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**DEVELOPMENT OF INNOVATIVE METHODS FOR
CONTROL OF INORGANIC, ORGANIC AND
RADIOCHEMICAL COMPOSITION OF LIQUID AND SOLID
WASTE**

ABSTRACT

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The dissertation consists of 128 pages, of which 12 pages with references and 9 pages with appendices. It contains 21 figures and 39 tables. The bibliographic reference covers 188 literary sources. **The numbering of the sections, figures, and tables in the abstract does not match those in the dissertation.**

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Abbreviations

EU – European Union

IL – Ionic liquid

MPC – Maximum permissible concentration

MS – Model solution

PAHs – Polycyclic aromatic hydrocarbons

PTEs – Potentially toxic elements

WWTP – Wastewater treatment plant

REE – Rare earth elements

POPs – Persistent organic pollutants

CRM – Certified Reference Material

DRC - Dynamic Reaction Cell

EY – Extraction yield

GC-MS – Gas chromatography with mass spectrometry

ICP-MS – Inductively coupled plasma mass spectrometry

IP – Ionization potential

LA-ICP-MS – Inductively coupled plasma mass spectrometry with laser ablation

LOD – Limit of detection

RP column – Reverse phase column

SF – Separation factor

I. Introduction

High levels of industrialization and anthropogenic activity in recent decades have led to a significant increase in waste generation. One of the environmental threats from the operation of landfills is the production of leachate, which contains high concentrations of both nutrients and toxic compounds (Abdel-Shafy et al., 2024, Minelgaite et al., 2019). Each landfill management plan provides for the implementation of approaches for its treatment, followed by final treatment in urban wastewater treatment plants (WWTPs). WWTPs generate tons of sludge annually (Giwa et al., 2023). Over 10 million tons of sludge are produced annually in the European Union (EU) (Anderson et al., 2021). In Bulgaria, 45,379 tons of sludge were produced from 90 urban WWTPs in 2023 (Valchev et al., 2024). A long-term EU goal and a fundamental principle of the circular economy is to reduce the amount of waste generated, and when this is unavoidable, to use it as a resource and achieve higher recycling levels. According to Eurostat data, in 2020 in Bulgaria the recycling process was below 10%, and its introduction as raw materials was below 5%.

Discharge of treated wastewater into a water body or its use as a source for irrigation is carried out after a strict assessment (Regulation No. 7, 2000, Regulation No. 6, 2000, Regulation No. 10, 2001, Regulation (EU) 2020/741). Modern approaches to management and disposal of WWTP sewage sludge is recycling into construction materials (Chang et al., 2020), landfilling, incineration, composting, reclamation of unused land, and the highest share in the EU, including Bulgaria, is its use as fertilizer in agriculture (Anderson et al., 2021). This application is regulated by the Bulgarian (Regulation on the procedure and manner for the utilization of sludge from wastewater treatment through their use in agriculture, 2004) and European (Council Directive 86/278/EEC, 1986) legislation, determining maximum permissible concentrations (MPC) for the potentially toxic elements (PTE) Cd, Cu, Ni, Pb, Zn, Hg, Cr and As, both in sludge and in soil. However, in order to determine not only toxic, but also beneficial for soil and plants (e.g. K, Ca, Mg, Fe, P, S, Se, etc.) properties, as well as to search for alternative ways of applications, it is necessary to determine the maximum number of elements. In the literature, the analysis of wastewater and sludge samples from WWTPs aims at the determination of a limited number of elements, mainly PTE (Chilian et al., 2022, Tuzen et al., 2022, Tytła, 2020). The most commonly used methods are ICP-MS (Li et al., 2015, Litvinov et al., 2022) and GFAAS (Chilian et al., 2022, Ozbek et al., 2016) for the determination of elements at micro- and trace levels and ICP-OES (Ali et al., 2014, Pöykiö et

al., 2019, Tytła et al., 2020) and FAAS (Chilian et al., 2022, Hammoudani et al., 2019) for elements at higher concentrations. In rare cases, the content of essential elements is determined (Ali et al., 2014, Litvinov et al., 2022). The routinely used approach is quantitative analysis after calibration for all analysed elements in an appropriate concentration range. However, in waste analysis, the sample matrix is often unknown, there are large variations between different sample types and between elements in the same type of sample (wt.-%-ng/kg), which necessitates the development of methods for preliminary “screening” analysis.

Along with PTE, sludge contains persistent organic pollutants (POPs) (Mohammed et al., 2021, Patel et al., 2015). Polycyclic aromatic hydrocarbons (PAHs) are one of the most common POPs, with 16 listed as priority pollutants by the US Environmental Protection Agency (US-EPA, 2007). The current European regulation for their use in agriculture does not specify MPCs for POPs. This document is almost 40 years old. A number of European countries are introducing stricter requirements and adopting MPCs for other heavy metals, as well as organic pollutants. In an EU working document from 2000 (EU Draft directive on sewage sludge, 2000), a MPC of 6 mg/kg was proposed, and in the Bulgarian regulation from 2004 - 6.5 mg/kg dry weight for the sum of PAHs. A large number of methods for the analysis of PAHs, including in WWTP sludge, have been described in the literature, which differ significantly both in the sample preparation step and the chromatographic method used (e.g. Blanchard et al., 2004, Qiao et al., 2018).

A specific group of pollutants that are not subject to research in waste according to the described regulations are radiochemical pollutants. As a result of climate anomalies on a global scale, their spread and redistribution in natural sites is possible. Classical approaches for the analysis of radiochemical pollutants are associated with the use of aggressive acids and bases or toxic organic solvents, which is a prerequisite for the creation of new quantities of hazardous waste.

Thus, the subject of this dissertation is the optimization of fast, accurate and sensitive methods for determination of a maximum number of elements in a wide concentration range, radiochemical contaminants after application a separation and concentration procedure using "green" reagents, as well as the content of PAHs, being priority pollutants in samples of liquid and solid waste.

II. Goals and tasks

Based on the literature review, the goal of this dissertation is to develop rapid, accurate and sensitive methods for determining inorganic composition, some radiochemical and organic contaminants in liquid and solid waste.

To achieve this goal, it was necessary to solve the following tasks:

- Optimization of instrumental parameters and sample preparation conditions for quantitative ICP-MS elemental analysis for determination of a maximum number of elements in waste samples;
- Development of a method for semi-quantitative ICP-MS analysis for the determination of elements in waste samples;
- Application of quantitative and semi-quantitative ICP-MS methods for the analysis of wastewater from the WWTP - Kubratovo;
- Fractionation of WWTP sewage sludge to establish the chemical species of the elements;
- Assessment of the possibility of the non-destructive LA-ICP-MS method for the determination of the content of chemical elements in WWTP sewage sludge;
- Development of a procedure for the extraction of radionuclides from aqueous solution using the ionic liquid 1-methyl-3-octylimidazolium saccharinate;
- Adaptation of a method for solid-phase extraction and GC-MS/MS determination of polycyclic aromatic hydrocarbons in WWTP sludge samples.

III. Experimental part

III.1 Description of the samples

III.1.1 Wastewater

Sampling of wastewater samples from the WWTP - Kubratovo was carried out three times - in the winter, the spring and the summer seasons of 2022 from the following points: WWTP inlet; sand trap outlet; fourth primary clarifier outlet; fifth bio-basin – aerobic zone, denitrification zone, biophosphorus zone; sixth secondary clarifier outlet; WWTP outlet. The wastewater samples were sampled in 1 L polyethylene bottles and stored at a temperature of about 4 °C. Before analysis, the samples were filtered through a 0.45 µm cellulose filter. Analysis was carried out directly and after acid digestion with HNO₃ and H₂O₂.

III.1.2 Solid waste

The following solid waste samples were analysed – wood ash, tailings and sewage sludge from WWTP, provided by the Forestry University.

Wood ash from the Svilosa TPP – (Wood ash):

“Svilosa” AD, through its main subsidiary “Svilocel” EAD, is the only producer in Bulgaria of sulphate bleached cellulose and its products. The analysed wood ash was obtained from the recovery of wood bark, a waste from wood processing at the plant. The ash is formed in a biomass combustion boiler. The installation is designed for recovery by incineration of waste (fresh and landfilled) wood bark from cellulose production and steam generation. About 10 000 t of wood ash is released annually from the wood bark combustion installation.

Tailings from Benkovski Tailings Reservoir 1 – (Tail - Benkovski):

The tailings pond came into operation in 1981 with an operation period until 2000. Flotation waste from the enrichment plant processing ore from the copper-porphyry deposit "Elatsite-Med" AD is deposited in the tailings pond "Benkovski 1". The main industrial element in the deposit is copper, and gold and molybdenum are secondary, with molybdenite concentrate being extracted only in separate periods of operation.

Tailings from the “Madzharovo–1” Tailings Facility - (Tail – Madzharovo):

The operation of the Madzharovo-1 tailings dam began in 1962 and ended in 1995. Waste from the Madzharovo lead-zinc enrichment plant was deposited there.

Sewage sludge from the Blagoevgrad municipal WWTP (Sewage sludge – Blagoevgrad):

The urban wastewater treatment plant in the city of Blagoevgrad was officially built in 2009 and is designed for 111 111 equivalent residents and an average daily water volume of 30 000 m³/day for the project period 2025. The wastewater treatment in the WWTP includes mechanical and biological treatment, as well as treatment of the formed sludge. Methods for compaction and dewatering are applied to the sludge and subsequent temporary storage in drying fields on the territory of the station. The analysed sludge was sampled from the sludge drying fields after biological treatment.

Sewage sludge from the WWTP of the company "ALMAGEST" AD, Ihtiman (Sewage sludge - Ihtiman):

In 2009, the company put into operation a plant for the production of ethanol from cereals. The WWTP treats a mixed flow of domestic, fecal and industrial water. The treated wastewater flow, together with rainwater, is discharged into the Mati Vir River. The analysed sewage sludge was sampled after the biological treatment stage.

III.2 Analysis of chemical elements in liquid and solid waste

III.2.1 Equipment

Destructive elemental analysis of wastewater and sewage sludge samples from the WWTPs is performed using a Perkin Elmer SCIEX DRC-e ICP-MS, and for non-destructive determination of chemical elements, LA-ICP-MS (ICP-MS connected to the New Wave Research Laser Ablation System was used (Table 1) is used.

Table 1. Instrumental parameters of ICP-MS/LA-ICP-MS.

ICP-MS		LA	
Parameter	Value	Parameter	Value
RF power	1100W	Wavelength	213 nm
Argon plasma gas flow	15 L/min	Pulse duration	5 ns
Auxiliary gas flow	1.2 L/min	Ablation mode	Raster, line
Nebulizer gas flow	0.9 L/min	ICP-MS Nebulizer gas flow	1.1 L/min
		LA Nebulizer gas flow	1 L/min
Lens voltage	7.5 V	Scanning speed	10 μ m/s
Pulse stage voltage	950 V	Beam diameter	100 μ m

Microwave digestion of the samples was performed with Microwave Reaction System (Anton Paar, Multiwave 3000).

The solid samples were homogenized using a Waring laboratory blender.

A 15 Ton Hydraulic Press, Specac, was used to prepare CPM tablets and powder samples.

III.2.2 Reagents and materials

- For acid digestion of the samples the following reagents were used: HF (47–51%, Fisher Chemicals, Ultra Trace Metal Grade, Loughborough, UK), HNO₃ (67–69%, Fisher Chemicals, Ultra TraceMetal Grade, Loughborough, UK), H₂ O₂ (30%, Fisher Chemicals, Ultra Trace Analysis Grade, Loughborough, UK), HClO₄ (70%, Chem-Lab NV Belgium)
- Standard solutions: ICP-MS multielement calibration standard solution-2 (Ultra scientific), consisting of 29 elements (Al, As, Ba, Be, Bi, Cd, Ca, Cs, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Ag, Na, Sr, Tl, U, V, Zn) with starting concentrations of 10 mg/L; ICP-MS multielement standard B (high-purity standards), containing 13 rare earth elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Yb) with starting concentrations of 10 mg/L; single-element standard solutions of Na, K,

Ca, Mg, Fe, Al, Si, P, Ti, Zr, Mo, Pd, Sn, Sb, Au, Hg, Hf, Y, Nb, Ru, Rh, Te, Ta, W, Re, Ir, Pt, Th, Be, Ge, Si, Sc, Tm, Os, S (Fluka), with starting concentrations of 1000 mg/L.

- To assess the accuracy of the results, the following CPM were used - LGC6177, Landfill Leachate—metals, UKAS Reference Materials, UK), Sewage sludges CRM 029 (Trace Metals—Sewage Sludge 2—Sigma-Aldrich, Laramie, WY 82070, USA) and ERM-CC144 (Sewage Sludge—elements, European Commission—Joint Research Centre Directorate F—Health, Consumers and Reference Materials, Geel, Belgium), coal standard reference material NBS 1632a (National Bureau of Standards, Washington. DC), lake sediment BCR-701 (trace elements, European Commission – Joint Research Centre Directorate F – Health, Consumers and Reference Materials Retieseweg 111, B - 2440 Geel, Belgium).
- For the pellets preparation of the solid samples the following binders were used: cellulose (Acros Organics), activated carbon (Chem-Lab NV Belgium), silica gel (Fisher Scientific UK), graphite (Acros Organics), H_3BO_3 (Valerus).

III.3 Optimization of a method for selective extraction and determination of radiochemical contaminants in an aqueous matrix

III.3.1 Equipment

- For the determination of radioisotopes, a Well-type HPGe detector Canberra gamma spectrometer with an energy resolution of 1.8 and an efficiency of 16% at 1332.5 keV, connected to a 4196-channel Canberra 35Plus analyser, was used.
- A Memmert climatic cabinet was used to heat the samples at different temperatures in order to establish the optimal extraction conditions.
- The pH of the samples was measured with a Sartorius PT-15 pH meter.
- To separate the organic from the aqueous phase, the samples were centrifuged using a Rotofix 32 A Hettich centrifuge.

III.3.2 Reagents

- To carry out the experiments, a solution of three radionuclides ^{241}Am , ^{137}Cs and ^{60}Co in 1 M HNO_3 was prepared (Laboratory of Radiochemistry at Sofia University "St. Kliment Ohridski") with specific activities of 80 Bq/mL ^{241}Am , 30 Bq/mL ^{60}Co and 40 Bq/mL ^{137}Cs .

- The ionic liquid 1-methyl-3-octylimidazolium saccharinate was synthesized and provided by the team of Prof. M. Bogdanov, Ph.D. (Sofia University "St. Kliment Ohridski").
- Phosphate buffer (Na_2HPO_4 , KH_2PO_4 , NaOH, HNO_3), universal buffer (Na_2HPO_3 , H_3BO_3 , citric acid, NaOH, HCl) and oxalic acid.
- 0.1 M NaOH and 0.1 M HCl were used for pH adjustment.
- CH_2Cl_2 was used for the regeneration of the ionic liquid.

III.4 Optimization of a method for solid-phase extraction and analysis of polycyclic aromatic hydrocarbons with GC-MS

III.4.1 Equipment

For the purposes of the experiment, the following were used: Elmasonic S10 ultrasonic bath, a ROTOFIX centrifuge, a solid-phase extraction system (CHROMABOND SPE vacuum manifold, Macherey – Nagel), and a concentrator by heating and purging with nitrogen (Stuart, SBHCONC/1).

The analysis of PAHs was performed using a gas chromatograph with a triple quadrupole mass spectrometer (GC-MS/MS, EVOQ GC-TQ, Bruker) with a weakly polar stationary phase Rxi-5Sil Ms (crossbond: 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane). The instrumental parameters are presented in Table 2.

Table 2. Instrument parameters of GC-MS / MS, EVOQ GC-TQ.

Column	Rxi®-5Sil MS, Capillary, (fused silica) Crossbond, 5% diphenyl/95% dimethyl polysiloxane
Column dimensions	30m*0.25mm
Eluent	He
Injector temperature, °C	280°C
Column temperature, °C	270°C
Analysis time	59.14 minutes

III.4.2 Reagents and materials

- For the preparation of model solutions and application of a standard addition method, a standard solution PAH-Mix 18 (10 mg/L, LGC Standards, DR Ehrenstorfer) was used, containing anthracene, acenaphthene, acenaphthylene, benzo[a]anthracene, benzo[a]pyrene, benzo[g,h,i]perylene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, naphthalene, perylene, pyrene, phenanthrene, fluoranthene, fluorene and chrysene in acetonitrile solution.

- The following organic solvents were used in the conditioning, elution, and extraction of CRM and sewage sludge samples of WWTP sludge: hexane (HPLC grade, 95%, Fisher Scientific), diethyl ether (HPLC grade, 99%, Fisher Scientific), dichloromethane (for Gas chromatography MS, Supelco), ethyl acetate (HPLC grade, Chem – Lab), acetone (HPLC grade, 99.8%, Fisher Scientific), methanol (HPLC grade, 99.8%, Fisher Scientific), acetonitrile (HPLC grade, 99.9%, Fisher Scientific).
- For the solid-phase extractions of PAHs from model solution and from extracts of sludge from WWTP and CPM LGC 6182, solid-phase extraction columns C18 (CHROMABOND C18 ec, 45 µm, 1 mL/100 mg) and HLB (Hydrophilic-lipophilic balanced N-vinylpyrrolidone-divinylbenzene, 1 mL/100 mg) were used.
- To assess the accuracy of the analysis, the CRM Sewage Sludge PAHs Reference Material LGC 6182 was used.

IV. Results and discussion

IV.1 Quantitative ICP-MS method for analysis of macro- and microelements in waste

IV.1.1 Optimization of instrumental parameters and calibration of the quantitative analysis method

Determination of macroelements at mg/kg levels in environmental and waste samples by ICP-MS under standard conditions is a difficult task. High concentrations and low ionization potential (IP) lead to high intensity of the analysed ions. Often, a saturated signal is obtained for these elements (typically 214025.68 cps in the PerkinElmer SCIEX ELAN DRC-e software). The linear range depends on the IP and the isotopic distribution of the isotopes. For this reason, they are routinely determined by ICP-OES or ICP-MS after multiple dilution. ICP-MS under cold plasma conditions is also applied. In all of the above approaches, at least two measurements of the sample are required. A possibility for simultaneous determination of macro- and microelements is the optimization and application of a dynamic bandpass parameter (Rejection Parameter a, RPa). For waste analysis, RPa coefficients for Na, K, Mg, Ca, Al, Ti, P, Si, Mn, Fe, and have been optimized. An optimization curve for ^{24}Mg is presented in Figure 1.

The advantage of the application of RPa is the possibility to reduce the signal of certain isotopes, which allows simultaneous determination of macro- and microelements. The reduction is obtained by application of a voltage between the pair of electrodes of the dynamic

reaction cell (DRC) (Tanner et al., 2002). The RPa value is optimized using a blank sample and a standard solution of the elements and can vary between 0.01 and 0.02 V. The value is chosen depending on the expected concentration interval of the element and the isotopic abundance of the respective isotope. The selected value in the optimization of ^{24}Mg at a concentration of 10 mg/L is 0.016V. At this RPa value, it is possible to determine both lower and higher concentrations without reaching a saturated signal.

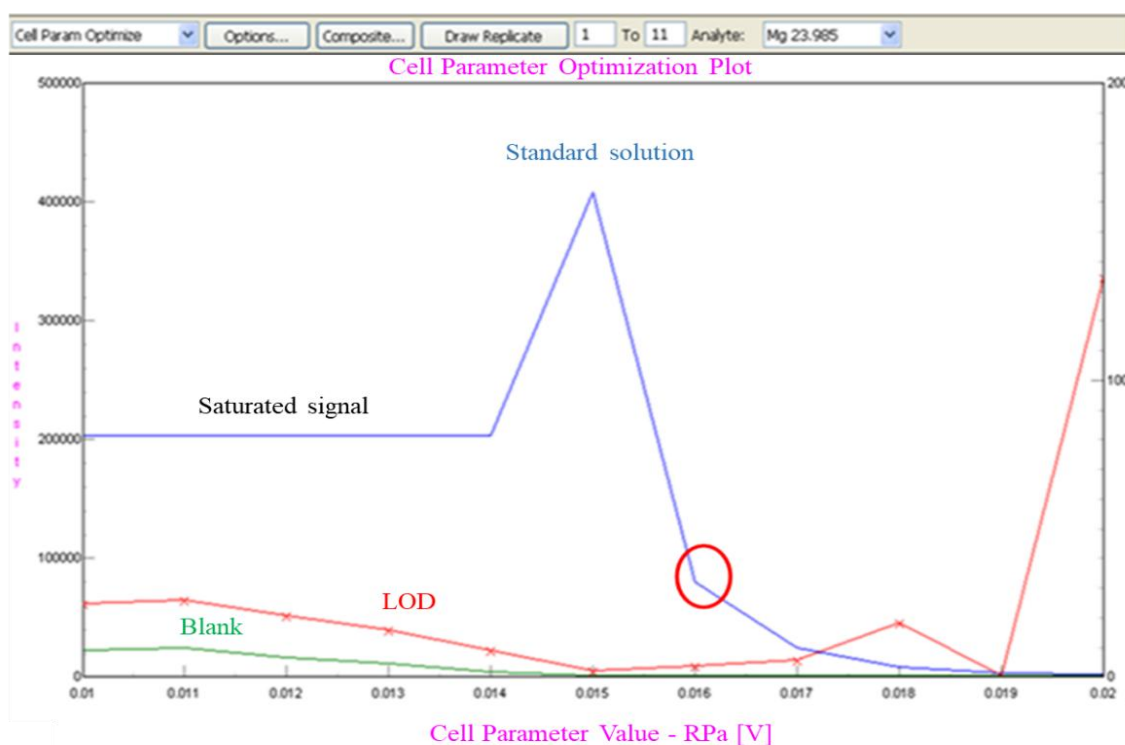


Fig. 1. Optimization curve of ^{24}Mg in concentration 10 mg/L.

Another matrix element in waste samples is sulfur. The use of the most common isotope ^{32}S has a serious interference from $^{16}\text{O}_2^+$, which leads to the impossibility of its use. Often for quantitative analysis of sulfur ^{34}S is used. Conducted measurements of sulfur using ^{34}S in surface water CRM SPS-SW2 show poor accuracy. For this reason, in the present work for the determination of sulfur DRC parameters – reaction gas flow [mL/min] and RPq [V] were optimized. Under DRC conditions, the following reaction occurs: $^{32}\text{S}^+ + \text{O}_2 \rightarrow ^{48}\text{SO}^+ + \text{O}$

The optimization of the reaction gas O_2 flow rate and the correction factor RPq was carried out with a blank sample (0.1% HNO_3 in deionized water) and a standard solution of S at a concentration of 1 mg/L in 0.1% HNO_3 . Values at which the oxide formation occurs to the highest extent (the standard solution has the highest intensity and the limit of detection (LOD))

has the lowest value) were selected. Optimization curve for O₂ flow is shown in Fig. 2, and for RPq in Fig. 3.

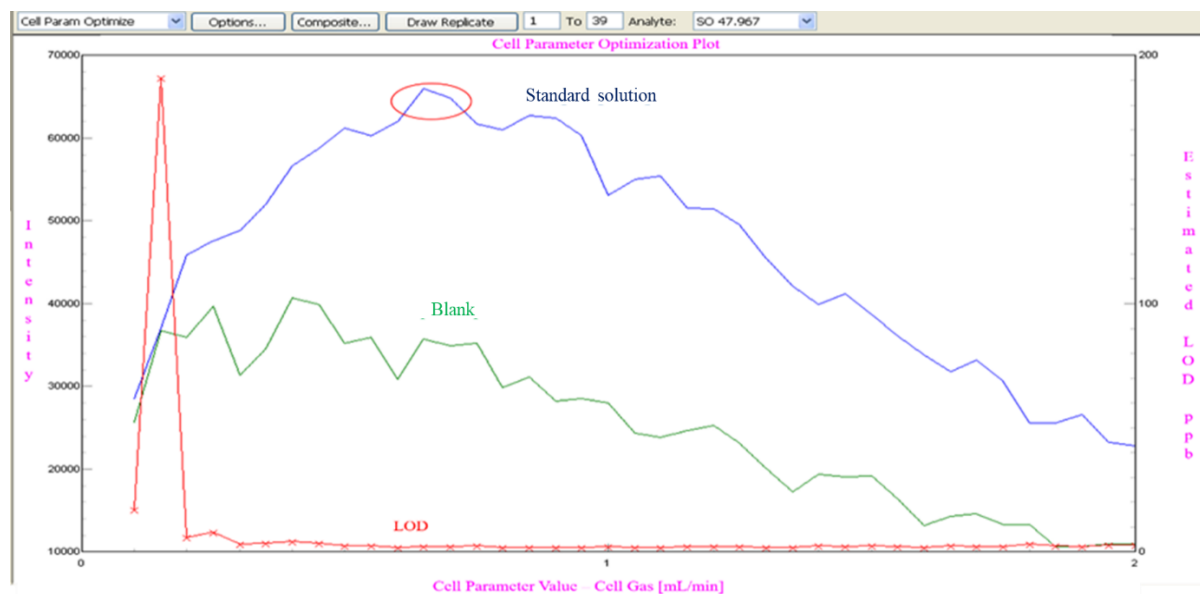


Fig. 2. Optimization of O₂ flow rate for formation and determination of ⁴⁸SO⁺ in the DRC.

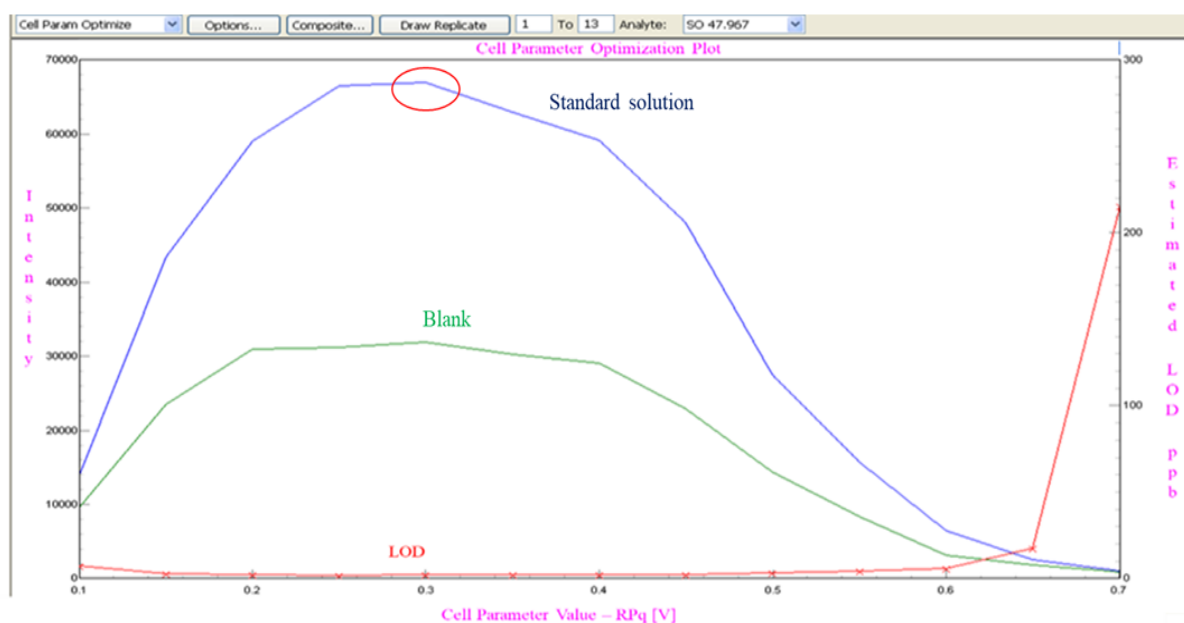


Fig. 3. RPq optimization curve for formation and determination of ⁴⁸SO⁺.

The optimization curves show that oxide formation occurs at highest rate at 0.7 mL/min O₂ and at RPq value 0.3 V.

The majority of trace elements were determined in standard mode, except for As and Se, whose isotopes are influenced by Ar-containing ionic associates. In order to eliminate interferences, As and Se were also analysed in DRC mode with reaction gas O₂ (Lyubomirova and Djingova, 2015). The optimized conditions are presented in Table 3.

Table 3. Instrumental parameters for the determination of macro- and microelements.

Macroelements			
Conditions	Isotope	RPa [V]	Linear interval
Cell-based	²³ Na	0.017	0.1-10 mg/L
	^{24,25,26} Mg	0.016/0.015/0.015	0.1-10 mg/L
	²⁷ Al	0.016	0.1-10 mg/L
	²⁸ Si (in liquid samples)	0.016	0.1-10 mg/L
	³¹ P	0.014	0.1-10 mg/L
	³⁹ K	0.017	0.1-10 mg/L
	^{42, 43, 44} Ca	0.014/0.013/0.015	0.1-10 mg/L
	^{46, 47,48,49} Ti	0.012/0.012/0.014/0.012	0.1-2 mg/L
	⁵⁵ Mn	0.012	0.1-2 mg/L
	^{54,56,57} Fe	0.014/0.017/0.014	0.1-10 mg/L
DRC	³² S→ ⁴⁸ SO	RPq – 0.3 V O ₂ rate – 0.7 mL/min	0.1-2 mg/L
Microelements			
Conditions	Isotope	RPq [V]/Oxygen rate [mL/min]	
DRC	⁷⁵ As→ ⁹¹ AsO	0.3/0.9	
	⁷⁷ Se→ ⁹³ SeO, ⁷⁸ Se→ ⁹⁴ SeO	0.3/1.5	
	⁸⁰ Se→ ⁹⁶ SeO, ⁸² Se→ ⁹⁸ SeO	0.3/0.9	
Standard conditions	^{6,7} Li, ⁹ Be, ^{10,11} B, ⁴⁵ Sc, ⁵¹ V, ^{52,53} Cr, ⁵⁹ Co, ^{58,60,62} Ni, ^{63,65} Cu, ^{64,66,68} Zn, ^{69,71} Ga , ^{72,74} Ge, ^{85,87} Rb, ^{86,88} Sr, ⁸⁹ Y, ^{90,92} Zr, ⁹³ Nb, ^{96,98} Mo, ¹⁰² Ru, ¹⁰³ Rh, ^{104,105,108} Pd, ^{107,109} Ag, ^{112,114,116} Cd, ^{113,115} In, ^{116,118,119,120} Sn, ^{121,123} Sb, ^{125,126,128,130} Te, ¹³² Cs, ^{136,138} Ba, ¹³⁹ La, ¹⁴⁰ Ce, ¹⁴¹ Pr, ^{144,146} Nd, ^{147,149,151} Sm, ^{151,153} Eu, ^{155,158} Gd, ¹⁵⁹ Tb, ^{161,162,163,164} Dy, ¹⁶⁵ Ho, ^{166,167,168} Er, ¹⁶⁹ Tm, ^{172,174} Yb, ¹⁷⁵ Lu, ^{176,180} Hf, ¹⁸¹ Ta, ^{182,184,186} W, ^{185,187} Re, ^{190,192} Os, ^{191,193} Ir, ^{194,195,198} Pt, ¹⁹⁷ Au, ^{200,202} Hg, ^{203,205} Tl, ^{204,206,207,208} Pb, ²⁰⁹ Bi, ²³² Th, ²³⁸ U		

I V.1.2 Optimization of the acid digestion – selection of acid mixture

The effectiveness of two acid mixtures for acid digestion was tested – 10 mL HF, 8 mL HNO₃ and 3 mL H₂O₂ and the same mixture, in combination with 5 mL HClO₄ on bituminous coal CRM – NBS 1632a and WWTP sewage sludge CRMs – ERM-CC144 and CRM 029.

To 0.25 g of a waste sample, 10 mL of conc. HF was added (in a parallel sample, 5 mL of conc. HClO₄ was also added) in polytetrafluoroethylene vessels and left for 24 hours. Then the mixture was digested by heating on a hot plate until the acid mixture was reduced to 2/3 of the initial volume. A new portion of 10 mL of conc. HF was added. If necessary, an additional portion of 10 mL of conc. HF was added to obtain clear solutions. The acid mixture was evaporated to almost dryness. New portions of 10 mL conc. HNO₃ and 3 mL of H₂O₂ were added. The solutions were heated until the volume was reduced to 0.5-1 mL. After cooling, the samples were transferred to polypropylene tubes and diluted to 50 mL with deionized water. Three parallel samples were prepared from each sample. A blank sample was prepared using

an identical procedure. The acid mixture HF-HNO₃-H₂O₂ with reduced volumes (1mL - 8mL - 3mL) was also used for microwave digestion.

The accuracy of each digestion procedure was assessed by calculating the percentage bias from the analysis according to formula 1:

$$(1) \quad \text{bias \%} = \frac{(C_{\text{exp}} - C_{\text{cert}})}{C_{\text{cert}}} * 100,$$

where C_{exp} is the experimental result obtained after the corresponding digestion procedure, C_{cert} is the certified concentration.

The data for the macroelements and selected microelements in CRM NBS 1632a, expressed as bias are presented in Figures 4 and 5.

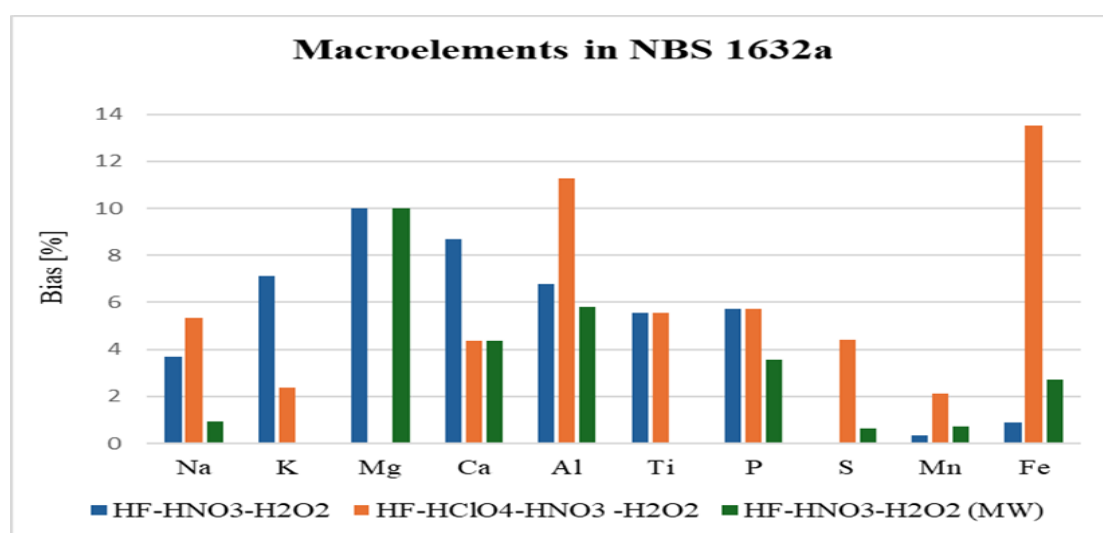


Fig. 4. Percentage bias for macroelements after application of different digestion procedures.

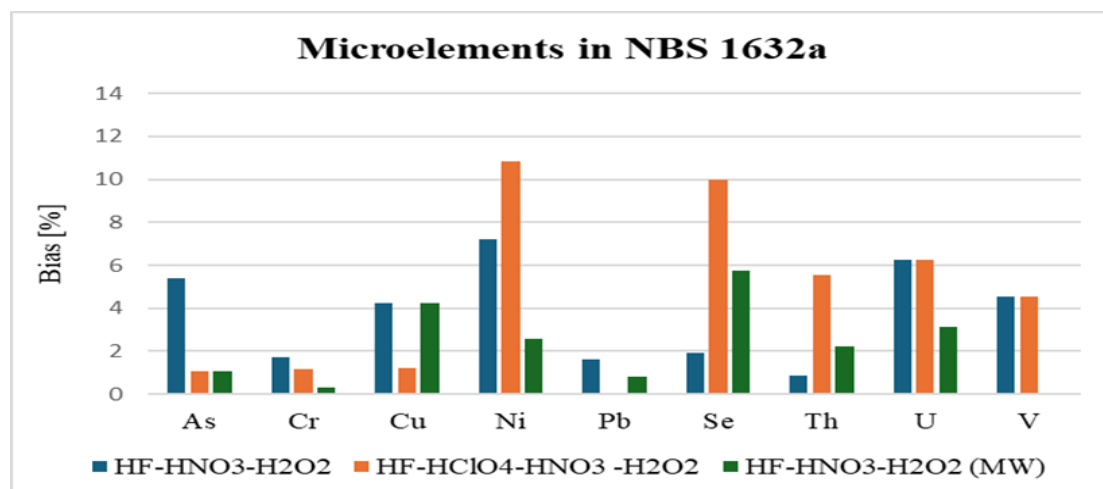


Fig. 5. Percentage bias for microelements after application of three acid digestion procedures.

The data show that complete digestion of the CRM is achieved with an acidic mixture HF-HNO₃-H₂O₂, both by open vessel and microwave digestion. Using all approaches high accuracy was achieved, and the reproducibility ranged from 0.5 to 10%. In order to proceed more efficient and faster digestion, with less reagent consumption, in the following experiments microwave digestion with 1mL HF, 8mL HNO₃ and 3 mL H₂O₂ was carried out

The digestion and quantification method were applied for the determination of chemical elements in different types of solid waste - wood ash, tailing and sewage sludge (Fig. 6).

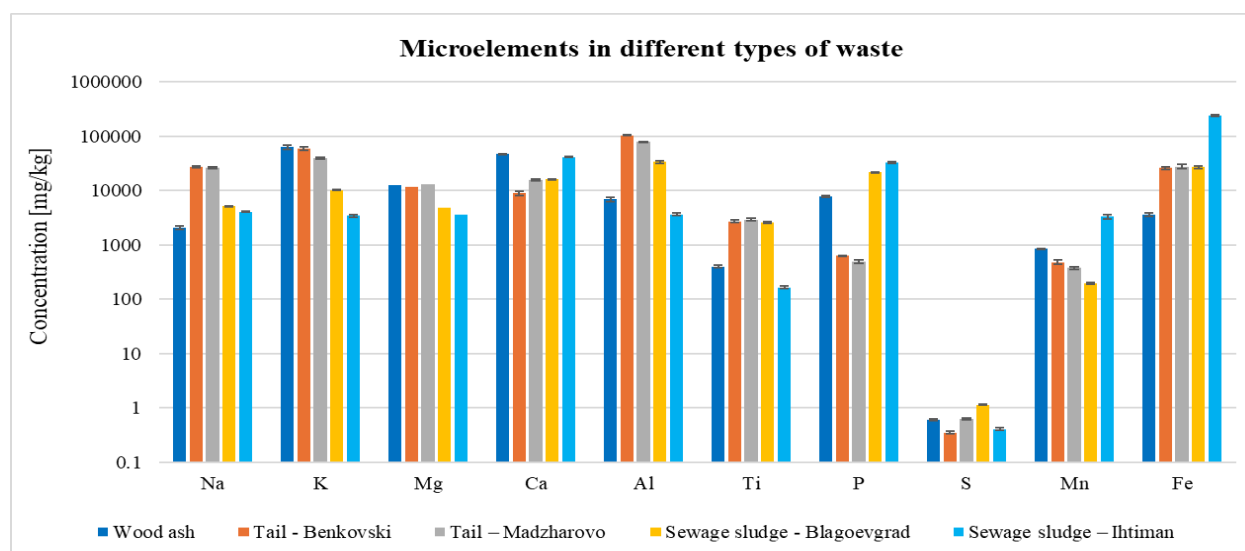


Fig. 6. Results for the concentrations of macroelements [mg/kg] in wood ash, two types of tailings and two types of WWTP sewage sludge.

The results showed variations, at least 2 times in the content of the elements in different types of waste, as well as some elements in the same type of waste

The large variations in the composition of waste samples, which is often unpredictable, initiated the following experiments to optimize a semi-quantitative method as a preliminary screening of waste samples.

IV.2 Semi-quantitative ICP-MS method of analysis

The analysis of waste samples is challenging due to the following problems: often the matrix and composition of the sample are unknown, there are large variations between different sample types and between individual elements (wt. %-ng/kg), CRM with appropriate matrix and composition are not always available, which makes it difficult to assess the accuracy. In addition, a preliminary semi-quantitative analysis is useful to obtain information on potential matrix or spectral interferences, the need to apply corrections, preparation of standard solutions

for calibration in an appropriate concentration range. To perform a semi-quantitative analysis, only a standard solution containing at least three elements and a blank sample are needed, and information is obtained for the entire mass spectrum (from ^6Li to ^{238}U). Typically, quantitative analysis is characterized by very accuracy, better than 10% (Chen et al., 2008), while the accuracy of the semi-quantitative method is worse and varies within quite wide limits, from below 10% (Amarasiriwardena et al., 1997, Chen et al., 2008) to 30-50% (Krzciuk, 2015).

IV.2.1 Calibration of a semi-quantitative method

The present study aims to evaluate the influence of the number of elements in the calibration step and the matrix of the calibration solution on the accuracy of the semi-quantitative method for the analysis of waste samples. Two approaches were used for calibration. The first one involved a seven-step procedure with a standard solution containing 69 elements (macroelements - Na, Mg, Al, Si, P, K, Ca, Fe and microelements - Li, Be, B, Sc, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, U). To test the influence of the number of elements used for calibration on the accuracy of the results for the 69 elements, determined in the model solutions, the calibration was performed seven times with an aqueous standard solution including 69, 50, 36, 27, 14, 8 and 3 elements. The steps of reducing the number of elements for calibration with a standard solution are presented in Fig. 7. After each step, the CRMs LGC6177, CRM 029 and ERM CC-155, and the sewage sludge samples were analysed.

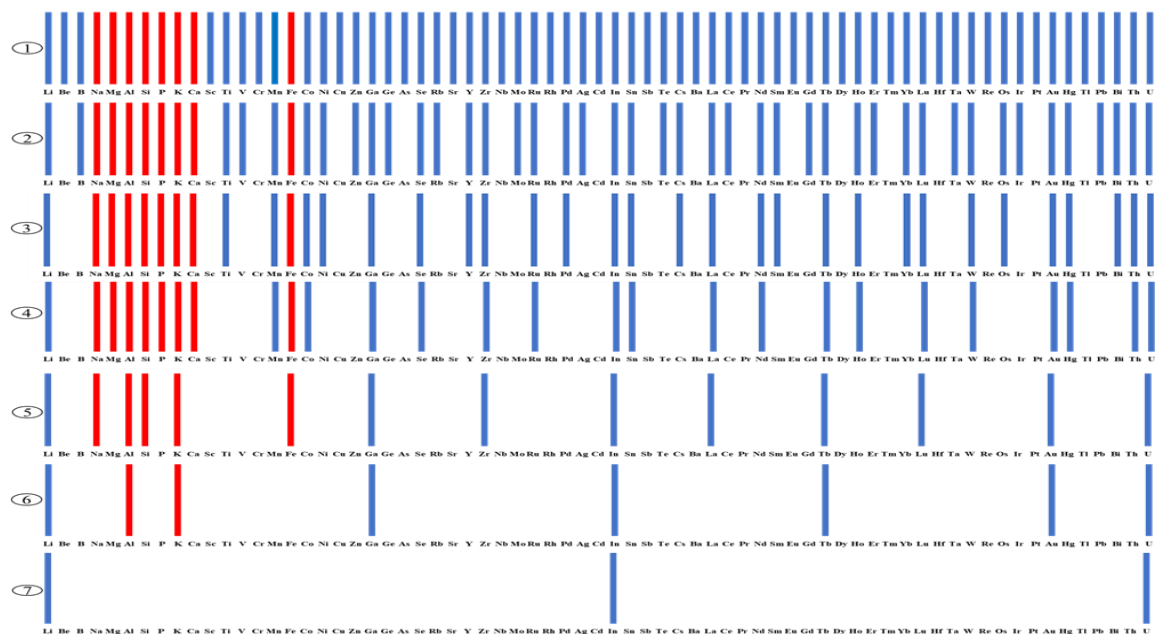


Fig. 7. Seven-step procedure used for calibration with aqueous standard solution.

In the second approach, the calibration was performed with a solution of the CRM 029, containing 29 elements (macroelements - Na, Mg, Al, K, Fe and microelements – Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb) with certified values. The calibration with CRM 029 and the measurement of wastewater and solid CRM and wastewater sludge samples was performed five times, using all the elements (29 elements) with certified values in the first step, and at each subsequent step one intermediate element was eliminated until three elements for calibration remained in the fifth step (Fig. 8).

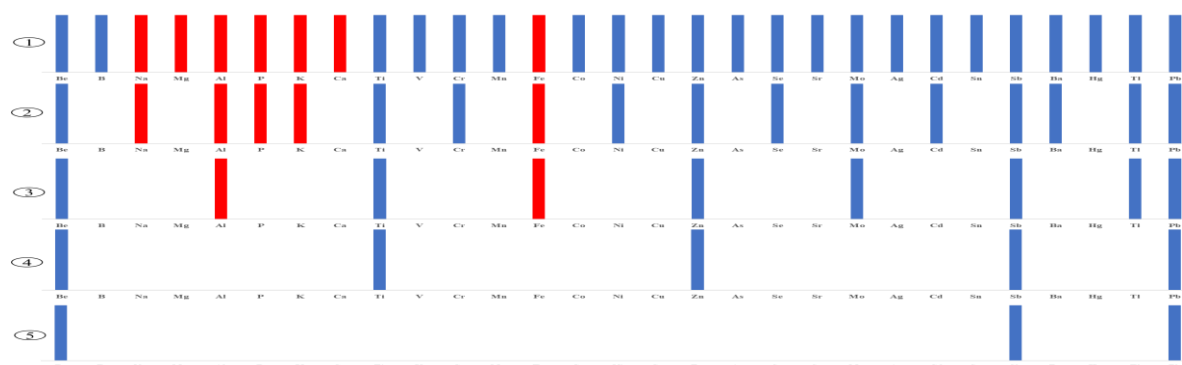


Fig. 8. Five-step procedure used for calibration using CRM 029.

In both figures, the macroelements are presented in red color, and the microelements in blue color on both figures. After each step, the CRMs LGC6177 and ERM CC-144, and the sewage sludge samples were analysed. The accuracy of their determination in each step of both approaches was assessed by calculation of percent bias, using formula 1.

IV.2.2 Optimization of instrumental parameters (RPa)

The application of the semi-quantitative method allows to obtain data on the content of all elements in the sample (from 6 amu to 240 amu, 69 elements) when calibrated with a minimum of three elements, calculated based on internal response factors (Response Factors RF, number of ions per unit concentration) (Chen et al., 2008; Jitaru et al., 2003). In the classical method provided by the manufacturer, the mass interval from 6 amu to 240 amu is divided into four intervals, presented in Table 4(A). When using this method, saturated signals are usually obtained for the matrix elements. For this reason, in the present work the standard method is modified by dividing the masses of the macroelements into separate mass intervals. The signal of their isotopes (^{23}Na , $^{24-26}\text{Mg}$, ^{27}Al , $^{28-30}\text{Si}$, ^{31}P , ^{39}K , $^{42-44}\text{Ca}$, $^{54,56-58}\text{Fe}$) was reduced by introduction of RPa parameter. The optimized mass intervals and the introduced RPa values for each mass interval are shown in Table 4 (B). In both cases, isotopes for oxygen (16, 17, 18) and argon (40, 41) ions were omitted to avoid detector overload (Jitaru et al., 2003).

Table 4. Mass interval partitioning and optimization of RPa [V].

A				
Starting mass [amu]	Final mass [amu]	Mass measurement time [ms]	Integration time [ms]	RPa [V]
6	15	50	500	0
19	39	50	1050	0
42	210	50	8450	0
230	240	50	550	0
B				
6	15	50	500	0
19	22	50	200	0
23	23	50	50	0.017
24	26	50	150	0.016
27	28	50	100	0.016
29	30	50	100	0.013
31	31	50	50	0.014
32	38	50	350	0
39	39	50	50	0.017
42	44	50	150	0.015
45	53	50	450	0
54	54	50	50	0.014
55	55	50	50	0
56	56	50	50	0.017
57	58	50	50	0.014
59	210	50	7600	0
230	240	50	550	0

IV.2.3 Calibration with a multi-element standard solution

To check the influence of the number of elements used for calibration on the accuracy of determination of 69 elements, sequential calibration was performed with 69, 50, 36, 27, 14, 8 and 3 elements. Calibration was also performed with three standard solutions with the following concentrations: Standard solution 1 – macroelements 1 mg/L, trace elements – 10 µg/L; Standard solution 2 – macroelements 5 mg/L, trace elements – 50 µg/L; Standard solution 3 – macroelements 10 mg/L, trace elements – 100 µg/L) in order to analyse the other two standard solutions and liquid and solid CRMs. The accuracy for each element is examined in terms of the percentage bias (relative bias, formula 1). The experimental results are compared to the certified value. The concentrations for the non-certified elements are compared to the data from the quantitative analysis. The results from the first, fourth and seventh steps are presented in Fig. 9. Average values of each of the standard solutions from at least 3 measurements are presented.

The obtained data show that when using the full set of elements present in the standard solution (69) for calibration (step 1), the percentage bias values are in the range of 0.1 and 11.3% (except for Se – 20.1%), comparable to quantitative analysis. The reason for the small

deviations is due to the fact that in this case no interpolation is performed, as the RF of each analyte is updated based on direct calibration. The deviation is only due to random bias from the measurement.

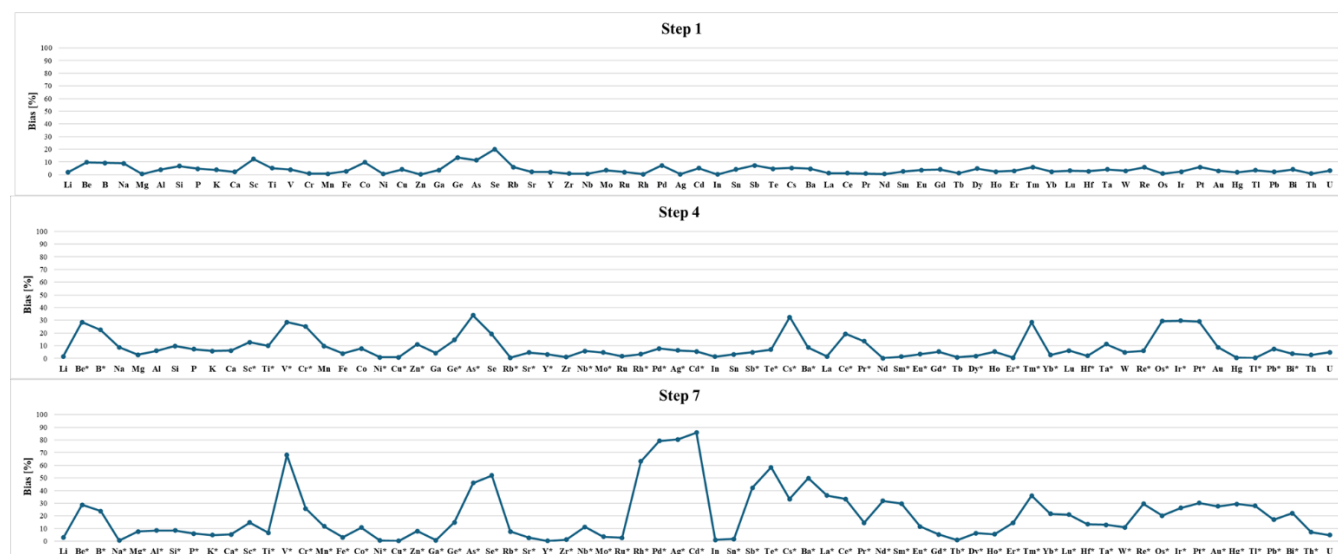


Fig. 9. Percentage bias [%] in the determination of 69 elements in aqueous standard solutions using different number of elements for calibration.

The accuracy of determination the concentration of macroelements (Na, Mg, Al, Si, P, K, Ca, Fe) is not significantly affected by their absence in the calibration. For them, the relative bias varies between 0.5 and 9.8%, and in some cases, it can even be lower when they are excluded from the calibration elements (e.g. for Na and P). The results for microelements show that the bias varies from 0.1 to 11% when the corresponding element is present in the method, except for Se – 21%.

The data also show that for a large number of the trace elements the accuracy does not change significantly when they are excluded from the calibration. The relative bias ranges from 0.1 to 15% for 28 elements (Sc, Ti, Mn, Co, Ni, Cu, Zn, Ga, Ge, Rb, Sr, Y, Zr, Nb, Mo, Ru, Sn, Pr, Eu, Gd, Tb, Dy, Ho, Er, Hf, Ta, W, Th). For the remaining elements, the bias increases when they are eliminated from the calibration step. For example, for Be, B, Cr, Yb, Lu, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, and Bi the bias is 15 and 30%, for As, Sb, Cs, Ba, La, Ce, Nd, Sm, Tm it reaches 30 – 50%, and for V, Se, Rh, Pd, Ag, Cd and Te is above 50%.

It is important to establish not only the relationship between the bias and the absence of a particular element from the calibration stage, but also from the elimination of elements with adjacent masses, i.e. narrowing the interpolation intervals. For 13 elements (Be, B, As, Se, Cs, La, Ce, Nd, Sm, Lu, Os, Ir, Pt) a sharp decrease in accuracy is achieved as a result of

their exclusion from the calibration and ranges from 13-54%, regardless of the presence of elements with adjacent masses. For others, such as V, Cr, Rh, Pd, Ag, Cd, Sb, Te, Ba, Pr, Eu, Tm, Yb, Hf, Re, Hg, Tl, Pb and Bi, the presence and absence of elements with adjacent masses after their elimination has a great influence. The bias varies in a wide range from under 1% (Cr, Ag, Cd, Ba, Eu) to 80–90% (Pd, Ag, Cd). The increase in the bias could be due to spectral interferences and the impossibility of correct interpolation under standard measurement conditions in a large interpolation range. For the remaining microelements (Sc, Ti, Mn, Co, Ni, Cu, Zn, Ga, Ge, Rb, Sr, Y, Zr, Nb, Mo, Ru, Sb, Eu, Gd, Tb, Dy, Ho, Ta, W, Au, Th, U) reducing the number of elements doesn't not significantly affect the accuracy of the results.

The evaluation of the obtained data shows that despite the theoretical possibility of obtaining quantitative results in calibration with three elements, in the determination of some micro- and trace elements, the bias is over 50%. The percentage bias from step 1 to step 4 is between 20 and 34% (Fig. 9). Based on these data, it can be assumed that the semi-quantitative analysis is successfully applied, with a bias of up to 30%, but for microelements it is necessary to provide narrower intervals and the presence of an element through at least 3–4 masses for calibration (as is the case, for example, in step 4). Reducing the number of macroelements does not affect the accuracy of their determination in a wide interval and any of them can be used for calibration.

IV.2.4 Analysis of landfill leachate reference material LGC6177

Figure 10(A) and 10(B) presents the percentage bias (%) obtained in Step 1 and Step 7 for the macroelements (B, Na, Mg, P, K, Ca, Fe in this case) and selected microelements in the reference material LGC6177 (landfill leachate) The values were calculated using certified values or concentrations obtained from quantitative analysis. The accuracy of the determination of non-certified elements was determined by standard addition method.

The analysed reference material has a complex matrix, which is a prerequisite for the presence of a number of matrix and spectral interferences that could have an impact during the analysis. However, relatively low deviations from the certified values of the macroelements were obtained. The calculated percentage bias varies between 0.3 and 18% in the presence and 0.8 and 20% in the absence of the given macroelements. For Mg and P, it is even lower when they are excluded from the calibration. The only exception is B, for which the percentage bias reaches 36% in its absence from the set of calibration elements.

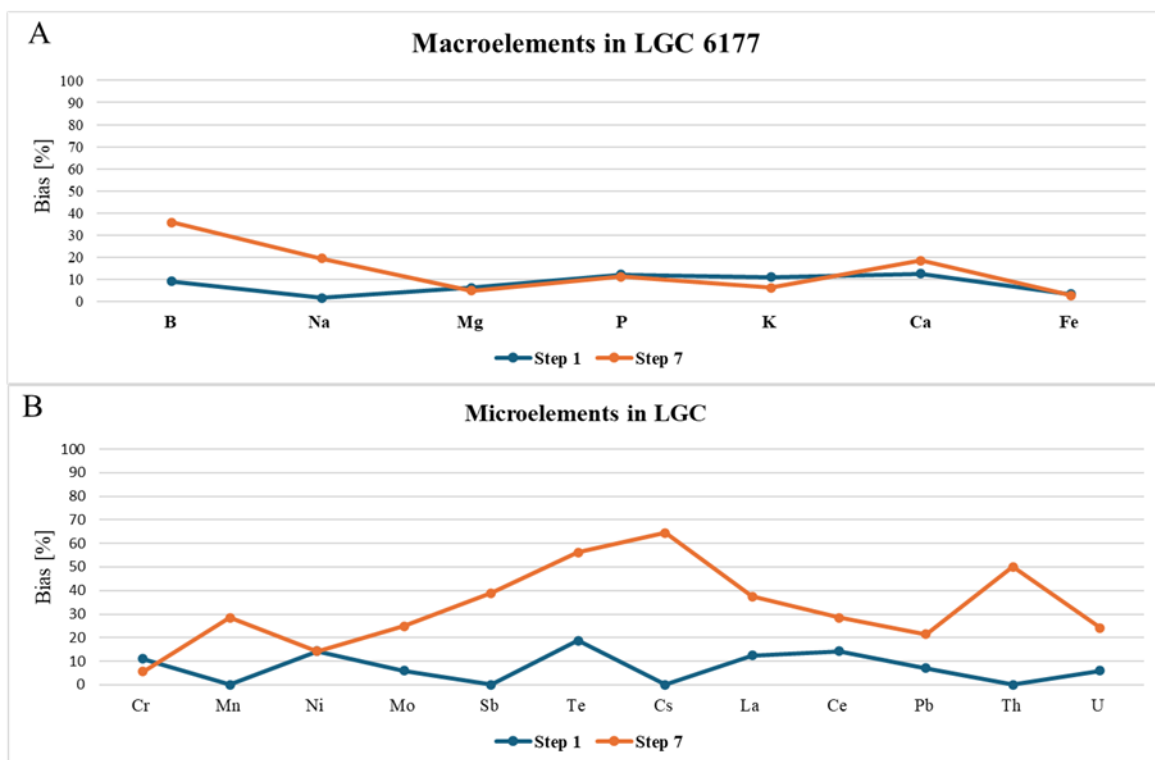


Fig. 10. Percentage bias [%] in semi-quantitative analysis of reference material LGC6177.

The intervals in which the bias varies in the determination of micro- and trace elements are much wider than those of macroelements. This is indicative of the dependence of the concentration of the element, as some of them are present at trace concentrations. Additionally, the presence of spectral interferences from matrix elements is expected for them. When microelements are included in the calibration, a percentage bias of over 30% is observed for elements present in concentrations between 0.09 and 0.6 $\mu\text{g/L}$ (for Se – 36.6 $\mu\text{g/L}$), with the highest being for As – 44%. In the absence of the elements, the deviation is over 30%. In addition to low concentrations, the presence of spectral interferences also contributes to the increase of the bias. Some rare earth elements (REE - Y, La, Ce, Pr, Nd, Eu, Tb, Ho and Tm), as well as In, Ta, Th, U, are present in concentrations below 1 $\mu\text{g/L}$. However, their determination is characterized by a bias of up to 50% except for Eu and Ho. The microelements Be, Re, Os, Pt are below LOD in both semi-quantitative and quantitative analysis. Other trace elements (Rh, Ag, Cd, Sm, Dy, Er, Yb, Lu, Ir, Au and Tl) have measurable concentrations (below 1 $\mu\text{g/L}$) when applying quantitative analysis, but are below the LOD of the semi-quantitative analysis due to its lower sensitivity. Comparison of the accuracy of the semi-quantitative analysis with literature data is impossible due to the lack of data for conducting semi-quantitative analysis of wastewater. However, the calculated range for percentage bias is

satisfactory, considering accuracy data from semi-quantitative analysis of natural waters – a percentage bias between 10% (Chen et al, 2008) and 50% (Jitaru et al., 2003).

For many of the trace elements, a sharp increase in the deviation was found after their exclusion, and for others - a strong dependence not only on their presence, but also on the presence of elements with adjacent masses. The factors that affect the accuracy of semi-quantitative analysis are complex and, in addition to the element's participation in calibration, the element concentrations, the presence of spectral interferences, and the presence of elements with adjacent masses have a major impact. In confirmation of this is the significant increase in the error in the determination of V, Pd, Sb, Te, Cs, Ba, Eu and Hg after excluding elements with adjacent masses, as already established for the aqueous standard solution. The presented results show the need for narrow interpolation intervals (for example, through 3–4 elements), when the aim of the analysis is to obtain satisfactory accuracy for micro- and trace elements present in concentrations close to the LOD. At large mass interpolation intervals, the results for them are only semi-quantitative and the bias can be over 100%.

IV.2.5 Analysis of certified reference materials from WWTP sewage sludge - CRM 029 and ERM-CC144

The results of the analysis of the solid CRMs after the calibration in the first and the seventh step with water standard solution are presented in Fig. 11 (CRM 029) and Fig. 12 (ERM-CC144) for the macro- and selected microelements.

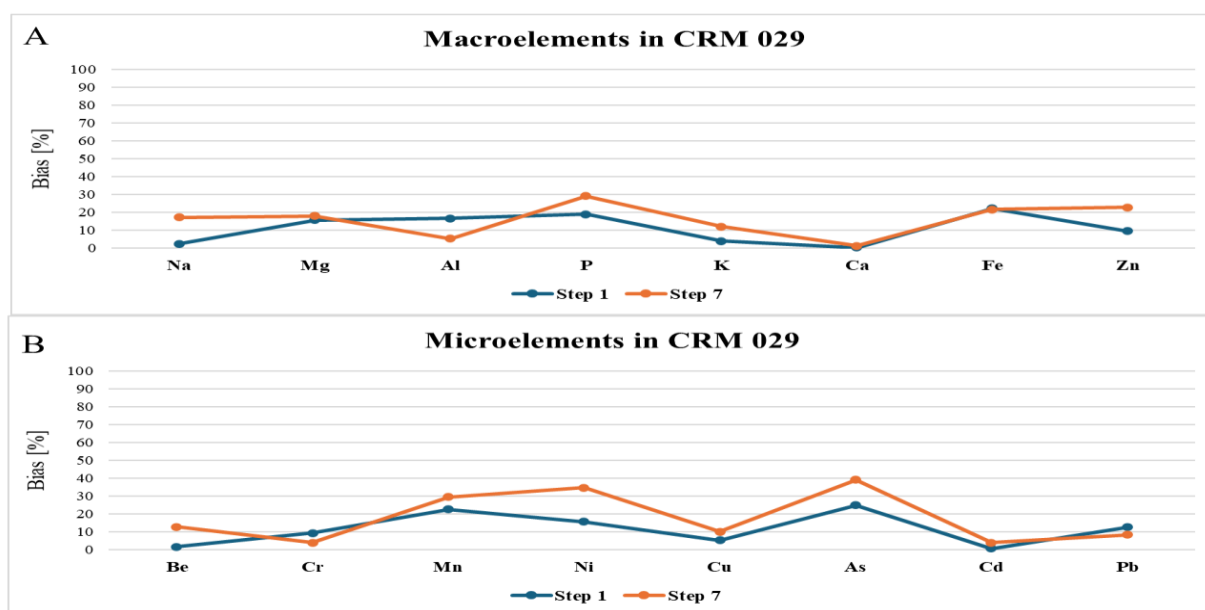


Fig. 11. Percentage bias [%] in semi-quantitative analysis of CRM 029, for the concentrations of macroelements and selected microelements, after sequential reduction of the elements, used for calibration of the method.

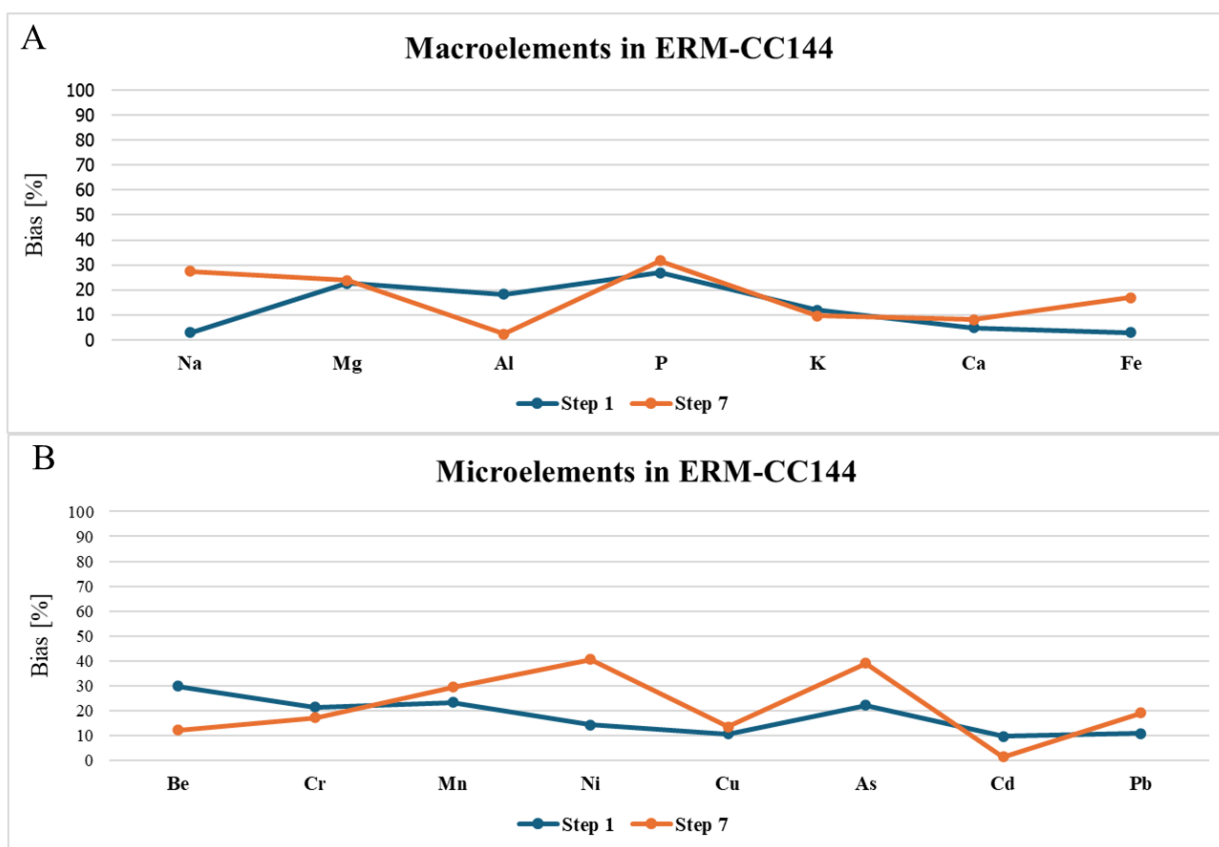


Fig. 12. Percentage bias [%] in semi-quantitative analysis of CRM ERM-CC144, for the concentrations of macroelements and microelements, after sequential reduction of the elements, used for calibration of the method.

The data show that the concentrations of the macroelements Na, Mg, Al, P, K, Ca and Fe in solid CRMs are characterized by a constant bias that does not depend significantly on the presence of the elements in the calibration, as already established for the liquid reference material LGC6177. The accuracy, calculated as a percentage bias, reaches a maximum value of 15% to 30%.

Regarding micro- and trace elements, it has been established that the accuracy depends on several factors. When they are present as calibration elements, the bias reaches 30% in both CRMs, and for some elements (Ti, V, Ga, As, Rb, Sn, U) it is up to 15% in one CRM and up to 30% in the other CRM. When they are eliminated from the method, the bias increases for most of the elements. Values up to 15% bias are obtained for a limited number of elements (Cr, Ga, Ge, Cd, La, Ce, Nd in both CRMs; Ti, Cr, Sr, Cs, Eu, Pb in CRM 029; Be, Zr, Ag, Sn, Dy in ERM-CC144). The percentage bias varies between 15 and 30% for 11 elements in both CPMs (B, Sc, Mn, Co, Cu, Zn, Rb, Y, Ba, Bi, Th), for Be, Se, Zr, Ag, Sn, Sb, Gd, Tb in CRM 029 and for Ti, V, Cr, Sr, Te, Pb in ERM-CC144. This shows that the correct determination by semi-quantitative method of about half of the elements is possible. Bias values between 30 and 50%

were obtained for Ni, As, Nb, Mo, Pr, Sm, Hf, Ta, W, Au, Hg in both CPMs, for V, Te, Dy, Ta, in CRM 029 and for Sb, Cs, Gd in ERM-CC144.

The difference in the bias for some trace elements in the two CRMs (e.g. Cr, Ag, Sb and some of the REE) with a similar matrix and, accordingly, similar spectral interferences, can be explained by their different concentration. A percentage bias of over 50% was found for Ho, Er, Yb, Lu in CRM 029 and for Eu and Tb in ERM-CC144, present in concentrations below 0.5 mg/kg. Obviously, the exclusion of elements present in trace concentrations leads to their determination with very poor accuracy. Indicative of the lower sensitivity of the semi-quantitative method than the quantitative method is the inability to determine the trace elements Sc, Se, Ho, Er, Yb, Tl, etc. in ERM-CC144. Despite the expectations of a strong influence of the matrix and, accordingly, the presence of strong spectral interferences in the absence of corrections in the semi-quantitative analysis, a greater influence on the accuracy is exerted by the concentration in which the given element is present.

The results of the analysis of solid CRMs give a reason to conclude that, thanks to the use of the RPa parameter for the macroelements, it is possible to determine about 40 elements with a bias up to 30%. For elements present in a concentration above 1 mg/kg, the bias varies in a certain interval that does not depend on the number of elements included in the calibration, for example, for Ti 0.5 – 16%, Cr 1 – 15%, Cd 1 – 14% in CRM 029 and Ti 10 – 32%, Co 1.5 – 23%, Cd 2 – 13% in ERM-CC144 (it is even possible to reduce the percentage deviation in the presence of a smaller number of elements). The reduction of the calibration elements most significantly reduces the accuracy of determination of elements in trace concentrations, for example, As, Se, Pr, Eu, Tb, Ho, Er, Yb, Lu, Hf, and Au, especially in the presence of spectral interferences (Se, V).

IV.2.6 Comparison of the accuracy of the results obtained after calibration with an aqueous standard solution and with a CRM solution

An analysis of the sewage sludge ERM-CC144 WWTP was conducted after calibration with CRM 029 in five steps. The calibration included 29 elements with certified values, with each subsequent step reducing their number up to 3 elements in the fifth step (Fig. 8).

When all elements are included in the calibration, the percentage bias reaches 16%. After their elimination, the bias decreases for As, Co, Hg, Mn and Ni, which is in the range of 30–45% for As and Hg, and in the range of 20–30% for Mn, Co and Ni.

To demonstrate the influence only of the matrix, not the number of calibration elements, Fig. 13 presents the values of the percentage bias of the certified elements from the steps in which they were used for calibration with both approaches.

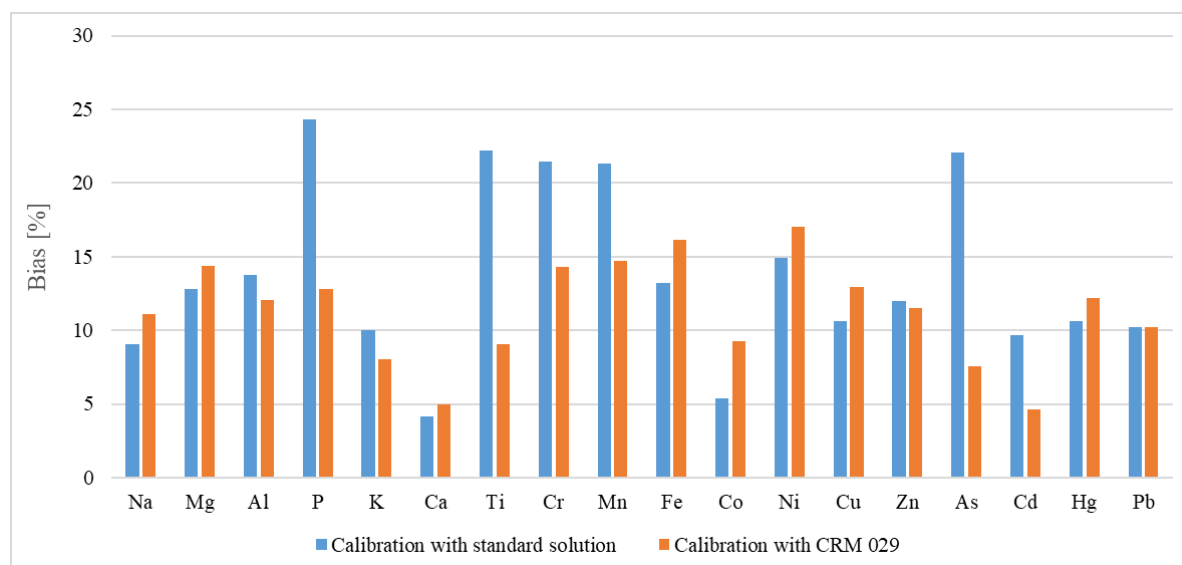


Fig. 13. Comparison of the mean bias [%] from the measurements of ERM-CC144 from the steps in which the elements were used for calibration.

The figure shows that the bias of the results obtained after calibration with CRM 029 have a maximum value of 15% (except Ni – 17% and Fe – 16%) and when using a standard solution – up to 25%. The statistical significance of the results obtained by calibration with an aqueous standard solution and CRM 029 was assessed by applying t-test. The data show that regardless of the applied calibration approach, good agreement between the experimental and certified values was obtained for Na, Ca, Co, Zn, Cd, Hg and Pb. A statistically significant difference is obtained for Mn and Ni in both approaches. For Al, P, K, Ti, Cr, Ti and As, and during calibration, a better match of the experimental and certified values is obtained when calibrating with CRM 029, and for Mg, Fe and Cu when calibrating with an aqueous standard solution. The conducted experiment shows that in the presence of a suitable CRM, with a matrix close to the studied sample and certified values for a sufficient number of macro- and microelements, it is advisable to carry out the calibration with CRM, in order to obtain better accuracy.

IV.2.7 Application of the semi-quantitative method for the analysis of sewage sludge

The developed semi-quantitative method was applied for the analysis of sewage sludge samples. Table 5 presents the results for the macroelements, and in Table 6 for micro- and trace

elements, obtained after calibration with a multielement standard solution (MSS) and with CRM, with the maximum number of elements in both approaches.

Table 5. Concentrations of macroelements [mg/kg] in WWTP sewage sludge after calibration with multielement standard solution (MSS), CRM 029 and quantitative analysis.

Elem.	Sewage sludge – Blagoevgrad			Sewage sludge – Ihtiman		
	Cal. with MSS	Cal. with CRM 029	Quantitative analysis	Cal. with MSS	Cal. with CRM 029	Quantitative analysis
Na	4386±175	3146±140	5124±105	3567±163	3004±108	4068±102
K	5745±270	9215±294	10251±230	3594±180	3836±192	3418±165
Mg	5616±224	7454±251	4845±208	2591±103	3191±112	3587±108
Ca	15854±634	16854±680	16029±610	35212±1300	39128±1410	41649±1180
Al	33252±1995	32690±1847	33449±1240	3479±208	3460±200	3643±190
P	15110±760	19511±810	21564±650	31477±1573	31085±1680	32806±1300
Fe	28112±1405	23622±1360	26804±1210	196024±9800	164158±8200	238603±10900

Table 6. Concentrations of micro and trace elements [mg/kg] in WWTP sludge after calibration with MSS, CRM 029 and quantitative analysis (QA).

Elem.	Sewage sludge – Blagoevgrad			Sewage sludge – Ihtiman		
	Cal. with MSS	Cal. with CRM 029	QA	Cal. with MSS	Cal. with CRM 029	QA
Li	10.1±0.8	8.2±0.7	9.0±0.5	0.20±0.09	0.23±0.07	0.24±0.04
Be	1.3±0.1	1.0±0.1	1.13±0.06	0.41±0.02	0.16±0.02	0.08±0.01
B	< 0.002	0.64±0.05	0.82±0.03	< 0.002	< 0.002	< 0.002
Ti	2052±102	2213±110	2573±91	113±6	162±8	166±7
V	86±7	58±6	82±5	74±6	83±6	80±4
Cr (500)	91±5	96±4	88±4	252±12	265±15	185±11
Mn	112±9	162±8	194±6	3070±245	3149±250	3316±329
Co	6.9±0.6	5.8±0.5	6.5±0.3	34±2	31±2	34±1
Ni (350)	37±2	26±1	29±1	150±6	97±4	108±3
Cu (1600)	208±9	221±8	229±7	437±19	471±21	504±12
Zn (3000)	1285±102	896±89	1108±60	459±35	500±38	670±30
Ga	7.9±0.7	10.0±0.7	10.8±0.5	2.3±0.2	3.4±0.3	3.8±0.2
As (25)	11±1	14±1	15±1	18±2	18±2	12±1
Yes	< 0.024	< 0.024	85±5	< 0.024	< 0.024	< 0.024
Rb	35±2	36±2	26.2±0.9	4.7±0.3	4.4±0.2	7.6±0.3
Sr	154±8	161±9	126±5	177±8	190±7	152±6
Y	7.0±0.4	8.2±0.5	7.1±0.4	0.98±0.5	1.2±0.4	1.1±0.2
Zr	33±2	39±3	30±1	5.1±0.3	6.3±0.4	5.2±0.1
Nb	3.3±0.2	4.0±0.2	2.1±0.1	0.67±0.04	0.88±0.04	0.71±0.02
Mo	4.3±0.2	6.6±0.3	3.5±0.2	9.7±0.5	16.3±0.7	13.2±0.2
Pd	0.98±0.09	0.98±0.09	1.41±0.06	0.39±0.03	0.25±0.02	0.45±0.03
Ag	1.28±0.06	3.1±0.4	3.5±0.2	1.2±0.1	0.91±0.09	0.93±0.04
Cd (30)	1.9±0.2	1.9±0.2	2.0±0.1	0.41±0.03	0.51±0.03	0.57±0.02
In	0.060±0.005	0.10±0.01	0.11±0.01	< 0.002	< 0.002	< 0.002
Sn	19.2±0.8	14.8±0.6	13.6±0.3	17.7±0.6	14.5±0.4	15.3±0.2
Sb	1.8±0.2	2.0±0.1	2.5±0.1	1.3±0.1	2.3±0.1	2.9±0.1
You	0.36±0.03	0.83±0.06	0.65±0.03	0.64±0.05	0.49±0.04	0.78±0.03
Cs	7.2±0.6	4.6±0.4	6.8±0.3	< 0.002	0.14±0.01	0.13±0.01
Ba	419±33	482±38	480±15	266±21	229±16	226±11
La	12.2±0.6	6.7±0.4	10.7±0.3	1.8±0.1	0.86±0.05	1.6±0.1
Ce	23±1	12.7±0.6	19.3±0.8	2.6±0.1	1.4±0.1	2.5±0.1
Pr	2.6±0.2	1.3±0.1	2.2±0.1	0.19±0.02	0.61±0.04	0.35±0.02
Nd	9.7±0.6	5.2±0.3	7.8±0.2	1.0±0.1	0.65±0.02	1.1±0.1

Sm	2.4±0.2	1.4±0.1	2.0±0.1	0.24±0.02	0.24±0.02	0.51±0.03
Eu	0.50±0.04	0.30±0.03	0.54±0.04	0.10±0.02	0.06±0.02	0.03±0.01
Gd	1.9±0.1	1.4±0.1	2.1±0.2	0.20±0.02	0.14±0.01	0.35±0.03
Tb	2.6±0.2	1.9±0.2	2.27±0.02	< 0.004	0.14±0.01	< 0.004
Dy	1.4±0.1	1.1±0.1	1.2±0.1	0.16±0.01	0.12±0.01	0.20±0.02
Ho	3.2±0.2	2.4±0.1	2.26±0.02	0.10±0.02	0.22±0.02	0.05±0.01
Er	0.72±0.06	0.73±0.06	0.68±0.04	0.12±0.02	0.06±0.01	0.10±0.01
Tm	4.7±0.2	3.1±0.2	0.16±0.03	2.5±0.2	2.7±0.2	0.69±0.04
Yb	0.46±0.03	0.54±0.03	0.65±0.04	0.06±0.02	0.04±0.01	0.15±0.01
Lu	< 0.004	< 0.004	0.10±0.01	< 0.004	0.06±0.01	0.02±0.01
Hf	0.82±0.07	1.1±0.1	0.60±0.03	0.16±0.02	0.14±0.02	0.09±0.02
Ta	0.26±0.02	0.42±0.03	0.14±0.01	< 0.004	0.06±0.01	0.015±0.001
W	< 0.002	13.6±0.5	5.0±0.2	< 0.002	10.0±0.5	5.5±0.2
Au	< 0.002	0.14±0.02	0.11±0.01	< 0.002	< 0.002	< 0.002
Hg (16)	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Tl	< 0.012	0.16±0.02	0.24±0.02	< 0.012	< 0.012	0.08±0.01
Pb (800)	42±3	43±2	32±1	10.5±0.9	11±1	9.6±0.4
Bi	1.2±0.1	1.5±0.1	1.8±0.1	3.5±0.3	2.7±0.2	2.2±0.1
Th	5.6±0.5	6.4±0.5	3.1±0.2	0.84±0.07	0.94±0.08	0.47±0.02
U	7.6±0.7	8.0±0.6	9.5±0.4	1.8±0.2	1.5±0.1	1.4±0.1

The concentrations of Ru, Rh, Re, Os, Ir and Pt are below the LOD in both the quantitative and semi-quantitative analysis and are not present in Table 6. The degree of closeness between the results of quantitative and semi-quantitative analysis varies for individual elements. It can be seen that for the main part of the elements they are relatively close. These results demonstrate the suitability of semi-quantitative analysis as a possibility for a rapid method for characterization of waste samples, for obtaining information on the content of matrix, micro- and trace elements with satisfactory accuracy when calibrated with only one standard solution, but by application of narrow mass interpolation intervals. For a small part of the trace elements, for example Mn, Zn, Mo, Sn and Th, significant differences are observed, regardless of the calibration method. Reducing the number of calibration elements leads to the most serious increase in the difference in the experimental results of semi-quantitative and quantitative analysis for elements present in concentrations close to the LOD, for example Mo, Rh, W, Tl and REE. In cases where high accuracy below 10% is required, quantitative analysis can be applied as a second step. The advantage of preliminary semi-quantitative analysis is the possibility of obtaining analytical data on the sample matrix, concentration ranges of individual elements, which provide information on the necessary concentration interval for calibration, appropriate sample dilution, the possibility of obtaining saturated signals, the presence of interfering elements, the need for correction, etc.

One of the most recommended approaches for managing WWTP sludge is its utilization as fertilizer in agriculture (Khnaïjer et al., 2020). The suitability of sludge for fertilizer is monitored according to Bulgarian and European legislation. The Bulgarian and European regulations specify maximum permissible concentrations for Cr, Ni, Cu, Zn, As, Cd, Hg and

Pb, the values indicated in brackets after the respective element in Table 6. Despite some differences in the experimental values obtained from semi-quantitative and quantitative analysis, the data clearly show that the concentrations of the potentially toxic elements are below the maximum permissible levels. The cited regulations do not specify a requirement for the accuracy of the methods. They only mention the instrumental methods (AAS, ICP-AES and ICP-MS) and the required LOD. This allows the use of a semi-quantitative ICP-MS method for the analysis of sludge intended for agricultural application. In case the concentrations determined are close to the MPC, it is necessary to apply quantitative analysis as a next step to achieve higher accuracy. In the literature, there are similar recommendations for the analysis of plant (Gałuszka et al., 2014) and soil samples (Laborda et al., 2001).

The data from the analysis of the studied sewage sludge samples show that the semi-quantitative method is a suitable approach for a quick assessment of the possibility of applying sewage sludge in agriculture with satisfactory accuracy. Another serious advantage of the optimized semi-quantitative method is that it offers a panoramic study of the concentrations of all elements present in the sample. Along with the data on the controlled potentially toxic elements, valuable information is simultaneously obtained for the microelements essential for the development of agricultural crops such as Na, P, K, Ca. This option reduces analytical work such as multiple dilution of samples, application of ICP-MS in cold plasma conditions, use of another analytical method, e.g. ICP-OES, which in all cases require at least two measurements of the samples with a set of standard solutions.

In summary, semi-quantitative analysis is suitable for rapid panoramic analysis of complex matrices such as sewage sludge. It allows simultaneous determination of concentrations of 69 elements with satisfactory accuracy. Higher accuracy can be achieved after calibration with a CRM with an appropriate matrix and the availability of certified values for both macro- and microelements. The best accuracy is obtained for macroelements when applying both calibration approaches – up to 30%. For microelements, the accuracy varies over a wide range depending on the concentration, their use in the calibration and/or the presence of adjacent masses. The worst accuracy is obtained for elements present in concentrations close to the LOD, due to the lower sensitivity of semi-quantitative compared to quantitative analysis.

Another significant factor affecting accuracy is the presence of uncompensated spectral interferences. Although the semi-quantitative method can be applied after calibration with three elements, for the analysis of waste samples with a complex matrix, it is recommended to use

more elements to narrow the mass intervals for interpolation, especially for the determination of trace elements. In order to reduce the analytical work of preparing a series of standard solutions with a full set of elements and at the same time obtain a satisfactory accuracy of up to 30%, it is preferable to calibrate with a larger number of elements, for example about 30 (Step 4 in Fig. 7).

IV.2.8 Application of quantitative and semi-quantitative ICP-MS method for analysis of wastewater from WWTP - Kubratovo

The developed semi-quantitative and quantitative ICP-MS methods of analysis were applied to the analysis of wastewater from the Kubratovo WWTP. Samples from the individual stages of the wastewater treatment process were studied: WWTP inlet (**influent**); sand trap outlet (**PZ**); outlet of the fourth primary clarifier (**PC**); fifth biobasin – aerobic zone (**Aerobic BB**), denitrification zone (**DZ**), biophosphorus zone (**Bio P**); outlet of the sixth secondary clarifier (**SC**); WWTP outlet (**effluent**).

The concentrations of all regulated elements (Regulation No. 12, 2002, Regulation No. H-4, 2012) in the influent and effluent during the studied seasons were below the MPCs, with the exception of Mn in the samples from the winter and spring sampling and Al during the spring sampling. The concentrations of all regulated elements in the influent and effluent during all three studied seasons are below the MPC in Regulation 12, with the exception of Mn in the samples from winter and spring sampling. These variations in the different stages are graphically presented as wastewater treatment efficiency, calculated using formula 2:

$$(2) \quad Eff. (\%) = \frac{(C_{infl.} - C_{effl.})}{C_{infl.}} * 100$$

where $C_{infl.}$ is concentration of the element in the influent, and $C_{effl.}$ is the concentration of the element in the effluent.

Figure 14 presents the treatment efficiency of macroelements in wastewater from the summer sampling.

The results show increased concentrations during the purification cycle, most significantly for P and Fe in the bio-basin zones. Such an increase is expected, having in mind that the activated sludge in bio-basins, used for a long period, has the ability to adsorb most elements. Due to the fact that activated sludge is used for a long period of time; it is possible to release a number of elements already adsorbed on it from a previous wastewater flow into the following flows. The concentrations of all macroelements in the effluent are lower

than those in the influent, which indicates that the treatment process is effective. A similar trend, even with a higher percentage of treatment efficiency, is also observed in the samples from the winter and spring sampling.

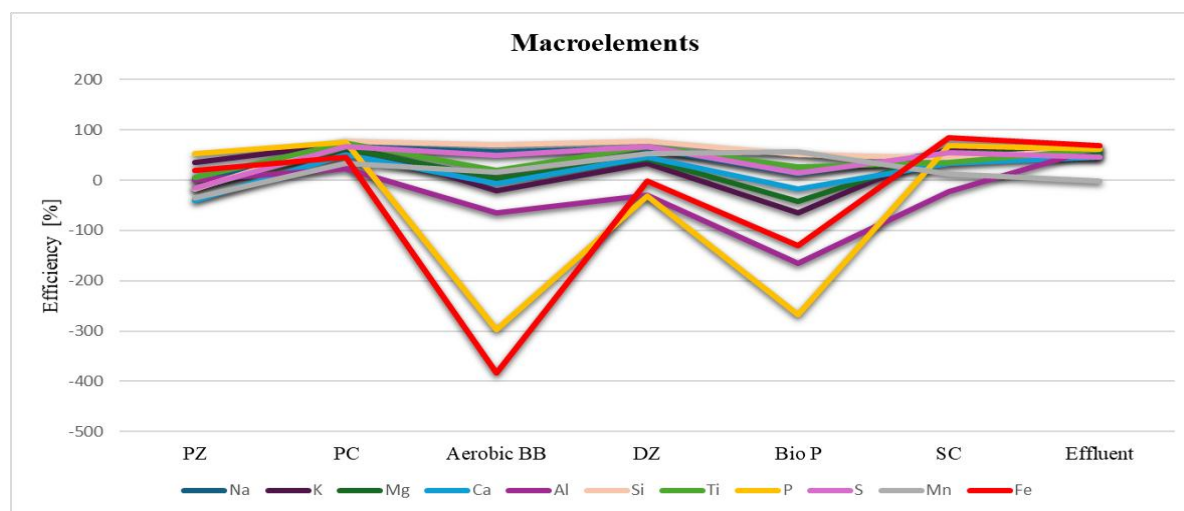


Fig. 14. Wastewater treatment efficiency [%] of macroelements in the samples from the Kubratovo WWTP from the summer season during the different stages of the treatment process.

Figure 15 shows the efficiency of wastewater treatment from the regulated elements in Regulation No. 12, 2002 and Regulation No. H-4, 2012.

From Figures 14 and 15, the efficiency of wastewater treatment can be traced. The data show that in the individual stages of the purification process, significant variations in the concentrations of the studied elements are observed. For example, after passing through the sand trap, the amounts of Cu, Se and Ba exceed the initial concentrations in the influent. The efficiency for Ba in the sand trap outlet (PZ) is -170%. An increase in the concentrations in different stages is also observed for B, Cu, Cd and Pb. However, despite the variations found, a positive efficiency was found in the effluent, with the exception of Cd (-78%) and Pb (-187%). However, their concentrations are far below the MPC (0.05 mg/L for both elements). The concentrations of Cd were 0.05 µg/L, 0.33 µg/L and 0.11 µg/L, and that of Pb is 0.23 µg/L, 0.28 µg/L and below <0.002 µg/L in the effluents from the summer, the spring and the winter season, which indicates that they did not represent an environmental problem. Positive efficiency at all stages was established for Cr, Co, Ni, Zn, As and U. At the outlet of the WWTP, all elements were lower or close to the concentrations of the elements before treatment, all of which were below the MPK, except for Mn and Al. For Mn, exceedances were observed both in the winter and spring seasons, and for Al in the spring season.

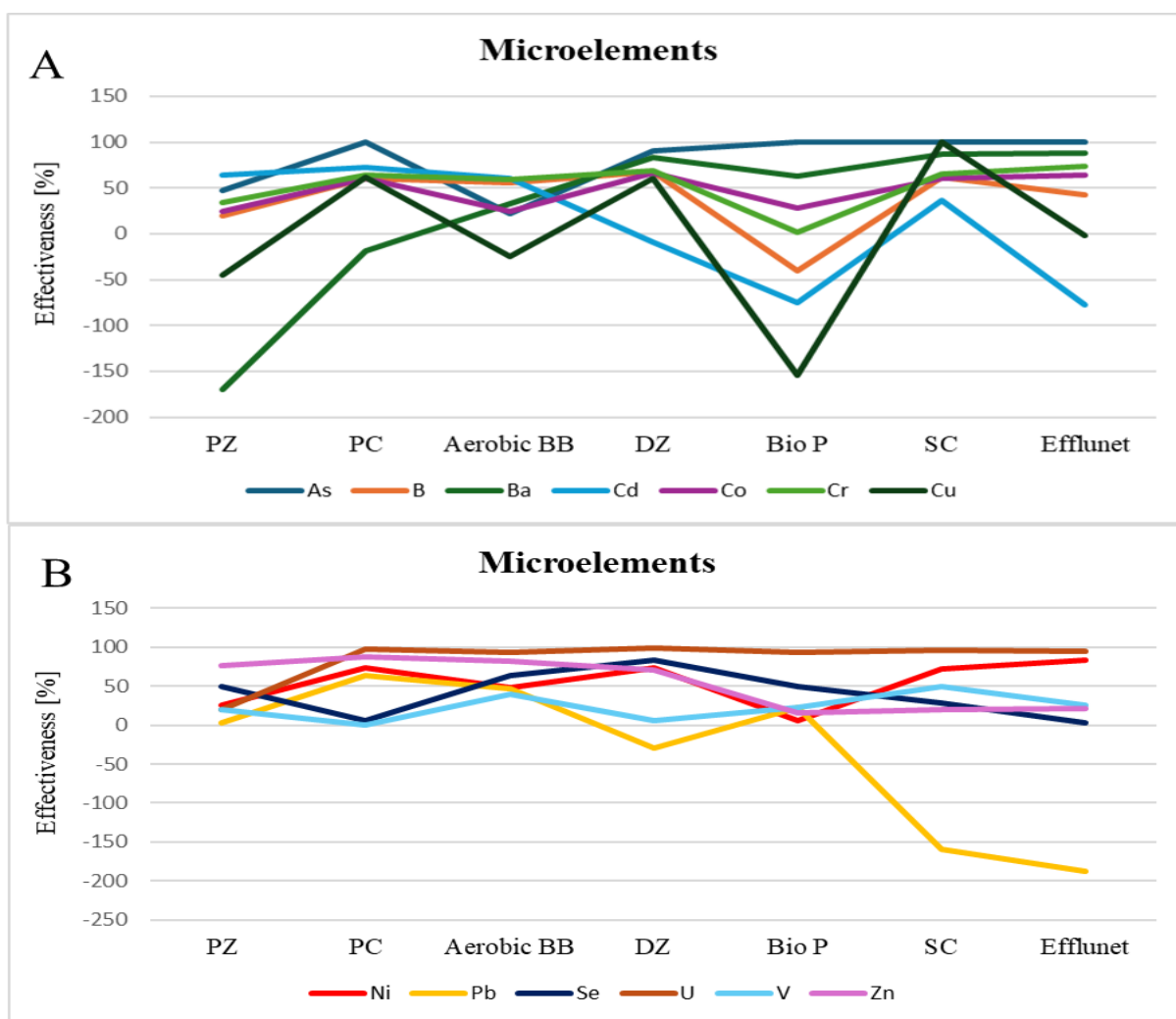


Fig. 15. Wastewater treatment efficiency [%] from potentially toxic elements included in Regulation No. 12 and Regulation No. H-4 (Al, U) in the samples from the Kubratovo - WWTP from the summer season during the different stages of the treatment process.

The data from the study show that the purification efficiency varies depending on the element. The concentration decreases to the greatest extent for Cr, Ni, As, Ba and U, for V, Mn, Se and Cu no change is observed, while for others – for example Cd and Pb, it is possible to increase. Lack of purification and/or increase in the concentration of elements has also been established in other authors (Shikuku et al., 2017), which may be due to desorption of the element from the activated sludge back into the water, probably due to its saturation over time and the accompanying competitive adsorption effects (Shikuku et al., 2017). A laboratory experiment with activated sludge from the WWTP – Kubratovo, taken from the denitrification zone showed similar effectiveness (Belouhova et al., 2022).

IV.3 Fractionation of WWTP sewage sludge

One of the most widely applied approaches for the management and disposal of sewage sludge in the EU is its reuse as fertilizer in agriculture. According to Bulgarian legislation (Regulation on the procedure and manner of utilization of sludge from wastewater treatment through their use in agriculture, 2004), the characterization of sewage sludge in terms of metal content includes the determination of heavy metals (Cd, Cu, Ni, Pb, Zn, Hg, Cr) and As, after aqua regia digestion, defined as extractable forms, for which MPC are specified. In addition, according to the regulation, extractable and exchangeable (extraction with 0.1 mol/L BaCl₂) species of K and P, as well as exchangeable species of Ca and Mg are examined, but no MPC are specified.

In the present work, in order to study the mobility and bioavailability of the controlled elements, in addition to the determined total concentrations, exchangeable species were determined after extraction with 0.1 mol/L BaCl₂ according to the regulation, a water-soluble fraction by extraction with deionized water, as well as BCR sequential extraction scheme to define their content in exchangeable (Fraction 1), reducible (Fe/Mn-oxides, Fraction 2) and oxidizable (organic and sulfide, Fraction 3). The accuracy of the results was assessed by analysis of the CRM BCR-701. The comparison between the experimental and certified values showed very good agreement.

The concentration of the elements in the respective extracts is presented as a percentage of the total concentration of the elements. Their percentage distribution in the extracts with deionized water, 0.1 mol/L BaCl₂ and BCR sequential extraction, as well as the sum of Fr.1, Fr.2 and Fr.3 from BCR extraction are presented in Fig. 16(A) - Sewage sludge - Blagoevgrad and Fig. 16(B) - Sewage sludge - Ihtiman. Mercury concentrations are not included because concentrations are below the LOD.

The results presented in Figure 16 show low rates of extraction of the elements using deionized water which is a sign for low mobility. The extracted fraction ranges from below 0.04% (Pb) to 7.9% (Ni). Despite the limited number of samples, it could still be concluded that the lowest leaching rates are found for Pb and Cr. These data indicate that there is no potential ecological threat when the sewage sludge is leached with water under natural conditions.

The extraction with BaCl₂ also shows a strong element dependence. The data show extraction below 1%, except for As (3.25%) in Sewage sludge-Ihtiman and extraction in the

range of 0.07% for Cr to 6% for Pb, except for Zn (17.5%) in Sewage sludge-Blagoevgrad. The results indicate a low degree of mobility of the potentially toxic elements.

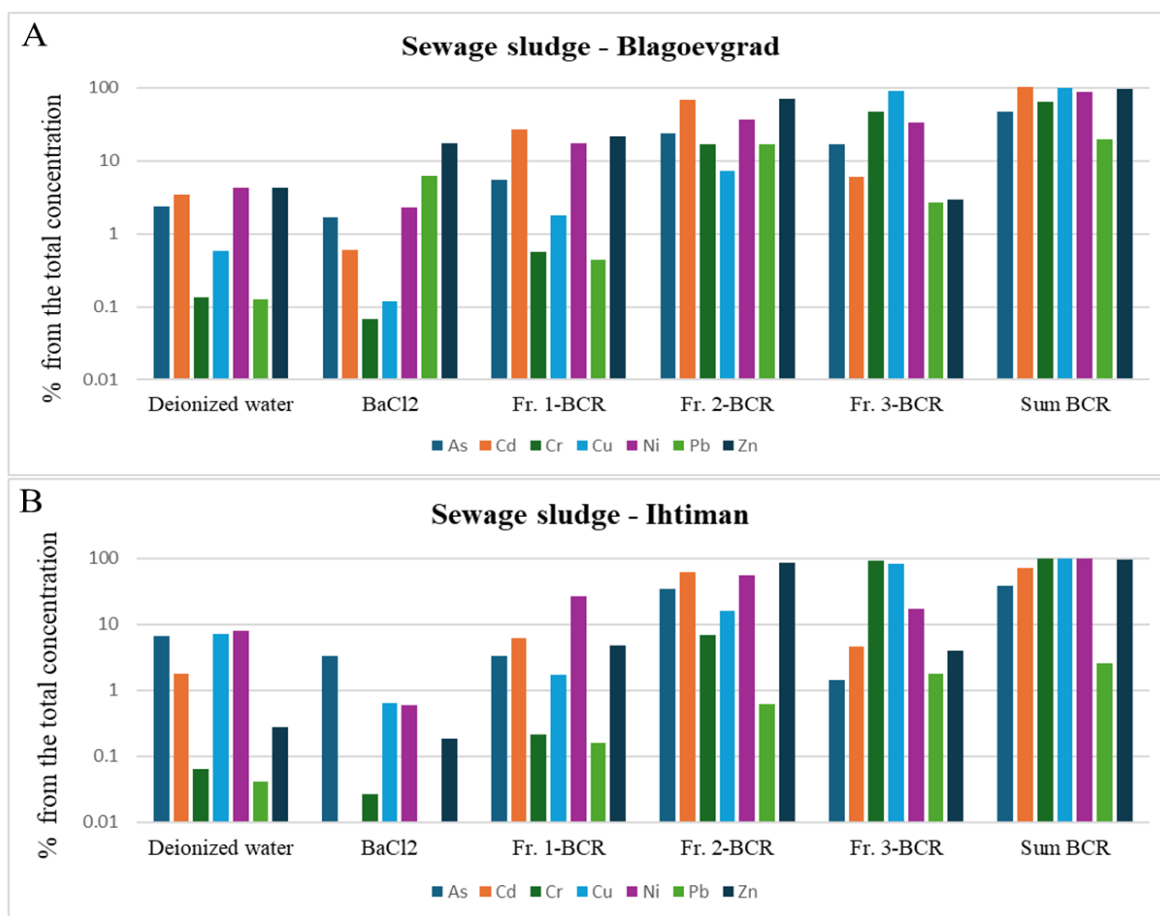


Fig. 16. Content of Cd, Cr, Cu, Ni, Pb and Zn and As, calculated as a percentage of the total concentration in the extracts with deionized water, 0.1 mol/L BaCl₂ and BCR sequential extraction, and the sum of Fr.1, Fr.2 and Fr.3 of BCR.

However, the data on the total extracted percentage by the BCRa sequential extraction procedure (Fr.1+Fr.2+Fr.3) show a high percentage of mobility. In the Sewage sludge-Blagoevgrad, quantitative extraction (from 95 to 100%) is established for Cd, Cu and Zn, Ni – 87% and Cr – 63%. Lower values are obtained for As – 46% and Pb – 20%. High total percentages are obtained for Cu, Cr, Ni and Zn (from 95 to 100%) and Cd – 72% and in the Sewage sludge-Ihtiman and a lower degree of extraction for As – 38.5% and Pb – 2.6%. Of interest is the distribution of toxic elements in the individual fractions. Figure 16(A) and 16(B) show a similar distribution of the studied elements in both sludges: Cu and Cr – mainly in the organic, and Cd, Ni and Zn predominate in the oxide fraction. Despite the lower recovery rates of As and Pb, the data again show a tendency for binding in the oxide fraction. Literature data on the fractionation of potentially toxic elements in sediments are scarce and contradictory.

Predominant binding in the organic fraction has also been found by other authors for Cu (Jakubus, 2020; Zaleckas et al., 2012) and Cr (Jakubus, 2020). For Cd, Ni and Zn, a predominant binding to the organic fraction was found, for Zn in the oxide fraction, as well as a high percentage of Pb binding in the oxide fraction, but predominantly in the organic fraction (Jakubus, 2020). For all elements, the lowest extraction percentage was found in the exchange fraction, indicative of high mobility and potential bioavailability of the elements. This result is consistent with the data from extraction with deionized water and BaCl₂. The percentage in this fraction varied between 0.15% (Pb) to 26% (Ni), which is within the ranges reported in the literature.

The fractionation of the essential elements K, Ca, Mg and P with 0.1 M BaCl₂, deionized water and the BCR extraction scheme are presented in Fig. 17. The extraction with 0.1 M BaCl₂ shows a low degree of ion mobility for all elements. Arranged by degree of mobility, the following order is obtained: P (0.42%-0.01%) < K (1.8%-5.1%) < Mg (8.3%-5.2%) < Ca (17.1%-8.0%) in both sludges. A higher degree of extraction for Ca, Mg and P was found in the sludge from a municipal WWTP (Sewage sludge – Blagoevgrad) - Fig. 17(A), and for K in an industrial wastewater treatment plant (Sewage sludge – Ihtiman) - Fig. 17(B).

As a result of the deionized water extraction the established order of mobility $P \sim K < Mg < Ca$ is confirmed, as well as the dependence of the extracted percentage of elements depending on the sample. The lowest mobility was found for P – 5.9% in the sludge from a municipal WWTP and 0.16% in the sludge from an industrial WWTP. Higher solubility in municipal WWTP is obtained for Ca (24.1% vs. 1.9%) and Mg (22.7% vs. 6.4 %), and in the industrial sludge for K (6.1% vs. 35%).

The results of the BCR sequential extraction showed the following general results:

- For K, a higher extraction was observed in the industrial sludge, the total extracted fraction being 21% in the municipal WWTP, compared to 66% in the industrial sludge. Despite this difference, in both sludges, K is mainly bound in the exchangeable fraction.
- High total calcium extraction percentage, reaching 100% in both sewage sludges and similar total percentages for Mg (68%, 73% respectively) was obtained. Both elements are associated mainly in exchangeable form, followed by oxide and organic forms.
- Low P extraction rate in the industrial sludge was obtained (total 1.4%), which is consistent with data from single extractions. In the sludge from the municipal WWTP,

a total extraction of up to 53% was observed, evenly distributed between the three studied BCR fractions.

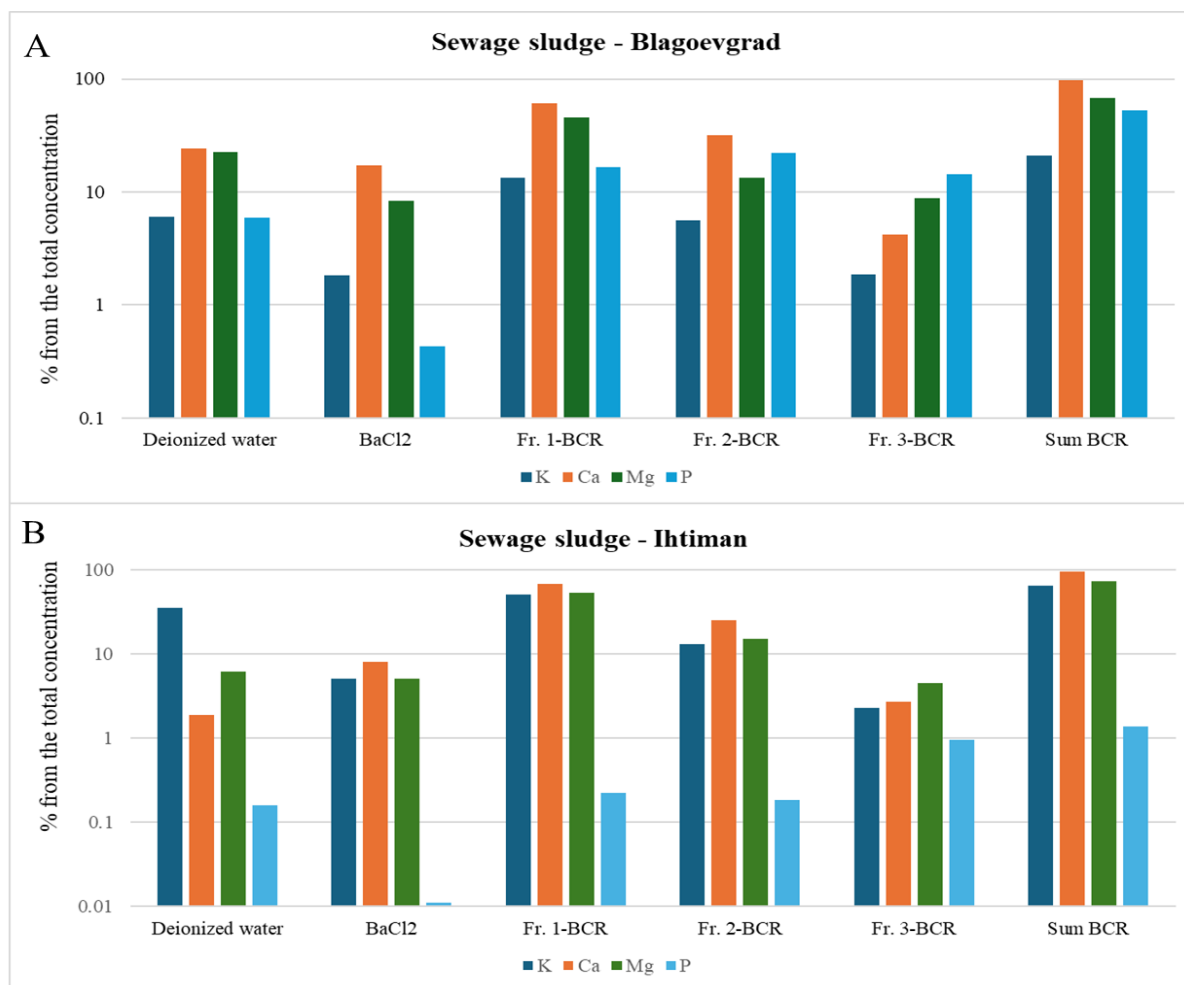


Fig. 17. Content of essential elements (K, Ca, Mg and P), calculated as a percentage of the total concentration in the extracts with deionized water, 0.1 mol/L BaCl₂ and BCR sequential extraction, and the sum of Fr.1, Fr.2 and Fr.3 of BCR.

Despite the limited number of samples, it could still be speculatively concluded that the mobility and bioavailability of the essential elements K, Ca and Mg are high and that the mobility of P is strongly dependent on the sample matrix.

IV.4 Assessment of the possibilities of the non-destructive LA-ICP-MS method for the analysis of WWTP sewage sludge

A commonly used method for elemental analysis of solid samples is LA-ICP-MS. Despite the significant advantages of the method, such as the lack of the need for acid digestion, the accuracy of the analysis can be affected by many factors, such as instrumental conditions (wavelength, pulse frequency, etc.), elemental fractionation, matrix effects, and sensitivity drift.

One of the most serious limitations for the application of the method to environmental sample analysis is the lack of solid CRMs with a matrix and geometry matching the samples which is required for quantitative calibration and accuracy assessment.

One approach that finds application for the analysis of powdered samples is the preparation of pellets with a suitable binder. To assess the possibility of waste tableting, in this study, pellets of CRM 029 and ERM-CC144, as well as WWTP sewage sludges samples (Sewage sludge – Blagoevgrad and Sewage sludge – Ihtiman) with different binders – H_3BO_3 , cellulose, graphite, activated carbon and silica gel were prepared. The ratio of sample to binder was studied – 1:5.

For the calibration of the LA-ICP-MS method of analysis, CRM from WWTP sludge – CRM 029 was used. The data obtained from the analysis of ERM-CC144, calculated as percentage bias, are presented in Table 7. The results of the analysis of heavy metals and arsenic, for which there are specified MPC and K, Ca, Mg and P, mentioned in the Regulation on the procedure and manner for the utilization of sludge intended for use in agriculture, are presented.

Table 7. Percentage bias [%], obtained by LA-ICP-MS analysis of ERM-CC144.

	H_3BO_3	Cellulose	Graphite	Activated carbon	Silica gel
As	28±1	95±4	90±2	90± 3	89± 2
Cd	11.0± 0.6	92±3	74±5	64± 6	96± 1
Cr	20±1	65±4	33±0.7	27.0 ± 0.6	43± 3
Cu	5. 6 ± 0. 3	50±2	11.2±0.3	28.3 ± 0.4	40± 2
Hg	75± 4	95±2	72±2	76± 5	92± 5
Ni	6.9± 0. 3	31±1	44±1	96± 1	86± 3
Pb	17.5±0.5	14.0 ± 0.9	10. 2 ±0.5	60 ±1	81± 3
Zn	16.8± 0.7	3 3 ± 1	4. 4 ±0. 1	17± 1	39± 1
K	22±1	20±1	19.8± 0.3	6.2± 0. 1	1 9 ± 1
Ca	6.2±0.3	6.45±0.5	8.0 ± 0.6	51± 2	77± 3
Mg	12.0±0.6	32±1	8. 8 ± 0.3	31.0 ± 0.4	35± 1
P	9.3±0.3	6.1±0.1	3.9± 0.2	22.3 ± 0.2	9.2± 0.4

During the pellet preparation, it was found that sufficient stability was not achieved for the pellets with activated carbon and silica gel binders. Probably for this reason, the resulting pellets had an unreproducible density, which led to evaporation of a different amount of aerosol and the results obtained had poor reproducibility and accuracy.

The results of the analysis of the other pellets showed that the most suitable binder is H_3BO_3 – the bias is below 25% for almost all of the studied elements, except for Hg and As.

The probable reason for the poor accuracy for these elements is their lower concentration and higher ionization energy. On the other hand, despite the careful grinding of the samples, as well as homogenization of the sample with the binders, it is possible that the pellets were not perfectly homogeneous, which is a key factor for high accuracy and reproducibility. The poor homogeneity of the pellets could be the reason for the obtained low and varying accuracy for some elements (for example Cu, Zn, Mg) in the pellets with cellulose and graphite.

The results obtained for the WWTP sewage sludge pellets are presented in Table 8. The values for the percentage bias were calculated using the concentrations, obtained from the quantitative analysis. The bias for the sewage sludge pellets showed lower accuracy than those of the CRM. The bias of the main part of the elements is over 50%, even reaching 100%. Again, the most suitable binding agent is H_3BO_3 . However, the bias for some elements, for example As, Ni, Cd, Ca. In addition, the data show different accuracy of these elements in the sewage sludge samples when using the same binding agent. This result could be explained by the different concentration of the elements.

Table 8. Percentage bias [%], obtained by LA-ICP-MS analysis Sewage sludge – Blagoevgrad and Sewage sludge – Ihtiman.

	Sewage sludge – Blagoevgrad			Sewage sludge – Ihtiman		
	H_3BO_3	Cellulose	Graphite	H_3BO_3	Cellulose	Graphite
As	37±1	98±2	84±5	61±1	48±2	96±2
Cd	15.1±0.8	96± 2	87±3	97±2	99±2	97±2
Cr	5.0±0.2	38±2	56±2	10.8±0.3	13.5±0.8	22±1
Cu	22.7±0.6	8.7± 0.8	11±1	3.6±0.3	34±5	9.5±0.2
Ni	39.6±0.9	31±1	91 ±4	34±1	9.8±0.4	64±2
Pb	8.1±0.7	13.7±0. 3	19.4 ±0.7	17.6±1.3	77±2	16±1
Zn	17.2±0.8	40±1	17.1±0.9	6.3±0.6	39±2	1.9±0.3
K	7.5±0.8	56 ±1	22±1	1.11±0.4	56±1	31±1
Ca	41±1	5.9±0.7	29±0.4	29±1	73±3	39±2
Mg	9.7±0.7	12.1±0.9	19.8±0.9	13.1±0.5	10.1±0.8	24±1
P	4.0±0.1	57±2	59±2	19±1	9.3±0.5	23±1

Thus, it can be concluded that LA-ICP-MS is not a suitable method for analysis of sewage sludge, regardless of the binder. The accuracy of the obtained results is comparable to that of semi-quantitative analysis, even in some cases it is worse. The main reasons for poor accuracy are the inhomogeneity of the samples, the possibility of different particle sizes within a sample and the difference between the samples and the CPM used for calibration, as well as the fact that a CPM is usually used for calibration in which the elements are in different concentrations than those in the analyzed samples.

IV.5 Analysis of radiochemical contaminants

One of the objectives of the present work is to investigate the possibilities of concentration of the radionuclides ^{241}Am , ^{60}Co and ^{137}Cs by extraction from a water matrix using 1-methyl-3-octylimidazolium saccharinate. This would allow their more precise determination by alpha- or gamma-spectrometric methods, in the study of surface water or wastewater samples. Their concentrations in various water sources are monitored regularly, as described in Regulation No. 9 of March 16, 2001 on the quality of water intended for drinking and domestic purposes.

IV.5.1 Optimization of the ionic liquid:aqueous phase ratio

A series of experiments were conducted to establish the optimal ratio between ionic liquid (IL):aqueous phase.

The results of the experiments are presented as extraction yield (EY, %), calculated by formula 3:

$$(3) \quad EY \% = \frac{A_i - A_f}{A_i} \cdot 100,$$

where A_i represents the initial activity in the sample under study, and A_f is the final activity in the aqueous phase (during the extraction of radionuclides from the aqueous phase with an ionic liquid or in the ionic liquid (during re-extraction of radionuclides from the ionic liquid);

as well as the separation factor (SF) – formula 4:

$$(4) \quad SF = \frac{X_1}{X_2},$$

where X_1 is the extraction yield of one radionuclide, and X_2 – of another.

As can be seen from Table 9, the extraction yield for all three radionuclides remains relatively constant at ratios of IL:MS from 1:3 to 1:5, while a subsequent increase in the volume of the MS leads to its decrease.

Table 9. Extraction yield EY [%] of radionuclides ^{241}Am , ^{60}Co and ^{137}Cs at different ratios between the ionic liquid (IL) and the model solution (MS).

Radio-nuclide	IL:MS 1:3	IL:MS 1:4	IL:MS 1:5	IL:MS 1:6	IL:MS 1:7	IL:MS 1:8	IL:MS 1:9	IL:MS 1:10
^{241}Am	46±2	44±1	42.5±0.9	30±1	26±1	24±1	14.0±0.5	9.0±0.1
^{60}Co	92±1	89±3	88±3	79±2	64.0±0.5	48.0±1.8	30.0±1.2	28±2
^{137}Cs	24±1	20.5±1.2	22.0±0.4	16.0±0.2	14.0±0.3	11.0±0.1	10.0±0.1	8±0.1

In the indicated interval, the extraction of ^{241}Am is achieved between 42%-46% and about 90% for ^{60}Co , even before optimizing the other factors affecting the extraction efficiency. The lowest degree of extraction is found for ^{137}Cs .

IV.5.2 Optimization of extraction time

The second group of experiments aimed to determine the appropriate contact time between the two phases. The shorter the time required to reach equilibrium, the more widely the radionuclide extraction procedure would find application.

The obtained results (Table 10) show that ^{137}Cs is in equilibrium under the established conditions from the beginning of the extraction and this does not change with time. ^{241}Am and ^{60}Co , on the other hand, require a longer contact time between the two phases in order to achieve the transfer of the maximum quantity of radioisotopes from the aqueous phase to the IL. The data in Table 10 show that after the third experiment, in which the sample was left to rest for 60 min, a plateau is reached for all three radionuclides (43.5% - 46.5% for ^{241}Am , about 90% for ^{60}Co and 20% for ^{137}Cs), therefore a longer contact period would be unnecessary.

Table 10. Extraction yield EY [%] of ^{241}Am , ^{60}Co and ^{137}Cs at different extraction times between the ionic liquid (IL) and the model solution (MS).

Radionuclide	15 minutes	30 minutes	60 minutes	90 minutes	120 minutes	720 minutes
^{241}Am	16.0±0.4	24±1	42.5±0.9	43.5±1.3	44.0±2.5	46.5±1.5
^{60}Co	74±1	85±2	88±3	90±3	88.0±0.9	89.0±1.4
^{137}Cs	15.0±0.2	16.5±0.9	22.0±0.4	20.1±0.5	19.5±1.0	20±1

IV.5.3 Optimization of the pH of the aqueous solution

The next group of experiments aimed to determine the influence of the pH of the aqueous phase on the extraction efficiency.

The studied radionuclides belong to different groups of chemical elements (^{137}Cs – s-element, ^{60}Co – d-element, ^{241}Am – f-element), therefore they exist in different forms under conditions of equal acidity of the medium, depending on their charge, stability and ability to form complexes. To determine the possible complexes formed between ^{241}Am , ^{60}Co , ^{137}Cs and the components of the buffer solution, the HYDRA - Hydrochemical Equilibrium Constant Database software, provided by the Royal Institute of Technology (KTH), Stockholm, Sweden (Fig. 18) was used.

From Figure 18 it can be seen that ^{137}Cs present in the solution in only two forms – Cs^+ and $\text{Cs}(\text{cit})^{2-}$ - complex ion of ^{137}Cs with citric acid from the universal buffer, regardless of the acidity of the medium.

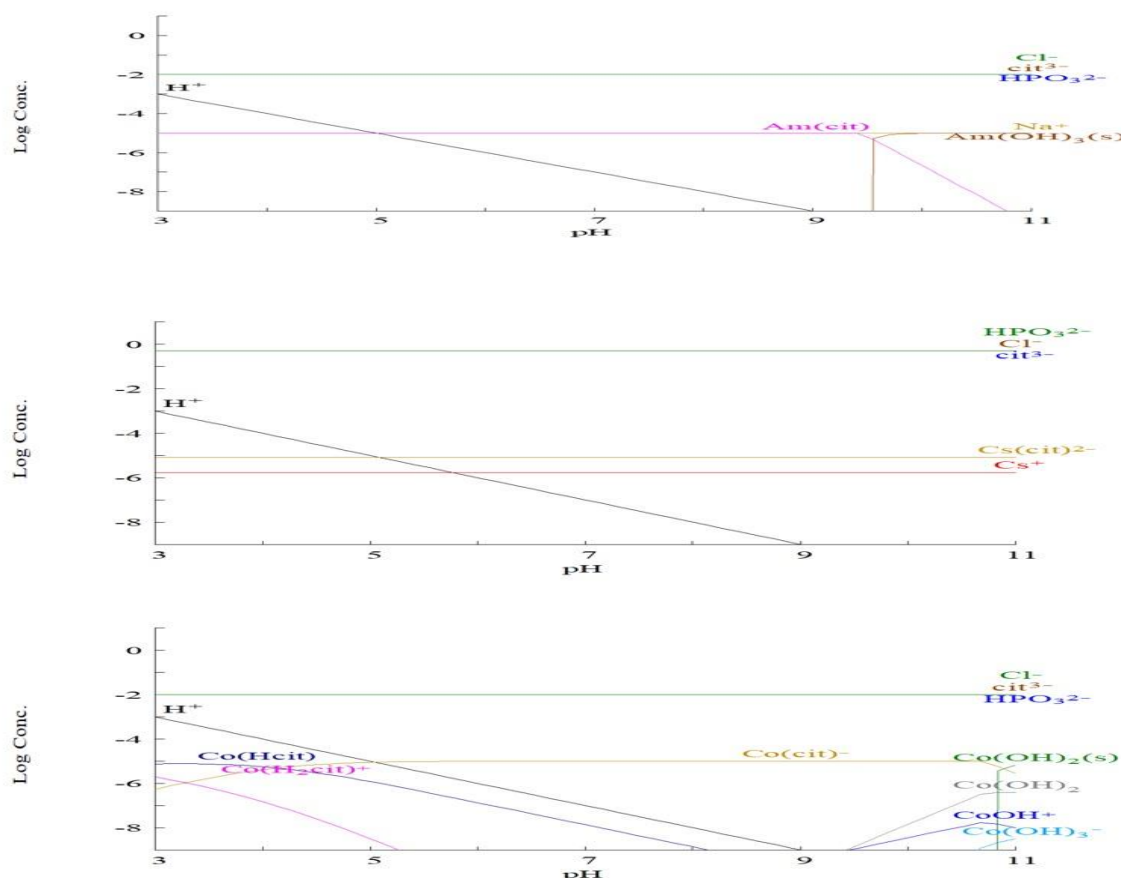


Fig. 18. Complexes at ^{241}Am , ^{60}Co and ^{137}Cs , formed in the presence of a universal buffer, at different acidity of the medium.

The results in Table 11, presenting the extraction yield when varying pH from 3 to 11, show an almost constant value for the transfer of Cs from the model solution to the IL – about 22% from pH=3 to pH=7, followed by a slight increase at pH=9 – 42% and a decrease at pH=11 (30.4% yield). Its preferential retention in the model solution, regardless of the change in experimental conditions, proves the affinity of ^{137}Cs to the aqueous phase.

Table 11. Extraction yield EY [%] for ^{241}Am , ^{60}Co and ^{137}Cs in the presence of a universal buffer at different acidity of the medium.

Radionuclide	pH=3	pH=5	pH=6	pH=7	pH=9	pH=11
^{241}Am	50±1	21.5±0.3	14.0±0.2	42.5±0.9	79.0±0.5	12.1±0.1
^{60}Co	61.0±0.9	64.0±0.5	70.5±0.6	88±3	68.0±0.8	12.0±0.6
^{137}Cs	22.0±0.3	22.5±0.8	25.3±0.4	22.0±0.4	42.0±0.9	30.4±0.2

^{60}Co , as a typical representative of d-elements, has a greater tendency to enter into various complexes. This is also confirmed by the data generated by HYDRA, presented in Fig. 18. They show that in the acidic range of the scale (from pH=3 to pH=7), ^{60}Co is present in the model solution in the form of complex citrate ions – $\text{Co}(\text{cit})^-$ – from pH=3 to pH=11; $\text{Co}(\text{Hcit})^-$ – from pH=3 to pH=8; $\text{Co}(\text{H}_2\text{cit})^-$ – from pH=3 to pH=5, while after pH=9.5, it begins to form complexes with hydroxyl anions – $\text{Co}(\text{OH})_2$, $\text{Co}(\text{OH})_3^-$, $\text{Co}(\text{OH})^+$. The experimental results also prove that ^{60}Co , unlike ^{137}Cs , is significantly affected by the change in the pH of the medium – a gradual increase in the extraction yield is observed at pH=3 (61%) to pH=7 (88%) and a drastic decrease at pH=11 (12%). From the above data, it can be concluded that the extraction of ^{60}Co , in the presence of a universal buffer, from the aqueous phase into an ionic liquid has the highest yields at neutral pH of the medium.

Although ^{241}Am is a good complexing agent, in combination with the universal buffer, it forms only two neutral compounds – $\text{Am}(\text{cit})$ (pH=3 to pH=11) and $\text{Am}(\text{OH})_3$ (pH=9.5 to pH=11). Unlike the other two radionuclides in the solution, which have a pronounced peak in recovery at a certain pH, the results for Am are rather zigzag – the highest recovery is obtained at pH=3 (50%) and pH=9 (79%), and the lowest – at pH=6 (14%) and pH=11 (12%). The hypothesis to which the obtained results lead is that the more forms of ^{241}Am are in contact with the IL phase, the higher the recovery will be achieved. In relation to ^{137}Cs and ^{60}Co , the inverse relationship is observed. Another interesting phenomenon is that the best results for extraction efficiency are obtained at pH values from 7 to 9. This is unusual for the nature of ^{241}Am , since usually its extraction is assisted by the participation of HNO_3 in high concentrations and various complexing agents (Yang et al., 2019).

IV.5.4 Determination of the influence of complexing agents on the efficiency of the reaction

After the experiments aiming the optimization of the acidity of the medium, the influence of various complexing agents on the extraction of radionuclides from the model solution was studied. For this purpose, universal buffer; phosphate buffer; universal buffer in combination with 0.5 M oxalic acid; phosphate buffer in combination with 0.5 M oxalic acid were used.

Using the HYDRA software, it was found that in the absence of organic anions, ^{137}Cs is present only in the Cs^+ state and increasingly smaller amounts of it pass into the ionic liquid phase, which was subsequently confirmed by the results of the gamma-spectrometric study – in the absence of citric acid in the system, the extraction yield is reduced by half (Table 12).

Table 12. Comparison of extraction yields EY [%] of ^{241}Am , ^{60}Co and ^{137}Cs in the presence of universal and phosphate buffer in the model solution.

Radionuclide	EY [%] in the presence of universal buffer	EY [%] in the presence of phosphate buffer
^{241}Am	42.5 ± 0.9	90 ± 3
^{60}Co	88 ± 3	22.0 ± 0.9
^{137}Cs	22.0 ± 0.4	10.8 ± 0.3

As can be seen in Figure 19, in the pH range from pH=6.5 to pH=8, achieved with phosphate buffer, ^{241}Am and ^{60}Co should also form mainly cations – Am^{3+} , AmNO_3^{2+} , AmCl^{2+} , AmOH^{2+} , AmOH_2^{2+} and Co^{2+} , CoCl^{2+} , CoOH^{1+} , respectively. This totally contrasts with their behaviour in the presence of a universal buffer at pH=7, where according to HYDRA they form $\text{Am}(\text{cit})$, Cs^+ , $\text{Cs}(\text{cit})^{2-}$, $\text{Co}(\text{cit})^-$, $\text{Co}(\text{Hcit})$.

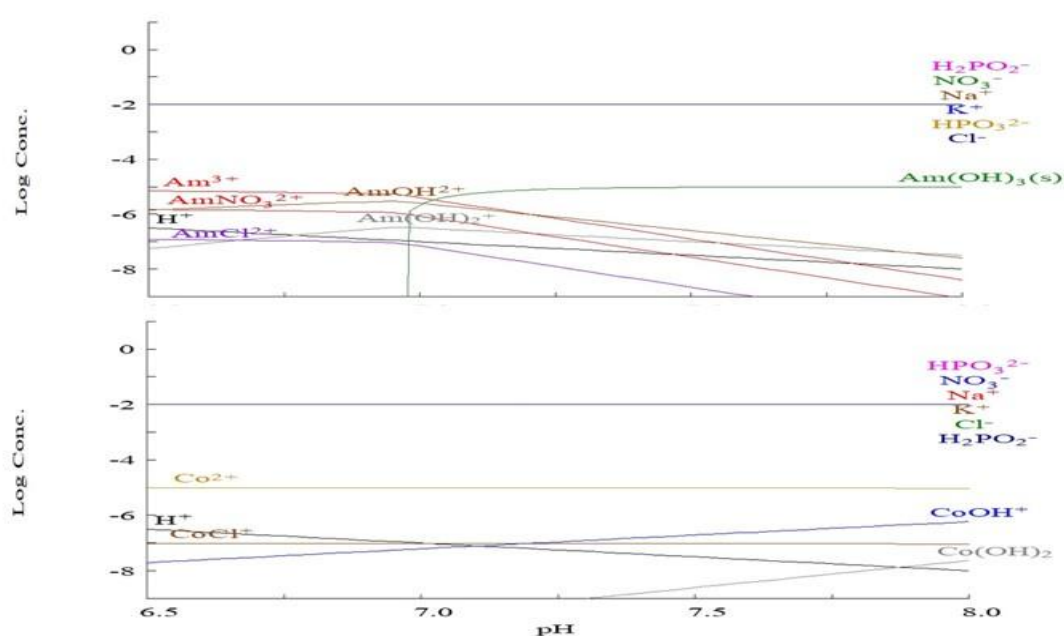


Figure 19. Complexes of ^{241}Am and ^{60}Co , formed in the presence of phosphate buffer, at pH between 6.5 and 8.

The experimental results show that the appearance of cations in the solution, due to the absence of citric acid from the system, favours an almost twofold increase in the extraction yield for ^{241}Am . In the case of the remaining two radionuclides, a drastic drop in values was observed – for ^{137}Cs – a twofold decrease, and for ^{60}Co – about four times (Table 12). This indicates that ^{241}Am is more soluble in the ionic liquid than in the aqueous phase when it is in the form of a complex ion rather than a neutral molecule. An inverse relationship is found for ^{137}Cs and ^{60}Co , which prefer the aqueous medium when in the presence of phosphate buffer. These findings lead to the conclusion that a potential quantitative extraction of ^{241}Am with the

used ionic liquid ($EY = 90 \pm 3\%$) from the aqueous phase would be possible in the presence of phosphate buffer alone, while in parallel, separation of the radionuclide from ^{137}Cs and ^{60}Co ($SF(^{137}\text{Cs}) = 8.5 \pm 0.2$; $SF(^{60}\text{Co}) = 4.1 \pm 0.1$) would occur, without the need for aggressive reagents and a special complexing agent.

In order to check the influence of another organic ligand in the extraction mixture, besides citric acid, samples containing universal buffer and oxalic acid - Fig. 20(a) or phosphate buffer and oxalic acid - Fig. 20(b) were prepared. According to the theoretical calculations of the software (Figure 20), in both samples a complex set of ions is obtained - ^{241}Am is present in one citrate and three oxalate complexes: $\text{Am}(\text{cit})$, $\text{Am}(\text{ox})_2^-$, $\text{Am}(\text{ox})_3^{3-}$, $\text{Am}(\text{ox})^+$ in a medium with universal buffer, and in the presence of phosphate – only as oxalate complex ions: $\text{Am}(\text{ox})_2^-$, $\text{Am}(\text{ox})_3^{3-}$, $\text{Am}(\text{ox})^+$.

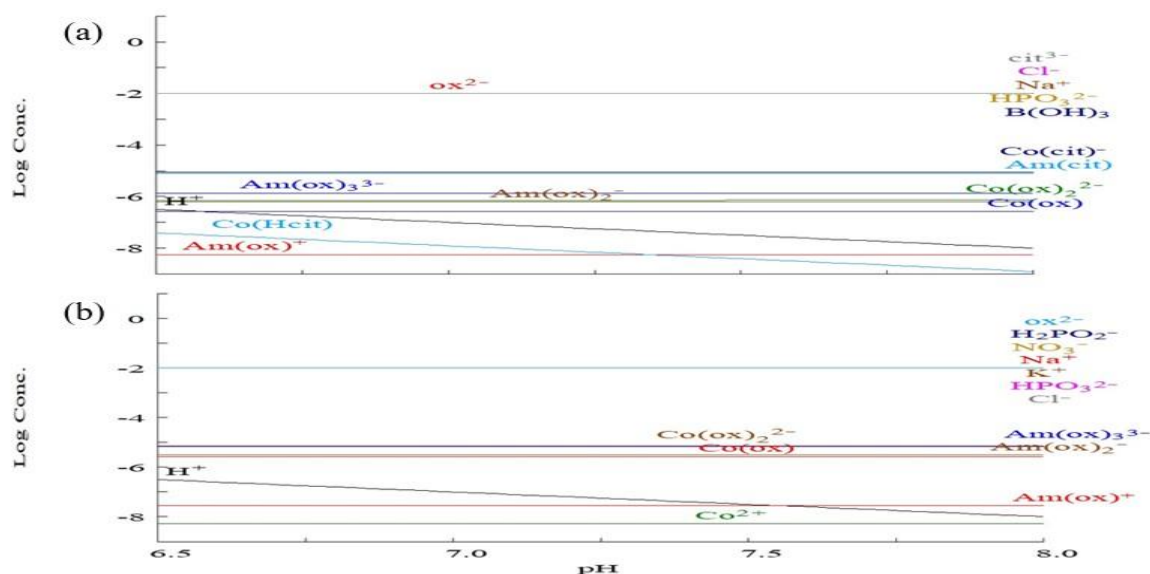


Fig. 20. Complexes at ^{241}Am , ^{60}Co and ^{137}Cs , formed in the presence of a universal or phosphate buffer, in combination with oxalic acid, at equal acidity of the medium.

The situation with ^{60}Co is similar – in the presence of a universal buffer solution in the aqueous phase it is present as $\text{Co}(\text{cit})^-$, $\text{Co}(\text{Hcit})$, $\text{Co}(\text{ox})$, $\text{Co}(\text{ox})_2^{2-}$, and in the presence of phosphate buffer and oxalic acid – Co^{2+} , $\text{Co}(\text{ox})$, $\text{Co}(\text{ox})_2^{2-}$. It is expected that ^{137}Cs will exist in the same forms as in the previous experiments. Comparing the results obtained from the experiments in the absence and presence of oxalic acid in the solutions (Table 13), it was found that the extraction yields for ^{241}Am and ^{137}Cs did not change significantly.

Table 13. Extraction yield EY [%] for ^{241}Am , ^{60}Co and ^{137}Cs in universal buffer in the presence and absence of oxalic acid and phosphate buffer in the presence and absence of oxalic acid.

Radionuclide	EY [%] in the presence of universal buffer	EY [%] in the presence of universal buffer + oxalic acid	EY [%] in the presence of phosphate buffer	EY [%] in the presence of phosphate buffer + oxalic acid
^{241}Am	42.5±0.9	43.0±1.2	90±3	92±2
^{60}Co	88±3	29.4±0.6	22.0± 0.9	2.0±0.1
^{137}Cs	22.0±0.4	21.1±0.7	10.8±0.3	12.0±0.5

The data on the extraction yield of ^{60}Co from the aqueous phase show that in the presence of a universal buffer with the addition of oxalic acid the extraction yield decreases threefold; in phosphate buffer the yield is 22.0±0.9% and in the presence of oxalic acid it is 2.0±0.1%. The obtained results confirm the possibility of preferential extraction of ^{241}Am from an aqueous matrix with other radionuclides by using 1-methyl-3-octylimidazolium saccharinate, in the presence of a suitable complexing agent in the solution and with a neutral pH of the medium. The advantages of this method are the relatively short sample preparation procedure – about 2 h. and the use of minimal amounts of environmentally harmful chemicals.

IV.5.5 Determination of the influence of temperature on extraction efficiency

With the last series of experiments conducted to optimize the extraction conditions, the temperature dependence of the extraction efficiency was tested. The extraction yield data are presented in Table 14.

Table 14. Comparison of extraction yield EY [%] for ^{241}Am , ^{60}Co and ^{137}Cs in universal or phosphate buffer, in combination with oxalic acid at temperatures of 25 °C, 30 °C and 35 °C.

Radionuclide	EY [%] in the presence of universal buffer	EY [%] in the presence of universal buffer + oxalic acid	EY [%] in the presence of phosphate buffer + oxalic acid
25 °C			
^{241}Am	42.5±0.9	43.0±1.2	92±2
^{60}Co	88±3	29.4±0.6	2.0±0.1
^{137}Cs	22.0±0.4	21.1±0.7	12.0±0.5
30 °C			
^{241}Am	36.5±0.9	30.0±0.5	63.0±0.7
^{60}Co	60.5±1.2	44.0±0.9	2.1±0.1
^{137}Cs	15.0±0.6	17.0±0.8	8.1±0.3
35 °C			
^{241}Am	28.1±0.5	14.3±0.7	42±1.7
^{60}Co	32.0±1.1	20.0±0.8	1.1±0.1
^{137}Cs	9.3±0.2	10.5±0.3	7.2±0.2

Contrary to our expectations, it turned out that with increasing temperature, the ability of the ionic liquid to extract radionuclides from the aqueous solution decreases significantly. In the solution containing phosphate buffer and oxalic acid, in which the extraction yield of ^{241}Am is highest at a temperature of 25 °C (92±2%), a sharp drop to about 40% was observed at T=35 °C. The same applies to ^{137}Cs and ^{60}Co in their optimal environment (for ^{60}Co – universal buffer, in the presence of oxalic acid; for ^{137}Cs – only in a universal buffer solution) – that of ^{137}Cs decreases from 21% at 25 °C to 10.5% at 35 °C. For ^{60}Co , the results show an even more critical drop – from 88% at 25 °C to 32% at 35 °C.

The obtained data categorically show that heating the extraction mixtures not only does not improve the extraction yield, but also leads to a decrease in the yields for all three radionuclides by the selected ionic liquid, regardless of the other experimental conditions.

IV.5.6 Ionic liquid regeneration

After the experiments conducted to establish the optimal conditions for the extraction of radionuclides from aqueous solution, the ionic liquid was subjected to regeneration. This proposal arose from a financial and environmental reasons. The ionic liquid is not a cheap means for extracting radionuclides from aqueous solutions. Also, relying on the principles of the circular economy, the possibility of purifying and reusing the ionic liquid was investigated, instead of its direct treatment as radioactive waste. 1-Methyl-3-octylimidazolium saccharinate has the ability to absorb water (50% wt./wt.) but is definitely more soluble in non-polar reagents. For this reason, dichloromethane was used to release the amount of water absorbed by the ionic liquid. Thus, the radionuclides would undergo another reconcentration process (from the IL into the aqueous phase), and the ionic liquid would be purified and reused. To ensure its absolute purification from radionuclides, a second extraction was carried out – with HNO_3 .

The results of gamma-spectrometric measurement of aliquot samples from both phases after each of the extractions confirm this assumption (Table 15).

Table 15. Extraction yield EY [%] of ^{241}Am , ^{60}Co and ^{137}Cs from the used ionic liquid using CH_2Cl_2 (1st purification step) and HNO_3 (2nd purification step).

Radionuclide	EY [%] with CH_2Cl_2	EY [%] with HNO_3
^{241}Am	92.7±2.6	99.7±1.3
^{60}Co	91.4±1.5	96.8±2.4
^{137}Cs	88.9±0.9	95.6±1.9

The data show yields of about 90% for the three radionuclides in the aqueous phase after the first extraction with dichloromethane and over 95% after the additional extraction with HNO₃.

Then, experiments were conducted to extract radionuclides from aqueous solution using the regenerated ionic liquid under the conditions selected based on the previous experiments. The results obtained show that the ability to separate and selectively extract ²⁴¹Am from the model solution using the purified ionic liquid differs from using a new ionic liquid within 2-3% (Table 16).

Table 16. Comparison between the extraction yields EY [%] of ²⁴¹Am, ⁶⁰Co and ¹³⁷Cs using unused and regenerated ionic liquid (IL).

Radionuclide	EY [%] with new IL	EY [%] with purified IL
²⁴¹Am	92±3	90.3±2.5
⁶⁰Co	2.0±0.1	2.0±0.2
¹³⁷Cs	12.0±0.5	10.0±0.4

IV.5.7 Optimization of the conditions for the determination of radionuclides in drinking water

After selecting suitable conditions for successful extraction of radionuclides from model solutions (ratio IL:aqueous phase 1:5; contact time – 60 minutes; pH=7; phosphate buffer medium in combination with 0.5 M oxalic acid; 25 °C), the possibility of their extraction from a real water sample of bottled mineral water was investigated. The data for the extraction yield are presented in Table 17.

Table 17. Extraction yield EY [%] of ²⁴¹Am, ⁶⁰Co and ¹³⁷Cs from drinking water.

Radionuclide	EY [%]
²⁴¹Am	89.4±2.3
⁶⁰Co	0.31±0.02
¹³⁷Cs	0.20±0.01

The presented data confirm the possibility of using the ionic liquid for selective extraction of ²⁴¹Am from water samples in which radioisotopes of other elements are also present. This procedure could be applied as sample preparation before using alpha- or gamma-spectrometric method for analysis of surface, drinking and wastewaters.

An advantage of the proposed method over other methods in the literature is the short sample preparation time, the use of smaller amounts of aggressive reagents, and the ability of the ionic liquid to be regenerated and reused.

IV.6 Development of a procedure for the determination of polycyclic aromatic hydrocarbons in sewage sludge samples

IV.6.1 Optimization of the GC-MS/MS instrumental parameters

In this study, a method for the extraction of polycyclic aromatic hydrocarbons (PAHs) – naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene – was optimized. The analysis of PAHs was carried out using a gas chromatograph with triple quadrupole mass spectrometer (GC-MS / MS, EVOQ GC-TQ, Bruker). The temperature conditions of the GC-MS/MS analysis were optimized by scanning (SCAN method) a standard solution at a concentration of 1 mg/L, prepared from CRM PAH-Mix 18 (10 mg/L, LGC Standards, DR Ehrenstorfer). The optimized conditions – retention times (tr) and temperature gradient were applied to selective ion monitoring (SIM) method for the quantitative determination of PAHs. The optimized temperature program is presented in Table 18, and the molecular ions and the corresponding retention times in Table 19.

Table 18. Optimized temperature program for PAHs analysis.

Temperature [°C]	Rate [°C/min]	Hold [min]	Total time [min]
60	-	1.00	1.00
180	7.00	2.00	20.14
270	6.00	20.00	55.14
290	10.00	2.00	59.14

Table 19. Molecular ions and retention times in the analysis of surfactants with the SIM method.

Compound	Molecular ion	Retention time, tr [min]
Naphtalene	128.1	10.291
Acenaphthylene	152.1	15.366
Acenaphthene	153.1	15.961
Fluorene	165.1	17.696
Phenantrene	178.1	21.547
Anthracene	178.1	21.774
Fluoranthene	202.1	26.731
Pyrene	202.1	27.620

The developed SIM method was calibrated with working standard solutions from the same CRM. The concentrations of the standard solutions are – 0.1 µg/L; 0.5 µg/L; 1.5 µg/L; 5 µg/L; 10 µg/L; 25 µg/L; 50 µg/L; 100 µg/L and 200 µg/L. For all analytes the coefficient of determination (R^2) was min. 0.99.

IV.6.2 Selection of a solid phase extraction column

PAHs are non-polar compounds. In their analysis, solid phase extraction (SPE) with a reversed-phase column (RP – column) is most often used, which enables their concentration and separation from other organic pollutants. C18 (CHROMABOND C18 ec, 45 μ m, 1 mL/100 mg), which is used in EPA Method 8310, and HLB (Hydrophilic-lipophilic balanced N-vinylpyrrolidone-divinylbenzene, 1 mL/100 mg) columns were selected, and their retention efficiency was checked under different conditioning and elution conditions.

IV.6.3 Optimization of the column conditioning and study of its capacity

The first step of the SPE procedure is sorbent conditioning. Experiments were conducted with solvents of different polarity - hexane, diethyl ether, dichloromethane and ethyl acetate with a volume of 10 mL. The standard solution of PAH-mix 18, from which the working standard solutions were prepared, is dissolved in acetonitrile. Therefore, 10 mL of acetonitrile was passed after each of the solvents used. After the conditioning step, the sample was applied. The capacity of the C18 and HLB columns was checked with standard solutions of PAHs containing 50 ng and 100 ng PAHs. The collected fractions were analysed by GC-MS/MS in SIM mode. The results of the analysis with 50 ng PAHs after conditioning of C18 column are presented in Fig. 21.

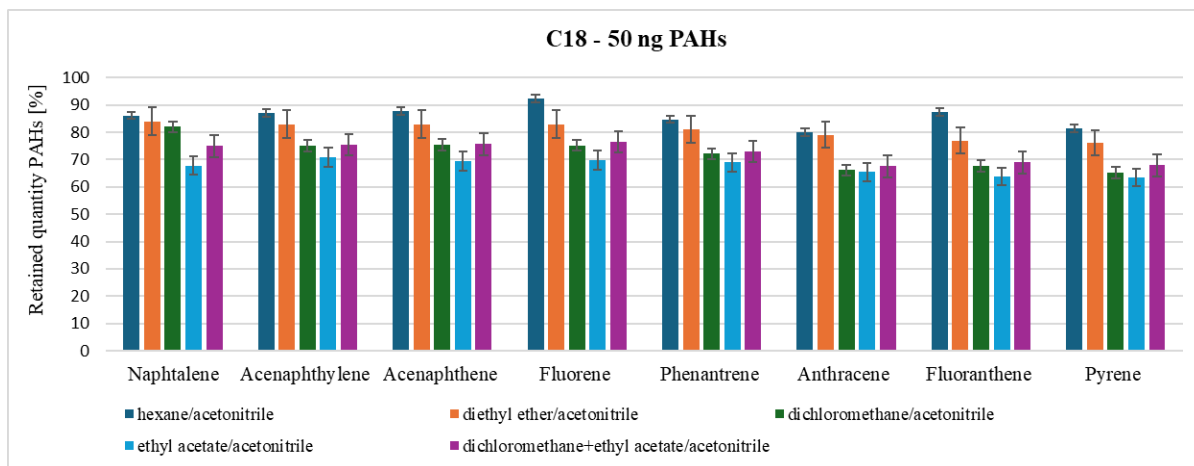


Fig. 21. Retained quantity of PAHs when loading 50 ng PAHs after conditioning of C18 column with hexane, diethyl ether, dichloromethane, ethyl acetate and a mixture of dichloromethane and ethyl acetate, followed by acetonitrile.

The measured concentrations in the C18 effluent showed incomplete retention of the analysed PAHs in the range from 66% to 83% when loading 100 ng PAHs and in the range from 64% to 92% when loading 50 ng PAHs.

Despite the unsatisfactory yields in all solvents used, it can be concluded that a decrease in retention is observed with increasing solvent polarity. The results with a mixture of dichloromethane and acetonitrile are comparable to the data from single solvents. Low retention was also found by Titato and Lanças, 2006, who compared C18 columns with 100, 200 and 300 mg resin and found low recoveries when performing the extraction with 100 mg. When using a column with 200 mg resin, the recoveries were in the range of 57-91%, and with 300 mg, 66-91%. Recoveries in the range of 76% to 95% were obtained by Kiss et al., 1996 with C18 column containing 360 mg resin when conditioned with dichloromethane-methanol.

The possibility of selective retention of PAHs was also studied using HLB reversed column. The data from the experiment with a quantity of 50 ng PAHs are presented in Fig. 22.

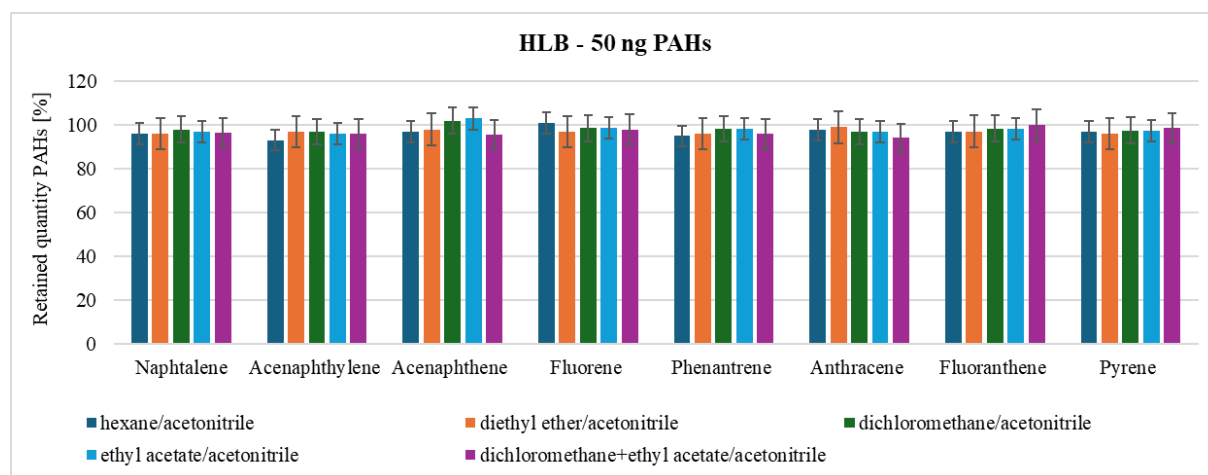


Fig. 22. Retained quantity of PAHs when loading 50 ng PAHs after conditioning of HLB column with hexane, diethyl ether, dichloromethane, ethyl acetate and a mixture of dichloromethane and ethyl acetate, followed by acetonitrile.

The results obtained after loading 50 ng and 100 ng PAHs indicate good column capacity, regardless of the quantity of PAHs. The obtained recoveries ranged from 89% to 102% at 100 ng and from 93% to 103% at 50 ng PAHs.

No dependence on the polarity of the solvent used for column conditioning was found. For this reason, all solvents used were also applied in the next optimization step of the PAHs elution step.

IV.6.4 Optimization of the elution step

Hexane, dichloromethane, ethyl acetate and a mixture of dichloromethane and ethyl acetate were used as eluents. In each experiment, the elution was carried out with the solvent used for conditioning. After conditioning the column with dichloromethane, an additional

experiment of elution with methanol was carried out, and after conditioning with ethyl acetate, in addition to ethyl acetate, methanol was also used as eluent.

Figure 23 presents the results for PAHs recoveries from the experiments on PAHs elution (50 ng) from C18 column. The data show that in the elution step, the polarity of the solvent used has a great influence on the recoveries. However, the relationship is not linear. Elution of PAHs from C18 column with the non-polar solvent hexane allows lead to recoveries in the range from 60% to 80% (Fig. 23). Quantitative elution is obtained when using a solvent with medium polarity - dichloromethane. The use of a mixture of dichloromethane and the more polar ethyl acetate leads to a decrease in the obtained recoveries. The dependence of the decrease in the obtained recoveries with increasing polarity of the eluents used is also preserved when eluting with the more polar solvents ethyl acetate, acetone and methanol. The recoveries decreased to 56%-74% with ethyl acetate, 44%-60% with acetone and in the range from 19% to 33% when the elution was done with methanol.

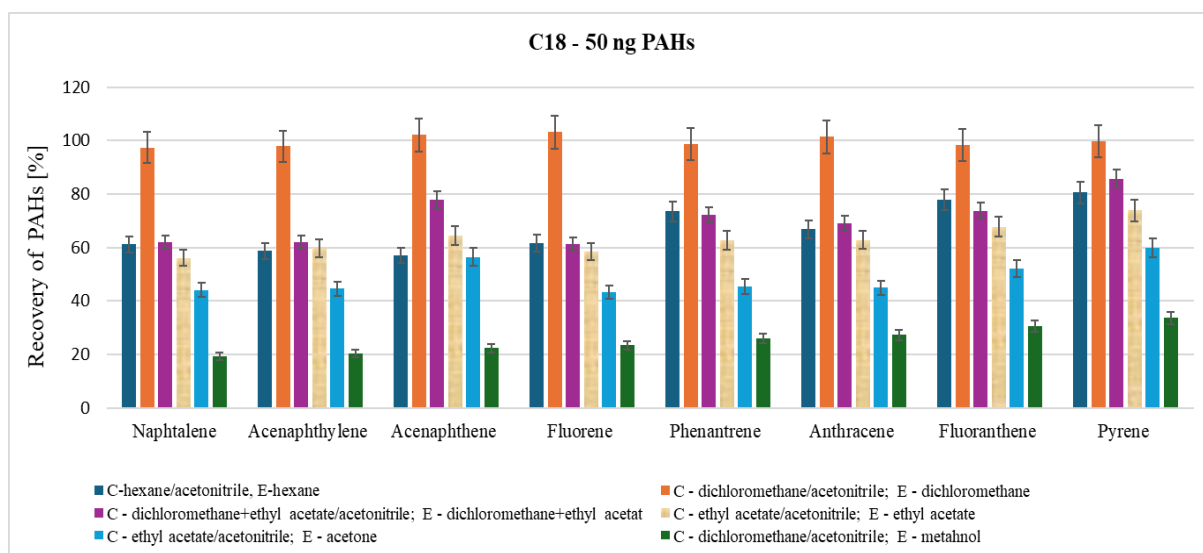


Fig. 23. Recovery for PAHs elution from C18 column with hexane, dichloromethane, ethyl acetate, acetone, dichloromethane+ethyl acetate and methanol.

The results for recoveries of PAHs after loading 50 ng on HLB column and elution with the same solvents are presented in Fig. 24.

The presented data for the HLB column also clearly confirmed the dependence of the recoveries on the polarity of the eluent used. The most non-polar eluent hexane is not applicable for elution. The results range from 3% to 72%, which is not related to the molecular weight and polarity of PAHs. Again, the highest recoveries were obtained when eluting with dichloromethane. The elution with a mixture of dichloromethane and ethyl acetate with this

column also lead to quantitative elution in the range from 95% to 98%. However, the use of more polar eluents also leads to a linear decrease in the obtained recoveries, which reach 20% with methanol.

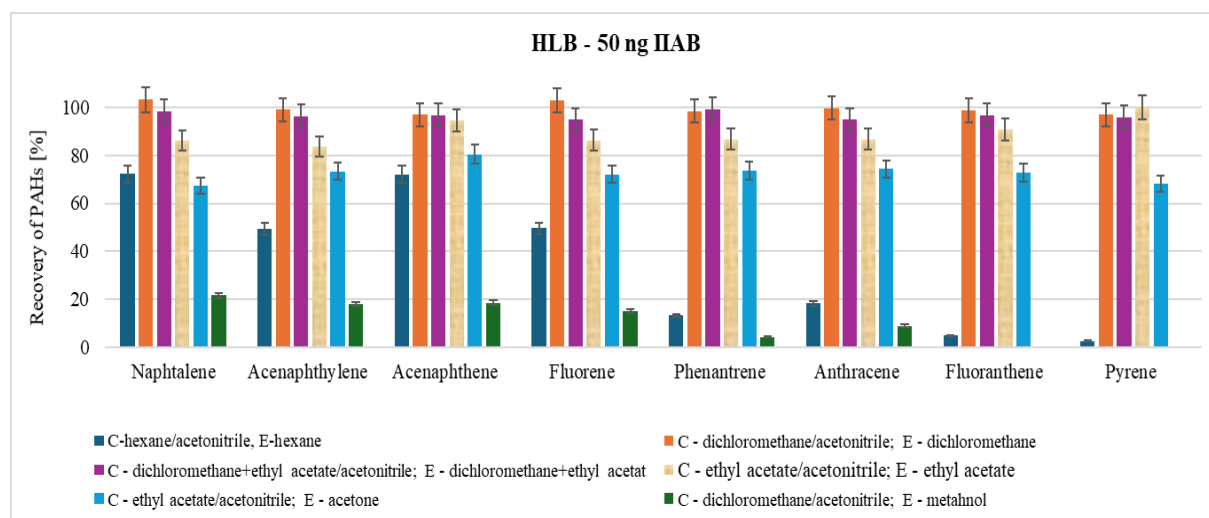


Fig. 24. Recovery for PAHs elution from HLB column with hexane, dichloromethane, ethyl acetate, acetone, dichloromethane+ethyl acetate and methanol.

Considering the higher retention of the analysed PAHs on HLB column, the choice of HLB over C18 for their separation and concentration is more suitable than C18 column. The highest recoveries were obtained after conditioning the column and elution of PAHs with dichloromethane or a mixture of dichloromethane and ethyl acetate.

IV.6.5 Extraction of PAHs from sewage sludge samples

The next step of the experiments was the optimization of the extraction of PAHs from sewage sludge samples. For the optimization of this step, two sewage sludge samples from the WWTP were used: Sewage sludge – Blagoevgrad and Sewage sludge – Ihtiman and CRM LGC 6182 Sewage Sludge. The sewage sludge samples were extracted directly and after addition the analyzed compounds from a standard solution, 0.1 mL of a standard PAHs solution at a concentration of 1000 µg/L was added to 1 g of the sewage sludge so that the concentration of PAHs in the samples increased by 100 µg/kg. Single extraction experiments were conducted with a series of solvents with different polarity – hexane, dichloromethane, acetone, methanol and a mixture of acetone and methanol.

The results of the extraction experiments conducted with CPM LGC 6182 Sewage Sludge are presented in Fig. 25 as analytical recovery (the ratio of the experimental concentration of the analyte to the certified value of the concentration).

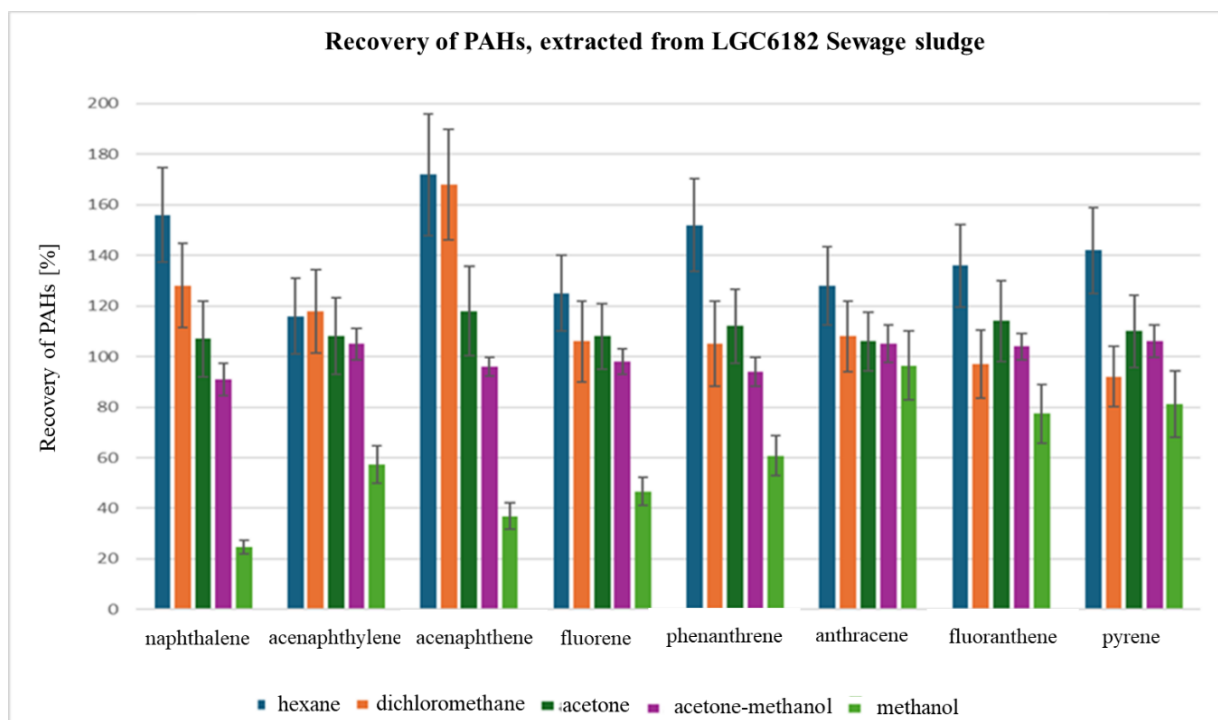


Fig. 25. Analytical recovery of PAHs, extracted from CPM LGC 6182 Sewage sludge.

The data from the extractions showed that the extraction with hexane lead to higher recoveries for all PAHs and the recoveries for naphthalene, acenaphthylenes and acenaphthene are higher than 100%, reaching 188% for acenaphthene after extraction with dichloromethane. These results could be explained by the presence of these analytes in the blank samples, in which high and non-reproducible concentrations of the respective PAHs were measured, therefore no correction of the results was made with respect to the blank sample.

When measuring a blank sample of acetone and methanol, no PAHs were found in the extracts. However, when extracting with acetone, the recoveries ranged from 107% to 118%. On the other hand, the extraction with methanol showed unsatisfactory recoveries in the range from 25% for naphthalene to 77% for fluoranthene, with the exception of anthracene – 96.5%. For this reason, extraction with a mixture of acetone and methanol was performed, which lead to satisfactory recoveries in the range from 91% to 106%.

The results of the extraction procedures performed on the WWTP sewage sludge samples are presented in Table 20 (Sewage sludge – Blagoevgrad) and Table 21 (Sewage sludge – Ihtiman). The results of the sewage sludge extractions confirm the data from the analysis of CRM LGC 6182 Sewage sludge. Again, the highest concentrations for all PAHs were obtained in the extracts with hexane and higher concentrations for naphthalene, acenaphthylene and acenaphthene in the extracts with dichloromethane. For the remaining PAHs, the

concentrations in dichloromethane and acetone extractions were relatively close. The lowest concentrations were obtained after extraction with methanol. Close concentrations were obtained in acetone and acetone:methanol (1:1) extracts.

Table 20. Concentrations of PAHs [$\mu\text{g/kg}$] in Sewage sludge – Blagoevgrad, obtained after extraction with hexane, dichloromethane, acetone, methanol and a mixture of acetone:methanol (1:1).

PAHs	hexane	dichloromethane	acetone	acetone: methanol	methanol
Naphthalene	162 \pm 18	160 \pm 15	106 \pm 11	102 \pm 5	54 \pm 5
Acenaphthylene	54 \pm 6	58 \pm 7	38 \pm 4	30 \pm 2	18 \pm 2
Acenaphthene	406 \pm 31	368 \pm 39	155 \pm 16	143 \pm 7	33 \pm 3
Fluorine	79 \pm 8	53 \pm 5	56 \pm 9	59 \pm 4	41 \pm 4
Phenanthrene	532 \pm 59	360 \pm 40	376 \pm 38	345 \pm 19	301 \pm 27
Anthracene	91 \pm 10	77 \pm 7	72 \pm 7	76 \pm 5	65 \pm 5
Fluorescent	202 \pm 39	188 \pm 19	190 \pm 18	185 \pm 8	152 \pm 12
Pyrenees	312 \pm 52	273 \pm 28	283 \pm 28	262 \pm 11	202 \pm 17
Σ PAH	1838	1537	1276	1202	866

Table 21. Concentrations of PAHs [$\mu\text{g/kg}$] in Sewage sludge – Ihtiman, obtained after extraction with hexane, dichloromethane, acetone, methanol and a mixture of acetone:methanol (1:1).

PAH	hexane	dichloro-methane	acetone	acetone: methanol	methanol
Naphthalene	1249 \pm 150	1273 \pm 130	1065 \pm 123	1023 \pm 52	549 \pm 47
Acenaphthylene	556 \pm 65	427 \pm 44	328 \pm 31	384 \pm 19	120 \pm 12
Acenaphthene	206 \pm 23	216 \pm 23	54 \pm 8	41 \pm 3	15 \pm 1
Fluorine	94 \pm 11	55 \pm 6	47 \pm 8	38 \pm 2	27 \pm 2
Phenanthrene	2136 \pm 251	1442 \pm 150	1345 \pm 154	1308 \pm 65	683 \pm 54
Anthracene	236 \pm 27	196 \pm 22	180 \pm 19	199 \pm 10	95 \pm 7
Fluorescent	1564 \pm 182	1390 \pm 141	1371 \pm 147	1227 \pm 61	653 \pm 52
Pyrenees	2882 \pm 340	1966 \pm 211	1972 \pm 218	1805 \pm 90	982 \pm 81
Σ PAH	8924	6965	6362	6028	3124

The accuracy of the obtained results was assessed using the spiked samples. The increase of the concentration of PAHs in the spiked sample Sewage sludge – Blagoevgrad is presented in Fig. 26. The data in Fig. 26 are analogous to the recovery results obtained for CRM LGC6182 Sewage Sludge with the respective solvent. Again, the extraction with hexane

lead to an increase of concentration more than 100 $\mu\text{g/kg}$ (in this case also 100%) for all analysed PAHs, and with dichloromethane – for naphthalene, acenaphthylene and acenaphthene. The data confirm the ineffective extraction with methanol. Extractions with acetone and a mixture of acetone:methanol (1:1) lead to an increase in concentration, adequate to the standard addition made. However, better reproducibility in the range of 4% to 7% was obtained after extraction with a mixture of acetone:methanol (1:1). The reproducibility of the results after extraction with acetone varied between 9% and 17%, therefore extraction with a mixture of acetone and methanol is recommended.

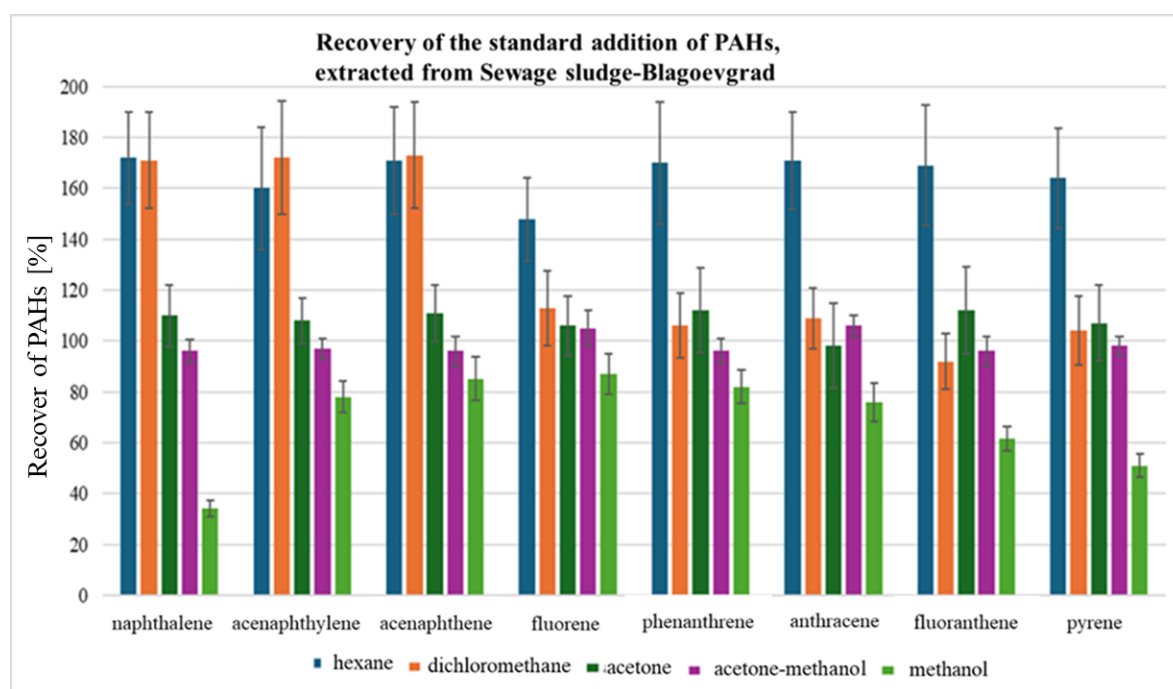


Fig. 26. Data on the increase in surfactant concentration during extraction with hexane, dichloromethane, acetone, methanol and a mixture of dichloromethane-methanol and acetone:methanol (1:1).

V. Conclusions

The research conducted within the framework of this dissertation work led to the following more significant conclusions:

1. A quantitative ICP-MS method for the determination of 70 elements in liquid and solid waste samples has been optimized for the first time. The concentrations of the macroelements Na, Mg, Al, Si, P, K, Ca, Ti, Mn and Fe were determined after optimization the RPa coefficient, and S – using DRC with reaction gas O₂.
2. An approach for complete digestion of various types of waste has been established by testing the effectiveness of different acid mixtures.
3. The influence of the number of calibration elements and the matrix on the accuracy of determination of the concentration of chemical elements in waste samples with a semi-quantitative ICP-MS method has been studied and established for the first time. To achieve an accuracy of up to 30%, it is recommended to provide the calibration with the presence of an element through a maximum of 3 to 4 masses
4. The developed methods enable rapid and accurate analysis and assessment of the chemical state of wastewater. A detailed assessment of the degree of purification of wastewater samples from the WWTP - Kubratovo during three seasons was made. It was found that the concentrations of all elements after the purification process are below the MPC in Regulation No. 12 of 2002, with the exception of a seasonal increase in Mn and Al.
5. An assessment of the binding of the regulated elements in WWTP sewage sludge based on fractionation was made. The mobility and bioavailability of both potentially toxic and essential elements were determined.
6. The possibilities for pelletization and non-destructive LA-ICP-MS analysis of the chemical elements in solid waste samples were tested. It was found that LA-ICP-MS is not a suitable method for the analysis of WWTP sludge samples.
7. A liquid-liquid extraction with the ionic liquid 1-methyl-3-oxyimidazolium saccharinate for the selective extraction of ²⁴¹Am from water samples (natural and waste), containing ⁶⁰Co and ¹³⁷Cs was performed for the first time.
8. A method for extraction from sewage sludge matrix, solid-phase extraction and GC-MS/MS determination of 8 PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene) in sewage sludge samples has been adapted.

VI. Literature

- Regulation No. H-4 of 14.09.2012 on the characterization of surface waters
- Ordinance No. 6 of November 9, 2000 on emission standards for permissible content of harmful and hazardous substances in wastewater discharged into water bodies
- Ordinance No. 7 of 14.11.2000 on the conditions and procedure for discharging industrial wastewater into the sewage systems of settlements
- Ordinance No. 9 of March 16, 2001 on the quality of water intended for drinking and domestic purposes
- Ordinance No. 10 of 3.07.2001 on issuing permits for the discharge of wastewater into water bodies and determining individual emission limits for point sources of pollution
- Regulation No. 12 of 18 June 2002 on the quality requirements for surface waters intended for drinking and domestic water supply
- Regulation on the procedure and manner of utilization of sludge from wastewater treatment through their use in agriculture. Adopted by Decree No. 339 of December 14, 2004, published in the State Gazette, issue 112 of December 23, 2004
- Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse
- Abdel-Shafy, H. I., Ibrahim, A. M., Al-Sulaiman, A. M., Okasha, R. A., Ain Shams Engineering Journal, 2024, 15(1), 102293. <https://doi.org/10.1016/j.asej.2023.102293>
- Amarasiriwardena, D., Durrant, S. F., Lászlity, A., Kvska, A., Argentine, M. D., Barnes, R. M., Microchem. J., 1997, 56(3), 352–372. <https://doi.org/10.1006/mchj.1997.1454>
- Anderson, N., Snaith, R., Madzharova, G., Bonfait, J., Doyle, L., Godley, A., Lam, M., Day, G. (2021). European Environmental Agency
- Belouhova, M., Yotinov, I., Schneider, I., Dinova, N., Todorova, Y., Lyubomirova, V., Mihaylova, V., Daskalova, E., Lincheva, S., & Topalova, Y., Processes, 2022, 10(3), 460. <https://doi.org/10.3390/pr10030460>
- Chang, Z., Long, G., Zhou, J. L., & Ma, C., Resour. Conserv. Recycl., 2020, 154, 104606. <https://doi.org/10.1016/j.resconrec.2019.104606>
- Chen, H., Dabek-Zlotorzynska, E., Rasmussen, P. E., Hassan, N., & Lanouette, M. (2008). Talanta, 74(5), 2008, 1547–1555. <https://doi.org/10.1016/j.talanta.2007.09.037>
- Gałuszka, A., Krzciuk, K., & Migaszewski, Z. M., Int. J. Environ. Sci. Technol., 2014, 12(10), 3071–3078. <https://doi.org/10.1007/s13762-014-0719-4>
- Giwa, A. S., Maurice, N. J., Luoyan, A., Liu, X., Yunlong, Y., & Hong, Z., Heliyon, 2023, 9(9). <https://doi.org/10.1016/j.heliyon.2023.e19765>
- Jakubus, M., Environ. Pollut. Bioavailab., 2020, 32(1), 87–99. <https://doi.org/10.1080/26395940.2020.1778541>
- Jitaru, P.; Tirez, K.; De Brucker, N., At. Spectrosc., 2003, 24, 1–11
- Khnajer, B., Cherkaoui, E., Khamar, M., & Nounah, A., E3S Web of Conferences, 2020, 150, 02004. <https://doi.org/10.1051/e3sconf/202015002004>
- Kiss, G., Varga-Puchony, Z., Hlavay, J. J. Chromatogr. A, 1996, 725(2), 261–272. [https://doi.org/10.1016/0021-9673\(95\)00940-x](https://doi.org/10.1016/0021-9673(95)00940-x)
- Krzciuk, K., Crit. Rev. Anal. Chem., 2015, 46(4), 284–290. <https://doi.org/10.1080/10408347.2015.1053106>
- Laborda, F., Medrano, J., Castillo, J.R., J. Anal. At. Spectrom., 2001, 16, 732–738
- Lyubomirova, V., Djingova, R., Compt. Rend. Acad. Bulg. Sci., 2015, 68 (7)
- Lyubomirova, V., Mihaylova, V., & Djingova, R., J. Food Compos. Anal., 2020, 93, 103595. <https://doi.org/10.1016/j.jfca.2020.103595>

- Mohammed, R., Zhang, Z.-F., Jiang, C., Hu, Y.-H., Liu, L.-Y., Ma, W.-L., Song, W.-W., Nikolaev, A., Kallenborn, R., & Li, Y.-F., *Toxics*, 2021, 9(4), 76. <https://doi.org/10.3390/toxics9040076>
- Shikuku, O. V., Achieng, O. G., Ng'eno, E., Okowa, M. G., Masitsa, A. G., Owuor, G. G., *Res. J. Chem. Sci.*, 2017, 7(8), 19-25
- Tanner, S. D., Baranov, V. I., & Bandura, D. R., *Spectrochim. Acta Part B: At. Spectrosc.*, 2002, 57(9), 1361–1452. [https://doi.org/10.1016/s0584-8547\(02\)00069-1](https://doi.org/10.1016/s0584-8547(02)00069-1)
- Titato, G. M., Lancas, F. M., *J. Chromatogr. Sci.*, 2006, 44(1), 35–40. <https://doi.org/10.1093/chromsci/44.1.35>
- Valchev, D., Ribarova, I., Borisov, B., Radovanov, V., Lyubomirova, V., Kostova, I., Dimova, G., Karpuzova, O., & Lazarova, S., *Environ. Sci. Eur.*, 2024, 36(1). <https://doi.org/10.1186/s12302-023-00837-x>
- Zaleckas, E., Paulauskas, V., & Sendžikienė, E., *J. Environ. Eng. Landsc. Manag.*, 2012, 21(3), 189–198. <https://doi.org/10.3846/16486897.2012.695734>

VII. Scientific publications on the topic of the dissertation

- Valentina Lyubomirova, **Iva Belovezhdova**, Rummyana Djingova, Petar Petrov, Ekaterina Todorova, Panoramic Semiquantitative Analysis for Multielement Characterization of Liquid and Solid Waste Samples, Processes, vol:11, issue:3379, 2023, doi:<https://doi.org/10.3390/pr11123379>, Ref, IF (2.8-2023), SCOPUS Quartile: Q2 (2023)

Citations in SCOPUS and Web of Science:

Xiaowei X., Dapeng, Zh., Jun, Zh., Zehua, Zh., Jing, H., Yi, W., Houhu, Zh., Qi, Y., *Identification and Assessment of Toxic Substances in Environmental Justice Cases*, *Toxics* 2024, 12 (12), 900; <https://doi.org/10.3390/toxics12120900>

- **Iva Belovezhdova**, Boyan Todorov, Milen G. Bogdanov, Recovery of radionuclides with ionic liquids. I. Selective extraction of ²⁴¹Am, Separation and Purification Technology, issue:262, 2021, doi:<https://doi.org/10.1016/j.seppur.2021.118303>, Ref, IF (7.278 - 2021), Web of Science Quartile: Q1 (2021)

Citations in SCOPUS and Web of Science:

Kanmazalp, S. D., Sagher, M., Dege, N., Içbudak, H., *Synthesis, Hirshfeld Surface, FTIR Analysis and Single Crystal X-Ray Structure of 2-Amino-3-Hydroxypyridinium Saccharinate*, *J. Struct. Chem.*, 2023, 64(6), 1137-1146

A β -ray irradiation resistant MOF-based trap for efficient capture of Th(IV) ion, Chen, X., Liu, X., Xiao, S., Xue, W., Zhao, X., Yang, Q., *Sep. Purif. Technol.*, 2022, 297, 121517

Jing, X., Sun, Z., Zhao, D., Sun, H., Ren, J., *A Mini-Review on Methods of Solvent Extraction Kinetics for Heavy Metal Ions*, *Russ. J. Appl. Chem.*, 2022, 95(2), pp. 155-166

VIII. List of presented scientific papers and posters on the dissertation

1. National Conference 135 Years of University Education in Inorganic Chemistry, "Fractionation of Potentially Toxic Elements in WWTP Sludges", **I. Belovezhdova**, V. Lyubomirova, 30-31.10.2024, Sofia, Bulgaria, poster presentation.
2. International Conference 100th Anniversary of the Department of Analytical Chemistry at Sofia University "St. Kliment Ohridski" with international participation, "Development of

- solid-phase extraction method for sample preparation and GC-MS/MS, **I. Belovezhdova**, V. Lyubomirova, 03-04.10.2024, Sofia, Bulgaria, poster presentation.
3. St. Kliment Days of Sofia University "St. Kliment Ohridski", "Optimization of sample preparation for GC-MS analysis of PAHs in solid waste samples", **I. Belovezhdova**, V. Lyubomirova, B. Todorov, 21.11.2023, Sofia, Bulgaria, poster presentation.
 4. RAD 2023 Eleventh International Conference on Radiation, Natural Sciences, Medicine, Engineering, Technology and Ecology, "Optimization of sample preparation for GC-MS analysis of PAHs in solid waste samples", **I. Belovezhdova**, V. Lyubomirova, B. Todorov, 19-23.06.2023, Herceg Novi, Montenegro, poster presentation.
 5. Clement Days of Sofia University "St. Kliment Ohridski", "Pollution with potentially toxic elements in effluent discharge zone - ranking and assessment of their impact on microbial communities", V. Yordanova, Y. Todorova, I. Yotinov, V. Lyubomirova, **I. Belovezhdova**, V. Mihaylova, Y. Topalova, 04.11.2022, Sofia Bulgaria, poster presentation.
 6. EMCEI-22, "Ranking of potentially toxic pollutants and their impact on microbial communities in water bodies receiving wastewater discharges", V. Yordanova, Y. Todorova, M. Beluhova, V. Lyubomirova, **I. Belovezhdova**, V. Mihaylova, Y. Topalova, 1-4.11.2022, Sousse, Tunisia, oral presentation.
 7. EXTREME "Advanced Studies in colloid and interface science", "Impact assessment of discharge of treated wastewater effluent on the chemical parameters of the water in the upper part of Iskar River", V. Lyubomirova, **I. Belovezhdova**, V. Mihaylova, Y. Todorova, R. Djingova, 11-14.07.2022, St. St. Constantine and Helena Resort, Bulgaria, oral presentation.
 8. EXTREME "Advanced Studies in colloid and interface science", "Evaluation of the effectiveness of wastewater metal removal in Kubratovo Wastewater Treatment Plant", **I. Belovezhdova**, V. Lyubomirova, M. Belukhova, Y. Topalova, 11-14.07.2022, St. St. Constantine and Elena Resort, Bulgaria, oral presentation.
 9. "Eleventh national conference on chemistry", "Determination of the element and anionic composition of surface waters and sediments – analytical methods (a comparative study)", V. Lyubomirova, V. Mihaylova, **I. Belovezhdova**, B. Todorov, A. Kamusheva, R. Djingova, 23-25.06.2022, Sofia, Bulgaria, poster presentation.
 10. "Eleventh national conference on chemistry", "Sample preparation techniques for multielement analysis of solid waste materials using LA-ICP-MS method", **I. Belovezhdova**, V. Lyubomirova, 23-25.06.2022, Sofia, Bulgaria, poster presentation.
 11. XX National Chemistry Conference for Undergraduates and PhD Students, "Investigation of Sample Preparation Possibilities for LA-ICP-MS Analysis of Waste Samples", **I. Belovezhdova**, V. Lyubomirova, 18-20.05.2022, Sofia, Bulgaria, oral presentation.
 12. "Colloid and interface research and innovations", "Analytical approaches for multielement characterization of liquid and solid waste samples", V. Lyubomirova, **I. Belovezhdova**, R. Djingova, P. Petrov, 18-21.07.2021, St. Constantine and Helena Resort, Bulgaria, oral presentation.
 13. XIX National Chemistry Conference for Undergraduates and PhD Students, "Investigation of the Possibilities of a Semi-Quantitative ICP-MS Method for Characterization of Liquid and Solid Wastes", **I. Belovezhdova**, V. Lyubomirova, 02-04.06.2021, Sofia, Bulgaria, oral presentation.
 14. Tenth Anniversary National Chemistry Conference, "Preconcentration of uranium in drinking water by using ionic liquid – 1-methyl-3-octylimidazolium saccharinate", **I. Belovezhdova**, M. Bogdanov, I. Svinyarov, B. Todorov, 26-28.09.2019, Sofia, Bulgaria, poster presentation.