±	1716
Salvia officinalis	87	±	7	5500	±	330	539	±	43	22200	±	1776	15700	±	1727
Salvia sclarea	244	±	20	6140	±	368	4180	±	334	19100	±	1528	19400	±	2134
Salvia sclarea	146	±	12	4820	±	289	208	±	17	34200	±	2736	20300	±	2233
Salvia tesquicola	210	±	17	6070	±	364	8370	±	670	23200	±	1856	12600	±	1386
Salvia tesquicola	177	±	14	8710	±	523	8910	±	713	27000	±	2160	13800	±	1518
Salvia verticillata	259	±	21	8010	±	481	9870	±	790	25000	±	2000	24000	±	2640
Satureja kitaibellii	83	±	7	5290	±	317	7190	±	575	15800	±	1264	25300	±	2783
Satureja parnassica	197	±	16	4850	±	291	3410	±	273	18400	±	1472	18800	±	2068
Satureja parnasica	177	±	14	6380	±	383	4390	±	351	22100	±	1768	18400	±	2024
Satureja subspicata	245	±	20	5590	±	335	6480	±	518	15000	±	1200	23800	±	2618
Scutellaria albida	156	±	12	6380	±	383	227	±	18	29200	±	2336	22800	±	2508
Scutellaria altissima	47	±	4	2290	±	137	2700	±	216	24100	±	1928	11000	±	1210
Scutellaria baicalensis	55	±	4	4150	±	249	2440	±	195	13500	±	1080	14600	±	1606
Thymus calcareus	73	±	6	3810	±	229	325	±	26	14200	±	1136	15800	±	1738
Thymus citriodorus ’Aureus’	396	±	32	6030	±	362	2910	±	233	27400	±	2192	17700	±	1947
Thymus citriodorus	144	±	12	3820	±	229	1620	±	130	20800	±	1664	15900	±	1749
Thymus commosus	266	±	21	3480	±	209	3500	±	280	19600	±	1568	12000	±	1320
Thymus commosus	89	±	7	3570	±	214	2010	±	161	19900	±	1592	13300	±	1463
Thymus ellipticus	237	±	19	3640	±	218	2090	±	167	21600	±	1728	14600	±	1606
Thymus marschallianus	75	±	6	6040	±	362	374	±	30	15200	±	1216	21700	±	2387
Thymus moldavicus	84	±	7	5300	±	318	333	±	27	17000	±	1360	19700	±	2167
Thymus vulgare	215	±	17	4710	±	283	721	±	58	24600	±	1968	16400	±	1804
Mentha x piperita citrata	5530	±	220	2770	±	140	1750	±	140	9720	±	875	16400	±	2460
Mentha x gracilis’Variegata’	3810	±	115	4320	±	215	12800	±	1025	15400	±	1385	19100	±	2865
RP	**150**			**200**			**2000**			**19000**			**10000**		

Table A1.11 Elemental content of essential elements in analysed samples determined by NAA as well as Reference plant data (in µg/g)

Plant	Concentration, µg/g																				
	Cr			Mn			Fe			Co			Zn			Br			Mo		
Agastache rugosa				109	±	4.4	215	±	2.2	0.39	±	0.039	56	±	2.2	58	±	2.3	0.35	±	0.04
Agastache urticifolia				106	±	4.2	405	±	4.1	0.38	±	0.038	75	±	3.0	34	±	1.3	0.8	±	0.09
Ajuga genevensis	3.9	±	0.46	112	±	4.5	520	±	5.2	0.38	±	0.038	32	±	1.3	18	±	0.7	1.7	±	0.19
Ajuga reptans				35.5	±	1.4	171	±	1.7	0.15	±	0.015	23	±	0.9	5	±	0.2	0.21	±	0.02
Betonica officinalis				98	±	3.9	158	±	1.6	0.34	±	0.034	25	±	1.0	9	±	0.4	0.28	±	0.03
Hyssopus officinalis (f. alba)				86	±	3.4	95	±	1.0	0.13	±	0.013	28	±	1.1	24	±	0.9	0.47	±	0.05
Hyssopus officinalis				77	±	3.1	121	±	1.2	0.11	±	0.011	28	±	1.1	28	±	1.1	0.34	±	0.04
Hyssopus officinalis				78	±	3.1	134	±	1.3	0.12	±	0.012	43	±	1.7	27	±	1.1	1.48	±	0.16
Lamium album				107	±	4.3	303	±	3.0	0.17	±	0.017	25	±	1.0	9	±	0.4	0.74	±	0.08
Lavandula angustifolia				12.7	±	0.5	118	±	1.2	0.14	±	0.014	19	±	0.8	27	±	1.1	0.74	±	0.08
Melissa officinalis ssp. *altissima*				108	±	4.3	121	±	1.2	0.13	±	0.013	18	±	0.7	16	±	0.6		±	
Melissa officinalis				86	±	3.4	212	±	2.1	0.17	±	0.017	14	±	0.6	22	±	0.9	0.6	±	0.07
Melissa officinalis				83	±	3.3	296	±	3.0	0.22	±	0.022	35	±	1.4	28	±	1.1			
Mentha verticillata				72	±	2.9	421	±	4.2	0.37	±	0.037	30	±	1.2	23	±	0.9	0.25		0.03
Nepeta pannonica				45	±	1.8	142	±	1.4	0.15	±	0.015	15	±	0.6	33	±	1.3	0.53	±	0.06
Nepeta parviflora				24	±	1.0	67	±	0.7	0.03	±	0.003	29	±	1.1	9	±	0.4	1.17	±	0.13
Nepeta transcaucasica				97	±	3.9	321	±	3.2	0.17	±	0.017	29	±	1.2	31	±	1.2	0.81	±	0.09
Ocimum basilicum				123	±	4.9	467	±	4.7	0.53	±	0.053	31	±	1.2	85	±	3.4	0.31	±	0.03
Origanum laevigatum				103	±	4.1	389	±	3.9	0.24	±	0.024	39	±	1.6	46	±	1.8			
Origanum tyttanthum				95	±	3.8	263	±	2.6	0.24	±	0.024	40	±	1.6	85	±	3.4			
Origanum virens				68	±	2.7	112	±	1.1	0.12	±	0.012	39	±	1.6	46	±	1.8			
Origanum vulgare	3.9	±	0.47	81	±	3.2	497	±	5.0	0.29	±	0.029	37	±	1.5	59	±	2.4	0.51	±	0.06
Origanum vulgare				71	±	2.8	493	±	4.9	0.38	±	0.038	33	±	1.3	60	±	2.4			
Origanum vulgare				33	±	1.3	86	±	0.9	0.13	±	0.013	28	±	1.1	8	±	0.3			
Origanum vulgare				88	±	3.5	126	±	1.3	0.17	±	0.017	35	±	1.4	36	±	1.4			
Origanum vulgare				52	±	2.1	97	±	1.0	0.15	±	0.015	26	±	1.1	24	±	1.0			

Table A1.11 Elemental content of essential elements in analysed samples determined by NAA as well as Reference plant data (in µg/g), continuation

Plant	Concentration, µg/g																				
	Cr			Mn			Fe			Co			Zn			Br			Mo		
Salvia nemorosa				127	±	5.1	542	±	5.4	0.35	±	0.035	37	±	1.5	27	±	1.1	0.49	±	0.05
Salvia officinalis				105	±	4.2	209	±	2.1	0.13	±	0.013	25	±	1.0	5	±	0.2			
Salvia sclarea				99	±	4.0	713	±	7.1	0.41	±	0.041	27	±	1.1	35	±	1.4	0.7	±	0.08
Salvia sclarea				101	±	4.0	191	±	1.9	0.18	±	0.018	32	±	1.3	10	±	0.4			
Salvia tesquicola				95	±	3.8	494	±	4.9	0.3	±	0.030	26	±	1.1	41	±	1.6	0.32	±	0.04
Salvia tesquicola				124	±	5.0	184	±	1.8	0.10	±	0.010	28	±	1.1	29	±	1.2			
Salvia verticulata				194	±	7.8	510	±	5.1	0.29	±	0.029	28	±	1.1	37	±	1.5			
Satureja kitaibelli				70	±	2.8	184	±	1.8	0.20	±	0.020	36	±	1.4	34	±	1.4			
Satureja montana				85	±	3.4	208	±	2.1	0.21	±	0.021	28	±	1.1	17	±	0.7			
Satureja parnassica				72	±	2.9	454	±	4.5	0.3	±	0.030	28	±	1.1	40	±	1.6			
Satureja parnassica				97	±	3.9	479	±	4.8	0.36	±	0.036	35	±	1.4	50	±	2.0			
Satureja subspicata				117	±	4.7	782	±	7.8	0.36	±	0.036	25	±	1.0	62	±	2.5			
Scutellaria albida				106	±	4.2	117	±	1.2	0.11	±	0.011	26	±	1.1	9	±	0.4	1.22	±	0.13
Scutellaria altissima				32.6	±	1.3	129	±	1.3	0.15	±	0.015	21	±	0.9	6	±	0.2	0.56	±	0.06
Scutellaria baicalensis				89	±	3.6	110	±	1.1	0.11	±	0.011	13	±	0.5	34	±	1.3	0.31	±	0.03
Thymus calcareus				47	±	1.9	211	±	2.1	0.13	±	0.013	33	±	1.3	2	±	0.1			
Thymus citriodorus ’Aureus’				163	±	6.5	1910	±	19.1	0.88	±	0.088	44	±	1.8	35	±	1.4			
Thymus citriodorus				123	±	4.9	653	±	6.5	0.31	±	0.031	29	±	1.2	12	±	0.5			
Thymus commosus	9.3	±	1.40	125	±	5.0	1230	±	12.3	0.64	±	0.064	39	±	1.6	53	±	2.1	1.2	±	0.13
Thymus commosus				100	±	4.0	191	±	1.9	0.17	±	0.017	29	±	1.2	57	±	2.3			
Thymus ellipticus				101	±	4.0	888	±	8.9	0.43	±	0.043	35	±	1.4	63	±	2.5			
Thymus marschallianus				78	±	3.1	182	±	1.8	0.11	±	0.011	42	±	1.7	2	±	0.1			
Thymus moldavicus				68	±	2.7	184	±	1.8	0.12	±	0.012	40	±	1.6	3	±	0.1			
Thymus vulgare	2.47	±	0.37	174	±	7.0	675	±	6.8	0.37	±	0.037	36	±	1.4	25	±	1.0			
Mentha x piperita citrata				81	±	2.4	297	±	21	0.12	±	0.013	28	±	1.6	9	±	0.3			
Mentha x gracilis’Variegata’	7.1	±	0.7	136	±	4	1110	±	66	0.53	±	0.026	46	±	2.7	34	±	1.4			
RP	**1.5**			**200**			**150**			**0.2**			**50**			**4.0**			**0.5**		

Table A1.12 Elemental content of Al, As, Rb, Sr, Sb, Cs, Ba, Hf, Th, and U in analysed samples determined by NAA as well as RP data (in µg/g)

Plant	**Concentration**																													
	Al			**As**			**Rb**			**Sr**			**Sb**			**Cs**			**Ba**			**Hf**			**Th**			**U**		
Agastache rugosa	372	±	15	0.65	±	0.03	7.3	±	1.10	49	±	4.9	0.05	±	0.005	0.05	±	0.005	28	±	2.8	0.06	±	0.007	0.07	±	0.003		±	
Agastache urticifolia	656	±	26	0.73	±	0.03	5.9	±	0.89	62	±	6.2	0.05	±	0.005	0.07	±	0.007	39	±	3.9	0.14	±	0.016	0.23	±	0.011	0.06	±	0.005
Ajuga genevensis	1090	±	44	0.97	±	0.04	8	±	1.20	63	±	6.3	0.05	±	0.005	0.08	±	0.008	66	±	6.6	0.07	±	0.009	0.23	±	0.012	0.04	±	0.003
Ajuga reptans	314	±	13	1.07	±	0.04	2.84	±	0.43	48	±	4.8	0.03	±	0.003	0.03	±	0.003	13	±	1.3	0.09	±	0.011	0.08	±	0.004	0.02	±	0.001
Betonica officinalis	222	±	9	0.97	±	0.04	18.2	±	2.73	23.6	±	2.36	0.02	±	0.002	0.09	±	0.009	10	±	1.0	0.10	±	0.011	0.10	±	0.005	0.02	±	0.002
Hyssopus officinalis (f.	180	±	7	0.54	±	0.02	4.45	±	0.67	64	±	6.4	0.02	±	0.002	0.02	±	0.002	13	±	1.3	0.01	±	0.001	0.02	±	0.001		±	
Hyssopus officinalis	167	±	7	0.38	±	0.02	6	±	0.90	88	±	8.8	0.04	±	0.004	0.03	±	0.003	42	±	4.2	0.02	±	0.003	0.03	±	0.001		±	
Hyssopus officinalis	216	±	9	0.56	±	0.02	8.4	±	1.26	56	±	5.6	0.04	±	0.004	0.03	±	0.003	19	±	1.9		±		0.03	±	0.002		±	
Lamium album	437	±	17	1.1	±	0.04	5.5	±	0.83	39	±	3.9	0.04	±	0.004	0.03	±	0.003	38	±	3.8	0.16	±	0.020	0.13	±	0.007	0.03	±	0.004
Lavandula angustifolia	157	±	6	0.9	±	0.04	13	±	1.95	28	±	2.8	0.03	±	0.003	0.02	±	0.002	13	±	1.3	0.03	±	0.004	0.04	±	0.002		±	
Melissa officinalis ssp.	173	±	7	0.13	±	0.01	7.5	±	1.13	100	±	10	0.03	±	0.003	0.033	±	0.003	31	±	3.1	0.04	±	0.005	0.03	±	0.001	0.01	±	0.002
Melissa officinalis	287	±	11	0.86	±	0.03	5	±	0.75	112	±	11.2	0.05	±	0.005	0.04	±	0.004	34	±	3.4	0.05	±	0.006	0.06	±	0.003	0.02	±	0.002
Melissa officinalis	437	±	17	0.18	±	0.01	5.1	±	0.77	60	±	6	0.07	±	0.007	0.05	±	0.005	67	±	6.7	0.11	±	0.013	0.10	±	0.005	0.03	±	0.003
Mentha verticillata	766	±	31	0.69	±	0.03	9	±	1.35	50	±	5	0.03	±	0.003	0.08	±	0.008	29	±	2.9	0.17	±	0.020	0.22	±	0.011	0.04	±	0.004
Nepeta pannonica	132	±	5	0.41	±	0.02	11.7	±	1.76	32	±	3.2	0.05	±	0.005	0.02	±	0.002	15	±	1.5	0.02	±	0.003	0.02	±	0.001		±	
Nepeta parviflora	107	±	4	0.88	±	0.04	10.5	±	1.58	176	±	17.6	0.02	±	0.002	0.02	±	0.002	54	±	5.4	0.01	±	0.002	0.02	±	0.001	0.01	±	0.001
Nepeta transcaucasica	532	±	21	0.9	±	0.04	7.3	±	1.10	64	±	6.4	0.04	±	0.004	0.06	±	0.006	79	±	7.9	0.14	±	0.017	0.13	±	0.006	0.03	±	0.003
Ocimum basilicum	804	±	32	1.32	±	0.05	10.6	±	1.59	58	±	5.8	0.04	±	0.004	0.07	±	0.007	25	±	2.5	0.25	±	0.030	0.26	±	0.013		±	
Origanum laevigatum	698	±	28	0.44	±	0.02	9.2	±	1.38	60	±	6	0.02	±	0.002	0.06	±	0.006	32	±	3.2	0.03	±	0.004	0.13	±	0.007	0.04	±	0.004
Origanum tyttanthum	489	±	20	0.34	±	0.01	9.2	±	1.38	36.6	±	3.66	0.03	±	0.003	0.05	±	0.005	13	±	1.3	0.11	±	0.013	0.11	±	0.005		±	
Origanum virens	86	±	3	0.26	±	0.01	9.1	±	1.37	49	±	4.9	0.01	±	0.001	0.01	±	0.001	23	±	2.3	0.02	±	0.003	0.01	±	0.000	0.01	±	0.001
Origanum vulgare	925	±	37	1.16	±	0.05	7.4	±	1.11	36	±	3.6	0.03	±	0.003	0.08	±	0.008	26	±	2.6	0.18	±	0.022	0.29	±	0.014	0.05	±	0.005
Origanum vulgare	926	±	37	0.44	±	0.02	12	±	1.80	32	±	3.2	0.04	±	0.004	0.10	±	0.010	21	±	2.1	0.09	±	0.011	0.23	±	0.011	0.04	±	0.004
Origanum vulgare	136	±	5	0.31	±	0.01	11.3	±	1.70	99	±	9.9	0.02	±	0.002	0.06	±	0.006	22	±	2.2	0.02	±	0.002	0.03	±	0.002	0.02	±	0.002
Origanum vulgare	105	±	4	0.36	±	0.01	13.4	±	2.01	36	±	3.6	0.02	±	0.002	0.03	±	0.003	1	±	0.1		±		0.02	±	0.001		±	
Origanum vulgare	155	±	6	0.41	±	0.02	10.6	±	1.59	46	±	4.6	0.03	±	0.003	0.03	±	0.003	16	±	1.6		±		0.03	±	0.001	0.01	±	0.001

Table A1.12 Elemental content of Al, As, Rb, Sr, Sb, Cs, Ba, Hf, Th, and U in analysed samples determined by NAA as well as RP data (in µg/g), continuation

Plant	**Concentration**																													
		Al			**As**			**Rb**			**Sr**			**Sb**			**Cs**			**Ba**			**Hf**			**Th**			**U**	
Salvia nemorosa	1270	±	51	0.86	±	0.03	16.3	±	2.45	41	±	4.1	0.03	±	0.003	0.10	±	0.010	40	±	4.0	0.16	±	0.020	0.19	±	0.010	0.05	±	0.005
Salvia officinalis	225	±	9	0.27	±	0.01	13	±	1.95	41	±	4.1	0.02	±	0.002	0.02	±	0.002	20	±	2.0	0.05	±	0.005	0.05	±	0.002		±	
Salvia sclarea	2100	±	84	0.51	±	0.02	11.3	±	1.70	55	±	5.5	0.04	±	0.004	0.11	±	0.011	49	±	4.9	0.38	±	0.045	0.31	±	0.016	0.07	±	0.007
Salvia sclarea	152	±	6	0.31	±	0.01	31	±	4.65	41	±	4.1	0.02	±	0.002	0.05	±	0.005	19	±	1.9	0.03	±	0.004	0.03	±	0.001		±	
Salvia tesquicola	752	±	30	0.74	±	0.03	13	±	1.95	45	±	4.5	0.04	±	0.004	0.08	±	0.008	38	±	3.8	0.16	±	0.020	0.14	±	0.007	0.04	±	0.004
Salvia tesquicola	245	±	10	0.25	±	0.01	25	±	3.75	44	±	4.4	0.03	±	0.003	0.05	±	0.005	15	±	1.5	0.06	±	0.007	0.10	±	0.005		±	
Salvia verticillata	1110	±	44	0.46	±	0.02	12.7	±	1.91	57	±	5.7	0.03	±	0.003	0.08	±	0.008	33	±	3.3	0.14	±	0.017	0.19	±	0.010		±	
Satureja kitaibellii	247	±	10	0.38	±	0.02	9.4	±	1.41	89	±	8.9	0.02	±	0.002	0.03	±	0.003	65	±	6.5	0.05	±	0.006	0.05	±	0.003		±	
Satureja montana	332	±	13	0.55	±	0.02	12.5	±	1.88	63	±	6.3	0.03	±	0.003	0.05	±	0.005	47	±	4.7	0.10	±	0.012	0.11	±	0.005		±	
Satureja parnassica	924	±	37	2.34	±	0.09	12.7	±	1.91	58	±	5.8	0.04	±	0.004	0.13	±	0.013	36	±	3.6	0.17	±	0.020	0.39	±	0.020	0.07	±	0.007
Satureja parnassica	900	±	36	0.49	±	0.02	11	±	1.65	66	±	6.6	0.02	±	0.002	0.09	±	0.009	71	±	7.1	0.09	±	0.011	0.15	±	0.008	0.03	±	0.003
Scutellaria albida	196	±	8	0.39	±	0.02	20.6	±	3.09	68	±	6.8	0.03	±	0.003	0.04	±	0.004	35	±	3.5	0.03	±	0.003	0.03	±	0.002		±	
Scutellaria altissima	222	±	9	0.36	±	0.01	4.3	±	0.65	44	±	4.4	0.01	±	0.001	0.03	±	0.003	26	±	2.6	0.04	±	0.004	0.03	±	0.002	0.01	±	0.001
Scutellaria baicalensis	236	±	9	0.91	±	0.04	9	±	1.35	44	±	4.4	0.02	±	0.002	0.04	±	0.004	21	±	2.1	0.05	±	0.006	0.03	±	0.001		±	
Thymus calcareus	269	±	11	0.56	±	0.02	3.9	±	0.59	429	±	42.9	0.02	±	0.002	0.03	±	0.003	119	±	11.9	0.02	±	0.002	0.06	±	0.003	0.04	±	0.004
Thymus citriodorus	3500	±	140	1.04	±	0.04	9.4	±	1.41	51	±	5.1	0.08	±	0.008	0.29	±	0.029	74	±	7.4	0.45	±	0.054	0.75	±	0.038	0.16	±	0.016
Thymus citriodorus	981	±	39	0.5	±	0.02	4.6	±	0.69	38	±	3.8	0.04	±	0.004	0.10	±	0.010	58	±	5.8	0.17	±	0.020	0.26	±	0.013	0.05	±	0.005
Thymus commosus	2350	±	94	1.23	±	0.05	9.5	±	1.43	46	±	4.6	0.05	±	0.005	0.22	±	0.022	70	±	7.0	0.50	±	0.060	0.59	±	0.030	0.14	±	0.014
Thymus commosus	404	±	16	0.44	±	0.02	10.7	±	1.61	39	±	3.9	0.03	±	0.003	0.03	±	0.003	50	±	5.0	0.11	±	0.013	0.12	±	0.006	0.02	±	0.002
Thymus ellipticus	1610	±	64	0.52	±	0.02	6.7	±	1.01	50	±	5	0.04	±	0.004	0.13	±	0.013	96	±	9.6	0.27	±	0.032	0.36	±	0.018	0.10	±	0.010
Thymus marschallianus	334	±	13	0.69	±	0.03	4.3	±	0.65	390	±	39	0.01	±	0.001	0.02	±	0.002	92	±	9.2	0.02	±	0.002	0.05	±	0.003	0.05	±	0.005
Thymus moldavicus	305	±	12	0.84	±	0.03	4.2	±	0.63	391	±	39.1	0.01	±	0.001	0.03	±	0.003	98	±	9.8	0.06	±	0.007	0.06	±	0.003	0.05	±	0.005
Thymus vulgare	988	±	40	0.82	±	0.03	13.5	±	2.03	58	±	5.8	0.04	±	0.004	0.10	±	0.010	71	±	7.1	0.15	±	0.018	0.21	±	0.010	0.06	±	0.006
Mentha x piperita citrata	456	±	5	0.12	±	10	6	±	17	67	±	8	0.11	±	7	0.05	±	7	17	±	6	0.07	±	11	0.09	±	5	0.02	±	11
Mentha x	1900	±	5	0.38	±	9	6.7	±	17	105	±	8	0.20	±	7	0.18	±	4	62	±	5	0.40	±	6	0.43	±	4	0.10	±	6
RP	**80**			**0.1**			**50**			**50**			**0.1**			**0.2**			**40**			**0.05**			**0.005**			**0.01**		

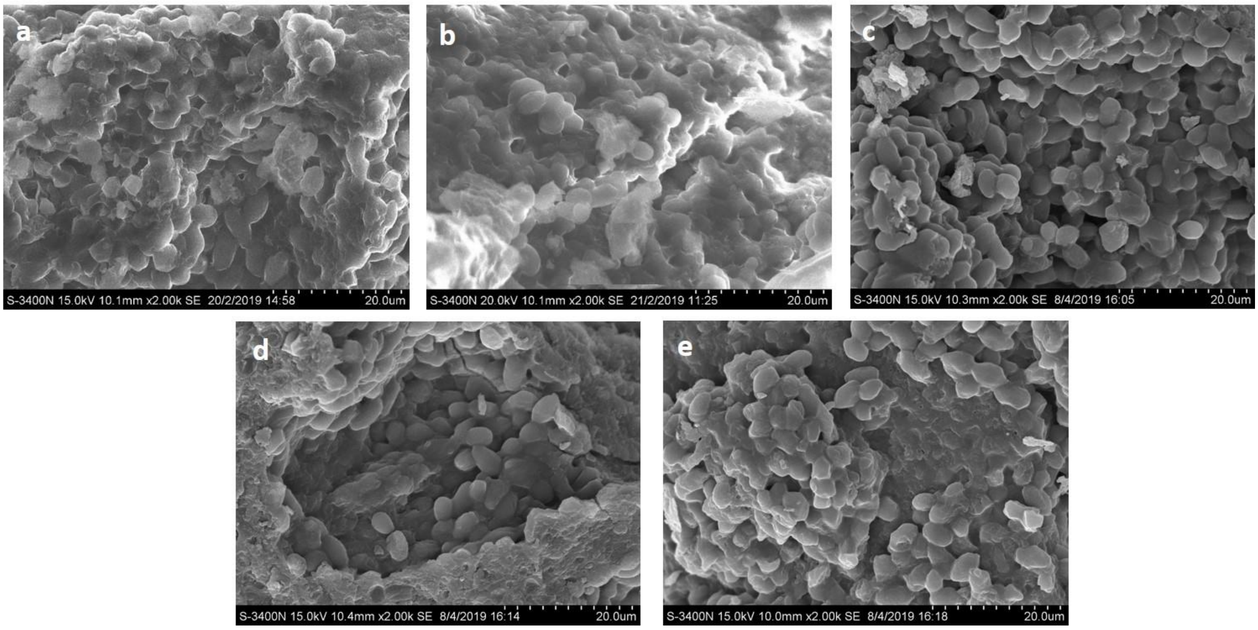


Fig. A1.1 The SEM images of *S. cerevisiae*: a) control, b) system Cr(VI)-Fe(III), c) system Cr(VI)-Fe(III)-Ni(II, d) system Cr(VI)-Fe(III)-Ni(II)-Zn(II) and e) system Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II)

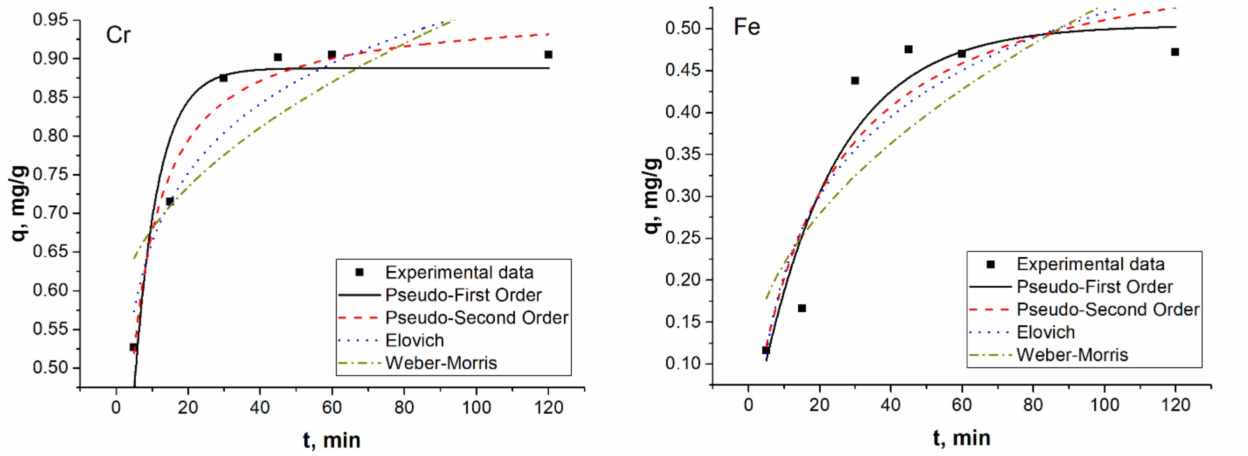


Fig. A1.2 Kinetics of the metal adsorption using *S. cerevisiae* in Cr(VI)-Fe(III) system

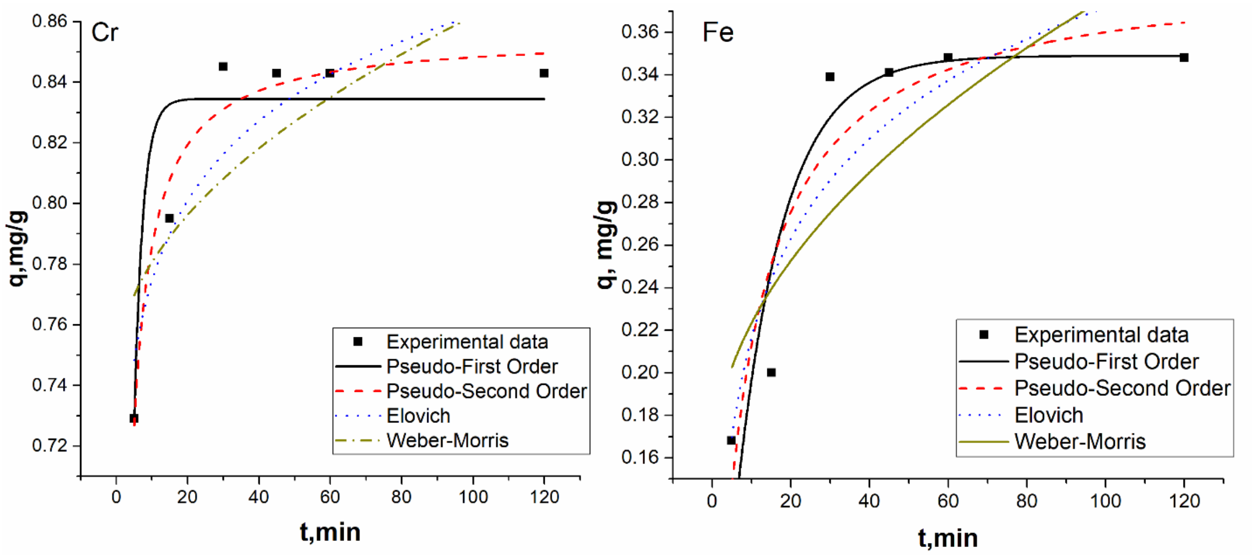


Fig. A1.3 Kinetics of the metal adsorption using *S. cerevisiae* in Cr(VI)-Fe(III)-Ni(II) system

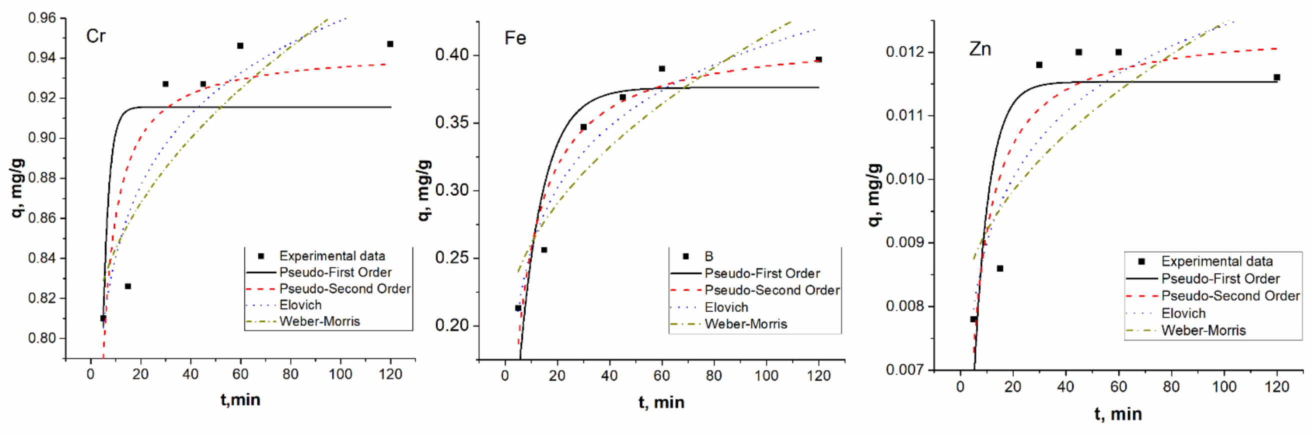


Fig. A1.3 Kinetics of the metal adsorption using *S. cerevisiae* in Cr(VI)-Fe(III)-Ni(II)–Zn(II) system

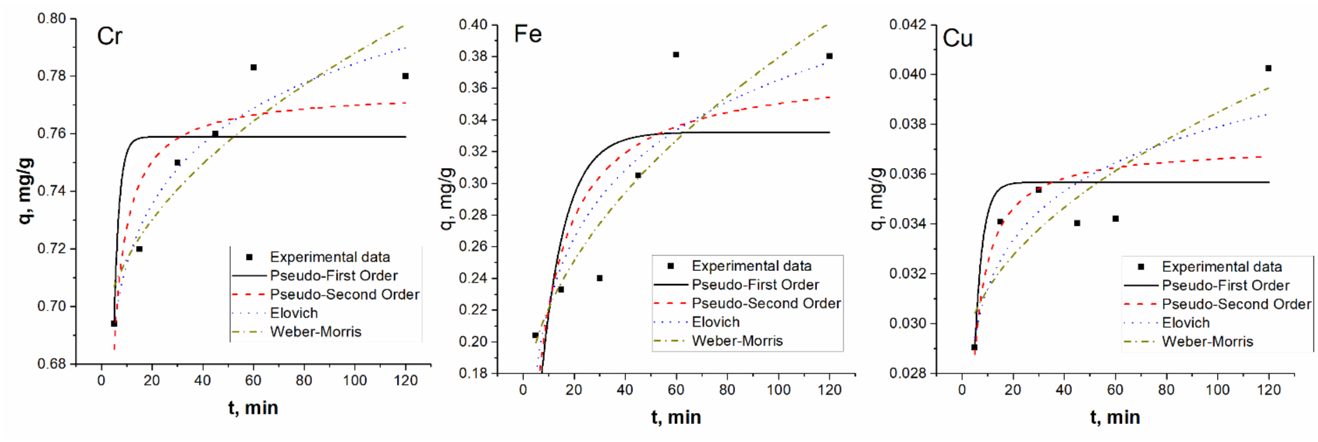


Fig. A1.4 Kinetics of the metal adsorption using *S. cerevisiae* in Cr(VI)-Fe(III)-Ni(II)–Zn(II)-Cu(II) system

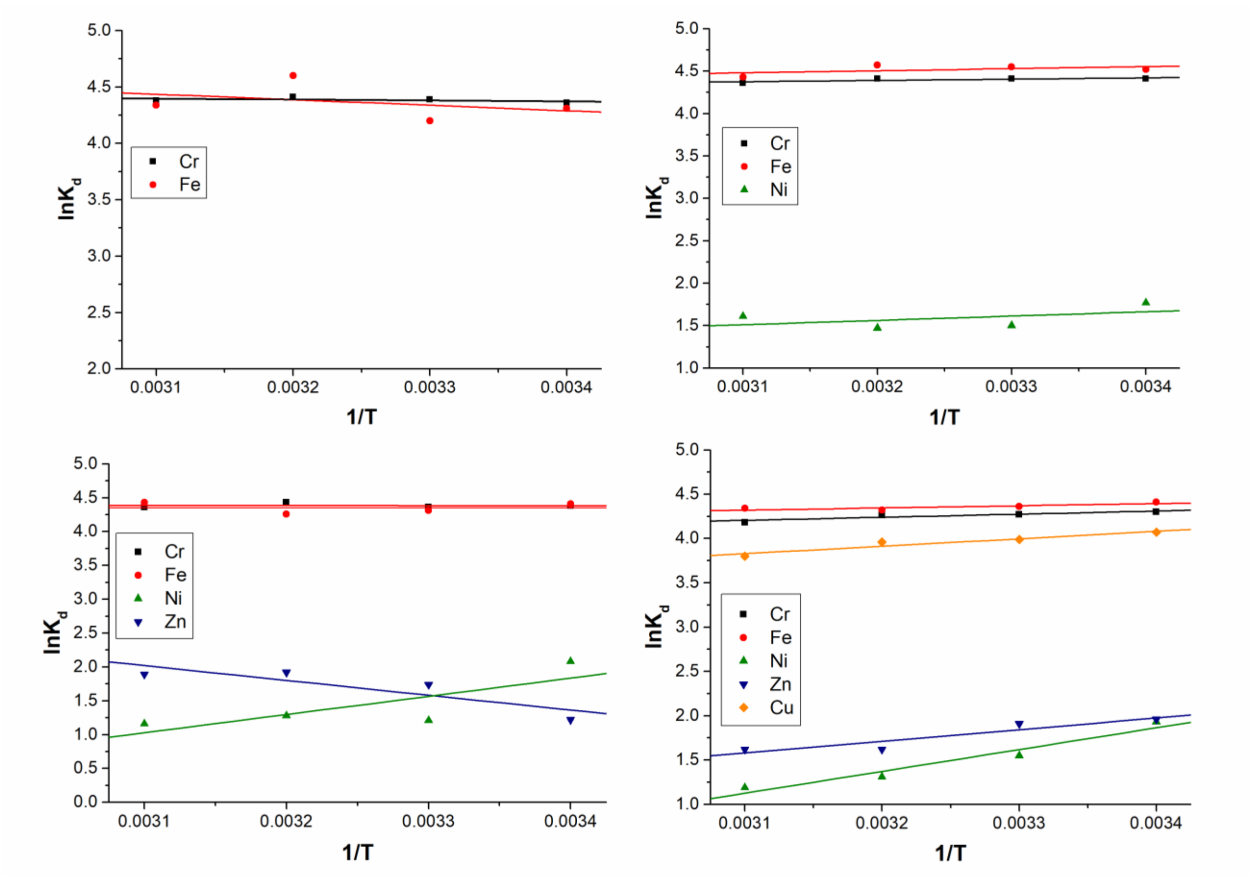


Fig. A1.5 lnK$_d$ versus 1/T

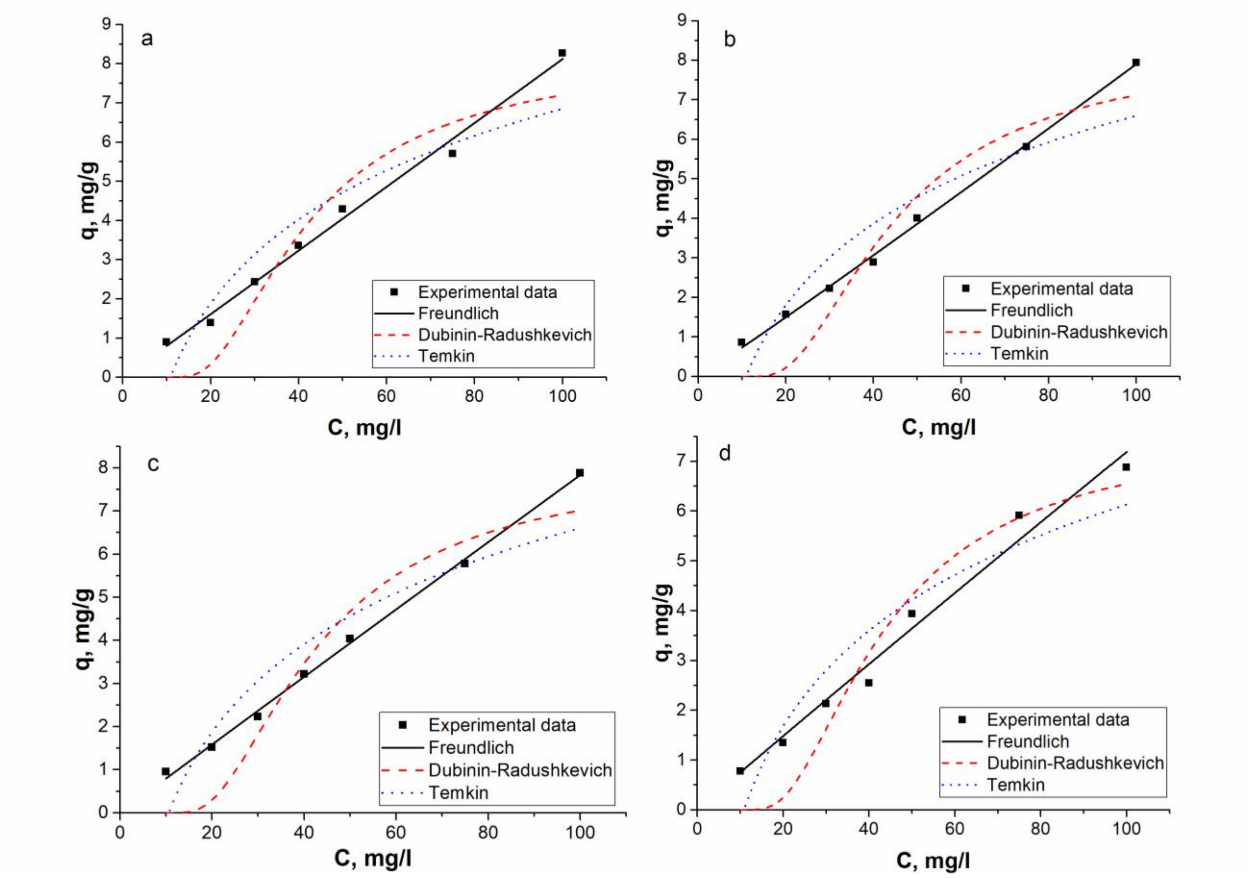


Fig. A1.6 The adsorption isotherms: a) Cr(VI)-Fe(III), b) Cr(VI)-Fe(III)-Ni(II), c) Cr(VI)-Fe(III)-Ni(II)-Zn(II) and d) Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) systems

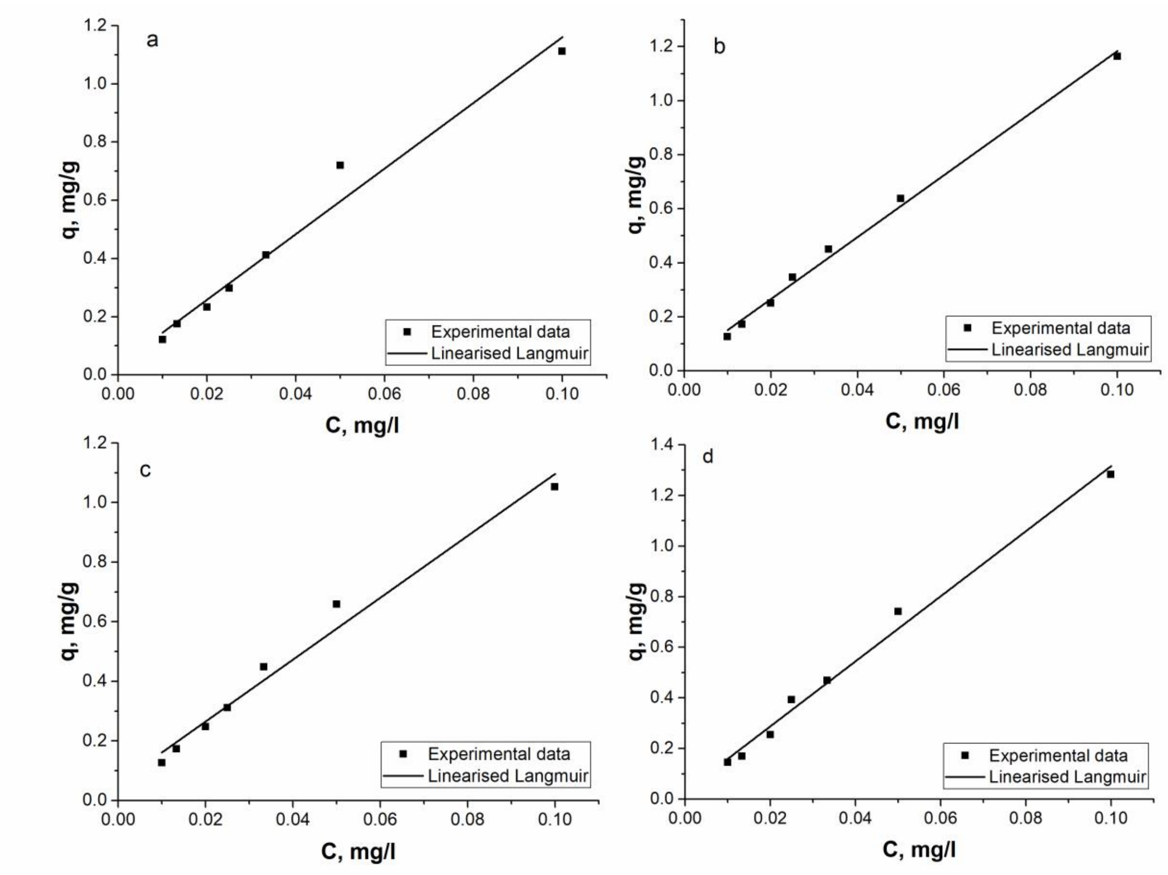


Fig. A1.7 The Langmuir adsorption isotherm: a) Cr(VI)-Fe(III), b) Cr(VI)-Fe(III)-Ni(II), c) Cr(VI)-Fe(III)-Ni(II)-Zn(II) and d) Cr(VI)-Fe(III)-Ni(II)-Zn(II)-Cu(II) systems

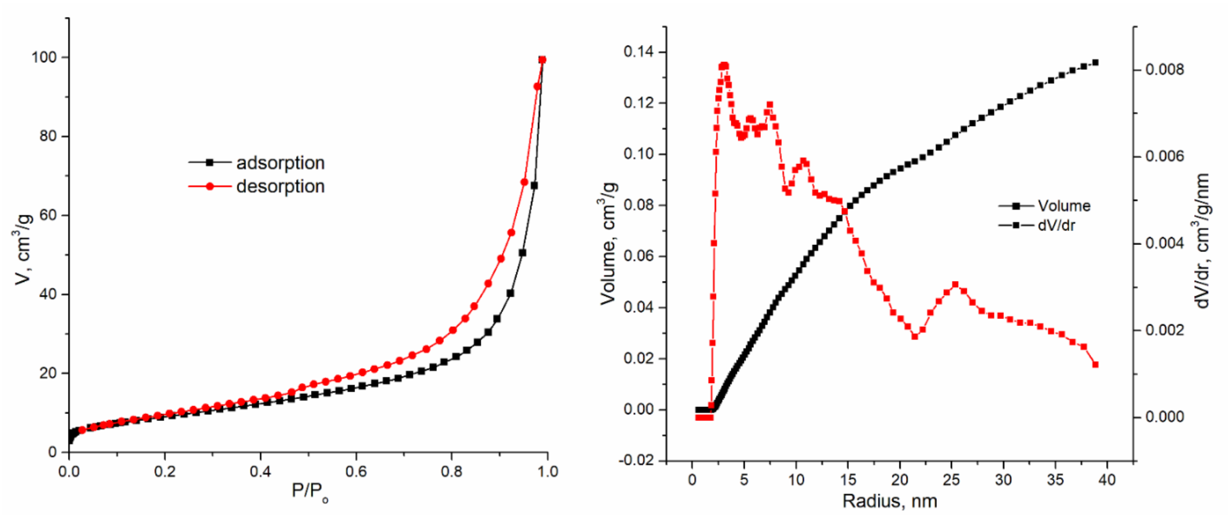


Fig. A1.8 Left: Adsorption-desorption isotherms of nitrogen at 77 k on bio-zeolite; right: Pore size distribution for bio- zeolite

Annex 2

Acts of implementation

Association of Aromatic and Medicinal Plant
Growers of Republic of Moldova

Republic of Moldova, MD 2004, 180, Stefan cel
Mare, Chisinau municipality, str, of. 915

AROMEDA

Asociaţia Cultivatorilor de Plante Aromatica
şi Medicinale din Republica Moldova

Republica Moldova, mun. Chişinău, str. Stefan cel
Mare. 180. Bir 915

Nr. 012/C
Din 02 aprilie 2021

ACT DE IMPLEMENTARE A REZULTATELOR ŞTIINŢIFICE

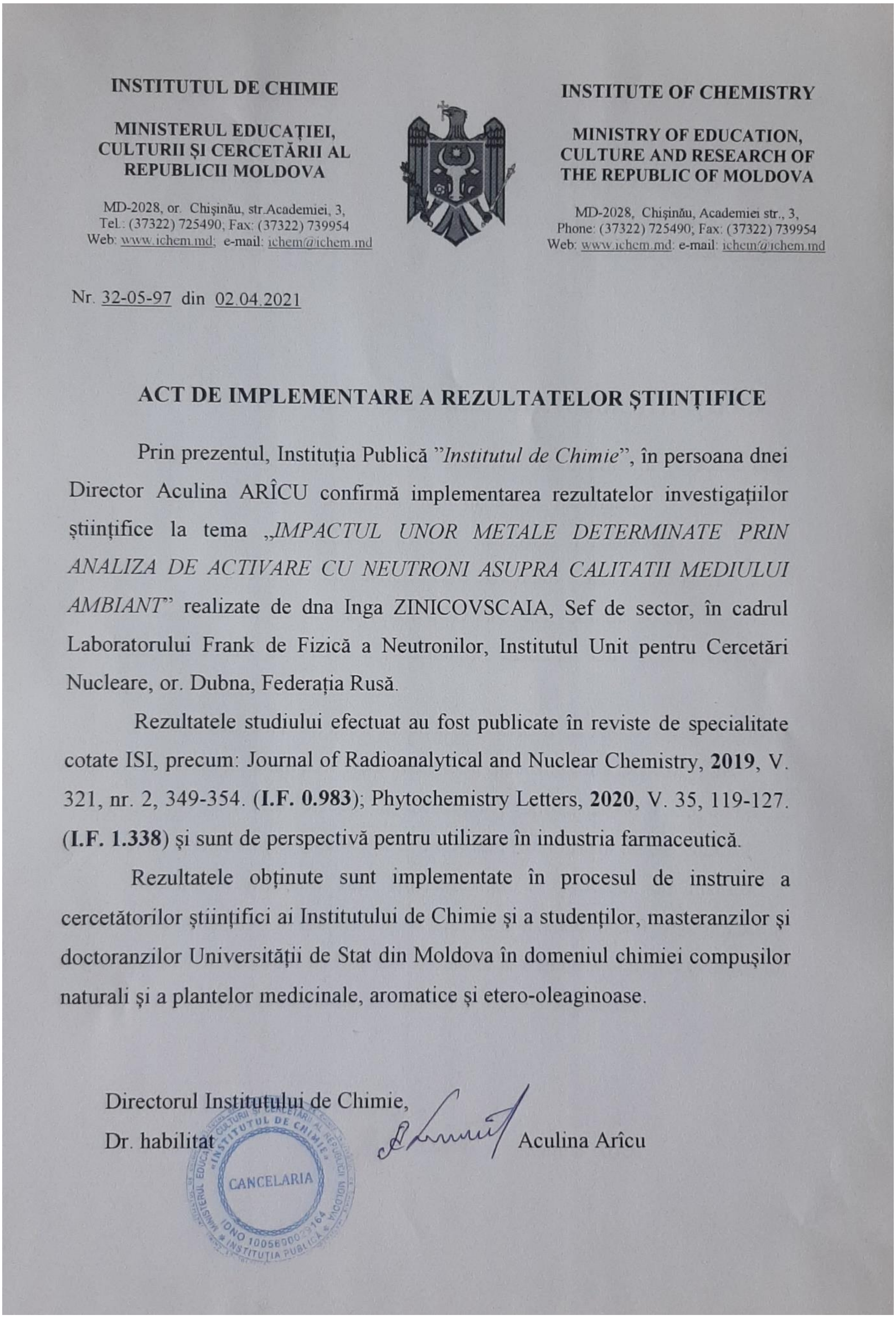
Prin prezentul, Asociaţia Cultivatorilor de Plante Aromatice şi Medicinale din Republica Moldova "AROMEDA", în persoana dlui Preşedinte Victor Melnic confirmă implementarea rezultatelor investigaţiilor ştiinţifice la tema „IMPACTUL UNOR METALE DETERMINATE PRIN ANALIZA DE ACTIVARE CU NEUTRONI ASUPRA CALITATII MEDIULUI AMBIANT" realizate de dna Inga ZINICOVSCAIA, Sef de sector, în cadrul Laboratorului Frank de Fizică a Neutronilor, Institutul Unit pentru Cercetări Nucleare, or. Dubna, Federaţia Rusă.

Rezultatele studiului efectuat pot fi implementate în procesul de instruire a producătorilor locali de plante medicinale, aromatice şi etero-oleaginoase.

Preşedintele Asociaţiei,
Dr. în biologie

Victor Melnic

02.04.2021



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Nr. 32-05-97 din 02.04.2021

ACT DE IMPLEMENTARE A REZULTATELOR ȘTIINȚIFICE

Prin prezentul, Instituția Publică "*Institutul de Chimie*", în persoana dnei Director Aculina ARÎCU confirmă implementarea rezultatelor investigațiilor științifice la tema „*IMPACTUL UNOR METALE DETERMINATE PRIN ANALIZA DE ACTIVARE CU NEUTRONI ASUPRA CALITATII MEDIULUI AMBIANT*" realizate de dna Inga ZINICOVSCAIA, Sef de sector, în cadrul Laboratorului Frank de Fizică a Neutronilor, Institutul Unit pentru Cercetări Nucleare, or. Dubna, Federația Rusă.

Rezultatele studiului efectuat au fost publicate în reviste de specialitate cotate ISI, precum: Journal of Radioanalytical and Nuclear Chemistry, **2019**, V. 321, nr. 2, 349-354. (**I.F. 0.983**); Phytochemistry Letters, **2020**, V. 35, 119-127. (**I.F. 1.338**) și sunt de perspectivă pentru utilizare în industria farmaceutică.

Rezultatele obținute sunt implementate în procesul de instruire a cercetătorilor științifici ai Institutului de Chimie și a studenților, masteranzilor și doctoranzilor Universității de Stat din Moldova în domeniul chimiei compușilor naturali și a plantelor medicinale, aromatice și etero-oleaginoase.

Directorul Institutului de Chimie,
Dr. habilitat Aculina Arîcu

CANCELARIA
IDNO 1005600029164

AGENŢIA NAŢIONALĂ
PENTRU SIGURANŢA
ALIMENTELOR
I.P. „LABORATORUL CENTRAL
DE TESTARE A BĂUTURILOR
ALCOOLICE/NEALCOOLICE
ŞI A PRODUSELOR CONSERVATE”

НАЦИОНАЛЬНОЕ АГЕНТСТВО
ПО БЕЗОПАСНОСТИ ПИЩЕВЫХ
ПРОДУКТОВ
П.У. «ЦЕНТРАЛЬНАЯ
ИСПЫТАТЕЛЬНАЯ ЛАБОРАТОРИЯ
АЛКОГОЛЬНЫХ/БЕЗАЛКОГОЛЬНЫХ
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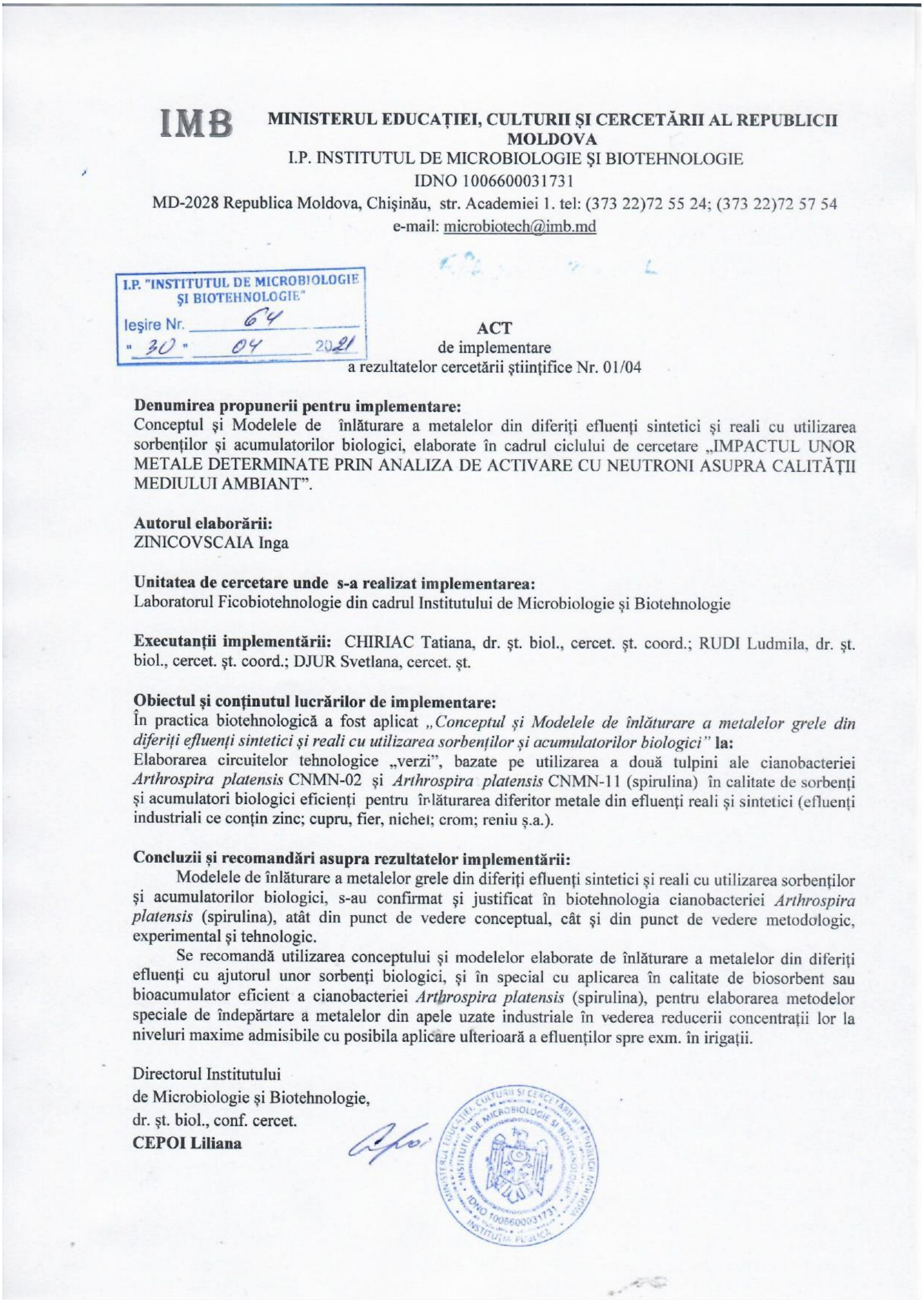
ACT DE IMPLEMENTARE
A REZULTATELOR ŞTIINŢIFICE

Prin prezentul, *Instituţia Publică „Laboratorul central de testare a băuturilor alcoolice/nealcoolice şi a produselor conservate”* confirmă implementarea rezultatelor investigaţiilor ştiinţifice la tema *„IMPACTUL UNOR METALE DETERMINATE PRIN ANALIZA DE ACTIVARE CU NEUTRONI ASUPRA CALITATII MEDIULUI AMBIANT”* realizate de dna Inga ZINICOVSCAIA, Şef de sector, în cadrul Laboratorului Frank de Fizică a Neutronilor, Institutul Unificat de Cercetări Nucleare, or. Dubna, Federaţia Rusă şi cercetător ştiinţific stagiar în cadrul Laboratorul Chimie Fizică şi Cuantică al Institutului de Chimie.

Rezultatele studiului efectuat pot fi implementate pentru analiza calităţii produselor agricole şi a vinurilor, şi determinarea provenienţei lor pe baza compoziţiei elementare.

Director **Lucia GHERDELESCU**



IMB **MINISTERUL EDUCAȚIEI, CULTURII ȘI CERCETĂRII AL REPUBLICII MOLDOVA**
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Ieșire Nr. 64
"30" 04 2021

ACT
de implementare
a rezultatelor cercetării științifice Nr. 01/04

Denumirea propunerii pentru implementare:
Conceptul și Modelele de înlăturare a metalelor din diferiți efluenți sintetici și reali cu utilizarea sorbenților și acumulatorilor biologici, elaborate în cadrul ciclului de cercetare „IMPACTUL UNOR METALE DETERMINATE PRIN ANALIZA DE ACTIVARE CU NEUTRONI ASUPRA CALITĂȚII MEDIULUI AMBIANT".

Autorul elaborării:
ZINICOVSCAIA Inga

Unitatea de cercetare unde s-a realizat implementarea:
Laboratorul Ficobiotehnologie din cadrul Institutului de Microbiologie și Biotehnologie

Executanții implementării: CHIRIAC Tatiana, dr. șt. biol., cercet. șt. coord.; RUDI Ludmila, dr. șt. biol., cercet. șt. coord.; DJUR Svetlana, cercet. șt.

Obiectul și conținutul lucrărilor de implementare:
În practica biotehnologică a fost aplicat *„Conceptul și Modelele de înlăturare a metalelor grele din diferiți efluenți sintetici și reali cu utilizarea sorbenților și acumulatorilor biologici"* **la:**
Elaborarea circuitelor tehnologice „verzi", bazate pe utilizarea a două tulpini ale cianobacteriei *Arthrospira platensis* CNMN-02 și *Arthrospira platensis* CNMN-11 (spirulina) în calitate de sorbenți și acumulatori biologici eficienți pentru înlăturarea diferitor metale din efluenți reali și sintetici (efluenți industriali ce conțin zinc; cupru, fier, nichel; crom; reniu ș.a.).

Concluzii și recomandări asupra rezultatelor implementării:

Modelele de înlăturare a metalelor grele din diferiți efluenți sintetici și reali cu utilizarea sorbenților și acumulatorilor biologici, s-au confirmat și justificat în biotehnologia cianobacteriei *Arthrospira platensis* (spirulina), atât din punct de vedere conceptual, cât și din punct de vedere metodologic, experimental și tehnologic.

Se recomandă utilizarea conceptului și modelelor elaborate de înlăturare a metalelor din diferiți efluenți cu ajutorul unor sorbenți biologici, și în special cu aplicarea în calitate de biosorbent sau bioacumulator eficient a cianobacteriei *Arthrospira platensis* (spirulina), pentru elaborarea metodelor speciale de îndepărtare a metalelor din apele uzate industriale în vederea reducerii concentrații lor la niveluri maxime admisibile cu posibila aplicare ulterioară a efluenților spre exm. în irigații.

Directorul Institutului
de Microbiologie și Biotehnologie,
dr. șt. biol., conf. cercet.
CEPOI Liliana

IDNO 1006600031731

Declaration about assuming responsibility

The undersigned, Inga Zinicovscaia, announce on my own responsibility that the materials presented in Doctor thesis are the results of proper investigations and scientific advances. Comprehend that in opposite case, follow to be accountable for the consequences in compliance with current legislation.

Signature

Data **25.10.2022**

CURRICULUM VITAE

ZINICOVSCAIA INGA

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STUDIES

2010-2013	"Study of the interaction of metals (Hg, Cr, Zn, Ag, Au) with *Arthrobacter genera* and *Spirulina platensis* PhD study, University of the ASM. Speciality: Chemistry (PhD in Chemistry, defence in 2013)
2008-2009	„ Epithermal neutron activation analysis for bacterial transformations of chromium" Master study, University of the ASM. Speciality: Chemistry
2004-2008	The influence of deltametrin pesticides on the process of radical self-treatment process of the aquatic environment" Moldova State University. Speciality: Chemistry and chemical technology

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WORK EXPERIENCE

21.07.2019 – present	Head of the Sector of neutron activation analysis, Joint Institute for Nuclear Research, Dubna, Russia/Institute of Chemistry/ Horia Hulubei National Institute of Physics and Nuclear Engineering
01.12.2015-21.07.2019	Senior researcher, Joint Institute for Nuclear Research, Dubna, Russia/Institute of Chemistry/ Horia Hulubei National Institute of Physics and Nuclear Engineering
10.04.2012-01.12.2015	Scientific researcher, Joint Institute for Nuclear Research, Dubna, Russia/Institute of Chemistry/ Horia Hulubei National Institute of Physics and Nuclear Engineering
17.09.2009-10.04.2012	Junior scientific researcher, Joint Institute for Nuclear Research, Dubna, Russia/Institute of Chemistry

21.10.2008-17.09.2009	Engineer, Joint Institute for Nuclear Research, Dubna, Russia/Institute of Chemistry
SPECIALIZATION	Neutron activation analysis, atomic absorption in the environment studies, materials science, biotechnology, nanotoxicology
AWARDS	
2010, 2012/13, 2015/16, 2019	Grant for young scientist and specialist of the JINR
2011, 2014, 2017	I.M. Frank fellowship in the field of nuclear physics
2013, 2015, 2021	Gold medal Euroinvent, Iasi, Romania
2016	Third JINR Prize for cycle of publications “Application of neutron activation analysis in wastewater treatment”
2017	Second JINR Prize in Applied Physics Research for work „Neutron activation analysis for ecological state assessment of coastal ecosystems of the Black Sea”
2018	Second JINR Prize in Applied Physics Research for work “Determination of the elemental content of Moldavian wines and soils by neutron activation analysis”
PARTICIPATION IN THE CONFERENCES	**Total 54**: Invited talk (1), plenary talk (6), oral presentations (35), poster presentations (3)
PUBLICATIONS	Papers (84), communications at scientific meetings (87)
REVIEWER	Reviewer for 39 international journals
EDITOR	Journals: Materials, Coatings
H-INDEX	Scopus (11), Web of Science (10), Scholar google (12)
LANGUAGES:	Russian-native, English, Romanian-fluent, German -moderately
PARTICIPATION IN PROJECTS	20.8009.5007.27 ”Mecanisme fizico-chimice a proceselor redox cu transfer de electroni implicate în sistemele vitale, tehnologice si de mediu” (Executor) 2018-2021 RFBR Grant 18-29-25023 мк "Study of metal biosorption and bioaccumulation from complex solutions using different type of biological sorbents." (Coordinator) 2019-2021 гг. RFBR Grant 19-015-00145 A "Study of the effect of metal nanoparticles on the reproductive function of female mice and the evaluation of the cognitive abilities of offspring exposed to nanoparticles during prenatal development" (Coordinator)