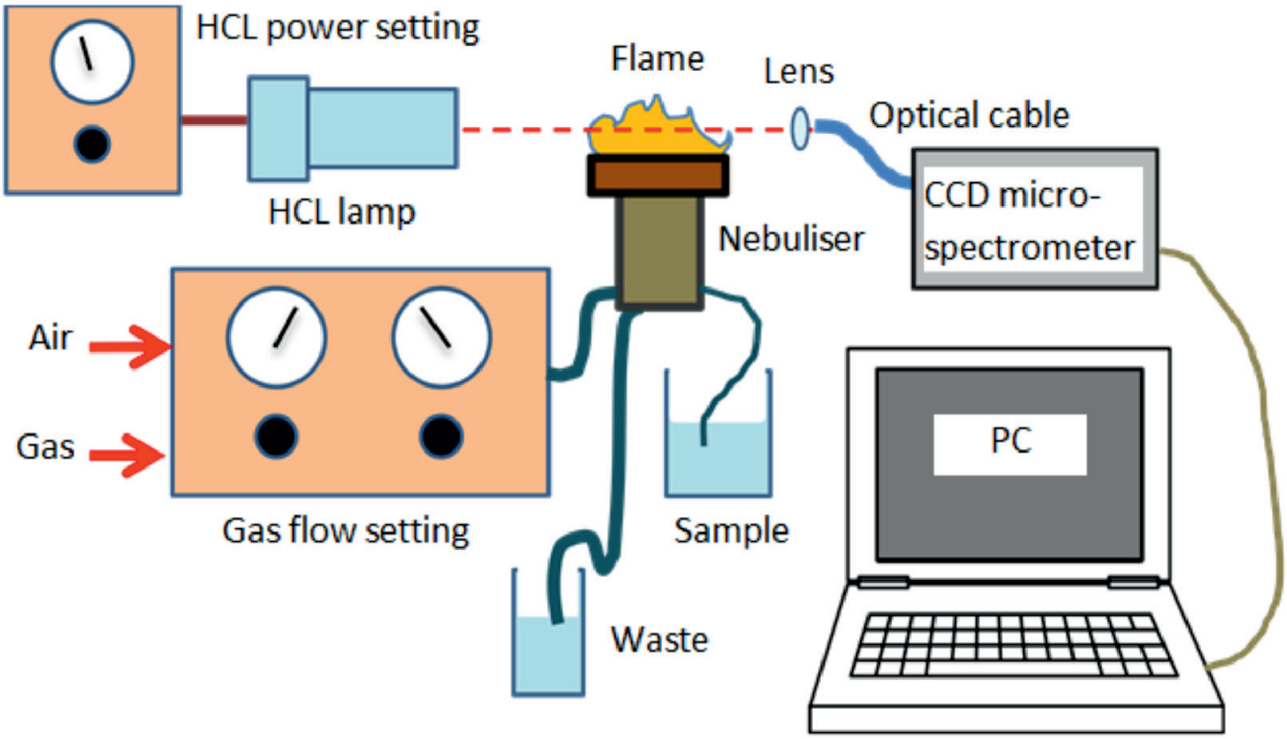
STUDIA UNIVERSITATIS
BABEŞ-BOLYAI



CHEMIA

3/2016
Tom II

STUDIA
UNIVERSITATIS BABEŞ-BOLYAI

CHEMIA

3/2016
Tom II

http://chem.ubbcluj.ro/~studiachemia/; studiachemia@chem.ubbcluj.ro
http://www.studia.ubbcluj.ro/serii/chemia/index_en.html

YEAR **Volume 61 (LXI) 2016**
MONTH **SEPTEMBER**
ISSUE **3, TOM II**

S T U D I A
UNIVERSITATIS BABEŞ–BOLYAI
CHEMIA

3
TOM II

Dedicated to Professor Emil Cordoş
on the Occasion of His 80th Anniversary

STUDIA UBB EDITORIAL OFFICE: B.P. Hasdeu no. 51, 400371 Cluj-Napoca, Romania, Phone + 40 264 405352

CUPRINS – CONTENT – SOMMAIRE – INHALT

Studia Universitatis Babes-Bolyai Chemia has been selected for coverage in Thomson Reuters products and custom information services. Beginning with V. 53 (1) 2008, this publication is indexed and abstracted in the following:

- Science Citation Index Expanded (also known as SciSearch®)
- Chemistry Citation Index®
- Journal Citation Reports/Science Edition

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 295-297)
(RECOMMENDED CITATION)

Prof. Emil Cordoş PhD

Professor Cordos is part of a student generation that makes the most representative connection between the group of professors that founded the chemistry department in early 1920 and the generation that graduates in early 1960 and developed the chemistry department in both fundamental and engineering chemistry. This generation was the last to have the chance of lectures of great chemists of this country like Raluca Ripan in Inorganic Chemistry, Ioan Cadariu in Physical Chemistry or Ioan Tanasescu in organic Chemistry. Also, these generation had a very good background in physics with Professor Mercea and mathematic with Professor Chis.

Professor Cordos became a student of the Faculty of Chemistry in 1954 and graduated in 1959 with a degree of merit (red diploma), specialty Inorganic Chemistry. He became a member of the Department of Inorganic and Analytical Chemistry led by Academician Raluca Ripan in 1960, when he obtained, by competition, a preparatory position followed in 1961 by a promotion to assistant.

His university career unfolded, until retirement, in the same department, by going through the consecrated steps of the academic hierarchy: lecturer in 1968, associate professor in 1976, professor in 1990 and consulting professor in 2005.

Professor Cordoş also occupied leadership positions at the Babes-Bolyai University, Faculty of Chemistry: Vice Dean, 1977-1981, Dean, Faculty of Chemistry, 1990-1992 and Head of Analytical Chemistry Chair, 1996-2002. As vice dean of the Faculty of Chemistry has contributed to the establishment of departments of chemical engineering. Professor Cordoş was, for a two years mandate, the first Dean of the Faculty of Chemistry after the events from December 1989. Needless to say that it was a much tensed mandate that required determination and very clear objectives. In spite of it, he managed to re-establish the traditional departments of the faculty, ensured a beginning of compatibility with top European university curricula and a smooth continuation, without major shocks, of the teaching process.

Specialization of Professor Cordoş in Analytical Chemistry is the Instrumental Analysis with emphasis on automated analytical instrumentation and its applications, mainly in spectrometric methods. He made the first steps in this field at the University of Illinois, USA, where he worked for three years as Fulbright fellow and postdoctoral research associate. As a matter of fact he defended his PhD in 1969 with a thesis entitled "Contributions to the automation of kinetic methods of analysis" using the experimental work done in the period of Fulbright grant. On those years the scientific research in all fields, including analytical instrumentation was advancing under the flag of computer assisted methods, and electronics on microchips. He understood that valuable research in the analytical instrumentation implies an interdisciplinary approach and proper infrastructure that could not rely only on resources and staff of a department in a Romanian university. Therefore, besides the remodeling of the Instrumental Analysis course he set up and organized research teams and groups within specific projects. In 1990 these collectives merged and, finally, in 1996 they became the Research Institute for Analytical Instrumentation, ICIA, a subsidiary of the National Institute of Research and Development in Optoelectronics. Those teams and the institute could and did address projects on very broad topic including spectrophotometry systems, from simple devices to systems of analysis based on radio frequency generated plasma, sensors based on ceramic semiconductor, robots specialized in automatic analysis and very complex national projects on environment, health, biofuels, and highly specialized methods of control for food and drugs. While the most research institutes from Cluj-Napoca are disappearing the institute founded by Professor Cordos is growing and providing jobs and attractive professional domains for many talented and hardworking people.

Professor Cordoş achievements in the fields of instrumental analysis, especially in spectrometric methods, are described in 207 papers, 130 of these being published in ISI indexed journals. He published five books: Electronics for Chemists, Ed. Stiintifica (1978), Atomic Absorption and Fluorescence Spectrometry, Ed. Academiei (1984), Analysis by Atomic Spectrometry, Ed. INOE (1998). Analysis by UV- Vis Molecular Absorption Spectrometry, Ed. INOE (2001), Analytical Atomic Spectrometry with Plasma Sources, Ed. INOE (2007); three chapters in books

and a chapter in a megaencyclopedia devoted to analytical chemistry into the third millennium. The encyclopedia had 14 volumes and 700 contributors as experts in their fields. Only two of them were Romanians. To these should be added the studies, methods and instrumentation resulted from more than 100 projects and research contracts or grants covered as project director and 12 patents. A special note for the spectrometric systems based on radio frequency generated plasma sources, made before 1990 and continued through a series of projects to date and for the environmental projects in European programs. Under his leadership were presented 23 doctoral thesis. Regarding the connection with Studia, Prof. Cordos, as Dean, was Studia's responsible editor in 1990-92. He published his first papers in Studia in 1962, volume VII.

Professor Cordos is member of many professional societies among them stands: American Chemical Society, Romanian Society of Chemistry, Society for Applied Spectroscopy, International Society of Environmental Epidemiology, EURACHEM Romania (founding member). He was president of the first subsidiary in Cluj-Napoca of the Romanian Society of Chemistry and is president and founder of PROANALITICA XXI.

Cluj-Napoca, iulie 2016

Prof. dr. Tiberiu Frentiu

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 299-310)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

DEVELOPMENT AND CHARACTERIZATION OF A METHOD FOR THE DETERMINATION OF TOTAL As IN WATER BY HYDRIDE GENERATION AND OPTICAL EMISSION DETECTION IN ARGON CAPACITIVELY COUPLED PLASMA MICROTORCH

SINZIANA BUTACIU[a], MICHAELA PONTA[a], EUGEN DARVASI[a], MARIA FRENTIU[b], HORVATH GABRIELA[a], TIBERIU FRENTIU[a*]

ABSTRACT. Arsenate was firstly prereduced to arsenite in 0.01 mol L^{-1} HCl (pH 2.00 ± 0.01) and 0.3% L-cysteine, than arsine was generated with 0.5% $NaBH_4$ solution stabilized in 0.5% NaOH and introduced into a capacitively coupled plasma microtorch (10 W, 150 ml min^{-1} Ar) for measurement of As 228.812 nm emission with the Ocean Optics QE65 Pro spectrometer of low resolution. The optimization steps for arsine generation and plasma operation are presented. Under optimal operating conditions, linearity of calibration curve covers the range 0 – 100 µg L^{-1}, while detection and quantification limits are 0.2 and 0.6 µg L^{-1} respectively. Thus, the proposed method is able for As quantification in drinking and groundwater at levels below maximum admitted concentration (10 µg L^{-1}). The method was validated by analyzing certified reference water samples containing 10 – 60 µg L^{-1} As with recovery of 99 ± 6%. (95% confidence level, n = 5 measurements). The analytical capability of the method was demonstrated in the analysis of test samples (drinking-, ground- and waste water) with As concentration in the range 0.6 – 80 µg L^{-1} As with a precision of 1.2 – 10.8%. The completely miniaturized instrument including the capacitively coupled plasma microtorch has analytical potential to be used for monitoring the quality of water sources.

Keywords: *arsenic, hydride generation, water, capacitively coupled plasma microtorch, optical emission spectrometry, miniaturized instrumentation*

[a] *Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos 11, RO-400028, Cluj-Napoca, Romania*
[b] *National Institute for Research and Development of Optoelectronics Bucharest, Research Institute for Analytical Instrumentation, Donath 67, 400293, Cluj-Napoca, Romania*
** Corresponding author: ftibi@chem.ubbcluj.ro*

INTRODUCTION

Arsenic is present in environment (water, soil, vegetation) as inorganic (arsenite, arsenate) and organic (monomethylarsonic, dimethylarsinc acid) species, with different toxicity. Thus, inorganic compounds are more toxic and several have been classified as Group I human carcinogens [1,2]. Besides carcinogenicity, exposure of humans to inorganic As species are associated to neurological, cardiovascular and hematological diseases or spontaneous abortion [1,3,4]. One of the sources with high risk of As exposure of population is long-term water consumption at concentrations above or even little below the safety threshold [5–8].

Evaluation of water quality in terms of As concentration and human health risk is of high concern, since more than 500 millions people living in different areas on the Earth like Bangladesh, West Bengal India, Taiwan, Northern Chile, China, Mexico, USA, and Central and Eastern Europe including Romania (700000 people) are chronically exposed *via* water consumption, which is naturally contaminated with arsenic [1,5,8]. In two previous studies, it has been found that soil and groundwater in the vicinity of waste ponds in the Baia Mare area, northern Romania, are polluted with arsenic, in this case as a result of the non-ferrous metal industry very active until 1990 [9,10]. Based on Water Framework Directive (WFD) 2000/60/EC and Groundwater Directive (GWD) 2006/118/EC, European Community established a Maximum Contaminant Level (MCL) of 10 μg L^{-1} As in drinking and groundwater [11–14].

The methods frequently used for As determination in water, foods and beverages are atomic absorption spectrometry in flame/quartz tube with/without hydride generation, electrothermal vaporization atomic absorption spectrometry using different preconcentration procedures to enhance sensitivity [15 – 20], molecular absorption spectrophotometry [21] or fluorescent spectroscopy based on ultra-small ZnO particles [22]. In addition, coupling a separation technique like high performance liquid chromatography with inductively coupled plasma mass spectrometry provides speciation of different As compounds [23]. Recent advances in spectrometric methods to enhance sensitivity for the determination of elements, including total As or its speciation, after chemical vapor/ hydride generation (HG), were reviewed by several authors [24–28]. A green derivatization approach based on UV photo-induced hydride generation in the presence of formic or acetic acid was successfully applied for the determination of As in surface water prior measurements by atomic fluorescence spectrometry or other spectrometric methods [29,30]. A new generation of miniaturized UV-Vis spectrometric systems which valorize microplasmas technology able to be interfaced with a microspectrometer, was successfully tested for the

determination of elements (Hg, As, Sb, Se and transitional metals) forming chemical vapor *via* classical or sono-/UV induced procedure [31–37]. In our laboratory it was recently developed a method for As and Sb determination in non- and biodegradable materials and soil by hydride generation capacitively coupled plasma microtorch optical emission spectrometry (HG-μCCP-OES) [38,39].

The aim of the study was to evaluate whether the HG-μCCP-OES method, which involves arsine generation from As(III) species in the presence of $NaBH_4$ in diluted HCl solution and L-cysteine, is suitable for the determination of total As in various water types. The method was optimized and characterized in terms of linearity, limit of detection and quantification, accuracy and precision. The analytical capability of the proposed method was demonstrated by analyzing samples of drinking water, groundwater and waste water with different As concentration. The novelty of the study lies in the fact that the method has been implemented for the first time on a miniaturized prototype model, which provides benefits related to low consumption of energy and argon for plasma generation and reduction of used reagents.

RESULTS AND DISCUSSION

Optimization of the working conditions for As determination in water by HG-μCCP-OES

The HG-μCCP-OES method was optimized in terms of general conditions for hydride generation (concentrations of HCl, L-cysteine, $NaBH_4$ and NaOH) and plasma operation (power, Ar flow rate). The optimization criterion was to get the highest emission signal at As 228.812 nm. The influence of conditions for arsine generation from As(III) species on emission signal is presented in Figures 1-4.

The examination of Figures 1–4 evidentiated the following optimal conditions in terms of reagent concentrations for arsine generation from As(III) species: 0.01 mol L^{-1} HCl in sample and carrier (pH 2.00±0.01); 0.3% L-cysteine in sample; 0.5% $NaBH_4$ stabilized in 0.5% NaOH.

The optimal conditions for plasma operation were previously found to be 10 W plasma power and 150 mL min^{-1} Ar for arsine purge and plasma sustaining, and 0 mm observation height [39].

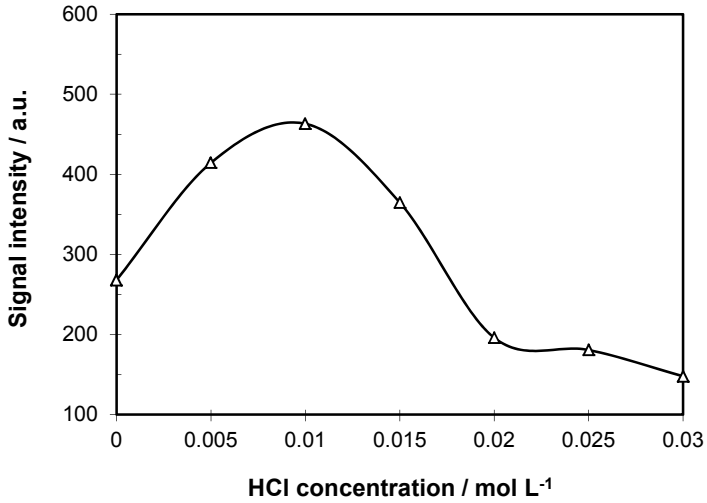


Figure 1. Influence of HCl concentration in sample on As 228.812 nm emission signal after arsine generation from As(III) species. Experimental conditions: 50 µg L^{-1} As; identical concentration of HCl in sample and carrier; 0.3% L-cysteine; 0.5% $NaBH_4$ stabilized in 0.5% NaOH; 10 W plasma power; 150 mL min^{-1} Ar as gas for arsine purge and plasma sustaining

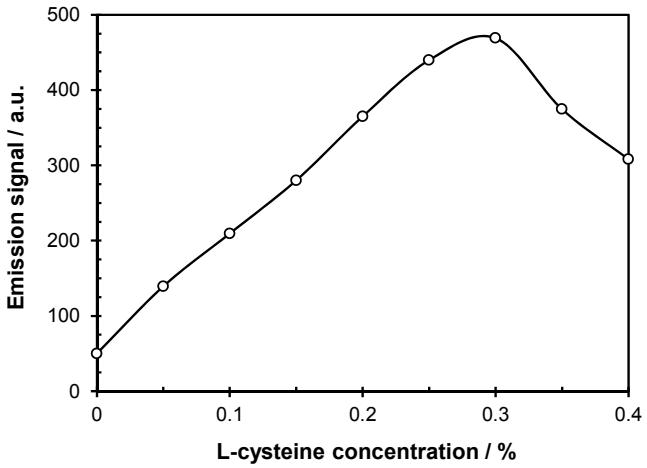


Figure 2. Influence of L-cysteine concentration in water sample on As 228.812 nm emission signal after arsine generation from As(III) species. Experimental conditions: 50 µg L^{-1} As; 0.01 mol L^{-1} HCl in sample and carrier; 0.5% $NaBH_4$ stabilized in 0.5% NaOH; 10 W plasma power; 150 mL min^{-1} Ar as gas for arsine purge and plasma sustaining

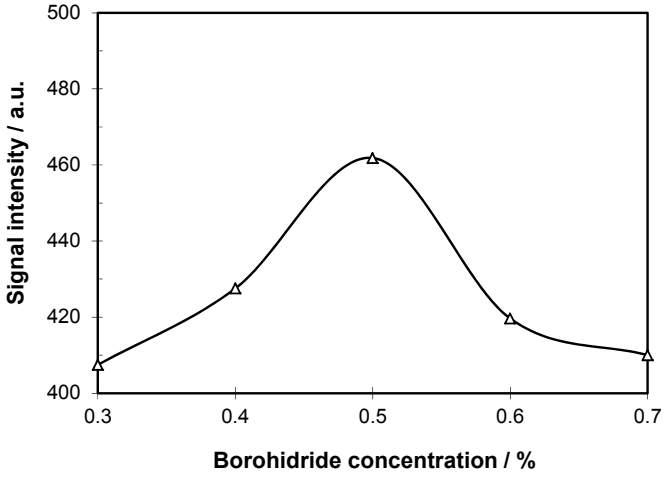


Figure 3. Influence of $NaBH_4$ concentration in hydride generation reagent on As 228.812 nm emission signal after arsine generation from As(III) species. Experimental conditions: 50 µg L^{-1} As; 0.01 mol L^{-1} HCl in sample and carrier; $NaBH_4$ solution stabilized in 0.5% NaOH; 10 W plasma power; 150 mL min^{-1} Ar as gas for arsine purge and plasma sustaining

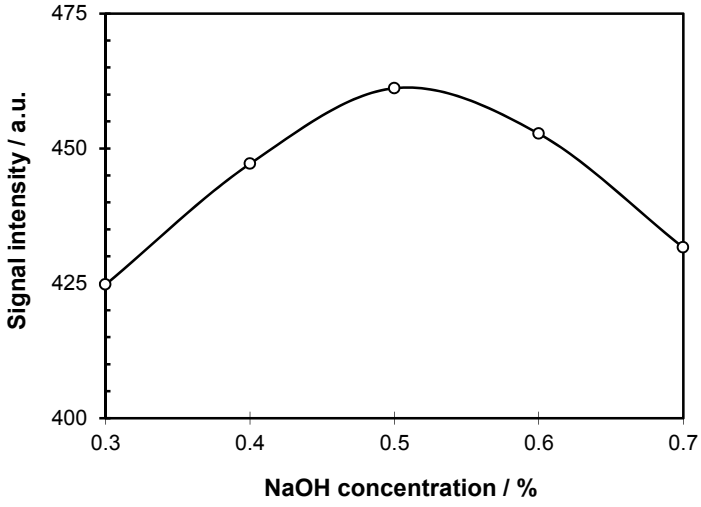


Figure 4. Influence of NaOH concentration in hydride generation reagent on As 228.812 nm emission signal after arsine generation from As(III) species. Experimental conditions: 50 µg L^{-1} As; 0.01 mol L^{-1} HCl in sample and carrier; 0.5% $NaBH_4$; 10 W plasma power; 150 mL min^{-1} Ar as gas for arsine purge and plasma sustaining

Figures of merit for As determination in water by HG-μCCP-OES

The HG-μCCP-OES method was assessed regarding linearity of the calibration curve, limit of detection and quantification, and accuracy. The limit of detection was calculated using the 3σ criterion from the parameters of the calibration curve ($3s_{y/x}/m$), where ($s_{y/x}$) was the standard deviation of residuals (3.4795) and (m) the calibration sensitivity (8.8917) over the range 0 – 100 μg L^{-1} As(III) in standards. Limit of quantification was considered to be 3 folds the limit of detection. The parameters of the calibration curve for As are presented in Fig. 5.

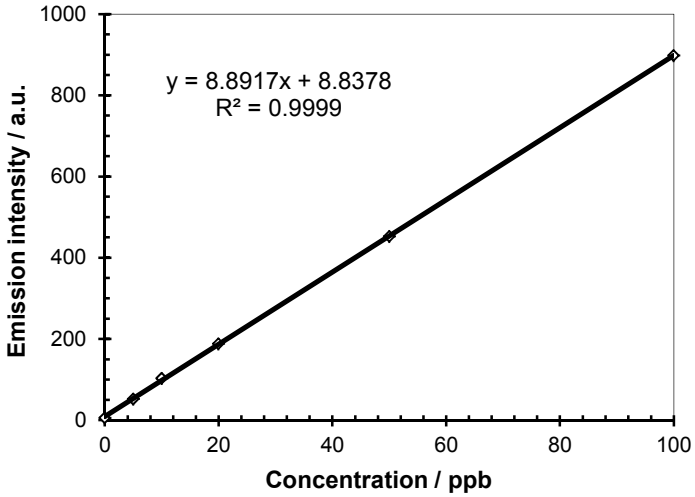


Figure 5. Calibration plot for As 228.812 nm in HG-μCCP-OES over the range 0 – 100 μg L^{-1} As

The limit of detection by HG-μCCP-OES was 0.2 μg L^{-1} and limit of quantification of 0.6 μg L^{-1}, above which As quantification in water is achievable. The limit of detection is 50 folds lower than the maximum concentration limit in drinking water and groundwater according to current guidelines [13,14,40] so that the HG-μCCP-OES method fulfils the demands for such kind of analysis.

Arsenic concentrations found in certified reference water samples by HG-μCCP-OES are presented in Table 1.

Data in Table 1 demonstrate that the HG-μCCP-OES method provides a good accuracy for As determination in drinking water and groundwater with recovery in the range 99±6%. No significant differences were between certified and found concentrations.

Table 1. Results (μg L^{-1}) obtained for As determination in certified reference samples of water by HG-μCCP-OES.

Certified sample	Type	Certified value±U	Found value±U [a]	Recovery/%[b]
LGC 6010	Drinking water	55±5	54±3	98±6
ERM-CA011b	Drinking water	10.15±0.34	10.25±0.85	101±8
SRM 1643e	Synthetic	60.45±0.72	60.55±0.72	100±1
ERM-CA615	Groundwater	9.9±0.7	10.0±0.5	101±5
BCR 610	Groundwater	10.8±0.4	10.5±0.5	97±5

[a] – Expanded uncertainty for 95% confidence level and (n=5 parallel measurements)
[b] – Recovery for 95% confidence level and (n=5 parallel measurements)

Analysis of test samples of water

The total As concentration found in several real samples of water are provided in Table 2.

Table 2. Total As in water samples by HG-μCCP-OES

Sample	Sample size	As/μg L^{-1}				RSD/%[a]
		Min	Max	Mean	Median	
Drinking water[b]	9	0.6	2.8	1.9	2.1	5.0 – 10.8
Non-contaminated groundwater[c]	13	3.2	9.1	5.8	5.4	1.8 – 8.0
Contaminated groundwater[c]	18	10.9	79.9	32.6	27.5	1.2 – 7.1
Waste water[d]	6	12.8	25.2	17.2	17.1	1.6 – 9.3

[a] – RSD - relative standard deviation (n = 5)
[b] – collected from private wells and water supply network
[c] – collected from western and south-western Romania (natural enrichment)
[d] – contaminated water (no treatment) from mining and non-ferrous metal industry

Data in Table 2 confirm that the HG-μCCP-OES method can be used for accurate determination of As in water of different origin. For concentrations in the range 0.6 – 80 μg L^{-1} precision of repeated measurements (n = 5) was between 1.2 – 10.8%.

CONCLUSIONS

It was developed and characterized a method for As determination in different types of water after hydride generation using a prototype system based on a capacitively coupled plasma microtorch coupled with a low resolution microspectrometer for optical emission measurements. The proposed

method developed on fully miniaturized instrumentation fulfils the required analytical performances for As determination in water at concentrations level much below that maximum admitted in drinking and groundwater. Under these circumstances the HG-μCCP-OES prototype system has promising perspectives to be used for monitoring As in diverse water sources.

EXPERIMENTAL SECTION

Reagents, standard solutions, CRMs and water test samples

Hydrochloric acid, 30% (m/m), ultrapur (< 5 ng L^{-1} As), $NaBH_4$ pro analysis (< 0.001% As), NaOH suprapur (99.99%), L-cysteine for biochemistry (< 0.0005% As), stock solution of As(V) 1000 μg mL^{-1} were purchased from Merck (Darmstadt, Germany). A solution of 3% L-cysteine in HCl (pH = 2.00±0.01) was used to prereduce As(V) to As(III), while a solution of 0.01 mol L^{-1} HCl (pH = 2.00±0.01) was used as carrier. The solution of 0.3% L-cysteine in HCl (pH = 2.00±0.01) was used as carrier of arsine into plasma. The solution of 0.5% $NaBH_4$ stabilized in 0.5% NaOH used as derivatization reagent was daily prepared. Calibration of HG-μCCP-OES was carried out with standards in the range 0 – 100 μg L^{-1} As after prereduction in the presence of 0.3% L-cysteine in HCl medium (pH 2.00±0.01) on water bath for 10 min. The HG-μCCP-OES method was assessed for by analyzing several certified reference materials of water (ERM CA011b Hard Drinking Water UK - Metals, LGC 6010 Hard Drinking Water, ERM-CA615 Groundwater, BCR 610 Groundwater, SRM 1643e Trace Elements in Water) acquired from LGC Promochem (Wesel, Germany). The applicability of the method was checked on real samples of drinking water, groundwater and waste water with As content below or above the maximum concentration level in drinking water.

The glassware was soaked in 5 mol L^{-1} HNO_3 for 12 h and rinsed with Milli-Q water.

Water sample preparation for As determination by HG-μCCP-OES

Determination of As in water samples in the presence of L-cysteine in dilute HCl solution involves two steps: (i) prereduction of As(V) to As(III) and (ii) arsine generation from As(III). After filtration, an aliquot volume of 10 – 40 mL sample was mixed with 5 ml 3% L-cysteine in 0.01 mol L^{-1} HCl and heated on water bath at 90±5 ºC for 10 min for prereduction of As(V). After cooling, pH was adjusted to (2.00±0.01) by potentiometric titration and diluted to 50 mL with HCl solution of (2.00±0.01) pH. In this way water samples had a final concentration of 0.3% L-cysteine and (2.00±0.01) pH. The samples of CRMs and calibration standards of As were prepared in the same way.

Operation of the HG-μCCP-OES equipments

The HG-μCCP-OES equipment is a miniaturized prototype consisting of a plasma microtorch (INCDO-INOE 2000 Bucharest, Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania), a free-running generator (13.56 MHz, 10 – 30 W, 15x17x24 cm^3 size) (Technical University Cluj-Napoca, Romania), a HGX-200 hydride generator (Omaha, Nebraska, USA) and a QE65 Pro Spectrometer (190 – 380 nm, 3648-element Toshiba CCD-array detector, 0.4 nm FHWM), (Ocean Optics, Dunedin, USA). Constructive and operation details are presented in Table 3.

Table 3. Constructive details and working conditions for the HG-μCCP-OES prototype equipment

Component	Description
Plasma microtorch	Capacitively coupled with Mo tip microelectrode of 1 mm diameter (Goodfellow, Cambridge, UK), quartz tube of 160 nm cut-off, 5 mm i.d., 25 mm length (H. Baumbach & Co. Ltd., Ipswich Suffolk, UK); Ar and arsine intake into plasma through channels of 0.75 mm; other constructive details are available in ref. [38]; 10 W plasma power; 150 mL min^{-1} Ar flow for arsine purging from hydride generator and plasma sustaining; 0 mm viewing height.
Radiofrequency generator	Free running, 10 - 30 W, 13.56 MHz (Technical University, Cluj Napoca, Romania).
Hydride generator	HGX-200 CETAC (Nebraska, USA). Flow rates: sample 7 mL min^{-1}; $NaBH_4$ solution 1 mL min^{-1}; carrier (HCl solution, pH 2.00±0.01) 1 mL min^{-1}, MasterFlex L/S peristaltic pump with 4-channels (Model 7535-04, Cole Parmer, Montreal, Canada).
Spectrometer	QE65 Pro Ocean Optics (Dunedin, USA), 190 – 380 nm spectral range; 0.4 nm FWHM, Toshiba CCD detector with 3648 pixels cooled at (– 20 ºC) with Peltier element, back-illuminated.
Signal processing	Spectrasuite software, peak height signal at As 228.812 nm, 10 s integration time, background correction using two points model.

Measurement of As emission by HG-μCCP-OES was carried out as follows: 1. registration of plasma background during pumping of 0.3% L-cysteine in HCl solution with pH (2.00±0.01) as blank; 2. background correction; 3. registration of the net spectrum containing the As 228.812 nm emission signal after aspirating standards/CRMs/test water samples in the HGX-200 generator. Memory effects were overcome by washing the sample channel of the generator with the following solution to be measured for 40 s. The emission spectrum of As in HG-μCCP-OES was already presented [38,39].

ACKNOWLEDGMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI–UEFISCDI, project number PN-II-PT-PCCA-2011-3.2-0219 (Contract no. 176/2012.)

REFERENCES

1. P. Ravenscroft, H. Brammer, K. Richards, "Arsenic pollution: a global synthesis", Wiley-Blackwell, John Wiley & Sons, Chichester UK, **2009**.
2. D. Panagiotaras, G. Panagopoulos, D. Papoulis, P. Avramidis, "Arsenic Geochemistry in groundwater system", Ed. D. Panagiotaras, Geochemistry - Earth's system processes, INTech Europe, Rijeka, Croartia, **2012**, chapter 2.
3. M. S. Bloom, S. Surdu, I. A. Neamtiu, E. S. Gurzau, *International Journal of Hygiene and Environmental Health,* **2014**, *217*, 709.
4. S. Surdu, M. S. Bloom, I. A. Neamtiu, C. Pop, D. Anastasiu, E. F. Fitzgerald, E. S. Gurzau, *Environmental Research,* **2015**, *140*, 657.
5. P. L. Smedley, D. G. Kinniburgh, *Applied Geochemistry,* **2002**, *17*, 517.
6. M. M. Rahman, Z. Dong, R. Naidu, *Chemosphere,* **2015**, *139*, 54.
7. A. Katner, M. Lackovic, K. Streva, V. Paul, W. C. Trachtman, *Journal of Public Health Management and Practice,* **2015**, *21*, S93.
8. A. E. Gurzau, C. Pop, A new public health issue: contamination with arsenic in private water sources (2012). Proceedings of the AERAPA, Conference.aerapa.conference.ubbcluj.ro/2012/Gurzau.htm.
9. E. A. Cordos, T. Frentiu, M. Ponta, I. Marginean, B. Abraham, C. Roman, *Chemical Speciation and Bioavailability,* **2006**, *18*, 11.
10. T. Frentiu, S. N. Vlad, M. Ponta, C. Baciu, I. Kasler, E. A. Cordos, *Chemical Papers,* **2007**, *61*, 6.
11. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. *Official Journal of European Communities,* **2000**, *L327*, 1.

12. European Union Directive 2006/118/EC of the European Parliament and of the Council of 12 December on the protection of groundwater against and deterioration. *Official Journal of European Communities*, **2006**, *L372/19*, 209.
13. Annex 3. To the Commission staff working document accompanying in the report from the Commission in accordance with article 3.7 of the Groundwater Directive 2006/118/EC on the establishment of groundwater threshold values. Information on the groundwater threshold values of the Member States. http://ec.europa.eu/environment/water/water-framework/groundwater/pdf/com_swd_annex_iii.pdf. (Accessed 24.12.2015).
14. E. Radu, R. Balaet, E. Vliegenthart, P. Schipper, *Environmental Engineering Research,* **2010**, *15*, 085.
15. S. Hassanpoor, G. Khayatian, A. R. J. Azar, *Microchimica Acta*, **2015**, *182*, 1957.
16. A. Khaligh, H. Z. Mousavi, H. Shirkhanloo, A. Rashidi, *RSC Advances*, **2015**, *5*, 93347.
17. A. C. Grijalba, L. B. Escudero, R. G. Wuilloud, *Analytical Methods*, **2015**, *7*, 490.
18. M. Shahlaei, A. Pourhossein, *Journal of Chemistry,* **2014**, article ID 912619**.**
19. H. Abdolmohammad-Zadeh, A. Jouyban, R. Amini, *Talanta*, **2013**, *116*, 604.
20. O. Acar, O. M. Kalfa, O. Yalcinkaya, A. R. Turker, *Analytical Methods*, **2013**, *5*, 748.
21. R. Gurkan, U. Kir, N. Altunay, *Food Analytical Methods,* **2015**, *8*, 1637.
22. S. K. Pal, N. Akhtar, S. K. Ghosh, *Analytical Methods,* **2016**, *8*, 445.
23. J. Sun, Z. G. Yang, H. W. Lee, L. Wang, *Analytical Methods,* **2015**, *7*, 2653.
24. A. R. Kumar, P. Riyazuddin, *International Journal of Environmental Analytical Chemistry,* **2007**, *87*, 469.
25. A. Gonzalvez, M. L. Cervera, S. Armenta, M. De la Guardia, *Analytica Chimica Acta,* **2009**, *636*, 129.
26. P. Pohl, P. Jamroz, *Journal of Analytical Atomic Spectrometry,* **2011**, *26*, 1317.
27. Z. Long, Y. M. Luo, C. B. Zheng, P. C. Deng, X. D. Hou, *Applied Spectroscopy Review,* **2012**, *47*, 382.
28. Z. Long, C. Chen, X. D. Hou, C. B. Zheng, *Applied Spectroscopy Review,* **2012**, *47*, 495.
29. Y. G. Yin, J. F. Liu, G. B. Jiang, *Trends in Analytical Chemistry*, **2011**, *30*, 1672.
30. Y. L. Wang, L. L. Lin, J. X. Liu, X. F. Mao, J. H. Wang, D. Y. Qin, *Analyst*, **2016**, *141*, 1530.
31. D. Luo, Y. Duan, *Trends in Analytical Chemistry*, **2012**, *39*, 254.
32. F. Pena-Pereira, I. Costas-Mora, V. Romero, I. Lavilla, C. Bendicho, *Trends in Analytical Chemistry*, **2011**, *30,* 1637.
33. X. Yuan, J. Tang, Y. Duan, *Applied Spectroscopy Reviews*, **2011**, *46*, 581.
34. S. Zhang, H. Luo, M.T. Peng, Y.F. Tian, X. D. Hou, X. M. Jiang, C. B. Zheng, *Analytical Chemistry*, **2015***, 87,* 10712.
35. K. Greda, P. Jamroz, D. Jedryczko, P. Pohl, *Talanta*, **201**5, *137*, 11.
36. T. Frentiu, S. Butaciu, E. Darvasi, M. Ponta, M. Senila, E. Levei, M. Frentiu, *Journal of Analytical Atomic Spectrometry,* **2015**, *30*, 1161.
37. T. Frentiu, S. Butaciu, E. Darvasi, M. Ponta, M. Senila, D. Petreus, M. Frentiu, *Analytical Methods*, **2015**, *7*, 747.

38. A. I. Mihaltan, T. Frentiu, M. Ponta, D. Petreus, M. Frentiu, E. Darvasi, C. Marutoiu, *Talanta*, **2013**, *109*, 84.
39. T. Frentiu, S. Butaciu, M. Ponta, E. Darvasi, M. Senila, D. Petreus, M. Frentiu, *Journal of Analytical Atomic Spectrometry*, **2014**, *29*, 1880.
40. Romanian Law no. 458/2002 on the quality of drinking water, published in Romanian Official Monitor, part I, 875, 2011.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 311-320)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

SIMULTANEOUS DETERMINATION OF CALCIUM AND MAGNESIUM IN NATURAL WATERS BY METHANE-AIR FLAME EMISSION AND FLAME ATOMIC ABSORPTION SPECTROMETRY USING A MICROSPECTROMETER

DARVASI EUGEN[a,*], MUNTEAN NORBERT[a], SZENTKIRÁLYI CSILLA[a]

ABSTRACT. The calcium and magnesium content of liquid samples has been determined directly by flame atomic absorption (FAAS) and flame atomic emission (FAES) spectrometry using the methane-air (M-A) flame. We measured simultaneously the intensity of the 554 nm wavelength molecular bands emitted by excited CaOH molecules and the decrease of light intensity of a hollow cathode lamp (HCL) absorbed by ground state Mg atoms. The simultaneous multiwavelength measurements enhanced by a charge-coupled device (CCD) microspectrometer allowed fast background correction for each studied element. The instrumental and flame parameters were optimized; the best results were obtained using a lamp current of 1 mA, and an observation height of 5 mm, in case of a reducing flame. The calcium and magnesium content of bottled water samples and water standard certified reference material (CRM) have been determined with standard addition method. The recovery for CRM was 97.80% for Ca and 98.51% for Mg. Under optimal working conditions the detection limits (according to the 3s criterion) were 25 $\mu g \cdot L^{-1}$ for Ca and 5.4 $\mu g \cdot L^{-1}$ for Mg.

Keywords: *methane-air flame, FAES, FAAS, CCD microspectrometer, Ca, Mg, simultaneous determination.*

INTRODUCTION

The most commonly used analytical method for the determination of Ca and Mg is complexometric titration [1], but this technique is time and reagents consuming. A good alternative for Ca and Mg determination in liquid samples is

[a] *Babeș-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany János, RO-400028 Cluj-Napoca, Romania*
** Corresponding author: edarvasi@chem.ubbcluj.ro*

flame atomic absorption (FAAS) [2, 3], flame atomic emission spectrometry (FAES) [4, 5], inductively coupled plasma optical emission spectrometry (ICP-OES) [6], inductively coupled plasma mass spectrometry (ICP-MS) [7], and electrothermal atomization atomic absorption spectrometry (ETAAS) [8]. Actually the trends of Green Analytical Chemistry (GAC) are gaining ground [9, 10]. In concordance with these principles the development of miniaturized instruments and methods are priorities. Using methane gas from the local gas network is economical and the energy consumption of a microspectrometer is very low (450 mA, 5V). The development of CCD microspectrometers allows the use of new signal acquision and calibration methods in atomic spectrometry [11, 12, 13, 14].

The aim of this study was to attempt a simultaneous use of flame atomic emission and absorption method for Ca and Mg determination in drinking water. In order to achieve this goal we optimized the flame and the instrumental parameters for the simultaneous FAES-FAAS determination in the M-A flame, and applied the results for the quantification of Ca and Mg in different bottled drinking water samples and water standard certified reference material.

RESULTS AND DISCUSSION

Optimization of analytical procedure

The wavelength selection in the spectral range (250-600 nm) was based on the analysis of the Mg HCL lamp and Ca emission signal (Fig. 1).

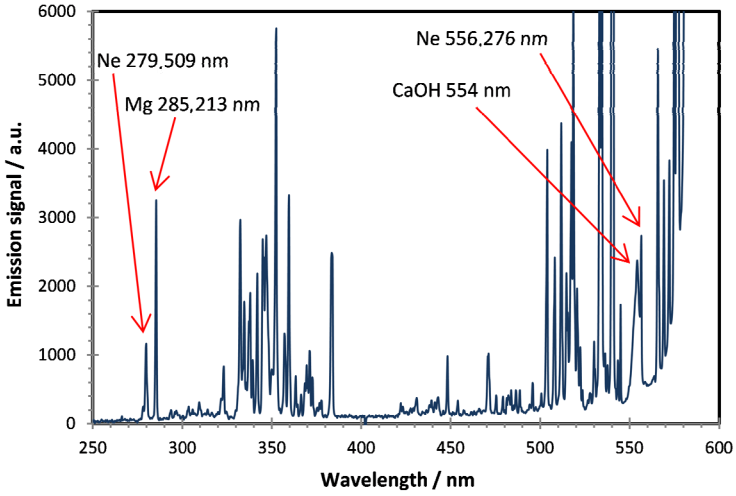


Figure 1. The Mg HCL lamp and Ca emission signal (I_{HCL}=1 mA, C_{Ca}=25 ppm, $t_{integration}$=5 s)

For the Mg determination we used the 285.213 nm line, and for Ca the 554 nm CaOH molecular emission band. The 422.672 nm Ca atomic emission line has a low intensity and the 622 nm molecular emission band could not be used because of interference with Ne emission lines.

After the flame and instrumental parameters optimisation, the measurements started with initial background correction. After the selection of integration time and average number, covering the HCL lamp light, we measured the flame background with a blank solution. This background signal was computer-subtracted from each later measured spectrum. The analytical signal was obtained after the second background correction of emission lines based on three wavelength measurements. (Fig. 2.)

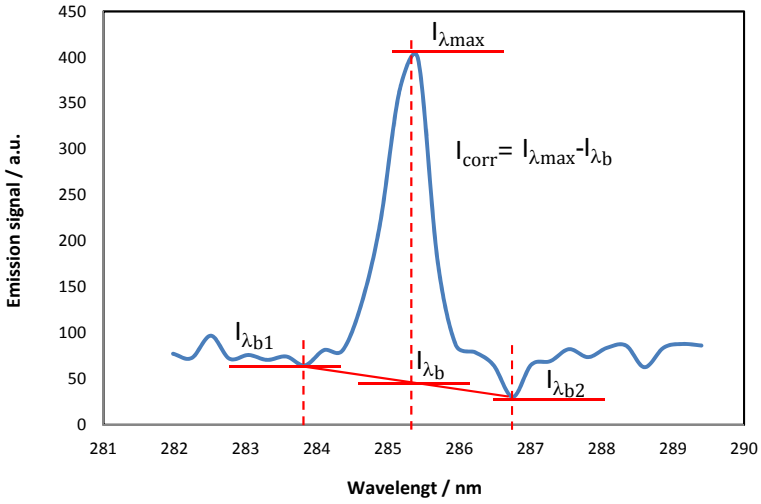


Figure 2. The background correction based on three wavelength measurements

Optimization of the flame and observation height

The flame composition and the observation height were optimised. The concentration of the calibration solution was of 5 mg·L^{-1} Mg and 25 mg·L^{-1} Ca. Two flame compositions (reducing and oxidizing) were used and measurements were performed at different observation heights. The HCL current was increased in the range 2.5–20 mA in 7 steps. The air flow-rate was kept constant 600 L/h and the flow rate of the methane was fixed at 56 L/h for oxidizing flame, and 66 L/h for reducing flame. The best results were obtained in case of reducing flame (air flow-rate 600 L/h and methane flow rate 66 L/h, at the observation height of 5 mm (Fig. 3).

Optimization of the hollow-cathode lamp current and integration time

The hollow-cathode lamp current and integration time was optimized by making calibration for each element at different lamp current intensities, between 0.5–2 mA, in 0.5 mA steps and different integration time (3, 4, and 5 s). In each case we followed the coefficient of determination (R^2), the sensitivity (m) and the detection limit (LOD).

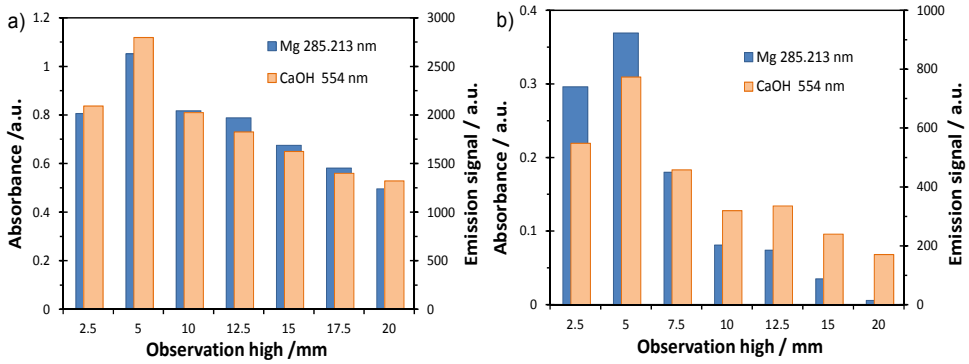


Figure 3. Signal at different observation height for (a) reducing (b) oxidizing flame

The results are presented in Fig 4. a), b), c). The measurements demonstrate the increase of calibration sensitivity with integration time, but if an integration time longer than 5 s is used, the time of analyses becomes too long. The detection limit for Mg decreases, and for Ca increases with the increase of lamp current. The 1 mA is an optimal value for both of them. The coefficient of determination (R^2) has the best value for 1 mA optimal lamp current for both elements.

We can consider that the optimal value of the lamp current for the simultaneous determination of Ca and Mg is 1 mA, and the best integrating time is 5 s.

Figures of merit

For Ca calibration we measured the intensity of CaOH molecular band at 554 nm. The signal and calibration curve are presented in Fig.5.

For Mg calibration the absorbance at 285.213 nm is calculated using the relation $A=-lg(p/p_0)$ where p_0 is the radiant power of the lamp in case of background measurement (blank solution) and p the radiant power of lamp in case of calibration solution measurement. The signal and calibration curve are presented in Fig.6.

The characteristics of calibration curves (coefficient of determination (R^2), calibration sensitivity (m)) and detection limits (LOD) according to the 3s criterion (3Sb/m) for Ca and Mg are given in Table I. For the determination of the standard deviation of the background (Sb) 10 independent measurements of the blank solution in the proximity of the analytical wavelength were used.

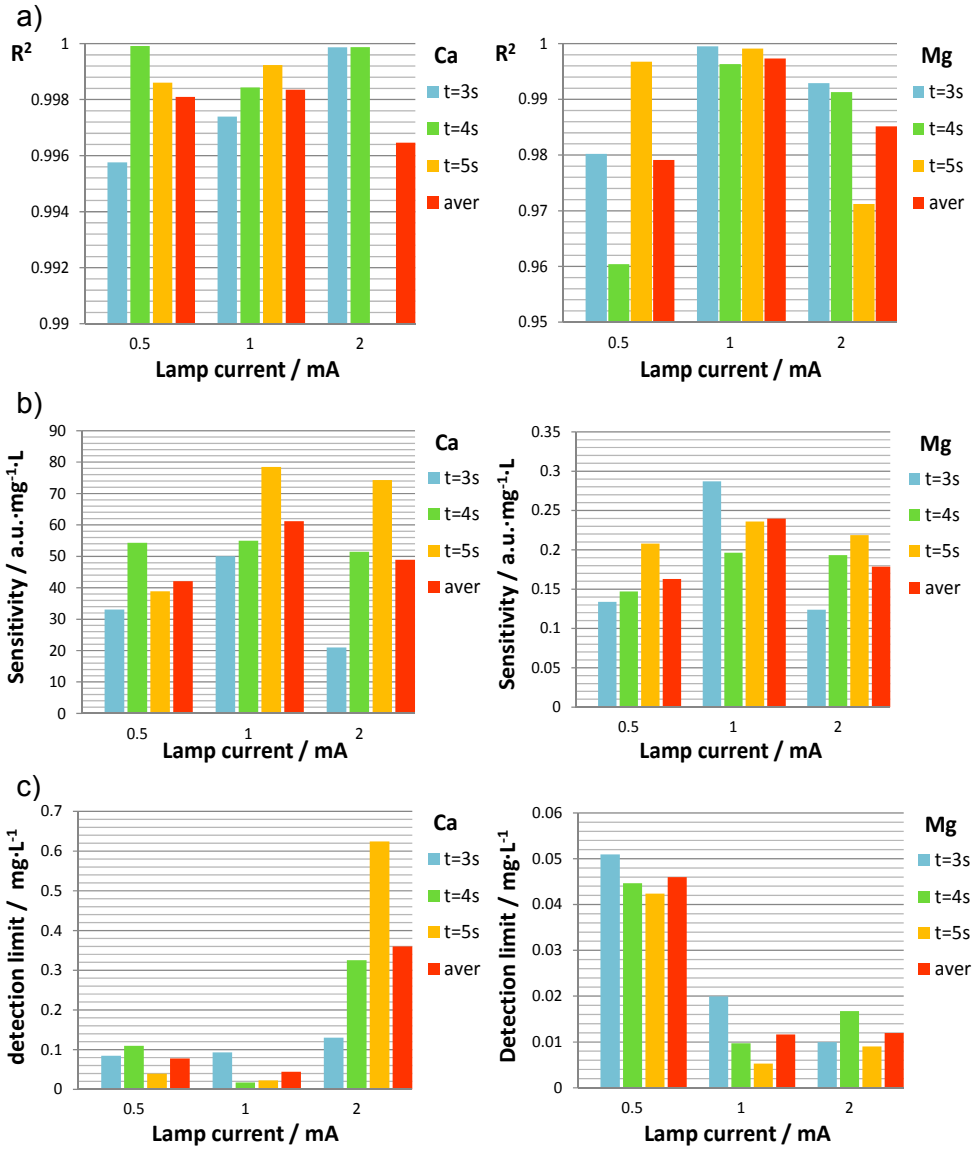


Figure 4. a) The coefficient of determination (R^2), b) the calibration sensitivity (m) c) the detection limit (LOD) for different lamp currents and integration times

As shown in table II. the detection limit in the presented CCD-based FAES-FAAS method is higher than in the case of ICP and Flame AAS, still it is substantially lower than the Ca and Mg concentration of natural waters.

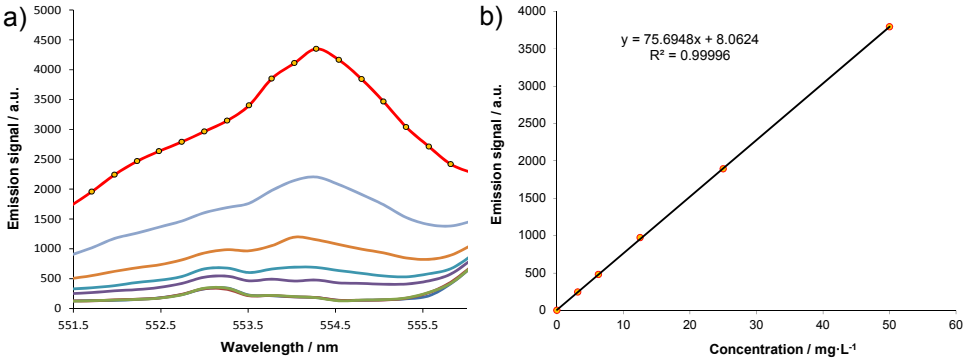


Figure 5. CaOH emission signal and calibration curve (554 nm)

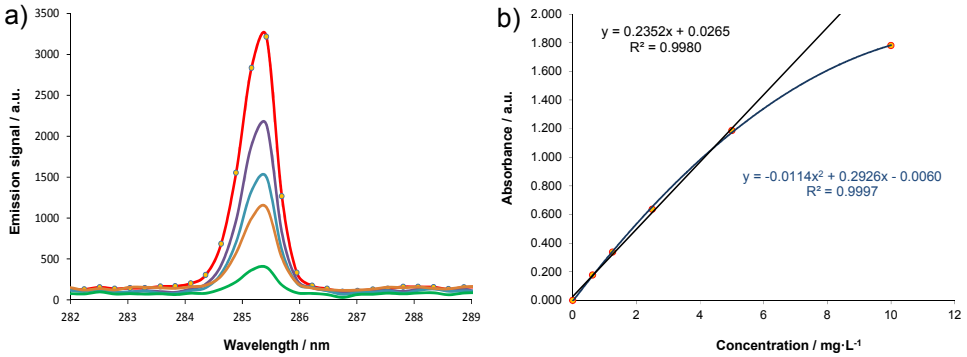


Figure 6. Mg lamp emission signal and calibration curve (285,213 nm)

Table I. Figures of merit for Ca and Mg determination

Element	λ (nm)	Dynamic range ($mg \cdot L^{-1}$)	Sensitivity (m) ($a.u. \cdot mg^{-1} \cdot L$)	RSD slope (%)	Coefficient of determination (R^2)	LOD ($\mu g \cdot L^{-1}$)
Ca	554	0.2-50	75.69	0.75	0.9999	25
Mg	285.2	0.05-5	0.235	2.55	0.9980	5.4

Table II. Comparison of CCD-based method with ICP and Flame AAS [15]

Element	Method	Wavelength (nm)	LOD (μgL^{-1})
Ca	CCD-based	554	25
	Flame AAS	422.7	1
	ICP	393.366	0.03
Mg	CCD-based	285.2	5
	Flame AAS	285.2	0.3
	ICP	279.553	0.1

Sample analysis

The analytical method was tested with different bottled waters (samples 1-5) and water certified reference material.

To minimize the formation of nonvolatile compounds the samples were diluted and treated with perchloric acid (1 mL/500mL). For analysis we applied the standard addition method. The concentration of the standard was 250 $mg \cdot L^{-1}$ Ca and 50 $mg \cdot L^{-1}$ Mg. A volume of 0.2 mL standard was added to 10 mL of diluted sample. Three additions of standard were made. The sample concentration was calculated based on the equation of regression line.

Table III. presents the results obtained for the determination of Ca and Mg in bottled waters. The average recovery was 101±4% for Ca and 115±22% for Mg.

The LOD calculated based on standard addition calibration sensitivity was found in the range of 15–28 $\mu g \cdot L^{-1}$ for Ca and 4,9–6,3 $\mu g \cdot L^{-1}$ for Mg. Table IV. presents the results obtained for the water certified reference material.

Table III. Bottled water analysis results (n=3)

Sample	Elements	$C_{cert.}$($mg\ L^{-1}$)	C ($mg\ L^{-1}$)[a]	Recovery (%)
Bottled water 1	Ca	47	57±1	121±2
Bottled water 2		17*	18±1	106±6
Bottled water 3		60*	58±2	97±3
Bottled water 4		9.5*	8.1±0.5	84±6
Bottled water 5		113*	112±1	99±1
Bottled water 1	Mg	6*	9±1	150±11
Bottled water 2		3*	3±1	100±33
Bottled water 3		31*	35±2	113±6
Bottled water 4		3*	3±1	100±33
Bottled water 5		41*	45±1	110±2

[a] Expanded uncertainty for 95% confidence interval

Table IV. Water certified reference material. (n=3)

Sample	Elements	$C_{cert.}$(mg L^{-1})	C (mg L^{-1})[a]	Recovery (%)
CRM	Ca	73.6±2.7	72.0±1.5	98±2
	Mg	14.78±0.48	14.56±0.51	98±3

[a] Expanded uncertainty for 95% confidence interval

CONCLUSIONS

In this paper an CCD-based FAES-FAAS original analytical method is presented, which allows the simultaneous determination of Ca and Mg atomised in methane-air flame. Measuring simultaneously the intensity of the 554 nm wavelength molecular bands emitted by excited CaOH molecules and the Mg atomic absorption of a HCL lamp light, we applied the FAES-FAAS methods together. A CCD microspectrometer with UV-VIS-NIR range for detection and background correction having been used, the method is in concordance with the principles of Green Analytical Chemistry (GAC).

The detection limit for Mg are 5.4 $\mu g \cdot L^{-1}$, the linear measuring range is 0.02–5 $\mu g \cdot L^{-1}$. The detection limit for Ca are 25 $\mu g \cdot L^{-1}$, the linear measuring range is 0.1–50 $\mu g \cdot L^{-1}$. The coefficient of determination R^2 >0.998 demonstrates a good linearity of the method.

The proposed method was successfully applied to analyse water samples. The recovery for CRM was 98±2 % for Ca and 98±3 % for Mg. The average recovery for bottled water was 101±4 % for Ca and 115±22 % for Mg.

EXPERIMENTAL SECTION

Reagents, Standard Solutions and CRM

Stock standard solutions of Ca and Mg (1000 $mg \cdot L^{-1}$) and analytical grade perchloric acid purchased from Merck (Darmstadt, Germany) were used. Double distilled water was used for all dilutions. For the CRM ERM CA011b (Hard drinking water) was used.

Samples

The bottled waters were purchased from supermarkets in Cluj-Napoca, Romania.

Instrumentation and analytical method

The FAES-FAAS equipment consisted of a HEATH EU-700-30 type Gas-flow and HCL lamp module, a pneumatic nebulizer chamber and a CCD microspectrometer (Fig. 7)

The pneumatic nebulizer chamber and gas-burner system that was used came from an AAS-1 (Carl Zeiss Jena) atomic absorption spectrometer. The burner-head was Mecker type. The air flow-rate was kept constant, 600 L/h, the flow rate of the methane being varied in 56-66 L/h interval.

The AAS lamp was a max 20 mA Activion Mg HCL lamp (Halstead Essex – England).

For optical detection a HR4000 Microspectrometer Ocean Optics CG–UV–NIR with the following parameters: 200–1100 nm spectral range, 50 μm entrance slit, Toshiba CCD detector with 3648 pixels, 1304 AP, FWHM: 1.5 nm was used. A collimating fused silica lens (5 mm diameter, 10 mm focal length) and a fibre optic QP 600 lm, 25 cm length (Ocean Optics, Dunedin, USA) assured the transmission of the optical signal.

Data acquision was performed using Spectrasuite soft (Ocean Optics); 0.1–20 s integration time, and computer-subtracted background correction.

Method validation

It was plotted the regression line for Ca (0–50 $mg \cdot L^{-1}$) and Mg (0–10 $mg \cdot L^{-1}$) standards. The equation of the regression line, the confidence limits and the coefficient of determination, R^2 were calculated with the least squares method.

The limit of detection (*LOD*) was calculated on the basis of 3 s criterion ($LOD=3s_B/m$), where m was the slope of calibration curve and s_B the standard deviation of 10 successive measurements of blank.

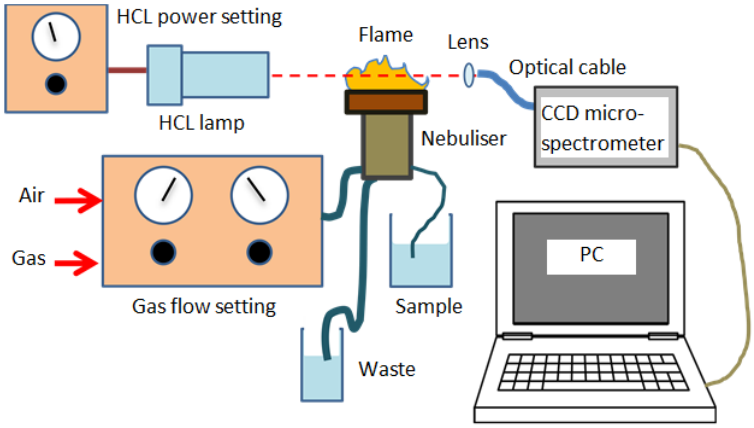


Figure 7. Schematic diagram of the CCD-based FAES-FAAS equipment

REFERENCES

1. A. Zsigmond, “Minőségi és mennyiségi analitikai kémia laborkönyv”, Editura Scientia, **2008**, chapter 1-3.
2. L. Noel, M. Carl, C. Vastel, T. Guerin, *International Dairy Journal*, **2008**, *18*, 899.
3. Leo M.L. Nollet, Leen S. P. De Gelder, “Handbook of Water Analysis”, Third edition, CRC Press, **2014**, chapter 15.
4. L. Kékedy Nagy, E. A. Cordoş, *Studia UBB Chemia,* **1999**, *44*, 137.
5. L.Kékedy-Nagy, E. A. Cordoş, *Studia UBB Chemia*, **1999**, *44*, 183.
6. C. Prohaska, K. Pomazal, I. Steffan, *Fresenius' Journal of Analytical Chemistry*, **2000**, *367*, 479.
7. D. Beauchemin, J. W. McLaren, A. P. Mykytiuk, S. S. Berman, *Anal. Chem.*, **1987**, *59,* 778.
8. Z. Kilic, O. Acar, M. Ulasan and M. Tlim, *Food Chem*., **2002**, *76*, 107.
9. C. Bendicho, I. Calle, F. Pena, N. Cabaleiro, I. Lavilla, *Trends Anal. Chem*., **2012**, *31*, 50.
10. C. Bendicho, I. Lavilla, F. Pena-Pereira, V. Romero, *J. Anal. At. Spectrom*., **2012**, 27, 1831.
11. C. Zheng, Y. He, S. Weia, X. Hou, J. Anal. At. Spectrom., **2005**, *20*, 60.
12. A. R. Zsigmond, T. Frentiu, M. Ponta, M. Frentiu, D. Petreus, *Food Chemistry*, **2013**, *141*, 3621.
13. T. Frentiu, E. Darvasi, S. Butaciu, M. Ponta, D. Petreus, R. Etz, M. Frentiu, *Microchem. J.* **2015**, *121*, 192.
14. T. Frentiu, E. Darvasi, S. Butaciu, M. Ponta, D. Petreus, A. I. Mihaltan, M. Frentiu, *Talanta*, **2014**, *129*, 72.
15. G. Tyler “AA or ICP – Which do you choose?” Varian Australia Pty. Ltd., ICP–3, Melbourne, Australia, **1991**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 321-332)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

EVALUATION OF THE ANALYTICAL CAPABILITY OF THERMAL DESORPTION ATOMIC ABSORPTION SPECTROMETRY METHOD USED FOR MERCURY DETERMINATION IN SEAFOOD

MARIN SENILA[a,*], OANA CADAR[a], ANDREJA DROLC[b], ALBIN PINTAR[b], LACRIMIOARA SENILA[a], TIBERIU FRENTIU[c]

ABSTRACT. Mercury is recognized as a highly toxic and widespread element in environment that can be transferred in the whole food chain. Thus, the content of mercury in foodstuff become of great interest. The aim of this paper is to assess the analytical capability and validation of the method for quantitative determination of total mercury (Hg) in seafood using thermal desorption atomic absorption spectrometry (TD-AAS). TD-AAS is a simple technique which does not require sample digestion prior to analysis. The main figures of merit such as selectivity, linearity, limit of detection (LoD), limit of quantification (LoQ), working range, accuracy and precision were studied and discussed in relation with the requirements in the Commission Decision 2002/657/EC and Commission Regulations 2011/836/EU and 2007/333/EC. Measurement uncertainty was estimated using top-down approach and was compared with the maximum uncertainty value calculated as specified in the Commission Decision 2002/657/EC. LoD estimated using 3s criterion was found to be 3.0 μg kg^{-1}, while LOQ 9.0 μg kg^{-1}. The recovery (%), estimated by using the certified reference material BCR-463 Tuna Fish, was 95 ± 5.0 %, whereas recovery (%) estimated using spiked samples was 92 ± 5.6 %. Standard deviation of repeatability (sr) was 5.6% (n=10 parallel samples), while standard deviation of within-laboratory reproducibility (sR) was 9.8 % (n=10 parallel samples), which correspond to HorRat's index for repeatability and reproducibility of 0.28 and 0.50, respectively. The estimated expanded relative uncertainty (k=2) was 15.6 %. The obtained figures of merit fulfil the requirements of the European legislation, and demonstrate

[a] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, 67 Donath, RO-400293, Cluj-Napoca, Romania*
[b] *National Institute of Chemistry, 19 Hajdrihova, SI-1000 Ljubljana, Slovenia*
[c] *Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos, RO-400028 Cluj-Napoca, Romania*
** Corresponding author: marin.senila@icia.ro*

that the laboratory can properly apply the method in order to achieve accurate results. The paper represents a model for the method validation in analytical laboratories in order to check the fit for purpose of analytical methods.

Keywords: *mercury, uncertainty estimation, validation, seafood, TD-AAS*

INTRODUCTION

Elemental mercury (Hg) and its organic and inorganic species are well-known as being highly toxic to the living organisms even in low concentrations, and have no known physiological function. In addition, Hg is recognised to have a high bioaccumulation factor [1-3]. Hg is released into environment through atmospheric paths, from both natural (volcanic emissions, oceans, vegetation, wetlands), and anthropogenic (mining, use of pesticides, burning of fossil fuels, chemical industry, etc.) sources [4-7]. Once released into environment, Hg persists for very long time, and circulates between atmosphere, water, sediments, soil and biota in different forms [8]. This behaviour has led to increased concentration of Hg in ocean water and ultimately, to its accumulation in seafood, which imply an increased interest for its determination at low concentrations.

Hg toxicity depends also on its chemical form. The principal chemical forms in the environment are: elemental Hg, divalent inorganic Hg, methyl Hg, and dimethyl Hg. Although both inorganic and organic species of Hg are toxic [9], the organic compounds were found to be the most toxic [1]. Studies in literature present Hg speciation in environmental and food sample both by chromatographic and non-chromatographic techniques [10-12]. However, Commission Regulation 1881/2006/EC [13] set the maximum level for total Hg in foodstuff, while Decisions 2007/333/EC and 2002/657/EC [14,15], impose strict requirements for the analytical performance and results interpretation for laboratories that analyse contaminants in foodstuff. Thus, the existence of highly precise and accurate analytical techniques with well-defined figures of merit for food analysis is a real need. The most commonly used analytical techniques for the determination of Hg in solid samples are based on wet digestion [16,17], but these techniques are time and reagents consuming. A good alternative to wet digestion is the use of reagent-free methods as they are based on thermal decomposition of solid samples or extraction and pre-concentration of liquid samples [5, 18-20]. Thermal desorption atomic absorption spectrometry (TD-AAS) is based on thermal decomposition of sample, mercury reduction using a catalyst, followed by Hg vapour trapping on a gold amalgamator. Subsequently, Hg is desorbed and

transported in a measuring cell where its concentration is measured by atomic absorption spectrometry [5]. Due to its advantages, this method has been employed for the determination of total Hg traces in food samples [21-25].

The aim of this study was to perform a detailed validation of total Hg determination in seafood by TD-AAS analysis applied according to EPA Method 7473 [26] in relation with the demands of the Decisions 2007/333/EC, 2011/836/EC and 2002/657/EC on the determination of toxic elements in food. When presenting the measurement results, it is necessary to evaluate their confidence intervals [27-29]. The estimation of measurement uncertainty was made, based on the evaluation of its uncertainty components, and their combining using the law of propagation of uncertainty. The paper is important for the routine analytical laboratories since it presents all the steps necessary to demonstrate the fit-for-purpose and to evaluate the measurement uncertainty for mercury determination in seafood.

RESULTS AND DISCUSSION

Method validation

The validation of the analytical procedure for Hg determination in seafood samples was performed by evaluating the main figures of merit: selectivity, linearity, limit of detection (LoD), limit of quantification (LoQ), working range, trueness/accuracy (including matrix effect), precision (repeatability) and measurement uncertainty.

Selectivity was verified by measuring blank samples (5 % HCl) in the absence of the analyte. Results revealed that there was no significant growth of absorbance signal at the wavelength of the Hg absorption (253.65 nm) when the blank samples were introduced into the instrument. The selectivity in this technique is assured both by the amalgamation step, which is a selective reaction for mercury, and by using of a characteristic wavelength for mercury [30]. The absorbance signal for released Hg in function of time, registered after amalgamator heating, is presented in Figure 1. The first peak corresponds to Hg measured in high sensitivity cell, while the second peak corresponds to low sensitivity cell (see Experimental section).

Linearity of the calibration curve constructed using 6 levels of concentration (0 - 0.050 μg Hg) was tested. The calibration curve was produced by injecting different weights of 100 μg L^{-1} or 1,000 μg L^{-1} Hg aqueous standards in 5 % HCl into the nickel-sampling boat. The calibration curve represents the absorbance in function of mass of injected Hg (ng). The determination coefficient, r^2 was 0.9991 and the residual error was smaller than 10%, indicating a good linearity of the method.

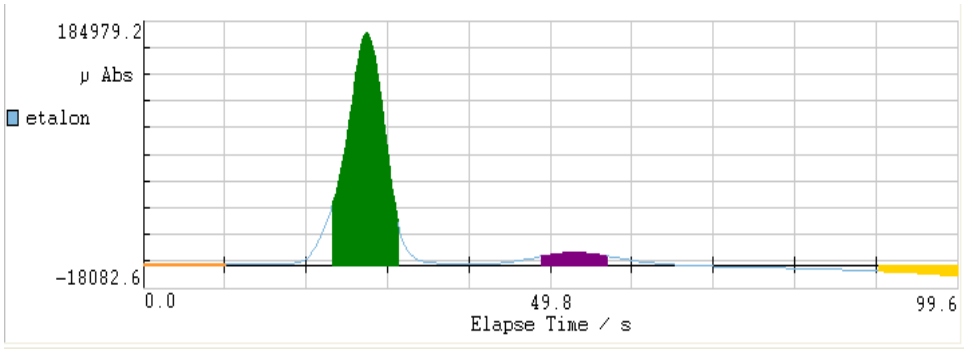


Figure 1. Typical absorbance signal for Hg determination

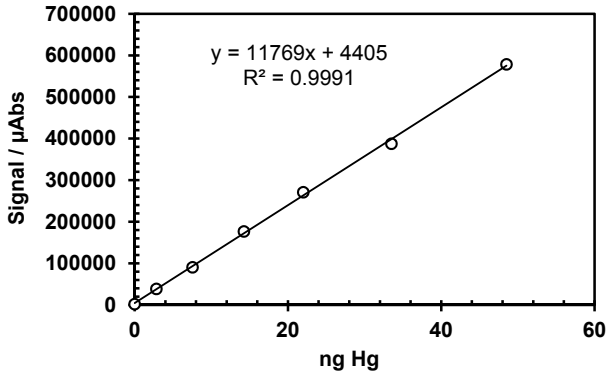


Figure 2. Calibration curve for Hg determination by TD-AAS

Limit of Detection (LoD) and Limit of Quantification (LoQ). LoD was calculated on the basis of 3 s criterion ($LoD=3s_B/m$), where m was the slope of calibration curve and s_B the standard deviation of 10 successive measurements of blank (5 % HCl). The LoQ was calculated as being $9s_B/m$. LoD was found to be 0.30 ng Hg, which means, for 100 mg sample a LoD of 3.0 μg kg^{-1}. LoQ was calculated to be 0.90 ng Hg (9.0 μg kg^{-1} if 100 mg of sample is analysed). This value was verified by analysing spiked solutions at the Hg content level equal to the evaluated LoQ. Relative standard deviation for ten replicates at this level of concentration was 17.5 % and recovery in confirmation of lower working range concentration was 90 %, what is satisfactory performance (targeted repeatability expressed as relative standard deviation (RSD) below 20 %, and recovery between 85-115 %). The method fulfils the requirements for Hg determination in foodstuffs (Decision 2007/333/EC): LoD and LoQ are less than one tenth and one fifth respectively, from the maximum level of 500 μg kg^{-1} Hg in fishery products (Decision 2006/1881/EC).

For the working range, at the lower end of the range, the restrictive factor is LoQ, while, at the upper end, limitations are imposed by various effects depending on the instrument response. For high sensitivity cell, the calibration curve is linear up to 50 ng Hg. If 100 mg sample is weighted and introduced in the system, the upper limit of working range is 5000 μg kg^{-1}, thus maximum level of 500 μg kg^{-1} Hg in fishery products and crustaceans (Decision 2006/1881/EC), can be easily measured by TD-AAS. Moreover, the upper limit of the working range can be extended by analysing less amount of sample or by using the low sensitivity cell of the instrument.

The *accuracy* of a method is acceptable if the mean analyte concentration measured in a CRM falls within ±10 % of the target value according to Commission Decision 2002/657/EC. Accuracy was studied by evaluating the recovery of a fish CRM (BCR-463 Tuna Fish). Thus, 5 parallel samples of CRM were analysed in order to determine the methods accuracy. Average recovery for fish CRM was 94 % with relative standard deviation of 5.0 % (n = 5 parallel samples). In addition, trueness was evaluated using the recovery for real fish samples spiked with known content of Hg. To each fish sample, amounts of 10 ng Hg were added. The recovery rate was calculated by taking into account the found concentrations in the enriched samples and the added concentration. The average recovery for spiked fish samples was 92% with a relative standard deviation of 5.6 % (n = 5 parallel samples). The results of Hg recovery for food CRM and spiked samples determined against aqueous curves confirmed that the method has no matrix effect.

The *precision* of Hg determination was verified in terms of compliance with the HorRat's index, calculated as the ratio of the relative standard deviation (RSD) found within the repeatability assay of test samples, and the predicted standard deviation (PRSD), calculated using the Horvitz's equation [31]:

$$PRSD = 2^{(1-0,5logC)} \quad (1)$$

where C is the half of the maximum mass fraction of Hg in fish tissue (2.5×10^{-7}) [9].

The repeatability of a method complies with requirements in Commission Decision (2007/333/EC) and Commission Regulation (2011/836/EU) if the HorRat index calculated as the RSD/PRSD ratio is less than 2 for Hg concentrations higher than 100 μg kg^{-1}. Precision was assessed both in terms of repeatability and reproducibility. For the repeatability study, the results were obtained by analysing 10 parallel samples by a single operator using the same equipment, while for the reproducibility study, a sample was measured in 10 different days by different operators using the same equipment. RSD for repeatability (RSD_r) was 5.6%, while RSD for reproducibility (RSD_R) was 9.8%

that correspond to $HorRat_r$ index of 0.28 and $HorRat_R$ index of 0.50, which denotes satisfactory performance. Summary of the results is presented in Table 1.

Table 1. Results of method validation for the measurement of Hg in seafood by TD-AAS method

Validation parameter	Results
Selectivity	No interfering signal
Linearity	$R^2 = 0.9991$
Limit of detection	3.0 μg kg^{-1}
Limit of quantification	9.0 μg kg^{-1}
Working range	9.0 – 5000 μg kg^{-1} (can be extended)
Trueness (recovery)	94% for CRM; 92% for spiked samples
RSD_r	5.6% (n=10 parallel samples)
RSD_R	9.3% (n=10 parallel samples)
$HorRat_r$ *index*	0.28
$HorRat_R$ *index*	0.50

Measurement uncertainty evaluation

In brief, the steps of the method are as shown in Figure 3.

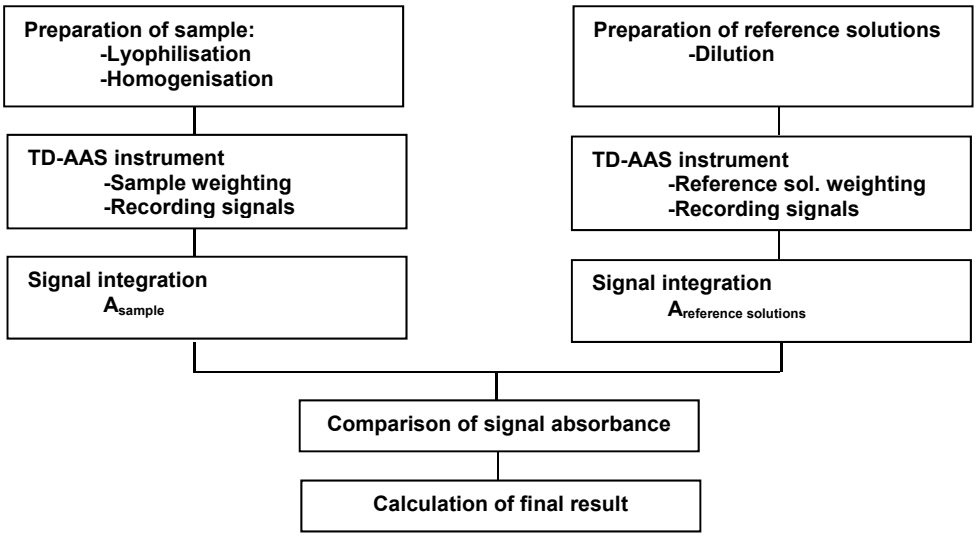


Figure 3. Experimental procedure for the measurement of Hg

Measurement uncertainty evaluation was based on method validation data (the "top down approach"), assuming that they comprise the total analytical procedure [26]. The identified main sources of measurement uncertainty were uncertainty of calibration reference materials (Ci), uncertainty of delivered volumes, uncertainty of weighted reference solutions and sample, uncertainty of the calibration curve, and accuracy and repeatability of the method, as presented in Figure 4 – cause and effects diagram (fishbone diagram).

Trueness of the method was determined by recovery study on CRM. The precision of the procedure represents a substantial source of measurement uncertainty and therefore requires detailed consideration in order to avoid over or underestimation of the combined uncertainty. Sources of uncertainty such as those arising from balances, volumetric measuring devices and influences of environmental conditions were covered by the within-laboratory repeatability. Following these assumptions, the total uncertainty of the method was composed of a contribution from the accuracy of the method (bias) and contribution from repeatability study in order to cover all the relevant uncertainty sources.

To estimate trueness of the method, recovery calculated from CRM analysis was used. Standard uncertainty associated to bias was calculated from Eq. 2:

$$u(B) = \sqrt{B^2 + u(C_R)} \tag{2}$$

where B is deviation from true value (140 μg kg^{-1}), u(C_R) is uncertainty of the certified reference material tested (80 μg kg^{-1}).

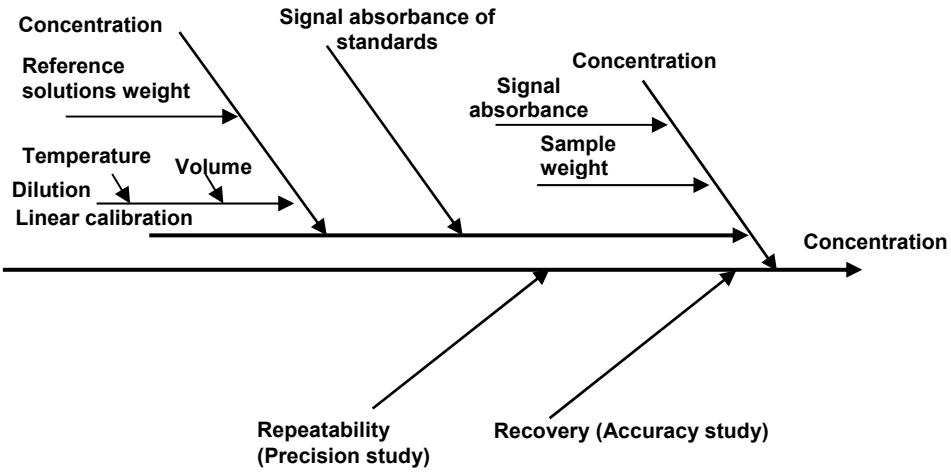


Figure 4. Cause and effects diagram (fishbone diagram) of uncertainties in measurement of Hg using TD-AAS

Uncertainty associated to method bias was calculated to be 160 μg kg^{-1} (5.6 %). Combined uncertainty was calculated following the Eq. 3:

$$u(Hg) = \sqrt{u(B)^2 + u(R_w)^2} \tag{3}$$

where $u(R_W)$ is the standard deviation resulted from within-laboratory repeatability study, for a real fish sample (average ± s = 329 ± 18 μg kg^{-1}). Before to be combined, the two components were transformed to relative standard uncertainties. Combined uncertainty u(Hg) was calculated to be 7.8%. The expanded uncertainty (U_E) resulted by multiplying u(Hg) by the coverage factor (k=2) which indicate the confidence interval expected to include 95% of results attributable to the measurand was 15.6%.

According to Commission Regulation (2011/836/ EU), the combined standard measurement uncertainty u(Hg) should be less than the maximum standard measurement uncertainty (Ut), calculated with the formula:

$$Ut = \sqrt{(\frac{LOD}{2})^2 + (\alpha\, c)^2} \tag{4}$$

where LoD is the limit of detection of the method (μg kg^{-1}); C is the concentration of interest (μg kg^{-1}); α is a numeric factor depending on the value of C (α = 0.18 for concentrations ranged between 51 - 500 μg kg^{-1} Hg). For the average concentration for the real sample of 329 μg kg^{-1} calculated Ut was 59 μg kg^{-1}, which represent 17.9%. Consequently, combined standard measurement uncertainty u(Hg) of 7.8% calculated for our method is well below Ut, which indicates satisfactory performance.

Real seafood samples analysis

Seafood samples were purchased from several supermarkets from Cluj-Napoca. In laboratory, the samples were lyophilised and analysed directly by TD-AAS. The average measured concentrations are presented in Table 2. The Hg concentrations ranged between 112 – 411 μg kg^{-1} wet weight (the higher Hg concentration was found in Hake), in the same order of magnitude with the results reported by Miclean et al. [32]. However, Hg concentrations in seafood samples were, in all cases, below the maximum level of 500 μg kg^{-1} wet weight set in Decision 2006/1881/EC, accordingly their consumption do not pose acute risks for consumers' health.

Table 2. Concentrations of total Hg measured in seafood samples (average ± U_E, k=2, n=5 parallel samples)

Sample type	**Hg** (μg kg^{-1} wet weight)
Hake *(Merluccius merlucius)*	411 ± 64
Shrimps (*Pandalus Borealis*)	310 ± 48
Pink shrimp *(Pandalus Borealis)*	112 ± 17
Squid *(calamarium)*	329 ± 51
Pangasius (*Pangasius buchanani*)	135 ± 21
Marbled rockcod (*Notothenia rossii*)	220 ± 34

CONCLUSIONS

The paper presents all the steps necessary to validate and to evaluate the measurement uncertainty for Hg determination in seafood using TD-AAS, a simple technique which require no sample digestion prior analysis. The studied figures of merit fulfil the requirements in terms of selectivity, linearity, LoD and LoQ, accuracy, and precision set out in the to the requirements in the Commission Decision 2002/657/EC and Commission Regulations 2011/836/EU and 2007/333/EC. The method was validated to be used for concentrations between 9.0 – 5000 μg kg^{-1}. TD-AAS techniques provide LoQ well below the maximum admitted concentration of Hg in seafood, which make it suitable to measure its concentrations at the imposed limits, and in addition for monitoring studies of Hg trace levels in seafood samples. Accuracy was studied by evaluating the recovery for a fish CRM and also by evaluating the recovery for spiked seafood samples. The recoveries for both CRM and spiked samples were in the target imposed by Commission Decision 2002/657/EC (90-110%). The precision of Hg determination was verified in terms of compliance with the HorRat's index calculated both for repeatability and reproducibility, and satisfactory results were obtained. Expanded uncertainty, estimated using top-down approach using the data from accuracy and precision studies, was 15.6% for a coverage factor k= 2. The combined standard uncertainty was less than the maximum standard measurement uncertainty calculated according to Commission Regulation (2011/836/ EU), indicating satisfactory performance of the method. It was demonstrated that the method can be applied in the laboratory for the designed purpose, determination of Hg in seafood by TD-AAS.

EXPERIMENTAL SECTION

Reagents, Standard Solutions and CRM

Stock standard solutions of mercury (1000 µg mL^{-1}) purchased from Merck (Darmstadt, Germany) was used for instruments calibration. Ultrapure water (18 MΩ cm^{-1}) obtained from a Millipore Direct Q3 (Millipore, France) and 30% (w/w) HCl ultrapur (Merck, Darmstadt, Germany) were used for all dilutions. A fish CRM BCR-463 (tuna fish) purchased from LGC Promochem (Wesel, Germany) was analysed to assess the accuracy of Hg determination. Oxygen (99.999%) for Hydra-C Analyzer supplied by Linde Gas SRL Cluj-Napoca, Romania was used.

Instrumentation and analytical method

The direct measurements of mercury from solid samples were carried out using an Automated Direct Hg Analyzer Hydra-C (Teledyne Instruments, Leeman Labs, USA). A block diagram of the instrument is presented in Figure 5 [33].

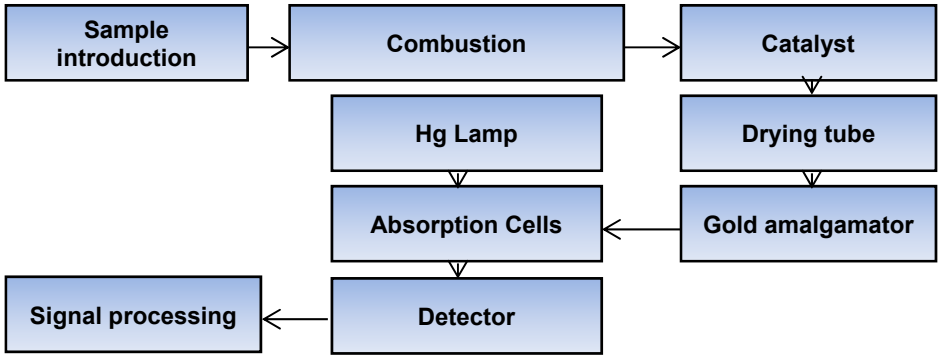


Figure 5. Block diagram of the TD-AAS instrument

The analyser includes a furnace module for the thermal decomposition of sample, an amalgamation trap, and a unit to measure the absorbance (AAS module). Determinations of Hg were performed using up to 100 mg dry sample weighted in nickel boats with a precision of ± 0.1 mg. The instrumental settings used for the Hg analyser for the all determinations are presented in Table 3.

Table 3. Instrumental setting for Hg determination in seafood using TD-AAS system

Parameter	Setting
Sample weight	100 ± 0.1 mg
Drying temperature/time	300°C / 45 sec.
Decomposition temperature/time	800°C / 150 sec.
Catalyst temperature	600°C
Catalyst Wait Period	60 sec.
Gold Trap temperature/time	700°C / 30 sec.
Measurement time	90 sec.
Oxygen Flow rate	300 min L^{-1}

Real seafood samples were purchased from supermarkets from Cluj-Napoca, Romania and were lyophilised prior to analysis using a FreeZone 2.5 Liter Benchtop Freeze Dry System (Labconco, USA).

ACKNOWLEDGMENTS. This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164 and Romanian Financing Authority CNCS – UEFISCDI, Partnership Program, VULMIN (No 52/2012).

REFERENCES

1. S. Ferreira, V. Lemos, L. Silva, A. Queiroz, A. Souza, E. da Silva, W. dos Santos, C. das Virgens, *Microchemical Journal*, **2015**, *121*, 227.
2. T. Frentiu, B.P. Pintican, S. Butaciu, A.I. Mihaltan, M. Ponta, M. Frentiu, *Chemistry Central Journal*, **2013**, *7*, 178.
3. M. Senila, E. Levei, L. Senila, G. Oprea, C. Roman, *Journal of Environmental Science and Health, Part A*, **2012**, *47*, 614.
4. E. Stanisz, J. Werner, H. Matusiewicz, *Microchemical Journal*, **2014**, *114*, 229.
5. M. Senila, E. Levei, L. Senila, O. Cadar, G. Oprea, C. Roman, *Studia UBB Chemia*, **2011**, *56*, 27.
6. T. Frentiu, S. Butaciu, E. Darvasi, M. Ponta, M. Senila, D. Petreus, M. Frentiu, *Analytical Methods*, **2015**, *7*, 747.
7. M. Hlodak, P. Matus, M. Urik, L. Korenkova, P. Mikusova, M. Senila, P. Divis, *Water, Air and Soil Pollution*, **2015**, *226*, 198.
8. United Nations Environment Programme (UNEP), Global Mercury Assessment, Geneva, Switzerland, **2002**, http://www.unep.org/gc/gc22/Document/UNEP-GC22-INF3.pdf, accessed on January 29, 2016.
9. T. Frentiu, S. Butaciu, M. Ponta, M. Senila, E. Darvasi, M. Frentiu, D. Petreus, *Food Analytical Methods*, **2015**, *8*, 643.
10. K. Leopold, M. Foulkes, P.J. Worsfold, *Analytica Chemica Acta*, **2010**, *663*, 127.

11. B.D. Barst, C.R. Hammerschmidt, M.M. Chumchal, D.C.G. Muir, J.D. Smith, A.P. Roberts, T.R. Rainwater, P.E. Drevnick, *Environmental Toxicology and Chemistry*, **2013**, *32*, 1237.
12. D.P.C. de Quadros, B. Campanella, M. Onor, E. Bramanti, D.L.G. Borges, A. D'Ulivo, *Spectrochimica Acta Part B*, **2014**, *101*, 312.
13. Decision 2006/1881/EC setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, L364, 5-24.
14. Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, L221, 8-36.
15. Decision 2007/333/EC laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Official Journal of the European Union*, L88, 29-39.
16. T. Frentiu, M. Ponta, C. Sarbu, *Chemosphere*, **2015**, *138*, 96.
17. F. Podolsky, V. Ettler, O. Sebek, J. Jezek, M. Mihaljevic, B. Kribek, O. Sracek, A. Vanek, V. Penizek, V. Majer, B. Mapani, F. Kamona, I. Nyambe, *Journal of Soil and Sediments*, **2014**, *15*, 648.
18. A. Zierhut, K. Leopold, L. Harwardt, M. Schuster, *Talanta*, **2010**, *81*, 1529.
19. G.M.A Leiva, S. Morales, R. Segura, *Water, Air and Soil Pollution*, **2013**, *224*, 1390.
20. M. Urik, M. Hlodak, P. Mikusova, P. Matus, *Water, Air and Soil Pollution*, **2014**, *225*, 2219.
21. S.L.C. Ferreira, V.A. Lemos, L.O.B. Silva, A.F.S. Queiroz, A.S. Souza, E.G.P. da Silva, W.N.L. dos Santos, C.F. das Virgens, *Microchemical Journal*, **2015**, *121*, 227.
22. C. Martins, E. Vasco, E. Paixao, P. Alvito, *Food Additives & Contaminants: Part B*, **2013**, *6*, 151.
23. D.P. Torres, M.B. Martins-Teixeira, *Food Additives & Contaminants: Part A*, **2012**, *29*, 625.
24. H.P. Vieira, C.C. Nascentes, C.C. Windmoller, *Journal of Food Composition and Analysis*, **2014**, *34*, 1.
25. R.F.L. Ribeiro, A. Germano, *Microchemical Journal*, **2015**, *121*, 237.
26. EPA Method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry.
27. L. Senila, A. Gog, M. Senila, C. Roman, L. Silaghi-Dumitrescu, *Revista de Chimie*, **2012**, *63*, 557.
28. M. Senila, E. Levei, L. Senila, *Chemistry Central Journal*, **2012**, *6*, 119.
29. A. Drolc, A. Pintar, *Accreditation and Quality Assurance*, **2012**, *17*, 323.
30. P. Konieczka, M. Misztal-Szkudlinska, J. Namiesnik, P. Szefer, *Polish Journal of Environmental Studies*, **2010**, *19*, 931.
31. W. Horwitz, L.R. Kamps, R.W. Boyer, *Journal of the Association of Official Analytical Chemists*, **1980**, 63, 1344.
32. M. Miclean, O. Cadar, C. Tanaselia, A. Gog, M. Senila, I.S. Groza, *Environmental Engineering and Management Journal*, **2012**, *11*, 133.
33. Teledyne Instruments Leeman Labs, “Hydra-C Automated Direct Hg Analyzer Operations Manual”.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 333-344)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

PRELIMINARY INVESTIGATION ON ARSENIC FRACTIONATION IN SOIL FROM OGOSTA RIVER FLOODPLAIN USING A SEVEN-STEP EXTRACTION PROCEDURE

MARIN SENILA[a,*], TSVETAN KOTSEV[b], ERIKA LEVEI[a], MARIUS ROMAN[a], VASSILKA MLADENOV[c], ZORNITSA CHOLAKOVA[c], LACRIMIOARA SENILA[a]

ABSTRACT. Arsenic (As) is a toxic element which can occur in increased concentrations mainly in areas affected by mining and ore processing activities. To assess the As fractionation in soils from the Ogosta River floodplain, a seven-step sequential extraction procedure (SEP) followed by As determination using ultrasonic nebulization inductively coupled plasma optical emission spectrometry (USN-ICP-OES) was applied. The SEP fractionate between the *(1)* ionically bound As; *(2)* strongly adsorbed As; *(3)* As co-precipitated with acid volatile sulphide, carbonates, Mn oxides, very amorphous Fe oxyhydroxides; *(4)* As co-precipitated with amorphous Fe oxyhydroxides; *(5)* extraction in 0,2M NH_4-oxalate buffer + ascorbic acid; *(6)* As associated with crystalline Fe oxides; *(7)* orpiment and remaining recalcitrant As minerals. No significant differences were found between the pooled amount of As concentrations in each extraction step and the total As concentration measured using a XRF spectrometer (recoveries rate of 90 –110%). Total As concentration in soils varied widely, in the range of 36 - 72300 mg kg^{-1}. The partitioning of As among the seven fractions in the six soil samples (%, medians and ranges) was: *(1)* 0.97 (0–4.8); *(2)* 12 (0–36); *(3)* 25 (12–44); *(4)* 8.7 (2.5–31); *(5)* 4.0 (0.2–25); *(6)* 34 (3.6–84); *(7)* 0.15 (0.02–1.1). Significant differences on As distribution in contaminated and uncontaminated soils were observed, the fractions of mobile species, were found to be predominant in highly contaminated soils in contrast to the low-As soils, where As contents were bound to the matrix.

Keywords: *arsenic fractionation, floodplain soil, contamination, ICP-OES, sequential extraction procedure, Ogosta River*

[a] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, 67 Donath, Cluj-Napoca, Romania*
[b] *National Institute of Geophysics, Geodesy and Geography, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str., bl. 3, 1113 Sofia, Bulgaria*
[c] *Sofia University "St. Kliment Ohridski", 15 Tsar Osvoboditel bul., 1000 Sofia, Bulgaria*
** Corresponding author: marin.senila@icia.ro*

INTRODUCTION

Environment contamination with toxic metals and metalloids may pose risks for the ecosystems and for the human health, therefore the assessment of theirs concentration and possible mobilization in soils is required [1-6]. Arsenic (As) is a metalloid well-known for its high toxicity, being categorized as a group I carcinogen in humans [7,8]. It enter into the environment is both from anthropogenic and natural sources. Anthropogenic sources of As are represented by mining and smelting of ferrous and base metal minerals, manufacturing of As-based chemicals, use of pesticides in agriculture, industrial wastes discharging, burning of fossil fuels, landfilling of industrial and municipal wastes [9]. All these sources have led to the increase of the As concentration in soils from affected areas [10].

Although the soil quality standards are typically based on total metal content, it is generally recognized that includes both bioavailable and non-bioavailable fractions [11]. Knowledge on metals distribution in different soil fraction is very important both for the assessment of risks for human health and for elaboration of remediation strategies of contaminated soil [12]. To obtain this, procedures for distinguishing among various binding forms of metals and metalloids in soils are necessary. The identification and quantification of metals/metalloids associated with predefined phases or soil was defined by IUPAC as “fractionation analysis” [13]. Different sequential extraction procedures (SEPs) were developed and applied in order to obtain the fractionation of elements (or species) and to estimate the quantities of elements that could be mobilized due to changes in chemical properties of the soils [14]. Selective chemical extractants, used in the SEPs, intend to replicate the conditions found in soils under different environmental scenarios in order to find the fraction of metals that can be released [15]. Many extraction procedures are based on sequential extraction scheme developed by Tessier et. al in 1979 [16]. Another selective extraction protocol widely used in the last years is the three-step BCR protocol [17], created to distinguish between exchangeable, reducible, oxidizable and residual fractions.

The majority of SEPs found in literature were developed for trace metals fractionations. The anionic nature of As species in soil led to a different behaviour of this element and thus the necessity of different SEPs. The information of SEPs used for As fractionation is still limited [7,14,18,19]. These SEPs vary in terms of types of extraction solutions and conditions of extractions, number of fractions and their sequence, but an accepted standardized SEP was yet not established. Thus, there is a need for developing and studying of analytical performances for SEPs special designed for As fractionation in soils in order to find the most appropriate procedure for this purpose.

As a result of historic mining activities and also due to the failure of a large tailing dam in 1964, the floodplain soil from Ogosta River valley, Bulgaria was highly polluted with As [20,21], thus an evaluation of its possible mobilization is of a high interest.

The aims of this study were: *(1)* to evaluate the main figures of merit of a seven-step SEP for As fractionation in soil; *(2)* to accomplish a preliminary assessment of the As fractionation in floodplain soil from Ogosta River valley, Bulgaria.

RESULTS AND DISCUSSION

Sequential extraction procedure (SEP) validation

The applied procedure was based on a modified SEP from Keon and co-workers [22] and included the steps presented in detail in the Table 2 (Experimental section).

The validation of the analytical procedure for SEP in soil was performed by evaluating the main figures of merit: limit of detection (LoD), precision in term of repeatability for the each step of extraction procedure and accuracy in terms of recovery from the soil CRM and by comparing the amounts of As extracted in each step with the total As concentration measured directly in soil samples using a XRF spectrometer. *LoD* was calculated on the basis of 3 s criterion ($LoD=3s_B/m$), where m was the slope of calibration curve and s_B the standard deviation of 10 successive measurements of blank [23]. LoD was 0.005 mg L^{-1} in extraction solutions, which means, for an extraction ratio of 1:100, a method LoD of 0.5 mg kg^{-1}. *Accuracy* was studied by evaluating the recovery of the soil CRM SRM 2709 San Joaquin Soil, New York, USA. Also, the pooled amount of As recovered from all the fractions using the SEP was similar (90–110%) to the total As concentration measured directly in soil samples using a XRF spectrometer. The recovery for CRM was 96 ± 5 %. *Precision* was studied in term of repeatability by analysing 3 parallel samples by a single operator using the same equipment. The standard deviations of repeatability, in all the steps of the procedure, did not exceed 5% of the respective means.

Concentrations of total As and metals in soil samples

In the Table 1 are presented the total As and metal concentrations measured in soil samples by XRF spectrometry. For comparison, the typical ranges and common values present in unpolluted soils and the average abundance in the earth's crust [24] are also showed. With the exception of samples S3 and S6, the concentrations of total As were much higher than the maximum concentration in Earth's crust in unpolluted soil and also much

higher than the typical range reported in literature [25]. In a similar study [26] carried out on soils from Tamar, England, it was reported total As concentrations in the range of 3.8 – 848 mg kg^{-1}. In other study on soils from China [27], were reported total As concentration in the range of 36.0 – 4172 mg kg^{-1}. Increased As concentrations in sediments and mine waste samples in mining areas from NW Spain ranged between 310 – 67000 mg kg^{-1} were also reported [28].

Table 1. Total metal and As concentrations in soil samples, typical range and the normal values in the unpolluted earth's crust (mg kg^{-1}, mean ± s, n=3 parallel measurements)

Sample	As	Fe	Mn	Mg	Al	Ca
S1	2460 ± 120	103500 ± 3100	14000 ± 440	10800 ± 240	23300 ± 360	72500 ± 2400
S2	1350 ± 67	135500 ± 4300	32000 ± 1000	7300 ± 130	13900 ± 220	70900 ± 2520
S3	33 ± 2.0	42400 ± 1000	1250 ± 50	7200 ± 140	21400 ± 310	10000 ± 430
S4	72300 ± 2900	296500 ± 14700	41200 ± 2100	17400 ± 1300	6400 ± 700	102500 ± 5500
S5	12700 ± 640	144800 ± 4900	24300 ± 800	21500 ± 1200	12300 ± 800	209800 ± 7300
S6	41 ± 3.0	43100 ± 1100	1300 ± 50	6100 ± 110	26900 ± 280	13800 ± 540
Range	33 - 72300	42400 - 296500	1250 - 41200	6100 - 21500	6400 - 26900	13800 - 209800
Typical range	0.1 - 50	7000 - 42000	20 - 10000	-	-	-
Earth's crust	40	50000	1000	-	81300	-

The concentrations of Mn and Fe (with the exception of samples S3 and S6) were higher than the Earth's crust maximum concentration and generally above the typical ranges. The high concentrations of total metals are an indicator for a contamination of the floodplain soils in the investigated area with metals coming from mining activities.

Arsenic fractionation in soil

The percentages of metals in each fraction are presented in Figure 1. It must be accepted that these results are operationally defined and redistribution and adsorption of As can occur during extraction steps; however the partitioning of the As into the different fractions provides an indication of their mobility and availability to the environment and to the living organisms [29].

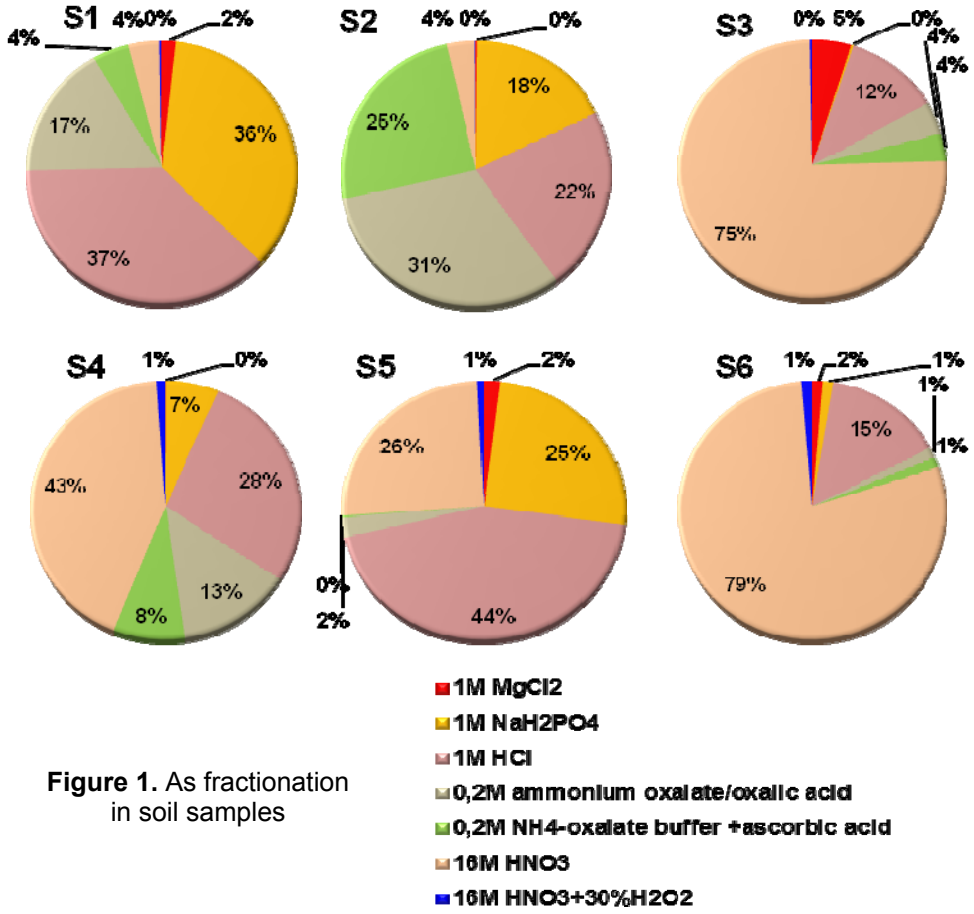


Figure 1. As fractionation in soil samples

The first extractant used in SEP (1 M $MgCl_2$) is a very weak extractant and only dissolve the As weakly (ionically) bound to the matrix. The As concentration extracted in this step varied from below detection limit (*bdl*) in sample S6 and 251 mg kg^{-1} in sample S5, and represented less than 5 % from the total As concentration in all analysed samples. The amounts of As extracted in the second fraction (1 M NaH_2PO_4) estimates the strongly adsorbed As and was found to be generally high, in particular for samples containing high amount of total As (S1, S2, S4, S5). Percent of As extracted in this fraction ranged between 0 - 36 % (median 12%). Our results are in agreement with those reported by Javed who found high percentage of strongly adsorbed As (16-29%) in sediment samples [30]. In the third fraction, extracted using 1 M HCl, As was found in amounts ranging between 5.8 –

19700 mg kg^{-1} (12 – 44%, median 25%). This fraction was the most dominant one in all soils contaminated with As (S1, S2, S4, S5). The 1 M HCl extracts As co-precipitated with acid volatile sulphide, carbonates, Mn oxides and very amorphous Fe oxyhydroxides. The results of fraction F3 showed that high percentages of As associated to this extraction step were observed mainly in samples rich in Mn (S1, S2, S4, S5). The 0.2 M ammonium oxalate + oxalic acid (F4), which solubilize the As co-precipitated with amorphous Fe oxyhydroxides. The percent of As extracted in this fraction was 2.5 – 31% (median 8.7%). Good correlations were observed between the As extracted in F4 with the amounts of Fe measured in the respective samples (higher extraction rate being observed in the samples S1, S2, S4). The extractant used in step 5 (0.2 M NH_4-oxalate buffer + ascorbic acid) dissolves As associated with crystalline Fe oxides. The As concentrations found in F5 were in the range of *bdl* – 5800 mg kg^{-1}. Also in this case percentages of As were well related to the amounts of Fe measured in the respective samples (higher extraction rate in the samples S1, S2, S4). The solution used in step 6 (16 M HNO_3) extracts the As co-precipitated with pyrite and As strongly bound to the matrix. This fraction was the most dominant As pool in the soils with low As contents (75% and 79%, of the total As for the samples S3 and S6, respectively). Surprisingly, also in the sample with the highest amount of total As (S4) an important percent of As was found to be soluble in this step, probably due to the contamination of floodplain soil in this sampling point with minerals containing arsenopyrite. In the last step of the SEP were measured orpiment and remaining recalcitrant As minerals using a hot mixture of 16 M HNO_3 + 30% H_2O_2. Amounts of As extracted in this step were between *bdl* – 760 mg kg^{-1}, representing only 0 – 1.1 % of the total concentration of As found in the sample. Our results highlight the importance of testing soils with different sources and contamination degrees, confirming thus previous studies found in literature [4, 7, 20, 21, 27, 28, 31].

Multivariate statistics

The varimax rotated factor loadings of principal components (PCs) for the total As and metal concentrations and As fractions are presented in the Table 2. The loadings in bold face correspond to variables with dominantly influence the selected latent factor. Two PC's with eigenvalues higher than 1 explains about 95% of the total variance of the system. The first component (PC1) exhibits 74% of the total variance with positive loadings on total As, Fe and Mn and As fractions F2, F3, F4, F5, F6 and F7 and negative loading on Al. This behaviour is explained by the influence of total As concentration

on the level of As in different fractions and also by the influence of Fe and Mn on As retention mechanisms in soil. The second component (PC2) explains about 21% of the variability and contains the Ca and Mg concentrations correlated with the most mobile fraction of As (F1) and partially correlated with fraction F2. This can be explained by the influence of Ca and Mg on the exchange capacity of the soil and thus on As mobilization.

Table 2. Factor loadings after Varimax rotation

	PC1	PC2
Total Fe	**0.827**	0.334
Total Mn	**0.657**	0.406
Total Mg	0.381	**0.807**
Total Al	**-0.666**	-0.493
Total Ca	0.140	**0.898**
Total As	**0.892**	0.157
As F1	-0.128	**0.886**
As F2	**0.707**	***0.555***
As F3	**0.879**	0.211
As F4	**0.910**	0.027
As F5	**0.912**	0.001
As F6	**0.900**	0.098
As F7	**0.899**	0.107

The dendrogram resulting from Agglomerative Cluster Analysis (AHC), created by using Ward's method and Euclidian distance for dissimilarity, is presented in Figure 2. The studied parameters were grouped in 3 clusters: cluster 1 contains total Al and indicate the lack of influence of this parameter on As, cluster 2 groups Ca, Mg and mobile fractions of As, F1 and F2, respectively, while cluster 3 contains total As, Mn, Fe associated to the As fractions F3, F3, F5, F6, F7 and confirm the results obtained by the PCA.

The obtained results suggests that in anthropogenic contaminated soils the As is found predominantly in fractions that can be relatively easily mobilised, whereas in soils with low As content high amounts of As were found to be retained mainly as insoluble forms under natural environmental conditions. Our results are in agreement with other previous results on As fractionation that revealed that As natural occurred in soils is immobilised in soil [29, 32]. Other authors [33] showed that aging favor the immobilization of As in soils, while in case of recent anthropogenic As inputs in soils a high percentage of As in more mobile fractions. In a previous study using a similar SEP (6 steps) to our study for As fractionations in sediments and mine waste

samples [28] it was shown that important amounts of As is incorporated into amorphous Fe oxyhydroxides. As a conclusion of that study, it was shown that arsenic fractionation is very much influenced by the extent of mining and ore processing.

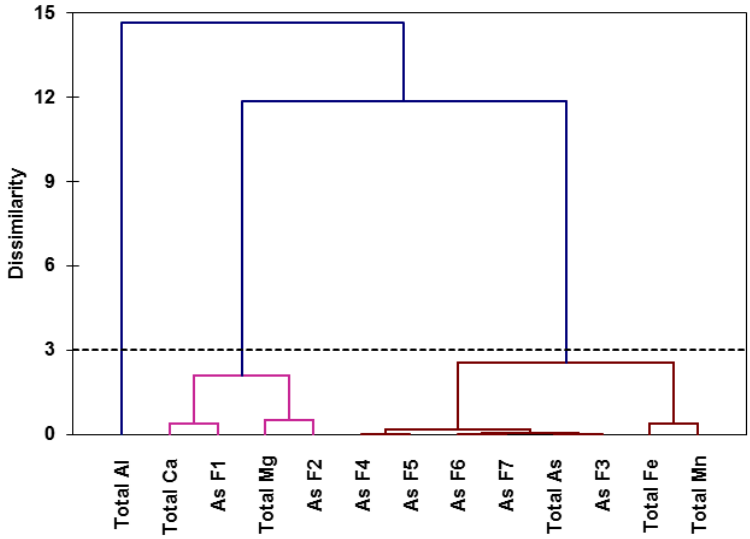


Figure 2. Dendrogram showing the clustering of total As, total metals concentration and As extracted in different fractions

CONCLUSIONS

A modified seven-step SEP was evaluated and applied for As fractionation in soil samples with total As concentrations varying between 36 - 72300 mg kg^{-1} collected in the Ogosta River floodplain. The results revealed that As distribution in the contaminated soils differs significantly from that of uncontaminated or soils with low As content. Thus, the fractions of mobile species, which are the most dangerous for the environment and biota, were found to be predominant in As contaminated soils in contrast to the percentages present in low-As soils, where As was to a high extent, strongly bound to the matrix. The most abundant fractions in contaminated soils were usually the F2 and F3 which represent the strongly adsorbed As and As co-precipitated with acid volatile sulphide, carbonates, Mn oxides and very amorphous Fe oxyhydroxides, respectively. However, As distribution showed significant differences between samples. Multivariate statistics (PCA and AHC) were used to find the correlations between As fractionation and the content of total As and other metals content in soil. Significant correlations

were found between total As concentration, Fe and Mn and the relatively immobile As fractions, while the most mobile As fractions (F1 and F2) were related to the content of Ca and Mg. The As fractionation in soils should be long-term monitored in order to assess if the aging and changes in soil properties will affect the As availability and its dynamics in natural ecosystem.

EXPERIMENTAL SECTION

Site description and sampling

The soil samples were collected from three sites in the Ogosta River basin upstream the "Ogosta" reservoir in 2009 (Figure 3). Site #1 N43°23.692', E23°06.338' and site #2 N43°24.557', E23°02.483' are located in highly contaminated sections of the Ogosta River floodplain close to the river banks. Samples S1, S2 and S3 are taken from prepared soil pit at site #1 from depths of 0-28 cm, 28-42 cm and 57-100 cm, respectively. The soil profile is well oxidized with no signs of Fe and Mn reduction. Samples S4 and S5 are from site #2 located at the river bank several kilometres upstream of the previous sampling area. The two samples were taken from the most contaminated layers of the floodplain sediments at depths 29-50 cm and 50-77 cm respectively. The upper part of the soil profile is well oxidized, but some ochre and grey-bluish spots in the lower part bellow 50 cm indicates frequently lowering of soil

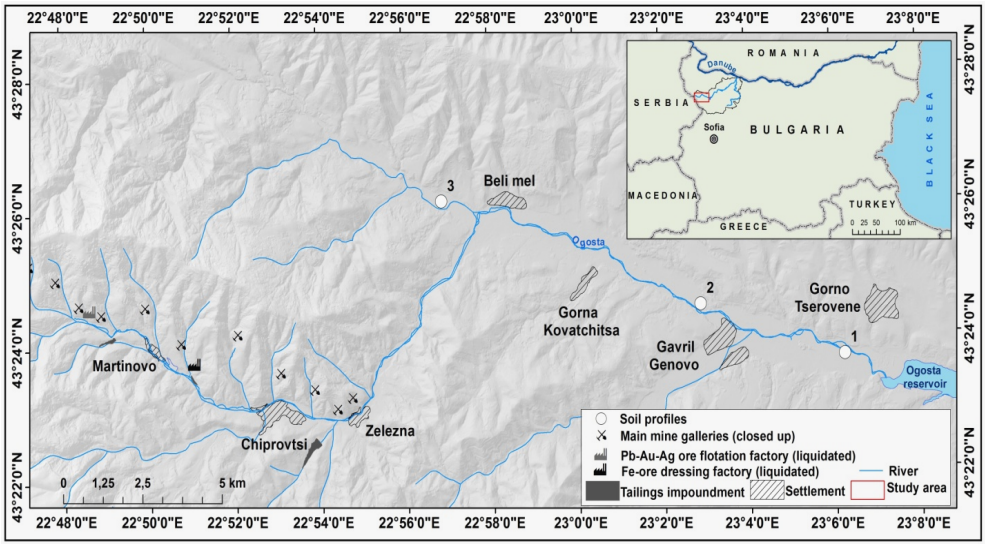


Figure 3. Study area and designation of soil sampling sites

redox potential. Sample S6 (well oxidized topsoil, 0-18 cm) is from the site #3 N43°26.028', E22°57.002' located in the floodplain of the Prevalska River. It is a tributary of the Ogosta River, which was not affected by the mining activities and where As levels and fractionation patterns in soil can be considered natural and serve as background.

For soil analyses, a representative part of each sample was air dried and then ground using a porcelain pestle and mortar. Fine fraction < 0.063 mm from each sample was obtained for chemical analyses.

Reagents and instrumentation

The solutions were prepared using reagents p.a. quality (Merck, Darmstadt, Germany) and ultrapure Milli-Q water provided by a Direct-Q purification system (resistivity > 18 MΩ cm^{-1}, Millipore, France). Stock multi-element standard solution containing Fe, Mn, Mg, Al, and Ca of 1000 μg mL^{-1} (Merck, Germany) and stock standard solution containing As 1000 μg mL^{-1} (Merck, Germany) were used to calibrate the ICP-OES for metals determination. All of the glassware was carefully cleaned, soaked in 10% (v:v) HNO_3 for 24 h and rinsed with ultrapure water prior to use. The reagents and operational conditions used in the SEP are summarized in Table 2. Three parallel sequential extractions were carried out using 1 g soil for each sample in order to separate the seven fractions. Soil was placed in 50 ml centrifugation tubes and 25 ml of the extraction reagents were added sequentially. After each extraction step, the slurry was centrifuged for 15 min at 4500 rpm using EBA 200 centrifuge (Hettich, Germany). Solution trapped in the remaining soil was collected in subsequent wash steps and combined with the corresponding extract.

Table 2 Reagents and operating conditions used in SEP (according to ref. [22]).

Step	Nominal target phase	Reagents	Shaking time and temperature
F1	*Ionically bound As*	Sample + 1M $MgCl_2$ pH=8	shaken 8h at 25 °C
		Residue + 1M $MgCl_2$ pH=8	shaken 8h at 25 °C
		Residue + Milli Q water	shaken 0.5 h
F2	*Strongly adsorbed As*	Residue +1M NaH_2PO_4 pH=5	shaken 16h at 25 °C
		Residue +1M NaH_2PO_4 pH=5	shaken 24h at 25 °C
		Residue + Milli Q water	shaken 0.5 h
F3	*As coprecipitated with acid volatile sulphide, carbonates, Mn oxides, very amorphous Fe oxyhydroxides*	Residue+1N HCl	shaken 1h at 25 °C
		Residue + Milli Q water	shaken 0.5 h

Step	Nominal target phase	Reagents	Shaking time and temperature
F4	*As coprecipitated with amorphous Fe oxyhydroxides*	Residue +0.2 M ammonium oxalate/oxalic acid, pH=3 Residue + Milli Q water	shaken 2h in dark (aluminium foil) at 25 ^{o}C shaken 0.5 h
F5	*As associated with crystalline Fe oxides*	Residue + NH_4-oxalate buffer (0.2M) + ascorbic acid (0.1 M) pH=3.25 Residue + Milli Q water	shaken for 0.5h at 96±3 ^{o}C at light shaken 0.5 h
F6	*As coprecipitated with pyrite and amorphous*	Residue +16 N HNO_3, Residue +16 N HNO_3, Residue + Milli Q water	shaken 2h at 25 ^{o}C shaken 2h at 25 ^{o}C shaken 0.5 h
F7	*Orpiment and remaining recalcitrant As minerals*	Residue +16 N HNO_3+30% H_2O_2	Boiling at least 2 h

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SWIS-CSNR 49164 and Romanian Financing Authority CNCS –UEFISCDI, Partnership Program, BIORESOL (No 91/2014). The survey in Bulgaria benefitted from the grant ВУ-Н3-04/05, 2005-2009, Programme "STIMULATION OF SCIENTIFICAL RESEARCHES IN HIGH SCHOOLS".

REFERENCES

1. A.G.F. Boim, L.C.A. Melo, F.N. Moreno, L.R.F. Alleoni, *Journal of Environmental Management*, **2016**, *170*, 21.
2. C. Gagiu, E.M. Pica, X. Querol, C.S Botezan, *Environmental Science and Pollution Research*, **2015**, *22*, 18885.
3. M.H. Kovacs, D. Ristoiu, *Studia UBB Chemia*, **2009**, *LVII*, 142.
4. M. Miclean, E. Levei, M. Senila, C. Roman, E. Cordos, *Revista de Chimie*, **2009**, *60*, 1.
5. M. Senila, E. Levei, L. Senila, G. Oprea, C. Roman, *Journal of Environmental Science and Health, Part A*, **2012**, *47*, 614.
6. M. Bizo, E.A. Levei, E. Kothe, M. Senila, C. Modoi, A. Ozunu, *Carpathian Journal of Earth and Environmental Sciences*, **2015**, 10, 195.
7. E.A. Cordos, T. Frentiu, M. Ponta, I. Marginean, B. Abraham, C. Roman, *Chemical Speciation & Bioavailability*, **2006**, *18*, 11.

8. M. Senila, C. Tanaselia, E. Rimba, *Carpathian Journal of Earth and Environmental Sciences*, **2013**, *8*, 145.
9. K.H. Goh, T.T. Lim, *Applied Geochemistry*, **2005**, *20*, 229.
10. T. Frentiu, S. Butaciu, M. Ponta, E. Darvasi, M. Senila, D. Petreus, M. Frentiu, *Journal of Analytical Atomic Spectrometry*, **2014**, *29*, 1880.
11. M. Senila, E.A. Levei, L.R. Senila, *Chemistry Central Journal*, **2012**, *6*, 119.
12. Z. Mester, C. Cremisini, E. Ghiara, R. Morabito, *Analytica Chimica Acta*, **1998**, *359*, 133.
13. D. M. Templeton, F. Ariese, R. Cornelis, L.G. Danielson, H. Munau, H. P. van Leeuwen, R. Lobinski, *Pure and Applied Chemistry*, **2000**, *72*, 1453.
14. K. Muller, B. Daus, P. Morgenstern, R. Wennrich, *Water, Air and Soil Pollution*, **2007**, *183*, 427.
15. M. Senila, *Journal of Environmental Health Science & Engineering*, **2014**, *12*, 108.
16. A. Tessier, P.G.C. Campbell, M. Bisson, *Analytical Chemistry*, **1979**, *51*, 844.
17. A.M. Ure, Ph. Quevaullier, H. Muntau, B. Griepink, *International Journal of Environmental Analytical Chemistry*, **1993**, *51*, 135.
18. W.W. Wenzel, N. Kirchbaumer, T. Prohaska, G. Stingeder, E. Lombi, D.C. Adriano, *Analytica Chimica Acta*, **2001**, *436*, 309.
19. J.M. Rosas-Castor, L. Portugal, L. Ferrer, J.L. Guzman-Mar, A. Hernandez-Ramirez, V. Cerda, L. Hinojosa-Reyes, *Analytica Chimica Acta*, **2015**, *874*, 1.
20. P. Mandaliev, C. Mikutta, K. Barmettler, T. Kotsev, R. Kretzschmar, *Environmental Science and Technology*, **2014**, *48*, 208.
21. C. Mikutta, P. Mandaliev, N. Mahler, T. Kotsev, R. Kretzschmar, *Environmental Science and Technology*, **2014**, *48*, 13468.
22. N.E. Keon, C.H. Swartz, D.J. Brabander, C. Harvey, H.F., Hemond, *Environment Science and Technology*, **2001**, *35*, 3396.
23. M. Senila, E. Levei, L. Senila, O. Cadar, G. Oprea, C. Roman, *Studia UBB Chemia*, **2011**, *56*, 27.
24. A. Giacomino, M. Malandrino, O. Abollino, M. Velayutham, T. Chinnathangavel, E. Mentasti, *Environmental Pollution*, **2010**, *158*, 416.
25. O. Abollino, A. Giacomino, M. Malandrino, E. Mentasti, *Water, Air & Soil Pollution*, **2007**, *181*, 149.
26. B. Palumbo-Roe, J. Wragg, M. Cave, *Environmental Pollution*, **2015**, *207*, 256.
27. S.W. Li, J. Li, H.B. Li, R. Naidu, L.Q. Ma, *Journal of Hazardous Materials*, **2015**, *295*, 145.
28. R. Larios, R. Fernandez-Martinez, R. Alvarez, I. Rucandio, *Science of the Total Environment*, **2012**, *431*, 426.
29. M.A. Yukseen, O. Gokyay, *Environmental Engineering Science*, **2006**, *23*, 125.
30. M.B. Javed, G. Kachanoski, T. Siddique, *Analytica Chimica Acta*, **2013**, *787*, 102.
31. M. Senila, E. Levei, M. Miclean, L. Senila, L. Stefanescu, S. Marginean, A. Ozunu, C. Roman, *Environmental Engineering and Management Journal*, **2011**, *10*, 59.
32. J.C. Novoa-Munoz, J.M.G. Queijeiro, D. Blanco-Ward, C. Alvarez-Olleros, E. Garcia-Rodeja, A. Martinez-Cortizas, *Science of the Total Environment*, **2007**, *378*, 18.
33. S. Liang, D.X. Guan, J.H. Ren, M. Zhang, J. Luo, L.Q. Ma, *Journal of Hazardous Materials*, **2014**, *273*, 272.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 345-353)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

VALIDATION OF A METHOD FOR DETERMINATION OF FREE GLYCEROL IN BIODIESEL

LACRIMIOARA SENILA[a*], MIRELA MICLEAN[a], OANA CADAR[a], MARIN SENILA[a], MELINDA KOVACS[a], MARIA-ALEXANDRA HOAGHIA[a, b]

ABSTRACT. In this study, validation of a method for determination of free glycerol from biodiesel samples by using gas chromatography coupled with flame ionization detector (GC-FID) and the measurement uncertainty estimation was described. The derivatization reaction with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was used to volatilize the glycerol prior to GC analysis. The 1,2,3-butanetriol was used as internal standard. Linearity, limit of detection, limit of quantification, precision and accuracy of the method were determined for the validation of the method. The limit of detection, estimated from chromatograms was 0.0006 % (w/w), while limit of quantification was 0.002 % (w/w). The recovery of free glycerol was determined by using certified reference material (CRMs) and was 102.4 ± 13.0 %. Also, the measurement uncertainty was estimated based on the bottom-up approach. The expanded uncertainty of the determination of free glycerol from biodiesel by GC-FID method was 16%.

Keywords*: free glycerol, validation, measurement uncertainty, GC-FID*

INTRODUCTION

Biodiesel is the most important biofuel in the world and a promising alternative to conventional diesel [1]. The properties of biodiesel are very similar to those of diesel; have high flash point and cetane number comparative to diesel and does not contain sulphur or aromatics [2, 3].

[a] *INCDO-INOE 2000 Research Institute for Analytical Instrumentation - ICIA, 67 Donath St. 400293 Cluj-Napoca, Romania*
[b] *Babes-Bolyai University, Faculty of Environmental Science and Engineering, 30 Fantanele, RO-400294 Cluj-Napoca, Romania*
** Corresponding author: lacri.senila@icia.ro*

Biodiesel (also known as fatty acid methyl esters) is obtained through transesterification of vegetable oil or animal fats with methanol using a catalyst. The most used vegetable oils for biodiesel production are: rapeseed, sunflower and soy oil [4, 5]. Currently, microalgae and used cooking oil are tested for biodiesel production [6, 7]. The transesterification reaction produces glycerol as the main by-product and unreacted triacylglycerides (TAGs), monoacylglycerides (MAGs) and diacylglycerides (DAGs) [8, 9]. Production of biodiesel is expected to increase in the next few years [10].

There are several analytical methods that ca be used for glycerol determination in fuels sample, based mainly on chromatographic techniques [11-14]. The European Standard EN 14105 describe the standardized procedure for the analysis of free glycerol, TAGs, MAGs, DAGs and total glycerol from fat and oil derivatives [15].

The method for determination of free glycerol and derivatives is based on transformation of these compounds in more volatile compounds by derivatization with MSTFA in presence of pyridine, followed by gas chromatography coupled with flame ionization detector (GC-FID) analysis on a non-polar column. The silylation reaction with MSTFA implies the replacing of acidic hydrogen with the more volatile trimethylsilyle derivatizing group.

Validation and uncertainty estimation of free glycerol determination in biodiesel is necessary to produce reliable analytic data related to compliance of biodiesel quality with the European standards [16]. Based on information obtained in the validation process (limit of detection and limit of quantification, linear range, accuracy and precision) the uncertainty of the method can be estimated. Therefore, identification and quantification of all uncertainty sources that occur in the method is obligatory [17,18].

The rules for estimation of uncertainty are established in the International Organization for Standardization (ISO) guide [19]. In EURACHEM document is defined how the ISO guide are applied in chemical measurements [20]. For the measurement uncertainty estimation there are two approaches: top-down or bottom-up. In bottom-up approaches all the uncertainty sources are estimated and included in the uncertainty, while in top-down approach only the major uncertainty sources are take into account [17].

The aim of this study was to validate and to estimate the uncertainty for the determination of free glycerol in biodiesel samples using the standardized method EN 14105 [15]. The measurements uncertainty was evaluated based on bottom-up approach.

RESULTS AND DISCUSSIONS

Validation of free glycerol determination method

The validation of the determination of free glycerol method was performed by evaluation of the main figures of merit: limit of detection (LOD),

limit of quantification (LOQ), working and linear range, accuracy and precision (both repeatability and reproducibility) according to the EURACHEM guide requirements [20].

The European standard EN 14214 requires the maximum limit of free glycerol of 0.02 % (w/w) in biodiesel in Europe [16]. The limit of quantification was targeted to be ten times smaller than the maximum amount of free glycerol (0.002 % (w/w)).

The LOD and LOQ were calculated using the 3s criteria by measuring the glycerol peak area in ten parallel samples with very low glycerol content. The LOQ for free glycerol was estimated to be nine times of standard deviation. LOD for free glycerol was established to be 0.0006% (w/w) and LOQ is 0.002 % (w/w). In Figure 1 is presented the chromatogram for a standard solution.

For evaluation of precision and accuracy of LOQ, six solutions of glycerol at a concentration of 0.002 % (w/w) were prepared and measured. The relative standard deviation (RSD) was 6.3 % and the recovery was 105%, and complies with the imposed target (RSD < 20% and recovery between 80 and 120%).

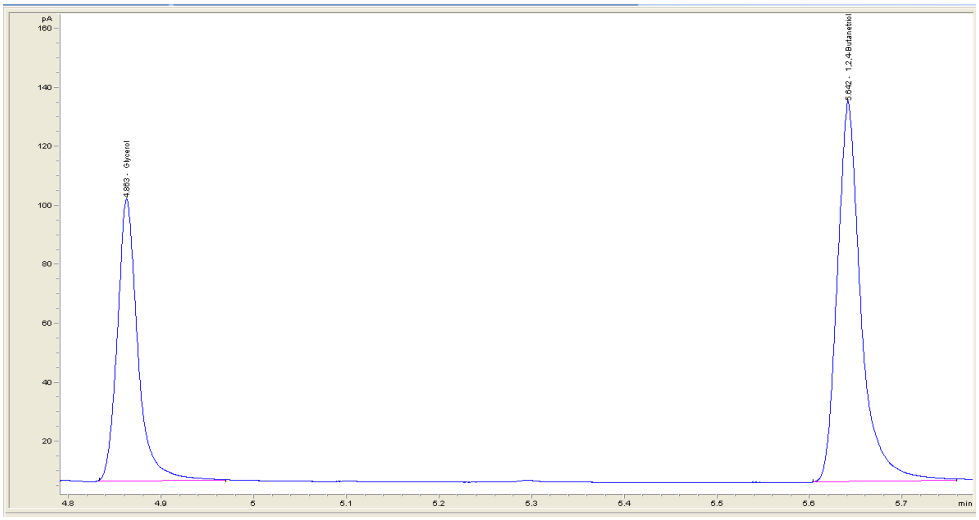


Figure 1. The chromatogram of the TMS derivatives of the standard solution

Working and linear range

Free glycerol was identified by comparison of the obtained retention time with the ones observed for standard solutions analyzed. Four calibration solutions (0.005 -0.05 mg of free glycerol) were prepared by diluting the stock solution of glycerol (500 µg.mL^{-1}). The derivatization procedure was applied for

each standard solution. For the lowest and the highest concentrations ten measurements were made for evaluation of homogeneity of variance. The standard deviation was used to calculate PG ratio (s_1/s_4) which was compared with the Fischer value F(9;9;0.99) = 5.35. The determination coefficient of r^2 >0.9999 proved the good linearity of the calibration curve. The statistical parameters of calibration curve for free glycerol were presented in Table 1.

Table 1. Statistical parameters of calibration curve for free glycerol

Parameter	a	b	S_y	S_{x0}	V_{x0}	r^2	PG
Free glycerol	-0.0027	1.22	0.013	0.0112	3.25	0.9999	4.3

a- intercept, b-slope, S_y – residual standard deviation, S_{x0} –standard deviation of the method (S_y/b), V_{x0} –coefficient of variation ($S_{xo}*100/X_{average}$), r^2- determination coefficient, PG- test value factor

Accuracy and precision

B100 Biodiesel (Soy-based) was used as certified reference material for evaluation of accuracy. For analysis of CRMs, the difference between certified value and measured value (Δm) must be lower than the expanded uncertainty obtained by combining the certified uncertainty (u_{crm}) with the uncertainty of the repeated glycerol measurements (u_m). The results obtained for analysis of CRM are presented in Table 2.

Table 2. Certified and measured values of free glyceron in B100 Biodiesel (Soy-based) CRM (mean ± expanded uncertainty, n = 6 parallel samples)

	Measured	Certified
Free glycerol	168 ± 28.1 mg kg^{-1}	164 ± 16.0 mg kg^{-1}

The methods recovery estimated by determination of free glycerol in CRM was 102.4 ± 13.0 %.

For estimation of the methods precision, the repeatability and reproducibility were determined. For the estimation of repeatability, six samples were analyzed. The limit of repeatability (r) represent the difference between two individual results, obtained by the same method and the same operator and must be below r = 0.1615*x + 0.0003, where x is the average of two results). The limit of repeatability (r) was 0.0012 % (w/w). The repeatability standard deviation was 0.00045 % (w/w).

The reproducibility of the method was determined by analyzing six real samples of biodiesel in ten different days using the same equipment. The limit of reproducibility (R) was 0.0062 % (w/w) and reproducibility standard deviation was 0.0025 % (w/w) and was below the reproducibility limit R = 0.1866*x + 0.0061. The precision of the method comply with that imposed by the EN 14214 standard (r < 0.002 % (w/w) and R below 0.0096 % (w/w)).

In Table 3 are presented the results obtained for validation of free glycerol from biodiesel samples.

Table 3. Results of method validation for the measurement of free glycerol

Validation Parameter	Results
Limit of detection	0.0006 %(w/w)
Limit of quantification	0.002 %(w/w)
Linear range	0.005 -0.05 mg
Accuracy for CRMs (recovery)	102.4 %
Precision (limit of repetability)	0.0012 %(w/w)
Precision (limit of reproductibility)	0.0096%(w/w)

Uncertainty estimation

For estimation of method's uncertainty all the uncertainty sources were identified and quantified and the combined uncertainty was calculated. The sources of uncertainty for glycerol determination are: the concentration of reference material, the concentration of internal standard, standard preparation (volumetric flasks, pipettes), the weight of samples, the uncertainty given by the calibration curve, precision of the method.

The concentration of glycerol is given on the certificate as 502.0 ± 2.5 ug mL^{-1}. Because in certificate is no additional information about the expanded uncertainty, a rectangular distribution is supposed. To obtain the standard uncertainty u_{rm} the value was divided by $\sqrt{3}$. The standard uncertainty u_{si} for internal standard was calculated by dividing by $\sqrt{3}$ the value given in certificate and the results is 2.89 ug.mL^{-1} (the concentration of 1,2,4-butanetriol given in certificate is 1003 ± 2.5 ug.mL^{-1}).

The preparation of stock and working solution for calibration curve gives a major source of uncertainty. The volumes of solution have three source of uncertainty: the uncertainty from the certificate of volumetric flask, the uncertainty given by the variation of the temperatures and standard deviation of repeated filling of the volumetric flask.

The weight of the sample and the analytic balance contribute also to the uncertainty of the method. The uncertainty given by the calibration curve (a linear function of first order) is calculated using Equation (1) [21,22]:

$$S_{x0} = \frac{S_y}{b} \bullet \sqrt{\frac{1}{N} + \frac{1}{m} + \frac{\left(\overline{y_0} - \overline{y}\right)^2}{b^2 \sum_{i=1}^{N} \left(x_i - \overline{x}\right)^2}} \quad (1)$$

where: S_y –residual standard deviation; *b*- is the calculated best-fit gradient of the calibration curve; *N* – number of repeat measurements made on the sample; *m* –the number of paired calibration points ($x_i y_i$); $\overline{y_0}$ – the mean of N repeat measurements of y for the sample; $\overline{y}$ – the mean of the y value for the calibration standards, x_i – a value on the x-axis; $\overline{x}$ - the mean of the x_i axis. In Table 4 is presented the uncertainty components for determination of free glycerol by GC-FID.

Table 4. Uncertainty components for determination of free glycerol by GC-FID

Source	Unit	Value	Standard uncertainty	Interven-tions	Total standard uncertainty	Relative uncertainty
Concentration of standard	μg mL^{-1}	502	1.443	1	1.4433	0.0029
Concentration of internal standard	μg mL^{-1}	1003	2.886	1	2.8860	0.0029
Pipette	μL	10	0.360	1	0.3600	0.0360
Pipette	μL	40	0.360	1	0.3600	0.0090
Pipette	μL	70	0.361	1	0.3610	0.0052
Pipette	μL	100	0.363	1	0.3630	0.0036
Pipette	μL	80	0.361	5	1.8050	0.0226
Pipette	μL	150	0.301	4	1.2049	0.0080
Pipette	mL	8	0.023	5	0.1150	0.0144
Pipette	μL	200	0.301	3	0.9036	0.0045
Weight of sample	g	0.1	0.00005	1	0.0001	0.0005
Equipment	%	100	0.0010	1	0.0500	0.0005
Calibration	mg	0.343	0.0112	1	0.0112	0.0327
Reproducibility	%	0.017	0.0025	1	0.0025	0.1479

After measurement of all relative uncertainty for each source of uncertainty, the combined standard uncertainty (U_c) is calculated by combining all the uncertainty components by using law of propagation of uncertainty. The combined standard uncertainty was calculated to be 8.0 %. The expanded uncertainty (U_E) is obtained by multiplying combined standard uncertainty by a coverage factor (k) which is 2 for level of confidence of 95 % [23]. In Table 4

can be observed that the biggest contributors to uncertainty comes from reproducibility and calibration curve.

The concentration of standard, concentration of internal standard and weight of sample has a low contribution to the uncertainty. The expanded uncertainty for free glycerol determination in biodiesel by GC-FID method is 16 %.

CONCLUSIONS

In this paper, a GC-FID method has been applied for the analysis of free glycerol in biodiesel samples according to the EN 14105 standard. The validation of this method and measurement uncertainty evaluation was made by quantification of all uncertainty sources based on bottom-up approach. The accuracy was studied by evaluating the recovery of biodiesel using certified reference material. It was demonstrated that the method can be applied for determination of free glycerol in biodiesel samples by GC-FID.

EXPERIMENTAL SECTION

All chemicals were analytical reagent grade. N-heptane and pyridine were purchased from Merck (Darmstadt, Germany). Glycerol (glycerin standard), 1,2,4-butanetriol (internal standard) and derivatization agent N-Methyl-N-(trimethylsilyl)trifluoroacetamide were purchased from Sigma–Aldrich. Biodiesel 100 (Soy-based) SRM 2772 was used as CRM for the validation procedure. The volumes were measured using calibrated glassware (Hirschman, Germany).

The transformation of free glycerol into more volatile silylated derivative is based on the procedures described in EN 14105 [15].

The method consists in weighting approximately 100 mg of samples in a 10 mL vial and mixing it with 80 µL 1,2,3-butanetriol, 100 µL pyridine and 150 µL MSTFA under continuous shaking. After keeping 15 min at room temperature, 8 mL n-heptane was added to the solution. The solution was analyzed by GC-FID.

Working standard solutions of glycerol 500 $\mu g.mL^{-1}$ were prepared by diluting glycerol stock standard solution (0.5 mg mL^{-1}) with pyridine. Calibration standard solution in the range of 0.005 - 0.05 mg (glycerol) were prepared by adding 10, 40, 70, 100 µL from working solution of glycerol. In each solution, 80 µL 1,2,3-butanetriol (as internal standard) and 150 µL MSTFA were added.

GC-MS analysis

Analyses were performed using a gas chromatograph (Agilent Technologies, 7890N GC) coupled with flame ionization detector (Agilent Technologies, 7683) and capillary column of 15 m length×0.32 mm I.D.×0.1 μm DB-5HT film thickness. The temperature program was as following: the initial oven temperature 50 °C, held for 1 min, from 50 to 180 °C via a ramp of 15°C/min, 180 to 230 °C at a ramp of 7°C/min and 230 to 370 °C at a ramp of 10 C/min for 15 min.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCS, project OPTRONICA IV (PN 16.40), Sectoral Operational Programme "Increase of Economic Competitiveness" Priority Axis II, (PN 1887, code SMIS-CSNR 49164) INOVA-OPTIMA and Romanian National Agency for Scientific Research, Project Number PN-II-PT-PCCA-2011-3.2-0452: "*Hydrogen production from hydroxylic compounds resulted as biomass processing wastes*" – HYCAT.

REFERENCES

1. J.V. Gerpen, *Fuel Processing Technology*, **2005**, *86*, 1097.
2. G. Baskar, R. Aiswarya, *Renewable and Sustainable Energy Reviews*, **2016**, *57*, 496.
3. E.F. Aransiola, T.V. Ojumu, O.O. Oyekola, T.F. Madzimbamuto, D.I.O. Ikhu-Omoregbe, *Biomass and Bioenergy*, **2014**, *61*, 276.
4. S. Semwal, A.K. Arora, R.P. Badoni, D.K. Tuli, *Bioresource Technology*, **2011**, *102*, 2151.
5. M.Y. Koh, T.I. Ghazi Mohd, *Renewable Sustainable Energy Reviews*, **2011**, *15*, 2240.
6. A. Gog, M. Roman, M. Tosa, C. Paizs, F.D. Irimie, *Renewable Energy*, **2012**, *39*, 10.
7. A.L. Ahmad, N.H. Mat Yasin, C.J.C. Derek, J.K. Lim, *Renewable Sustainable Energy Reviews*, **2011**, *15*, 584.
8. G. Knothe, *Journal of the American Oil Chemists' Society*, **2000**, *77*, 825.
9. M. Berrios, M.A. Martin, A.F. Chica, A. Martin, *Applied Energy*, **2011**, 88, 3625.
10. A.N. Dias, M.B.R. Cerqueira, R. Rodrigues de Moura, M.H.S. Kurz, R.M. Clementin, M.G.M. D'Oca, E.G. Primel, *Fuel*, **2012**, *94*, 178.
11. Y. Chen, H.L. Teo, Q. Liu, T. K. Lee, *Clinical Biochemistry*, **2015**, *48*, 897.
12. P.H.G.D. Dias Diniz, M.F. Pistonesi, M.C.U. de Araujo, B.S. Fernandez Band, *Talanta*, **2013**, *114*, 38.
13. L. Jiang, A. Zheng, Z. Zhao, F. He, H. Li, *Bioresource Technology*, **2015**, *196*, 194.
14. L.R. Miguel, M. Ulberth-Buchgraber, A. Held, *Journal of Chromatography A*, **2014**, *1338*, 127.

15. EN 14105:2011. Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, triglyceride content, European Committee for Standardization.
16. EN 14214:2008, Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods, European Committee for Standardization
17. A. Drolc, A. Pintar, *Accreditation and Quality Assurance*, **2012**, *17*, 323.
18. M. Senila, E. Levei, L. Senila, O. Cadar, G. Oprea, C. Roman, *Studia UBB Chemia*, **2011**, *56*, 27.
19. International Organization for Standardization, ISO/IEC Guide 98-3:2008, Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)
20. EURACHEM, 1998, The fitness for purpose of analytical methods, Eurachem LGC, Teddington European Environment Agency, http://www.eea.europa.eu/ data-and-maps/indicators/progress-inmanagement-of-contaminatedsites/ progress-in-management-of-contaminated-1.
21. M. Senila, E. Levei, L. Senila, O. Cadar, M. Roman, M. Miclean, *Studia UBB Chemia*, **2015**, *60*, 137.
22. ISO 8466-1, 1990, Water Quality – Calibration and evaluation of analytical methods and estimation of performance characteristics - Part 1, International organization for standardization.
23. M. Senila, A. Drolc, A. Pintar, L. Senila, E. Levei, *Journal of Analytical Science and Technology*, **2014**, *5*, 37.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 355-364)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

THE INFLUENCE OF POLLUTED SOIL AERATION IN THE PROCESS OF IN SITU BIOLEACHING

IOANA MONICA SUR[a,*], VALER MICLE[a], TIMEA GABOR[a]

ABSTRACT. The influence of aeration of soils polluted with heavy metals by using *Thiobacillus*-type microorganisms was studied using soil samples contaminated with heavy metals (Cu 4074 - 7550 $mgkg^{-1}$, Zn 5870 - 9310 $mgkg^{-1}$, Cd 36-50 $mgkg^{-1}$, Pb 15000 - 42890 $mgkg^{-1}$), from Romplumb, Baia Mare. The variation of the metal concentration extracted by bioleaching and aerated bioleaching was monitored for 16 weeks. The soil samples treated by bioleaching (Cu: 9 - 53%; Zn: 9 - 62%; Cd: 9 - 24%. Pb: 31 - 71%) have obtained a lower efficiency than the soil samples treated by aerated bioleaching (Cu: 34 - 70%; Zn: 36 - 76%; Cd: 17 - 38%. Pb: 44 - 78%), but there are percentage differences between the two processes (Cu: 17 - 27%; Zn: 14 - 27%; Cd: 8 - 14%, Pb: 7 - 13%).

Keyword: *bioleaching, contaminated soils, heavy metals, in situ, microorganisms.*

INTRODUCTION

Soil pollution with heavy metals in Baia Mare is recognized today as a significant problem and is a major risk to human health and the environment [1 - 3]. High concentrations of heavy metals in soils are related to anthropogenic pollution sources, mining and metallurgy [4].

Heavy metals pollution has a cumulative and residual character, which means that pollutants accumulate slowly, being the result of a permanent and long-term exposure of the soil to the action of these pollutants [5, 6].

[a] *Technical University of Cluj-Napoca, Faculty of Materials and Environmental Engineering, Department of Environmental Engineering and Sustainable Development Entrepreneurship, 103-105 Muncii Ave, Cluj-Napoca, Romania*
** Corresponding author: ioanamonica_berar@yahoo.com*

Bioleaching, or bacterial leaching, consists in the extraction by solubilization of the metallic elements from contaminated soil using bacteria. This method does not destroy (eliminate) the pollutants, but it favors their segregation from the contaminated environment, the microorganisms having the property to oxidize the metals, transforming them into a more soluble form [7 – 8].

Thiobacillus ferrooxidans was successfully adapted to leaching of both Cu, Ni, Fe, Zn, Cd [9 - 12].

There are several studies in the literature related to bioleaching of metals and metalloids using different type of microorganisms [13 - 23]. *Thiobacillus ferroxidans* give high yields in case of bioleaching of Cu, Cd and Zn [13, 14, 16, 18, 20 - 23], while *Thiobacillus thiooxidans* was proven to be efficient in the extraction of Cd, Cr; Cu, Mn, Ni, Pb, Zn [15, 17, 19]. However, bioleaching is still under study in order to determine the influence of the different parameters which can result in a higher yield and efficiency.

The preliminary experimental laboratory results have shown high extraction yields: Fe: 100%; Cr: 11- 25%; Cu: 40 - 100%; Zn: 55 - 94%; Cd: 17 - 60% și Mn: 32 - 66% [18].

The present researches and studies are focused on in situ bioleaching; thus, it was considered a necessity to develop a research that approaches to the method of treating the soil polluted with heavy metals by in situ bioleaching applying also one of the main parameters (soil aeration) which leads to high treatment efficiency.

The main advantage of in situ bioleaching is that the entire process happens without soil excavation, so the transportation costs are eliminated and the transport does not pollute the environment. In this way, capital and energy costs are low [24].

The purpose of this paper is to observe the influence of aeration in the extraction of heavy metals from polluted soils by in situ bioleaching, using *Thiobacillus ferrooxidans* type of microorganisms in 9K medium.

The paper tracks how aeration influences the in situ bioleaching process, applied to extract heavy metals from soil used in the experimental research. This is evidenced by variation in metal concentration depending on time and depth.

RESULTS AND DISCUSSION

Variation of extracted metal concentration

Copper is showing a very good extraction after only 4 weeks of experiment in the depth 0 – 10 cm. In the case of the sample extracted from 10 – 20 cm depth, it can be seen a decrease of Cu achieving maximum extraction after 8 weeks (Fig. 1).

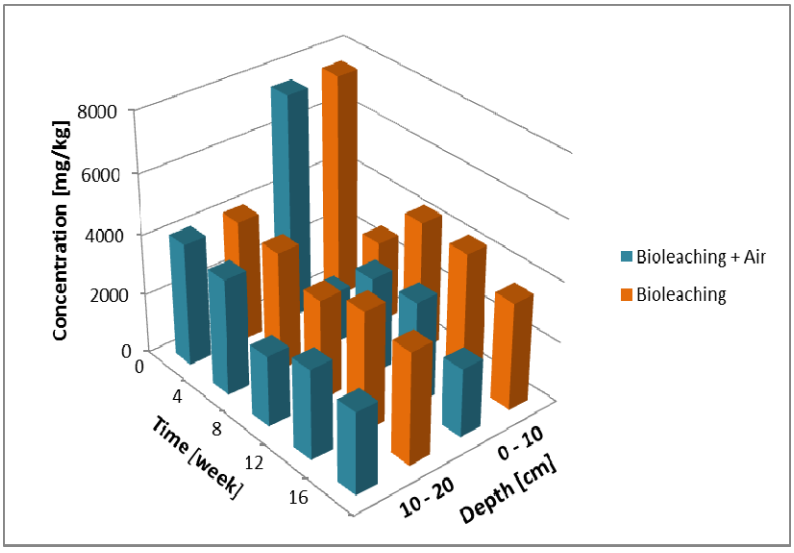


Figure 1. Variation in time of copper concentration in soil

We have seen maximum Zn extraction (Fig. 2) after 8 weeks of experiment, on both soil samples, regardless of the depth of sampling and the decontamination method applied (bioleaching or bioleaching + aeration).

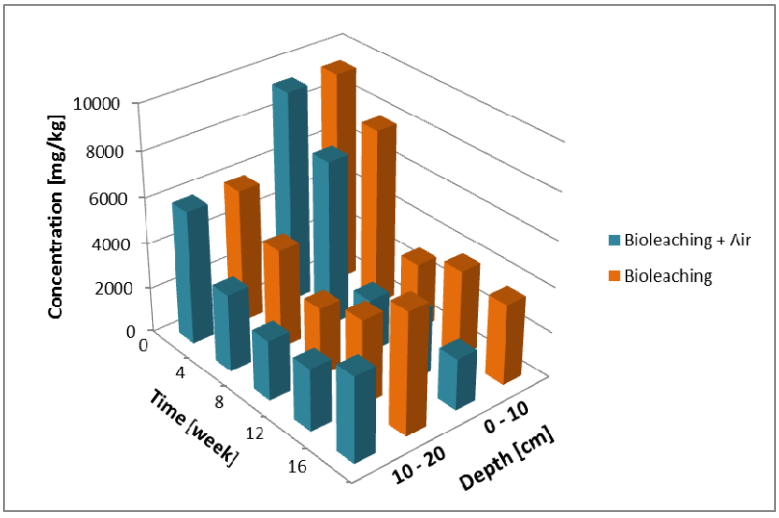


Figure 2. Variation in time of zinc concentration in soil

Cadmium and lead both have a good extraction after 8 weeks of experiment, regardless of the sampling depth (Fig. 3).

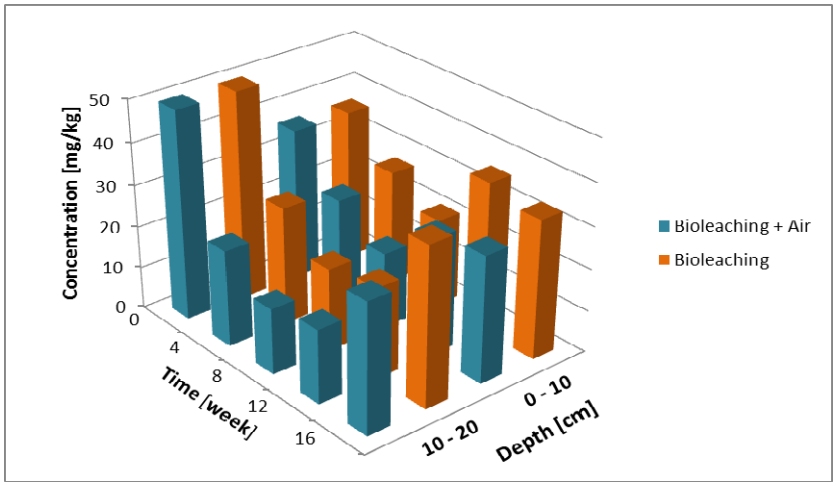


Figure 3. Variation in time of cadmium concentration in soil

The soil samples submitted to bioleaching + aeration show smaller concentration of lead than the soil samples submitted only to bioleaching during the entire period of experiment, regardless of the depth of sampling (Fig. 4).

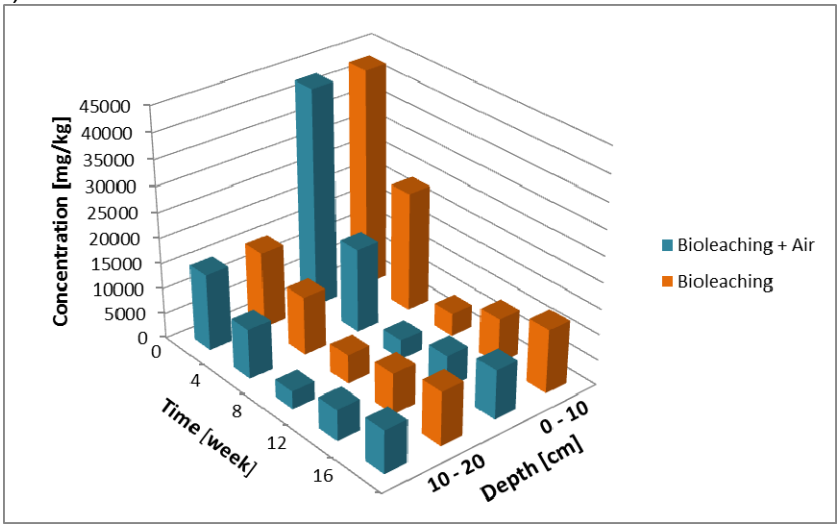


Figure 4. Variation in time of lead concentration in soil

The increase of Cu, Zn, Cd, and Pb metal concentration after 12 weeks is due to the metabolic activity of the microorganisms.

Both experiments have the same extraction trend, with a better extraction for the aerated soil samples.

Efficiency of extraction process

Evaluation of the extraction process efficiency for the metals in the soil after 16 weeks was carried out by determining the final yield of each element, by the following formula [25]:

$$\eta = \frac{C_i - C_f}{C_i} 100 \ [\%] \tag{1}$$

where: η final yield, in %;

C_i – initial metal concentration in soil sample, in mgkg^{-1};

C_f – metal concentration in soil sample at final treatment time, in mgkg^{-1}.

Analyzing figure 5 can be seen that Cu, Zn and Pb are showing a very good extraction (70%), Cd having the lowest leaching (17%).

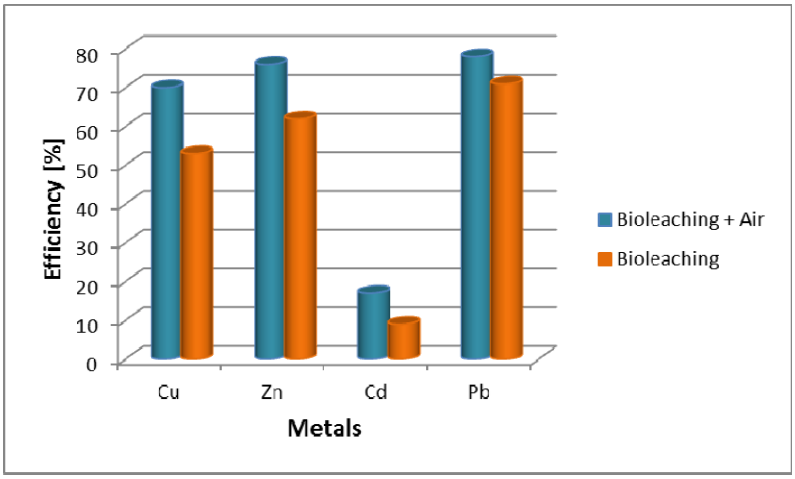


Figure 5. Efficiency of metals extraction from depth 0 – 10 cm

In figure 6 is showing that lower yields have been obtained (34 - 44%) compared with the surface sample. This is due to the fact that the metals from the surface sample were washed with leached solution in the moment when were added on top of the soil samples.

Comparing the two methods, the extraction efficiency of metals is much higher if aeration is introduced in the process (Cu: 17 - 27%; Zn: 14 - 27%; Cd: 8 - 14%. Pb: 7 - 13%).

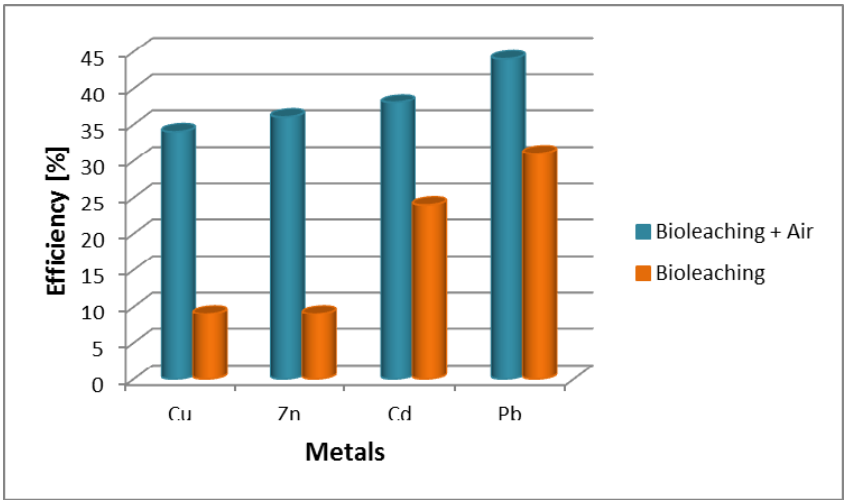


Figure 6. Efficiency of metals extraction from depth 10 – 20 cm

The Cu (34 - 70%) extraction yields are similar with the ones presented by Couillard and colleagues [21], but lower when compared with other results from the literature [14, 19, 22, 23].

Zinc (36 - 76%) has a lower extraction yield compared with the studies of Blais and colleagues (88 - 97%) [19].

The extraction yield for Cd (17 - 38%) is considerably lower than the ones from other experiments (80%) [19, 21, 23].

Lead has an extraction yield better than the one obtained in the experimental researches of Blais and colleagues (10 - 54%) [19].

CONCLUSIONS

The variation in the extracted concentration of heavy metal from soil samples shows a linear decrease of metals in soil, a good extraction being obtained after eight weeks of leaching.

The bioleaching solution used has a better efficiency for the extraction of metals such as Pb, Zn, Cu, but lower efficiency in the case of Cd extraction.

The extraction efficiency is higher for surface soil layer (0 - 10 cm depth) in case of all metals which have migrated underground due to the bioleaching solution that was added weekly over the soil samples.

Higher concentrations of metals compared to the samples subjected only to bioleaching were extracted from the samples subjected to both bioleaching and aeration.

The quantity of metal in the soil shows the same variables, whichever method is applicable (bioleaching or bioleaching + aeration) and regardless of the depth which was taken from the ground.

The results indicated that the bioleaching of metals is achieved by the growth of *Thiobacillus* bacteria type. After 16 weeks of treatment of bioleaching + aeration, heavy metals were extracted from soil metals, as follows: Cu: 34 - 70%; Zn: 36 - 76%; Cd: 17 - 38%; Pb: 44 - 78%.

The extraction efficiency of metals is much higher if aeration is introduced in the process (Cu: 17 - 27%; Zn: 14 - 27%; Cd: 8 - 14%. Pb: 7 - 13%).

The experiments carried out have led to the conclusion that soils can be decontaminated using in situ bioleaching and if aeration is used, higher yields are obtained.

EXPERIMENTAL SECTION

Sampling

Soil samples in natural state used for this lab experiment were collected from Romplumb, Baia Mare. The sampling point is located in the vicinity of the technologic gas chimney area and laboratories. From the research area were taken two soil samples in natural condition, as follows: **sample 1**, depth: 0 – 10 cm and **sample 2**, depth 10 – 20 cm. The samples in natural or undisturbed state were collected in a special container that keeps the soil natural settlement mode [26 –28].

Culture media

Remediation of contaminated soils was performed using *Thiobacillus ferrooxidans* type microorganisms (140 x 10^6 cells/mL), selected from the soil sampling area (Baia Mare). These microorganisms were grown on a 9K type nutrient medium with the following solutions: $(NH_4)_2SO_4$ - 3 g; KCl - 0.1 g; K_2HPO_4 - 0.5 g; $MgSO_4 \cdot 7H_2O$ - 0.5 g; $Ca(NO_3)_2 \cdot 4H_2O$ - 0.01 g; $FeSO_4 \cdot 7H_2O$ - 44.2 g is dissolved in distilled water (1 L), yielding a solution with pH = 2.5 [29].

Experimental

The soil samples taken in their natural state were transferred to glass containers (Lxlxh=150mmx150mmx300mm) and placed on a drainage layer of 30 - 45 mm gravel sort, depending on the depth of the sample in order to reconstruct the profile of the soil. Bioleaching solution – 9K medium, was added over the prepared soil samples, the soil samples being dipped in the bioleaching solution, to which was added on a weekly basis 100 ml of solution.

The process of cleaning the polluted soils with heavy metals was done by: bioleaching and bioleaching + aeration, the two processes being performed independently. Both experiments were done in the same conditions: pH=5, constant temperature (26 ± 2°C) and soil dipped in bioleaching solution. In the case of in situ bioleaching with aeration was introduced a perforated tube which was connected to a compressor, achieving 8 bar aeration pressure for 8 hours/a day on the entire duration of the experiment (16 weeks). Concentration from week 0 represents the quantity of pollutant present in the soil before beginning the remediation process.

After the start of the experiment, soil samples (10 g) were collected once per month for four months (16 weeks) in order to establish the speed of metal extraction from soil in the process of in situ leaching and to observe the influence of soil aeration in the process of bioleaching.

Metal concentration in soil samples were analyzed by inductive coupled plasma atomic spectrometry - ICP-AES (Optima 5300DV, Perkin Elmer) of the Research Institute for Analytical Instrumentation (ICIA-Cluj-Napoca).

ACKNOWLEDGMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-PT-PCCA-2013-4-1717.

REFERENCES

1. ***, Annual report on the state of the environment in Romania in 2007, the National Agency for Environmental Protection, Bucharest, **2008**.
2. M. Coman, V. Oros, B. Fălăuş, R. Pop, *ProEnvironment/ProMediu,* **2010**, 3, 155–158.
3. C. S. Cociorhan, V. Micle, V. Oros, I. M. Berar-Sur*, ProEnvironment/ProMediu*, **2011**, 4, 179–186.

4. F. Damian, G. Damian, R. Lăcătuşu, G. Macovei, G. Iepure , I. Năprădean, R. Chira, L. Kollar, L. Raţă, D. C. Zaharia, *Carpth. J. of Earth and Environmental Sciences*, **2008**, *3(1),* 85–98.
5. L. Miloiu, "Actual status of the environment in the Baia Mare and Copalnic basin"–PhD Thesis (in Romanian), Oradea, **2008**.
6. I. M. Sur, V. Micle, G. Plopeanu, *Ecoterra – Journal of Environmental Research and Protection,* **2012**, *31*, 105–109.
7. I. M. Sur, *Acta Technica Napocensis,* **2015***, 4(4), 61–67.*
8. ***, Feasibility study – Eco Industrial Parc SEPA Baia-Mare (in Romanian), Baia Mare, **2012**.
9. L. J. Mason, N.M. Rice, *Minerals Engineering*, **2002**, *15*, 795–808.
10. S. Kiss, D. Paşca, M. Drăgan-Bularda, "Enzymology of disturbed solis Developments in Soil Science", Editura Elsevier, **1998.**
11. T. Volke Sepúlveda, J.A. Velasco Trejo, D.A. de La Rosa Pérez, "*Suelos contaminados por metales y metaloides: muestreo y alternatives para su remediación*", Méxic, **2005.**
12. S. N. Groudev, I. I. Spasova, P. S Georgiev, *International Journal of Mineral Processing*, **2001**, *61(1*), 301–308.
13. X. Xuehui, Y. Xuewu, N. Liu, C. Xiaoguang, A. Awad, L. Jianshe, *BioMed Research International*, **2013**, 1–10.
14. D. B. Nakade, *International Journal of Advance Research in Engineering and Technology* (IJARET), **2014**, *5(1),* 52–58.
15. R. Naresh Kumar, R. Nagendran*, J Hazard Mater*, **2009**, *169*, 1119–1126.
16. H.-L. Liu, B.-Y. Chen, Y.-W Lan, Y.-C. Cheng, *Appl Microbiol Biotechnol*., **2003**, *62*, 414–420.
17. B.-Y. Chena, H.-L. Liu, Y.-W. Chenb, Y.-C. Chengb, *Process Biochemistry*, **2004**, *39*, 735–745.
18. I.M. Berar-Sur, V. Micle, S. Avram, M. Şenilă, V. Oros, *Environmental Engineering and Management Journal*, **2012**, *11(8*), 1389–1393.
19. J. F. Blais, R. D. Tyagi, J. C. Auclair, *Water Research*, **1993**, *27*, 101–110.
20. A. T. Lombardi, G. Jr. Oswaldo, *Water Research*, **2002**, *36*, 3193–3202
21. D. Couillard, M. Chartier, G. Mercier, *Journal of Water Science*, **1994**, *7,* 251–268.
22. R. C. Ivănuş, G. Bică, *Studia Universitatis "Vasile Goldiş" Series: Economic Sciences*, **2007***, 12,* 109–117.
23. R. C. Ivănuş, "Bioleaching Solubilization" (in Romanian), Universitaria Press, Craiova, Romania, **2005**.
24. Abhilash, B. D. Pandey, K. A. Natarajan, "Microbiology for Minerals, Metals, Materials and the Environment", CRC Press Taylor&Francis Group, **2015.**
25. A. Măicăneanu, H. Bedelean, M. Stanca, "Natural zeolites–Characteristics and applications in environmental protection" (in Romanian), Presa Universitară Clujeană, **2008**.
26. ***, STAS 7184/1-84, Soils. Sampling for soil and agrochemical studies (in Romanian).
27. ***, Order no. 148 of 09/21/1997, approving the procedure of environmental audit, Official Gazette no. 303 of 06.11.1997 (in Romanian).

28. ***, Order no. 756 of 11.03.1997 approving the regulations on environmental pollution assessment, Official Gazette no. 303 of 11/06/1997 (in Romanian).
29. M. P. Silverman, D. G. Lundgren, *Journal of Bacteriology*, **1959**, *77*, 642–647.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 365-378)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoş
on the occasion of his 80th anniversary

EARLY WARNING SYSTEMS FOR DISASTER RISK REDUCTION: A CASE STUDY IN NORTH-WEST OF ROMANIA

EMIL ROMAN[a], LUCRINA ŞTEFĂNESCU[a], KOZMA KIS ELISABETA-EDITA[a], DAN CHIRIBUCĂ[b], ADRIANA DÎNCU[c], VIOREL ARGHIUŞ[a], ALEXANDRU OZUNU[a,*]

ABSTRACT. Timely and effective warning sets the basis for building the prevention and awareness culture necessary for disaster risk reduction. The paper pursues to assess the awareness level of communities downstream some hydrotechnical facilities in NW Romania in terms of the risks these communities are exposed to and the availability of early warning systems. The research is based on the awareness on early warning systems of a community located downstream of some hydrotechnical facilities on the Somesul Cald River, NW Romania (Central-Eastern Europe), using the social investigation methodology, namely the questionnaire, applied to a group of 516 respondents from the risk-prone area, by the CATI (Computer Assisted Telephone Interviewing) method. The findings reveal the need to increase awareness of population and improve risk communication, as well as to conduct preparedness activities within the local community in order to build their resilience to disasters and improve the knowledge of population on the existing early warning systems.

Keywords: *early warning systems, disaster risk reduction, community awareness, resilience, Romania*

[a] *Babeş-Bolyai University, Faculty of Environmental Science and Engineering, Research Institute for Sustainability and Disaster Management based on High Performance Computing, 30 Fantanele str., RO-400294, Cluj-Napoca, Romania str., RO-400028, Cluj-Napoca, Romania*
[b] *Babeş-Bolyai University, Faculty of Sociology and Social Work, 128-130, 21 Decembrie 1989 Blvd. RO-400604 Cluj-Napoca, Romania*
[c] *Romanian Strategy and Evaluation Institute, 150 Turzii Street, Cluj-Napoca, Romania*
** Corresponding author: alexandru.ozunu@ubbcluj.ro*

INTRODUCTION

Early Warning Systems (EWS) are specially designed monitoring devices and key elements of risk reduction, used to mitigate the effects of natural and technological disasters on humans, property, environment, livelihoods, etc. [1, 2, 3]. Their main goal is to reduce injuries and death toll, economic losses, and social impacts of disasters by providing information that enables people and organizations to prepare for emerging disasters [4] and they are worldwide promoted by international initiatives, along with the development of risk prevention culture [5, 6, 7]. Whereas one of the priorities of the Hyogo Framework was to identify, assess and monitor disaster risks and enhance early warning, the new international framework for disaster risk reduction (DRR) focuses on enhancing multi-hazard early warning systems [8].

As human population represents the most important component of the disaster management cycle, effective EWS need to actively involve the communities at risk, to facilitate public education and awareness, to communicate and disseminate warning messages, and to ensure a constant state of preparedness [9]. EWS are integrated in the preparedness plans to monitor and predict the occurrence of hazards [10]. The disaster preparedness in Romania is currently conducted in a very general manner, so that population has general knowledge of a wide range of natural and technological disasters. Moreover, although information on early warning measures and systems is available, it does not always reach the target groups in need of emergency protection. Public education and awareness on risk scenarios and action models is one of the first steps towards this goal and towards risk prevention and mitigation [11, 12, 13], and should at least include details on the sender of the warning messages, content, timing, and the media used to communicate risk messages [14].

Among the driving factors causing disasters, floods and flash-floods are common phenomena in the temperate-continental climate, affecting human settlements throughout the Romanian territory every year. During the 2000-2009 period alone, the total national flood losses summed up to 4,215 billion US $ [15]. In order to mitigate the negative effects associated with floods and flash-floods, especially during the second half of the 20th century, a series of flood control works were carried. Among these works, most efficient are the reservoirs provided with flood storage capacities. There are currently more than 1,400 reservoirs in Romania, with an estimated volume of 3,700 mil. m^3. Besides their benefits derived from the specific functions (flood control, water supply, fishing, tourism, etc.), the reservoirs imply also negative aspects. Among these, flash-floods caused as a result of dam failures and/or events consisting in sudden high discharges, although very rare, are catastrophic and draw the media's attention, requiring national and even international assistance.

The main objective of this research is to determine the extent to which the communities located downstream of such high-risk facilities are informed on the alarm systems in place and, therefore, prepared to cope with disasters.

EXPERIMENTAL SECTION

The research was conducted in the Somesul Cald River basin (the main course of the Somesul Mic River), also called Somesul Mic River downstream of the confluence with the Somesul Rece River in Gilau. The largest community in the case study area is the city of Cluj-Napoca, the second largest city in Romania, 324,576 inhabitants [16].

The high rainfall amounts in the upper basin, the steep slopes and sparse vegetation in certain areas, all lead to serious floods, causing significant damages and negative impacts on soil due to erosion and excessive humidity. Therefore, flood risk is the specific type of risk along the Somesul Mic River. Several reservoirs were built (Fantanele, Tarnita, Somesul Cald, Gilau I and II, Floresti I and II, and Cluj), but Fantanele and Tarnita are worth mentioning in term of flood control. Their location is represented in Fig. 1 (upper image), together with the longitudinal section diagram (bottom image). However, despite their flood-control function, these facilities induce disaster risks by possible dam failures.

At global level, the dam-related failures and accidents are caused by the loss of stability and sustainability of construction or foundation in the first place (80%), while the operational failures are less frequent causes (14%) [18].

Dam failure can occur due to the singular or factor-related effect of the following elements, and the following are most likely to occur in the study area: exceed of the spillway evacuation capacity; loss of dam stability (landslides, dumping); loss of construction sustainability; foundation instability (in depth landslides, foundation surface landslides, foundation settling, plastic strains, infiltration through foundation or dam, increase of loads, cracking); sudden increase of the water volume in the lake; human error or deliberate actions (e.g. terrorist attacks).

In the case of the Somesul Cald reservoirs, the scenarios considered for the modelling of flash-flood waves resulting from Fantanele dam failure were the following: scenario 1 - full lake and 100 % failure; scenario 2 - 50 %, medium failure.

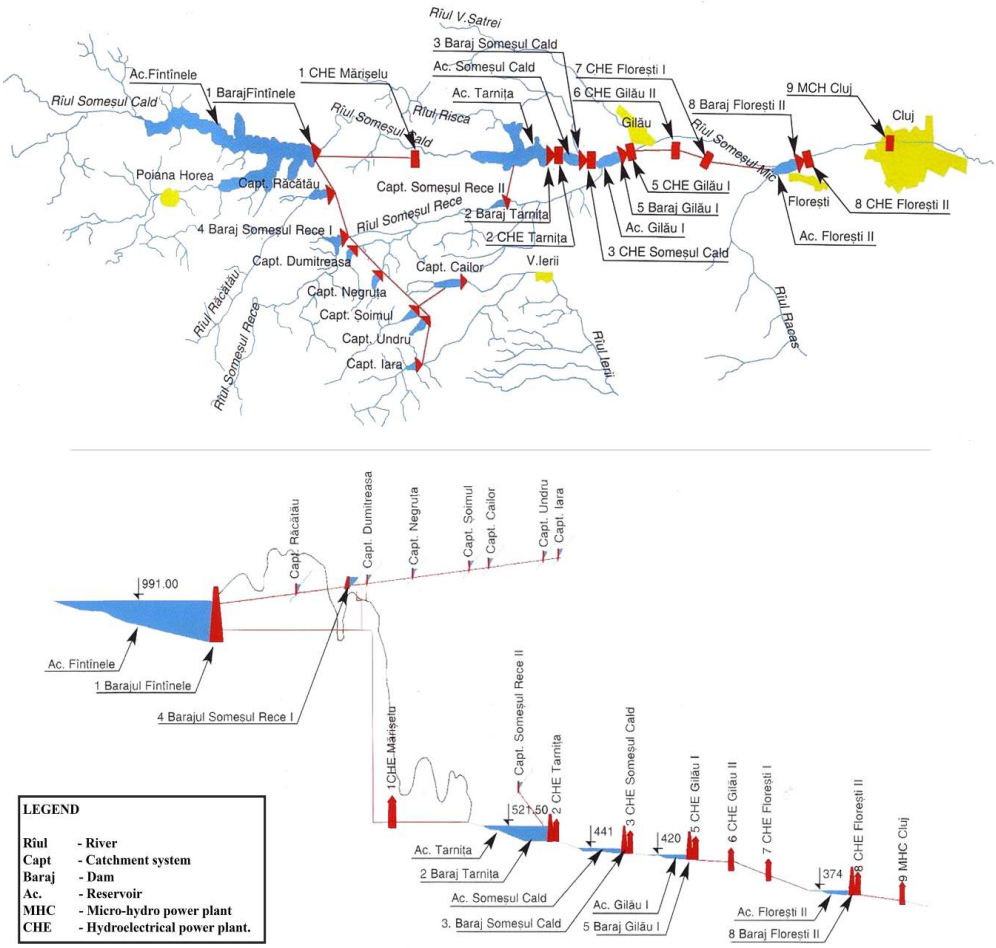


Figure 1. The structure of Somesul Cald (Mic) hydropower facilities system (upper image) and longitudinal section diagram (bottom image) [17]

Considering the worst case scenario of an accident, the 100 % failure of the upstream Fantanele dam, this would lead to the formation of a flash-flood wave in the case study area that would affect approx. 200,000 persons living in the downstream localities. This situation is rather hypothetical, considering that the rockfill dams never fail suddenly or to a 100 % extent. The average velocity of flood propagation to the border of the Cluj-Napoca municipality is estimated to be approximately 60 km/h. The impact with the communities located immediately downstream in the path of the flash-flood occurs within

several minutes from the occurrence of the accident. As the city of Cluj-Napoca is located at approximately 47 km, the first impact would occur in approximately 45 minutes [18]. Therefore, one may notice the particular significance of organizing the warning and alerting of population from the areas subjected to risk.

Response plans for the management of hydrotechnical disasters are in place in the Somesul Cald River basin. These plans are drafted based on the modelling of flash-floods resulting after dam failure.

In case of dam failure, the existing warning, alerting and response plans are applied. The Cluj hydropower plant branch will warn all the entities involved in flood emergency management, according to the informational flow block diagram provided by the Romanian Waters National Administration [19].

For the prompt warning of population, alarm systems and devices are timely provided in localities, economic operators and public institutions. The local and central public administration authorities, the heads of public institutions and managers of companies that are considered sources of risk, regardless of their form of ownership, provide special annual budget amounts for the development of civil protection activities, according to Romanian legislation in this field [20, 21, 22, 23]. The number, type and location of alarm devices are determined by the General Inspectorate for Emergency Situations (IGSU), based on audibility analyses.

The alarm should be pertinent, reliable and stable and should ensure the successful warning of population:

- pertinent – ensure the timely warning of population by alarm means and systems that can be activated immediately at the occurrence of aerial attacks or disasters;
- reliable – send the signals intended for warning of population through specific means by the assigned personnel based on the decision of the emergency situation committees presidents;
- stable - warn the population and economic operators under any circumstances.

In order to ensure the warning and alerting of population and to secure the facilities downstream of the dams, a warning and alerting plan and an adequate technical system are implemented. For this warning and alerting system to work in a timely manner, information is necessary regarding the state of hydrotechnical facilities and their behavior over time. These data are provided by the hydrometeorological informational system.

The warning for dam failure in the case study area is conducted by the Cluj hydropower plant branch, according to the existing plans and following the approved information and warning flow chart (Fig. 2).

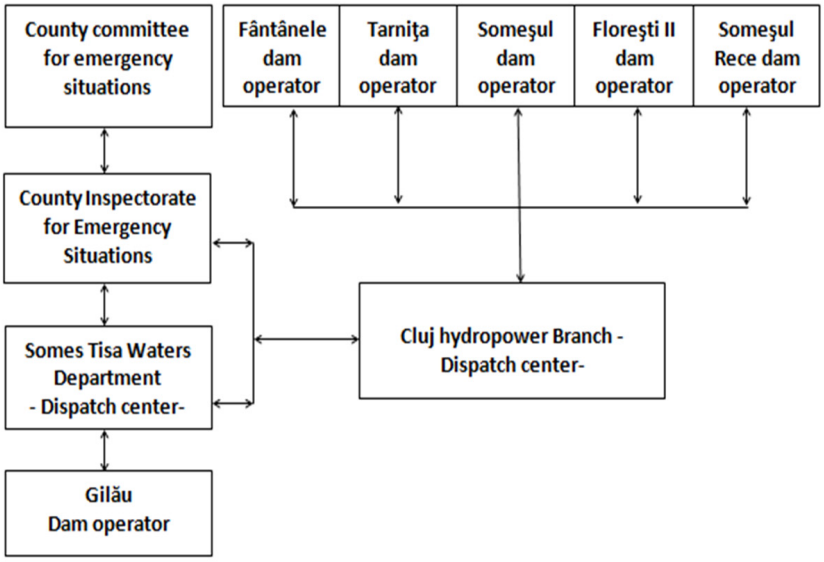


Figure 2. The notification and warning flow chart in the event of dam failure in the case study area

The population, public institutions and economic operators are alerted by acoustic signals emitted by means of the alarm devices and messages sent by the central and local radio and television stations, and, if possible, by rediffusion and radio-amplification.

The acoustic alarm signal for emergency situations “ALARM IN CASE OF DISASTERS” is composed of 5 sounds, 16 seconds each, separated by 10 second pauses. In case of compressed air sirens, the signal is composed of 5 sounds 8 seconds each, separated by 5 second pauses.

The Somesul Cald hydropower plant is equipped with a sound alarm system composed of:

- a warning and alerting system with electronic sirens within the Hydropower Cluj dispatch center composed of a station and 10 electronic sirens located in the flood-prone areas of the dams (Fig. 3);
- centralized alarm systems in the localities downstream of the dams: Gilau, Floresti and Cluj-Napoca;
- mobile systems mounted on vehicles, mobile police crews.

Figure 3. Location of alarm sirens in the case study area (adapted from Google maps)

The information and decision-making system represents an essential element of the emergency situations management and includes the subsystems ensemble designed for observation, detection, measurement, recording, storage and processing of specific data, alarm, information, gathering and communication of information and decisions by all factors involved in the prevention and management of an emergency situation.

The local public administration authorities, as well as the management of the economic operators and institutions located in risk-prone areas have the responsibility to take over from the central and local monitoring stations the necessary meteorological and hydrological data and warnings in order to take preventive and responsive actions.

MATERIALS AND METHODS

The investigation methodology used in the research is CATI – Computer Assisted Telephone Interviewing. In order to assess the information and preparedness level of the population, a public survey was conducted by the use of the CATI method on the population from the Cluj-Napoca, Gilau and Floresti localities. The survey was carried out by the Romanian Strategy and Evaluation Institute (IRES) in February 2013.

The survey was conducted on adult population (+ 18 years) living in the three localities and the sample group was of the simple probabilistic type.

516 respondents were interviewed, distributed approximately to an equal extent between the urban area (Cluj-Napoca – 51% of the cases) and the rural area (Floresti and Gilau – 49% of the cases). The error margin was 4.5%.

The questionnaire was structured into 4 distinct modules. The first introductory module included 2 general, non-targeted assessment indicators of the satisfaction regarding the standard of living and one assessment indicator of the generalized confidence, all with the purpose to define a comprehensive framework for the processing and analysis of the results achieved in the main modules (2 and 3). The 2nd module included a set of indicators regarding the opinions, level of information and expected behavior in emergency situations caused by natural and technological risks. The assessment of the subjective perception of the accident risks addressed a double reference (household and place of residence) and was conducted by means of a Lickert type scale with 7 categories. Value 1 was assigned to the total absence of risk and value 7 was assigned to the certainty or near certainty that the respective risk would occur. Module 3 included a set of indicators regarding the state of health, relevant for the assessment of the social vulnerability in the investigated area. Module 4 included socio-demographic data (gender, age, nationality, marital status, education, type of household, number of persons in the household, incomes, etc.) significant for the identification of the assessed behavior variation (perception of risks, information level, behavior response in emergency situations), depending on various subpopulation categories.

RESULTS AND DISCUSSION

Regarding the population perception of hydrotechnical accident risk, the survey highlighted a rather low level of public concern (Fig. 4). Only 16% of the population considered this risk to be very high (5%) or certain (11%). 41% of the interviewed population stated that the risk was very low (13%) or nonexistent (38%). However, it is worth mentioning that when the intensity of population fear towards a certain type of risk was considered (frequency of responses appreciating a certain type of risk as being very high or high), the investigated population stated that the hydrotechnical accident risk was equally high to that of public utility failure or nuclear accidents and failures. When considering the absence of population's fears (frequency of responses appreciating a certain type of risk as being nonexistent or very low), the hydrotechnical accident was perceived as an event inducing a higher risk of occurrence than industrial accidents (50% of the responses appreciated the respective risk to be nonexistent or very low) and almost equal to the earthquake risk (45% considered this risk to be nonexistent or very low).

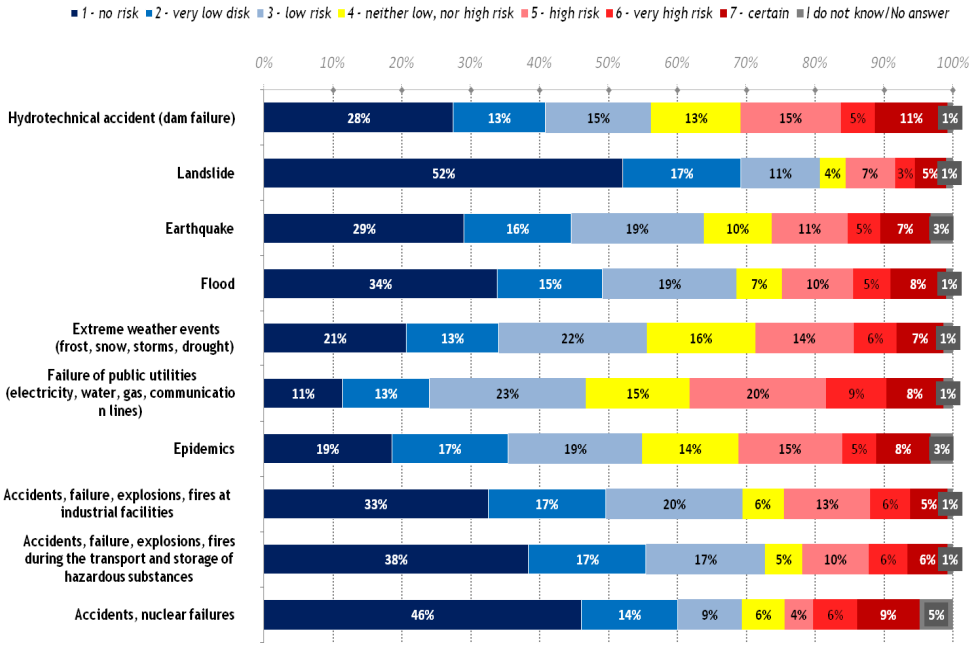


Figure 4. Population opinion regarding certain emergency situations that might damage their households

The assessment of risk perception of a population without any professional knowledge is inherently partial, fragmentary and one-dimensional, considering only the likelihood of a disaster (assessed subjectively and influenced by multiple psychological and socio-cultural variables). Such assessment did not include the gravity of consequences associated with the event occurrence. The public perception of natural or technological disaster risks was also influenced by the previous occurrence of such events. At common sense level and at popular rationale level rather that at logical level, the fact that a disaster never took place in a certain area was a sufficient proof of the very small or nonexistent chances to occur in the future.

The subjective risk perception regarding the occurrence of a disaster should be associated with the interest for the data on such risks. The questionnaire used in the survey did not include sufficient indicators to test such a hypothesis, but the data enabled us to notice the absence of an association relation between the perception of risk and the level of information of the investigated population.

Regarding the extent to which the population was informed on possible disaster risks, the existing warning methods, the knowledge of the signals and response behavior in case of disasters, the data analysis confirmed

one of the hypotheses that set the basis of this research: the activities regarding the information of population were insufficient. More than 80 % of the respondents stated that they have received no information on the risks that might cause disasters in their town/village, and neither on the warning and alarm methods and devices used in case of disasters (Fig. 5).

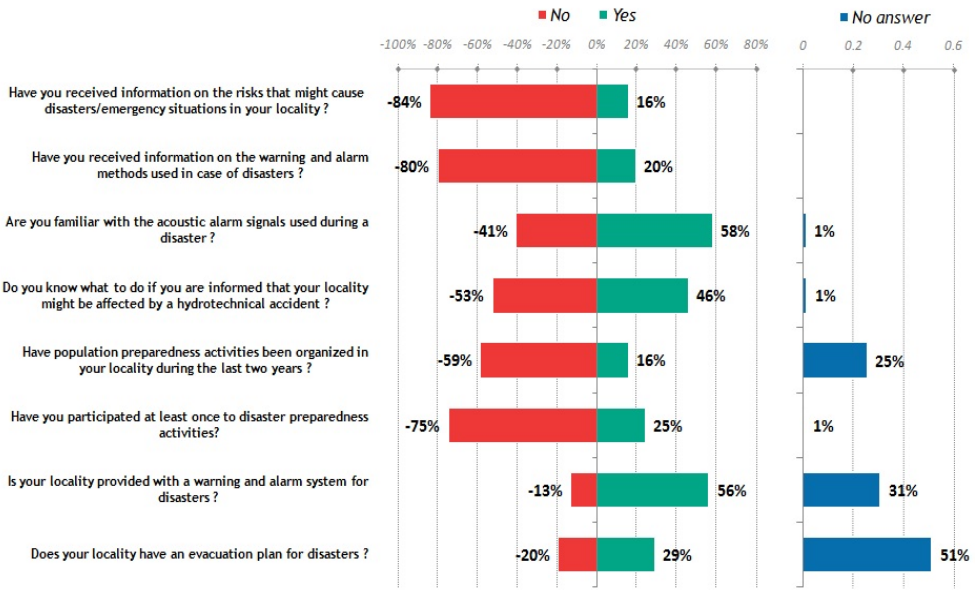


Figure 5. The level of information and preparedness of population for disasters

The main sources of information regarding risks causing disasters and the warning and alarm methods used in the event of disasters were, according to the opinions expressed by the respondents: local mass media, (52%), the city hall (41%), school and Emergency Situation Inspectorates (27 %), the Internet (25%) and NGOs (8%) (Fig. 6).

The data indicated an insufficient use of at least three entities. These had social functions (school), attributes related to the developed activities (Emergency Situations Inspectorates) and a potential to be used as a communication channel in communication campaigns (the Internet), all these enabling and ensuring the improvement of public information.

The low degree of population information was also confirmed by another indicator: despite the fact that almost 60% of the respondents declare that they were familiar with the alarm signals and the existence of a warning and alarm system of population in case of disasters within their localities, only 4% have indicated correctly the acoustic warning signal used to alarm the population in case of disasters.

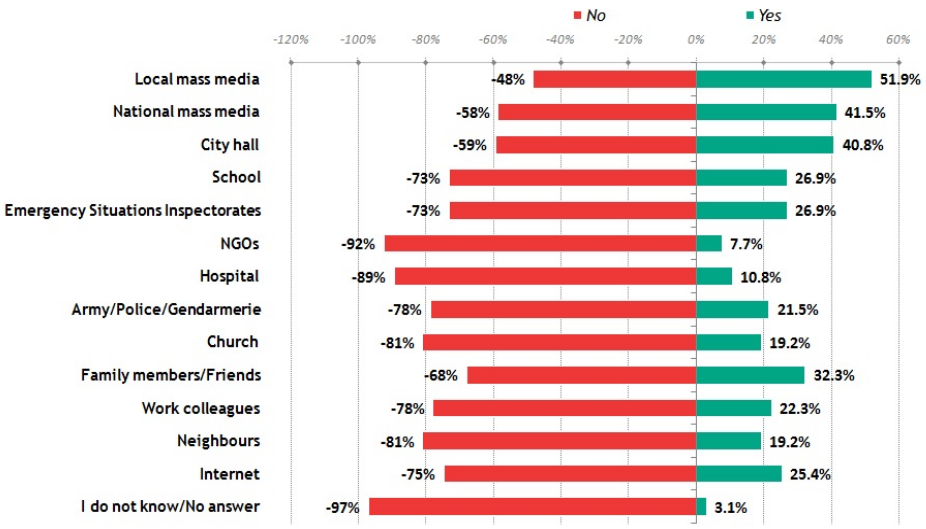


Figure 6. The information sources regarding the risks generating disasters

From the practical point of view, considering also the number of randomly correct answers, the 4% percentage signified that the level of recognizing the acoustic signal by the population was zero, with all the consequences that resulted in the event of a disaster.

The lack of population knowledge of the alarm signals represents, in itself, a powerful “alarm signal” for the public authorities and signifies the need to immediately initiate some public communication campaigns regarding the disaster information and preparedness of population. Moreover, the percentage of the population declaring that they knew what to do in the event of a hydrotechnical accident was less than half of the total investigated population (46%). In terms of the expected behavior in the event of a disaster, almost half of the subjects would choose incorrect actions. A relevant example was given by the high number (23%) of persons who declared that in case of a hydrotechnical accident they would call the emergency number 112 to receive detailed instruction, as this behavior would increase the risk that the emergency communication lines were blocked in a real situation by those waiting for instructions, therefore becoming inoperable or difficult to access for emergency response purposes.

Overall, the data indicated poor knowledge of the national (local) emergency management system, poor communication and engagement of the public institutions, as well as insufficient knowledge regarding the unitary response concept drafted at local level.

The collaboration between the community and the disaster preparedness administrative structures is essential in organizing and implementing a feasible and efficient warning system to reduce the negative effects of disasters and increase community resilience. The current research is not meant to be a comprehensive and unquestionable indicator. However, when asked directly about the quality of collaboration between the community and local authorities in the field of disaster preparedness, 63% of the respondents appreciated that this was completely missing or it was very poor.

CONCLUSIONS

One of the main conclusions resulting from the above data is that, although there is a technical alarm system implemented within the localities downstream of the hydrotechnical facilities in the Somesul Cald Valley, its efficiency is not yet confirmed. However, the nature of the causes for this situation is not technical, but it is related to the public information, communication and preparedness component. A technical system cannot be effective unless doubled by a good management of activities, by a unique concept of implementing a coherent decisional informational system and by dissemination of information to all members of the community. It is necessary that the community members understand the risks, as well as the disaster risk reduction measures, warning methods and modes of action, in order to mitigate the effects and reduce the losses.

The emergency situations management authorities should conduct population awareness building activities regarding the risks they are subjected to, while the communication, information and preparedness efforts should be directed towards these risks.

The degree to which population is informed on the existing risks, as well as the level of preparedness should be enhanced, with the active involvement of the local public authorities, the competent institutions, NGOs, and mass-media. Building communities' awareness on risks in their locality and providing them the necessary information, as well as organizing preparedness activities and encouraging active involvement are all necessary steps to be taken immediately to increase their resilience to disasters.

The revision of school curricula is also necessary, to insert topics regarding the preparedness and protection in the event of disasters, at all levels of education. Considering the special role that children have in disseminating information they learn in schools to their families, the school should provide complete and pertinent information on this topic.

Disaster preparedness is currently conducted at a general level, so that information on a wide range of natural and technological disasters is available to population. Moreover, although information is available, it does not always reach the target groups and those that should be familiar with the disaster reduction measures.

For a better protection of population in the event of hydrotechnical accidents, the implementation of several disaster risk reduction measures is necessary, such as organizing information and building awareness campaigns on types of risks and their manner of occurrence, as well as on the protective measures to be taken in such events. Organizing warning and alerting, evacuation and intervention exercises and practicing the alarm signals and the correct disaster behavior would also lead to increased community resilience to disasters.

Media campaigns on the alarm signals are also needed in the case study area, together with actions to replace the existing acoustic alarm signals with a unique one, doubled by messages sent through media and other communication ways. It is essential to correctly inform the population on the responsibilities of the 112 emergency services and their role in disaster management to increase the efficiency of response operations and, more importantly, not to hinder them.

REFERENCES

1. E. Intrieri, G. Gigli, F. Mugnai, R. Fanti, N. Casagli, *Engineering Geology*, **2012**, *147-148*, 124.
2. J. Birkmann, D.C. Seng, N. Setiadi, *Environmental Science & Policy*, **2012**, *27(1)*, S76.
3. B. Balis, M. Kasztelnik, M. Bubak, T. Bartynski, T. Gubała, P. Nowakowski, J. Broekhuijsen, *Procedia Computer Science*, **2011**, *4*, 96.
4. UNEP, Early Warning Systems: State-of-Art Analysis and Future Directions, Division of Early Warning and Assessment, *United Nations Environment Programme*, Nairobi, Kenya, **2012**, 63.
5. L. Alfieri, P. Salamon, F. Pappenberger, F. Wetterhall, J. Thielen, *Environmental Science & Policy*, **2012**, *21*, 35.
6. P. Gasparini, G. Manfredi, J. Zschau, *Soil Dynamics and Earthquake Engineering*, **2011**, *31*, 267.
7. F. Thomalla, T. Downing, E. Spanger-Siegfried, G. Han, J. Rockström, *Disasters*, **2006**, *30(1)*, 39.
8. UN, Sendai Framework for Disaster Risk Reduction 2015–2030, *United Nations General Assembly*, **2015**.
9. UNISCDR, EWC *Third International Conference on Early Warning, From concept to action*, Bonn, Germany, **2006**.

10. R. Palliyaguru, D. Amaratunga, D. Baldry, *Disasters*, **2014**, *38(1)*, 45.
11. A. Ozunu, F. Senzaconi, C. Botezan, L. Ştefănescu, E. Nour, C. Balcu, *Nat. Hazards Earth Syst. Sci.*, **2011**, *11*, 1319.
12. A. Ozunu, A. Gagiu, C. Costan, E. Nour, *NATO Science for Peace and Security Series - E: Human and Societal Dynamics*, **2011**, *80*, 3.
13. E.E. Kozma Kis, L.T. Deaconu, E. Roman, L. Ştefănescu, M. Meltzer, C. Pop, A. Ozunu, *Advances in Environmental Sciences Bioflux*, **2013**, *5(2)*, 158.
14. G.S. Leonard, D.M. Johnston, D. Paton, A. Christianson, J. Becker, H. Keys, *New Zealand Journal of Volcanology and Geothermal Research*, **2008**, *172*, 199.
15. V. Arghiuş, C. Botezan, A.C. Gagiu, I. Samara, F. Senzaconi, A. Ozunu, *Environmental Engineering and Management Journal*, **2011**, *10(1)*, 17.
16. INS, *National Institute of Statistics*, **2011**.
17. HIDROCONSTRUCTIA, *Development of River Somesul Mic Study*, **2000**, available at: http://www.hidroconstructia.com/dyn/2pub/proiecte_det.php?id=294&pg=48.
18. ISPH, The Warning – Alarm Plan of Population, economic and social objectives located downstream of the reservoirs, in case of accidents in the hidrotechnical facilities in the Somesul Mic river basin 2005-2015, (in Romanian), *S.C. Institutul de Studii şi Proiectări Hidroenergetice S.A.,* ISPH (Hydrothechnical Studies and Design Institute), Bucharest, **2005**.
19. ANAR, Romanian Waters National Administration Official website, http://www.rowater.ro/Continut%20Site/Flux%20informa%C5%A3ional%20pentru%20situa%C5%A3ii%20de%20urgen%C5%A3a.aspx, **2013**.
20. Law 481 of November 8th regarding the civil protection, *Romanian Official Gazette*, No. 1094 of November 24th, **2004**.
21. Emergency Governmental Decision no. 21 of April 15th, regarding the National Management System for Emergency Situations, *Romanian Official Gazette*, Part I, No. 361 of April 26th, **2004**.
22. Emergency Governmental Decision no. 1 regarding some measures in the field of emergency situations management and for the amendment and supplement of EGD 21/2004 regarding the National Management System for Emergency Situations, *Romanian Official Gazette*, Part I, No. 88 of February 4th, **2014**.
23. Ordinance 88 of August 30th, 2001 regarding the setting up, organization and operation of public services for emergency situations, *Romanian Official Gazette*, No. 544 of September the 1st, **2004**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 379-389)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoş
on the occasion of his 80th anniversary

A RISK ASSESSMENT STUDY FOR LOCAL CRITICAL INFRASTRUCTURES USED IN HAZMAT TRANSPORTATION

ANDREI RADOVICI[a,*], EMIL ROMAN[a], ZOLTÁN TÖRÖK[a], ALEXANDRU OZUNU[a]

ABSTRACT. Any kind of perturbation or disruption in the usual activity of the critical infrastructures (CI) in the transport sector will have immediate impact on vital social functions, health, safety, security, environment and economy, but also on other infrastructures which are dependent on the systems previously mentioned. In the recent years events occurred during the road transportation of hazardous materials have caused important losses both to humans and the environment, therefore it is strongly recommended to study the possible outcomes of such events in the process of critical infrastructure management. The complexity of an urban environment might be challenging because different variables (like traffic congestion, vehicle routes, road condition, presence of people, specific weather conditions, etc.) are contributing decisively to the effects of a possible accident, but also on the authorities response capacity. This study is focused on showing which areas in Cluj-Napoca Municipality are more prone to be affected by possible outcomes of an accident which involves a propane cargo truck. Using specific software it is possible to generate a risk map which can be a good tool to improve the decision making process for authorities.

Keywords: *critical infrastructure, hazmat transportation, modeling, risk, roads*

INTRODUCTION

A city, as a system, is prone to different types of changes in its components and in the relations between them, in order to comply with the current requirements of society. These changes support mainly the economic growth, but potential actions taken against some key aspects in the system can generate major effects in its operating capacity.

[a] *Babeş-Bolyai University, Faculty of Environmental Science and Engineering, 30 Fântânele str., RO- 400327, Cluj-Napoca, Romania*
[*] *Corresponding author: andrei.radovici@ubbonline.ubbcluj.ro*

With the increasing number of personal cars and also the increasing demand of fuel, the refueling stations network has grown dramatically in the developing cities. The process of delivering gasoline, diesel and LPG (liquid petroleum gas) to those refueling stations may consist in a risk factor for both humans and environment, but also for buildings and other infrastructures- of which some may be critical infrastructure (roads for example).

The previously mentioned petroleum compounds fall into the category of hazardous materials (hazmat - any substance or material capable of causing harm to people, property and the environment [1]) and it is known that the transportation of such substances is very well regulated with the purpose of minimizing possible economic and human losses.

Modern societies are more and more dependent on hazmats, but the inhabitants and their goods, located in the vicinity of the roads used for hazmats shipments, face the risk of suffering adverse consequences of an accident [2].

In a study, conducted in the United States, it is mentioned that a person is more likely to be killed by a lightning than by a hazardous accident in transportation [1]. Still, in recent years at international level, transportation of such explosive, corrosive, infectious, flammable, poisonous or radioactive products has caused catastrophic losses to economy and the environment [3].

In a report of Federal Motor Carrier Safety Administration [4] it is estimated that hazardous materials involved in highway crashes have a societal cost impact of more than $1 billion per year.

More than half of the total number of accidents involving hazmats transportation took place on roads [5], while the more susceptible elements are the junctions of roads and highways [6]. Human error seems to be an important triggering factor in all hazmats incidents.

Considering the fact that an urban environment is increasing the likelihood for an event to occur, mostly because of heavy traffic congestion and high population density, it is very important to study all the possible outcomes of an accident and the risk associated with hazmat transportation. In the risk management process, it is also essential to have an idea on the possible effects of an accident in order to protect the most vulnerable parts of the system and also to optimize the response of authorities in the given situation.

A graphic tool, as a map which includes a representation of risk associated with hazmat transportation, can be very helpful in order to optimize the decision making process. The initiative to create such a map may be difficult because most of the previous studies were focused primarily on routing optimization [3].

From another point of view, the Critical Infrastructure Management is, or should be strongly related to the Risk Management of hazmats transports, because unwanted effects of an accident can have destructive impact especially

on roads and on residential buildings situated in the vicinity of those roads, but also on other assets, buildings (hospitals, police stations, airports, etc.) or networks (electricity, water, etc.) which are considered Critical Infrastructures at local, regional or national level.

STUDY AREA

Cluj-Napoca Municipality houses a population of 324,576 people (2011 Census) with an average population density of 1,808 per square kilometer. The city is situated at the intersection of three European routes: E60, E81 and E576. At least 38 refill stations have been identified as operational in the city (out of which 7 are selling LPG), which gives us a ratio of 1 gas station per 8,541 people, ratio which is slightly higher than other developed cities in Romania (consequence of geographic limitations). The spatial distribution of those refill stations can be consulted in Figure 1).

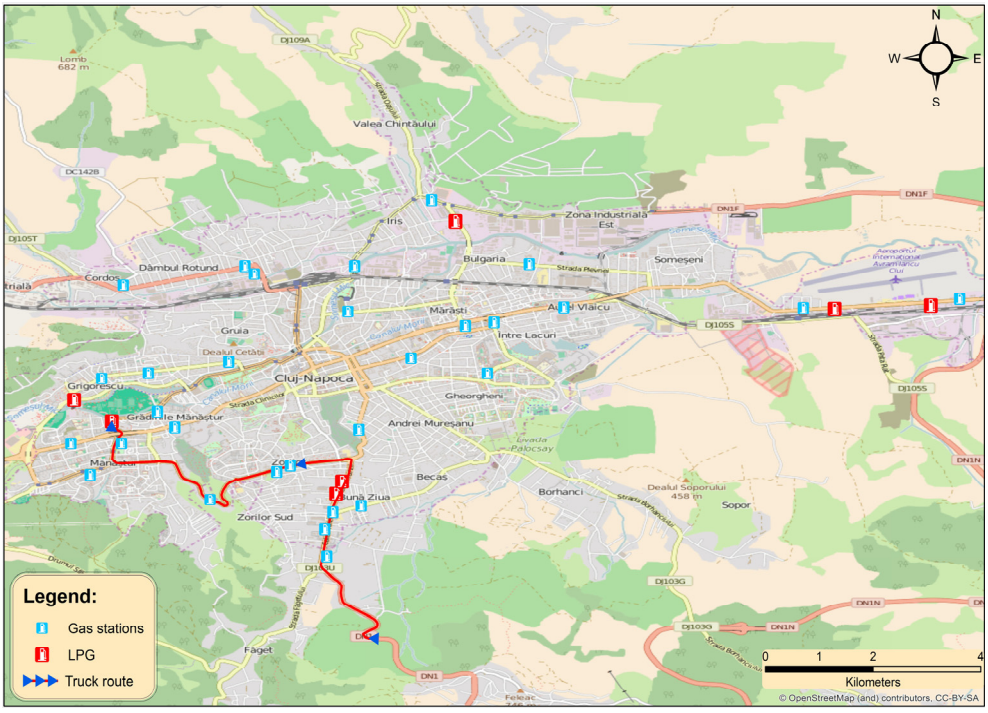


Figure 1. Spatial localization of refueling stations in Cluj-Napoca Municipality

When analyzing the previous map it is clearly deductible that the refueling stations which also provide LPG have been situated at the approximate periphery of the city and are almost equally distributed. This study will be focused on one of these stations, as the cargo trucks which are supplying it with LPG are crossing through some very dense populated areas in the city.

The lack of data available on the entire route of the cargo truck, from the city boundary to the refueling station and also some software limitation regarding the number of polygons (buildings) taken into account when running the simulation, have constrained the size of the area proposed for a detailed analysis. Also, in the procedure of selecting the best area for the study, factors like population density, traffic congestion, junctions, infrastructures, etc. have been taken into account. After a proper analysis of the entire route, the area represented in Figure 2 has been selected for analysis.

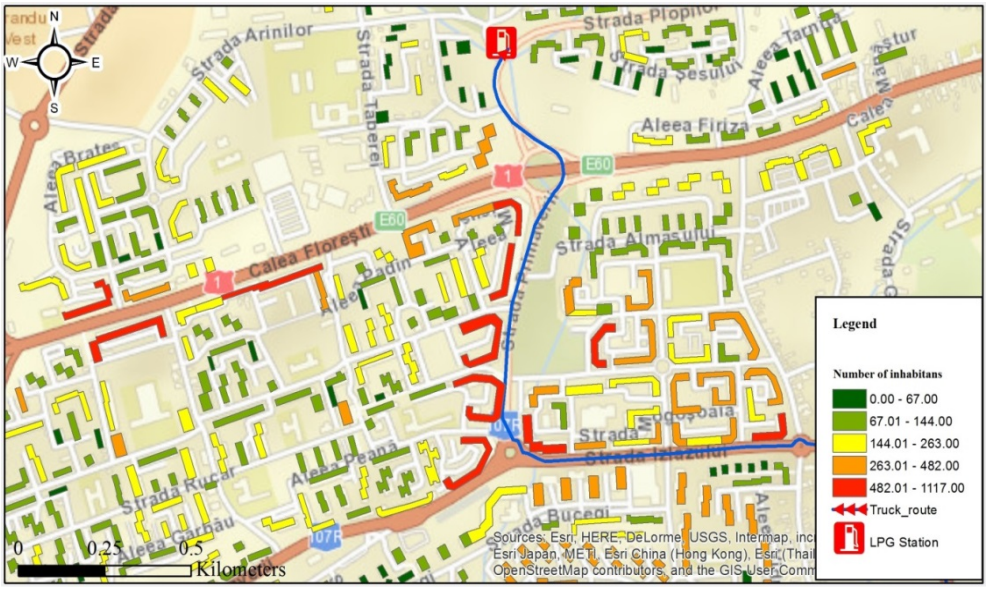


Figure 2. Study area

CRITICAL INFRASTRUCTURES

The term “Critical infrastructure” has gained a large amount of popularity in recent years and it is still a subject of debate in literature, but also for policymakers at international, national or local level.

The meaning of the term has suffered some concept reconsiderations because, in the beginning, it was referring only to “Infrastructures so vital that their incapacitation or destruction would have a debilitating impact on defense or economic security” [7], but more recent, in EU, it was transposed in the Council Directive 2008/114/EC [8] and defined as an “asset, system or part thereof located in Member States which is essential for the maintenance of vital societal functions, health, safety, security, economic or social well-being of people, and the disruption or destruction of which would have a significant impact in a Member State as a result of the failure to maintain those functions”.

The understanding of the definition given in Directive 2008/114/EC [8] can be made in such a manner that allows defining CI at local level by excluding the term of “Member State” and bringing up into discussion other levels of administration (local, regional, etc.).

Based on that understanding, in a previous study, a number of 24 Critical Infrastructures have been identified in Cluj-Napoca Municipality, grouped in 14 activity sectors [9]. When dealing with such a great number of CI, it is recommended to set a hierarchy to see which are more important in order to reduce the associated risks. A dependency-based classification shows that the most important CI at this local level is the *public road network* [9], because all the other infrastructures are dependent on it in order to maintain their functions. This is a strong argument which supports the necessity of a risk assessment study for roads as CI. Another solid argument for determining the possible consequences of an accident involving hazardous materials transported on roads, can be deducted by analyzing Figure 2: the route of the LPG cargo truck intersects the route of the European road E60 which is considered European Critical Infrastructure (“critical infrastructure located in Member States the disruption or destruction of which would have a significant impact on at least two Member States [8]) since it connects 6 UE Member States.

Other identified CI situated in the vicinity of the transport route, which can be directly related with the destructive effects of an accident are the energy distribution network, water distribution network, wastewater network, gas distribution network, IT networks, hospitals, commercial centers, financial institutions, recreational areas and public transport utilities.

RISK ASSESSMENT

Accidents involving the road transport of LPG can have multiple consequences based on the physical circumstances of the accident but also on intrinsic behavior of the LPG. The scenarios identified as possible in the

area of study are as follows: a) *LPG release*- following an accident, LPG leaks from a ruptured tank or a pipeline and gets dispersed into the atmosphere. From this point 4 other possible scenarios may result: b) LPG gets dispersed into atmosphere and forms a vapor cloud where the lower flammable limit is reached and it can result in an *unconfined vapor cloud explosion;* c) LPG vapors are ignited by an ignition source and result in a *flash fire.* Following these events it is possible for the flame to reach the release point in the LPG truck and to form a d) *jet fire* as a consequence to highly pressurized gas. The thermal radiation resulted from the jet fire can create a proper environment for e) *boiling liquid expanding vapor explosion (BLEVE)* which is caused by the rupture of the vessel containing a pressurized liquid (LPG) above its boiling point [10]. In a trunk tanker accident from Kannur, India, the entire previously detailed sequence has developed [11] which clearly demonstrate that the disaster mechanism can be replicated in the particular conditions of this study.

The worst case scenario impacting/affecting the people and structures implies a BLEVE explosion, scenario that was selected in this risk assessment. The three major effects of a BLEVE are the overpressure (caused by the vessel burst), projection of vessel fragments and thermal radiation (due to fireball).

Regarding the characteristics of the cargo trucks used to deliver LPG on the investigated route, it is supposed that there are two types of road tankers, in accordance with the European Agreement concerning the International Carriage of Dangerous Goods by Road [12]. These two categories of tankers differentiate each other by the nature of insulation: vacuum insulated or polyurethane insulated. In Table 1 some characteristics of a road tanker (polyurethane insulated) are presented, with the addition that the same type of tanker was involved in at least two other BLEVEs: in 2002 in Tivissa and in 2011 in Zarzalico [13]. Both accidents took place in Spain and in both cases there have been reported casualties and serious damages to buildings. The serious accident rate per km, regarding road tankers failures, has been estimated at a value of $2.2*10^{-7}$ [14].

Table 1. Characteristics of a road tanker [13].

Item	**Value**
Total length	14.04 m
Inner diameter	2.34 m
Outer diameter	2.6 m
Nominal total volume	56.5 m^3
LNG capacity	21000 kg
Maximum pressure service	7 bar
Vessel material	Stainless steel

Item	Value
Vessel thickness	4 mm(body)/6mm (bottom)
Insulation	Polyurethane (130mm)
Envelope	Aluminum (2 mm)
Safety valves	3 (two at 7 bar, one at 9.1 bar)

The rate was derived from data for accidents involving vehicles of over 4 tones in weight, for the period 1997-2008. A serious accident was defined as one for which cost of repair was at least £10,000 [14].

To run a simulation of a potential accident concerning LPG transport and to perform a quantitative risk analysis in the area, the "RiskCurves" software developed by TNO was used. The same software was used in the risk mapping process (displaying individual risk contours, F-N graph, overpressure contours, etc.). Models used by the RiskCurves software are based on existing models described in literature (Colored Book Series by TNO) or "may have been adapted to more recent theoretical insights" (RiskCurves Manual).

In order to perform a societal risk analysis, the RiskCurves Software requires data regarding the number of inhabitants or density distribution in the studied area and also day/night-time population. In this case the data used in the simulation is the result of the 2002 Census.

RESULTS AND DISCUSSION.

Once all the parameters described in the scenario have been introduced into the software for analysis, the individual risk (IR) map presented in Figure 3 has been generated.

According to the individual risk contours illustrated in the map, it can be noticed that a large area marked with green has an individual risk of death between 10^{-7} - 10^{-8} y^{-1}. A substantially smaller, yellow-marked area, with IR between 10^{-6} - 10^{-7} y^{-1} is also obtained on the map and it is considered to be the result of meteorological conditions (a higher probability of S-W, W wind directions) and a higher density of population.

The acceptable individual risk limit values used for land-use planning purposes, accepted in several EU member states, are 10^{-5} y^{-1} upper and 10^{-6} y^{-1} lower limits [15, 16]. In this case the individual risk does not exceed the above mentioned limits.

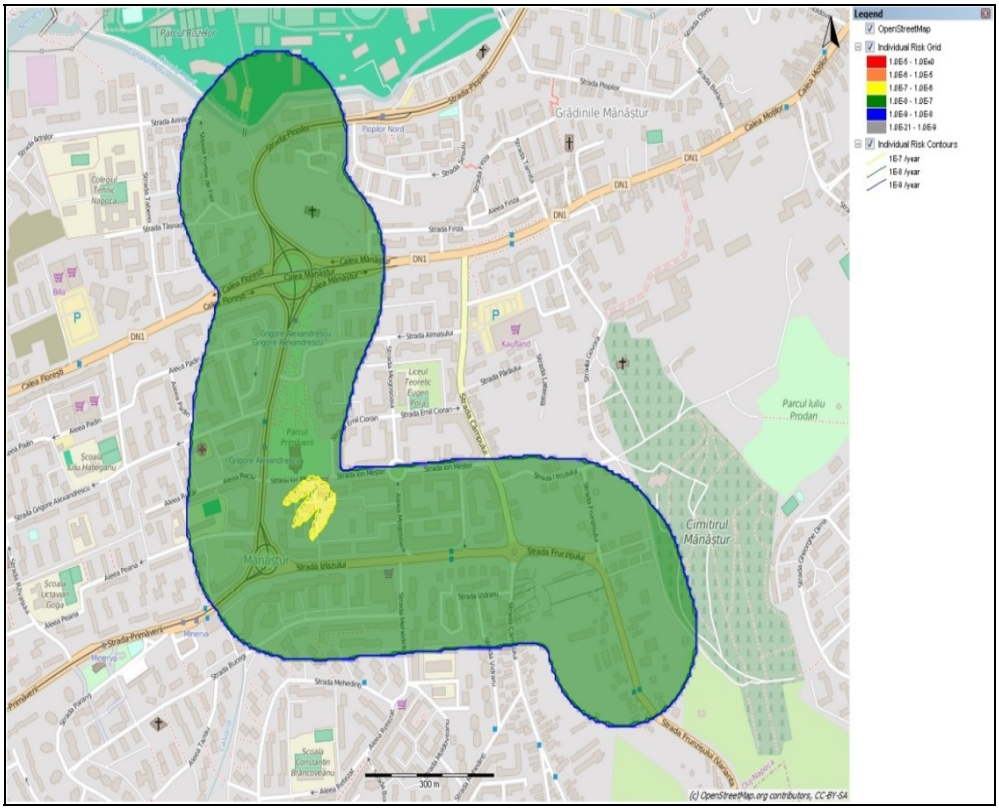


Figure 3. Individual risk map

The analysis of the societal risk curve (F-N graph), presented in Figure 4, clearly shows that the possible number of fatalities is exceeding the tolerable region, which means that the transport of LPG on the actual route should be reevaluated.

The contours for possible effects, detailed in Figure 5, are indicating that it is expected to have 1% lethality (due to heat radiation) up to 180 meters from the center of the fireball. The 10 kW/m^2 heat radiation contour is indicating the distance (329 m from the accident) on which it is possible for the exposed population to suffer 3rd degree burns. Also, on a radius of 170 m it is predictable to have damages to structures and metallic equipment due to a 37.5 kW/m^2 heat radiation.

The distance to the threshold overpressure of 100 mbar (due to vessel burst effects) has been calculated at 27.5 meters, distance at which the buildings made of reinforced concrete can suffer mild damage and multi-storey brick buildings suffer medium damage.

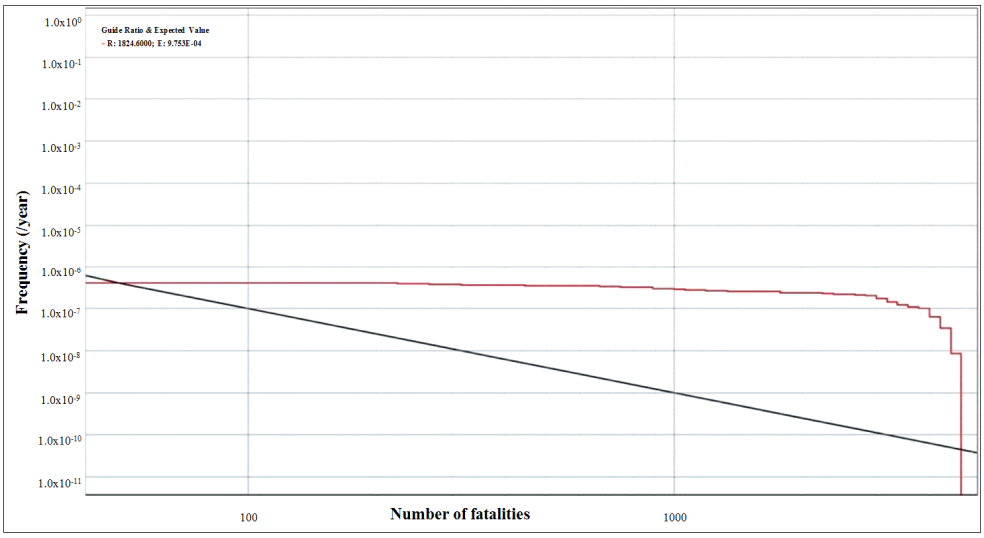


Figure 4. Societal risk curve (F-N graph)

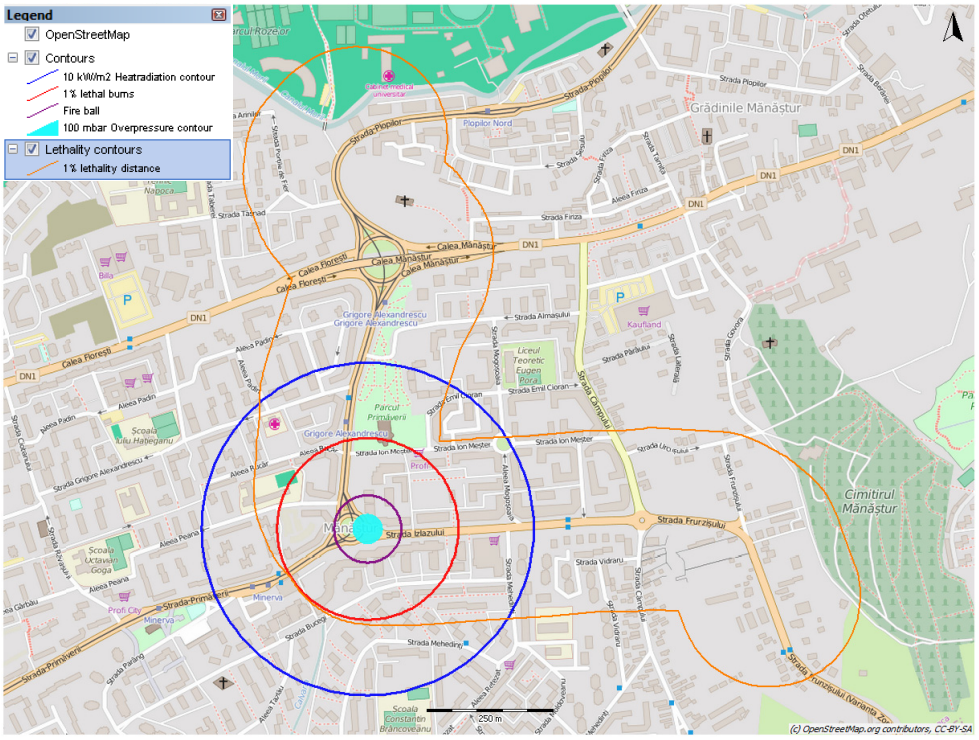


Figure 5. Physical effects map

At a distance of 100 meters from the center of the explosion windows shattering and consequent injuries are expected due to overpressure. It is also imperative to note, that on a small but important area (approximately 2.2 meters from the deflagration) the overpressure values are extremely high (7.36 bar), values at which even the road surface and underground utilities can suffer significant damages.

CONCLUSIONS

The results of this study are showing that the risk associated with the transport of the LPG on roads is generating significant risk for both population and Critical Infrastructures.

The outputs of this paper (IR map, F-N graph and the physical effects map) can be useful tools in the planning process of disaster response and in the risk management process.

This study was focused mainly on the direct physical effects of an accident on population and Critical Infrastructures, without the explicit calculation of the likelihood of transportation accidents. Indirect effects caused by the temporary disruption of facilities should be assessed in future studies. Also, the routing of the LPG cargo trucks is an important matter which needs to be debated, in order to provide a better alternative route with a lower, acceptable societal risk level.

ACKNOWLEDGEMENTS

This research was conducted using the research infrastructure purchased within the POSCCE Project entitled “Development of Research Infrastructure for HPC-Based Disaster Management” – MADECIP, SMIS CODE 48801/1862, co-financed by the European Union through the European Regional Development Fund.

REFERENCES

1. US Department of Transportation, “PHMSA (Pipeline and Hazardous Materials Safety Administration) brochure, **2012**, http://www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/phmsa_infopack.pdf (accessed 05.01.2016).
2. V. Vedat, Y.K. Bahar, *Risk Analysis*, **2001**, *21*, 1109.
3. T. Fan, W.C. Chiang, R. Russell, *Transportation Research Part D*, **2015**, *35*, 104.

4. FMCSA (Federal Motor Carrier Safety Administration), “Crashes Involving Truck Carrying Hazardous Materials”, **2004**, US OIM, http://ntl.bts.gov/lib/51000/51300/51302/fmcsa-ri-04-024.pdf (accessed 06.01.2016).
5. A. Oggero, R.M. Darbra, M. Munoz, E. Planas, J. Casal, *Journal of Hazardous Materials*, **2006**, *A133*, 1.
6. K.C. Uday, K.P. Jigisha, *Process Safety and Environmental Protection*, **2013**, *91*, Issue 4, 275.
7. B. Clinton, “US Executive Order 13010”, Federal Register, Washington D.C., **1996**.
8. European Council, “Council Directive 2008/114/EC”, Official Journal of the European Union, **2008**, L 345/75.
9. A. Radovici, L. Muntean, A. Ozunu, *Ecoterra*, **2015**, *12*(4), 75.
10. T. Kletz, “Critical Aspects of Safety and Loss Prevention”, Butterworth–Heinemann, **1990**, 43-45.
11. N. Bariha, I.M. Mishra, V.C. Srivastava, *Journal of Loss Prevention in the Process Industries*, **2016**, 40, 449.
12. United Nations, “ADR: European Agreement Concerning the International Carriage of Dangerous Goods by Road”, **2010**, https://www.unece.org/fileadmin/DAM/trans/danger/publi/adr/adr2011/English/VolumeI.pdf (accessed 16.01.2016)
13. E. Planas, E. Pastor, J. Casal, J.M. Bonilla, *Journal of Loss Prevention in the Process Industries,* **2015**, *34*, 127.
14. Z. Chaplin, “Derivation of an on-site failure rate for road tankers”, HSL internal report RSU/SR/2009/10*,* in: HSE, “Failure Rate and Event Data for use within Risk Assessments”, **2012**, chapter 3.2.2, http://www.hse.gov.uk/landuseplanning/failure-rates.pdf (accessed 11.05.2016).
15. N.J. Duijm, “Acceptance criteria in Denmark and the EU”, **2009**, http://www2.mst.dk/udgiv/publications/2009/978-87-7052-920-4/pdf/978-87-7052-921-1.pdf (accessed 31.01.2016).
16. V.M. Trbojevic, “Risk criteria in EU”, **2005**, http://www.risk-support.co.uk/B26P2-Trbojevic-final.pdf (accessed 31.01.2016).

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 391-400)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

CHEMICAL COMPOSITION OF SOME ROMANIAN BOTTLED NATURAL MINERAL WATERS

ERIKA ANDREA LEVEI[a,*], MARIA-ALEXANDRA HOAGHIA[a], MARIN SENILA[a], MIRELA MICLEAN[a], CLAUDIU TANASELIA[a], ELFRIDA MARIA CARSTEA[b]

ABSTRACT. Considering the increase of bottled mineral water consumption all over Europe, there is a growing interest related to its chemical composition and quality, especially for the elements that are not regularly monitored. This study reports the chemical composition of 21 bottled natural mineral waters available on the Romanian market. The studied mineral waters have low mineral contents (50-500 mg/l), except one, that was found to be rich in salts (>1500 mg/l). Generally, high bicarbonate contents (>600 mg/l) were found in the carbonated and partially degassed mineral waters, while the contents of sulfates and chlorides were low. The Piper diagram revealed that most of the waters are Ca-HCO_3 type. Compared to the threshold limits, all samples comply with the legislated limits for natural mineral waters and the majority complies also with the requested standards for drinking water. The determined parameters were found to be in good agreement with those reported on the label.

Keywords: *bottled natural mineral water, chemical composition, major and trace elements*

INTRODUCTION

Water may contain essential, non-essential or toxic substances with beneficial or harmful effects on consumers' health [1, 2]. Although more expensive than tap water, the consumption of bottled water increased considerably in the last decades, due to its association with purity and naturalness, to supposed good quality, taste and odor, and to effective advertising campaigns [3-7].

[a] *INCDO INOE 2000 Research Institute for Analytical Instrumentation, 67 Donath Street, RO-400293, Cluj-Napoca, Romania*
[b] *National Institute of R&D for Optoelectronics, 409 Atomistilor, RO-077125, Magurele, Romania*
** Corresponding author: erika.levei@icia.ro*

Therefore, in addition to data available on the products label, detailed information on the chemical composition of water would help consumers to make an informed choice among products and brands.

The average per capita consumption of bottled waters in Europe has an increasing trend, the most consumed being natural mineral and spring waters, representing about 97 % of the market volume in 2012 [8, 9]. Bottled waters available on market are classified as natural mineral, spring and table water. Natural mineral waters are microbiologically wholesome waters, characterized by their purity at source and their mineral content [10]. It may be subjected to separation of undesirable elements by filtration, decanting or treatment with ozone-enriched air and to total or partial elimination of free carbon dioxide by physical methods, but the addition of bacteriostatic substances or disinfecting treatments are prohibited [10]. Similar to natural mineral water, spring water must come from a specified underground source, be microbiologically safe at source without disinfection and must comply with the drinking water standards [9]. Table waters may originate from groundwater, surface water or municipal supply and are generally treated or disinfected to comply with drinking water standards [9].

Romania holds about 60 % of Europe's mineral water resources, although only about 20 % of these resources are exploited [8]. The tradition of mineral waters in these region dates back to Roman times, but its consumption is first mentioned in a 16th century document. Starting from 1806 the mineral waters from Borsec were bottled at industrial scale. Presently, in Romania, 66 varieties of natural mineral waters are recognized [11, 12], but five brands cover more than half of the market share [13].

The chemical composition of mineral waters has a large natural variation, determined mainly by the different geological settings, but different compounds may leach from the bottle material [14-16]. As bottled water consumption increased, several studies were conducted to determine the composition of mineral waters all over the world [4, 5, 17-28].

The objective of the study was to determine and compare the chemical composition of some bottled natural mineral waters available on the Romanian market in order to provide consumers information that may assist them in choosing the type of water for their daily consumption.

RESULTS AND DISCUSSION

The certified and measured values for the analyzed certified reference materials (CRMs) SRM 1643e: trace elements in water (NIST) and ERM-CA015 Hard drinking water–anions (LGC) are presented in Table 1. The measured values were found to be in agreement with the certified values, the recoveries ranging between 95-108 %.

The pH, electrical conductivity (EC), major elements and total mineral content determined as dry residue (R), together with the threshold limits established by the Romanian legislation [29] harmonized with the European legislation [30] are reported in Table 2, while the micro and trace elements contents in Table 3.

Table 1. Measured and certified concentration of metals in the certified reference materials

Parameter	Certified value	Measured value	Recovery (%)
SRM 1643e trace elements in water / (μg/l)			
Ca	32300±1100	32800±1400	102
Mg	8037±98	7990±420	99
Na	20740±260	20500±550	99
K	2034±29	2030±140	100
Al	141.8±8.6	135±11	95
As	60.45±0.72	59.8±2.2	99
B	157.9±3.9	165±8	104
Ba	544.2±5.8	583±28	107
Cd	6.568±0.073	6.4±0.4	97
Cr	20.40±0.24	19.8±1.2	97
Co	27.06±0.32	27.1±2.0	100
Cu	22.76±0.31	23.0±1.5	101
Li	17.4±1.73	18.4±1.1	106
Mn	38.97±0.45	39.6±2.9	102
Ni	62.41±0.69	63.1±4.6	101
Pb	19.63±0.21	19.2±1.1	98
Se	11.97±0.14	11.4±0.7	95
Sr	323.1±3.6	333±22	103
Zn	78.5±2.2	80.1±4.6	102
ERM-CA015 hard drinking water / (mg/l)			
Cl^-	247±8	241±8	98
F^-	1.3±0.1	1.4±0.1	108
NO_3^-	45±3	43±3	96
SO_4^{2-}	247±7	251±15	102

The charge balance errors based on the percentage difference between the total positive charge and the total negative charge, calculated according to Guller [3] was below ± 5 % for each sample.

Generally, the majority of parameters vary up to two orders of magnitude, and a few up to three orders of magnitude. The pH ranged between 6.22-7.72 and 4.46-5.85 for still and carbonated waters, respectively. In case of mineral waters with pH <6.5, corrosion may occur, favoring the release of metals

from pipes [19, 21]. The EC ranged from 85 to 2440 µS/cm, indicating a high variability of the total dissolved solids content in the studied mineral waters, thus suggesting their different source. According to dry residue, all studied mineral waters have low mineral contents (50-500 mg/l), except for one, that was found to be rich in mineral salts (>1500 mg/l). Generally, high bicarbonate contents (>600 mg/l) were found in the carbonated and partially degassed mineral waters, while high contents of sulfate (200 mg/l) and chloride (>200 mg/l) were not found.

Table 2. Dominant chemical composition (mg/l), pH, electrical conductivity (µS/cm) and dry residue (mg/l) of bottled natural mineral waters together with the threshold limits

Sample	pH	EC	R	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	NO_3^{-}	F^{-}	SO_4^{2-}
1	5.85	1630	990	257	77.6	4.30	2.50	2.36	1196	0.10	0.15	17.1
2	7.49	344	167	47.2	15.5	0.59	1.29	0.12	220	0.71	0.05	5.81
3	5.77	1650	998	163	56.2	93.8	33.4	23.7	1098	4.20	1.10	5.88
4	6.22	94	82	9.66	3.13	3.01	2.27	0.46	49	3.70	<0.01	6.70
5	6.05	2440	1580	326	101	77.5	16.2	17.3	1806	0.56	0.35	13.7
6	7.21	495	258	56.4	28.6	3.16	0.92	0.97	305	4.51	0.22	8.64
7	5.66	1090	640	152	45.9	19.3	3.64	4.10	732	2.10	0.38	12.0
8	6.92	85	66	9.80	3.11	3.00	1.30	0.45	49	4.55	<0.01	5.72
9	5.75	1450	930	296	11.2	24.3	3.80	7.76	976	0.95	0.32	31.7
10	7.72	315	140	62.5	1.73	1.11	0.57	0.17	171	3.21	0.03	7.40
11	4.46	124	61	18.8	3.67	1.47	0.78	0.72	66	3.20	<0.01	2.51
12	7.19	116	68	15.8	3.52	1.24	0.84	0.35	64	2.93	<0.01	2.70
13	5.28	1080	665	78.3	28.9	88.0	3.21	25.3	598	0.24	<0.01	36.7
14	5.76	1070	660	79.0	29.3	89.2	3.35	24.3	606	6.20	<0.01	32.8
15	5.20	507	282	92.0	7.39	2.73	0.55	1.28	336	2.40	0.03	15.2
16	7.20	477	264	85.0	7.73	1.56	0.39	0.83	293	2.46	<0.01	13.9
17	5.61	1060	681	93.0	41.1	60.3	9.07	12.7	703	2.04	<0.01	1.20
18	5.83	1030	676	91.5	40.3	58.2	8.51	12.0	693	2.20	<0.01	2.00
19	5.77	2050	1360	215	34.5	181	20.8	56.0	1361	3.24	<0.01	13.8
20	5.33	1070	623	45.6	12.1	140	7.14	118	375	18.2	0.12	31.6
21	7.55	984	574	49.2	12.6	132	7.26	107	368	22.8	<0.01	34.5
ML*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**50**	**5**	**-**
MAC**	**6.5-9.5*****	**2500**	**-**	**-**	**-**	**200**	**-**	**250**	**-**	**50**	**-**	**250**

*ML-maximum limit for natural mineral waters according to Directive 2003/40/EC [30],
**MAC-maximum admitted concentration according to Directive 98/83/EC [31],
***For bottled still water pH>4,5 while for bottled waters naturally rich in or artificially enriched with carbon dioxide, the minimum value may be lower [31].

Table 3. Trace metals content (μg/l) of bottled natural mineral waters together with the threshold limits

Sample	Li	Cr	Mn	Co	Ni	Cu	Zn	Se	Sr	Ba
1	6.9	<0.6	7.80	0.6	14.7	<2.0	2.8	2.2	350	47.4
2	<1.2	<0.6	<1.0	<0.4	<0.9	<2.0	<2.2	<1.4	49.6	6.9
3	400	<0.6	114	<0.4	<0.9	<2.0	24.0	1.6	536	943
4	1.3	<0.6	<1.0	<0.4	<0.9	<2.0	<2.2	<1.4	33.6	2.7
5	199	<0.6	119	0.8	2.5	<2.0	14.5	3.4	509	215
6	5.3	<0.6	<1.0	<0.4	<0.9	<2.0	<2.2	<1.4	73.5	17.3
7	63.1	<0.6	13.3	<0.4	<0.9	<2.0	20.0	<1.4	460	70.4
8	1.4	<0.6	<1.0	<0.4	<0.9	<2.0	<2.2	<1.4	57.8	4.5
9	92.6	<0.6	18.0	0.6	4.1	<2.0	48.8	2.9	1020	152
10	<1.2	<0.6	<1.0	<0.4	<0.9	<2.0	<2.2	<1.4	318	70.9
11	<1.2	<0.6	<1.0	<0.4	<0.9	<2.0	30.6	<1.4	14.0	17.1
12	<1.2	<0.6	<1.0	<0.4	<0.9	<2.0	7.1	<1.4	14.5	16.5
13	34.4	<0.6	213	0.7	<0.9	<2.0	7.3	<1.4	443	87.9
14	35.4	<0.6	200	0.7	<0.9	<2.0	<2.2	<1.4	424	83.7
15	1.4	<0.6	1.8	<0.4	<0.9	<2.0	66.4	<1.4	51.1	17.8
16	<1.2	<0.6	1.2	<0.4	<0.9	<2.0	<2.2	<1.4	43.1	14.7
17	190	<0.6	1.6	<0.4	1.0	<2.0	32.6	<1.4	652	136
18	181	<0.6	1.5	<0.4	<0.9	<2.0	4.6	<1.4	675	140
19	405	<0.6	3.2	<0.4	1.4	<2.0	3.7	2.4	1320	2.0
20	271	<0.6	18.6	<0.4	<0.9	<2.0	11.7	2.2	247	134
21	229	<0.6	25.0	<0.4	<0.9	<2.0	3.0	1.9	255	136
ML*	**-**	50	500	-	20	1000	-	10	-	1000
MAC**	**-**	50	50	-	20	2000	-	10	-	-

*ML-maximum limit for natural mineral waters according to Directive 2003/40/EC [30],
**MAC-maximum admitted concentration according to Directive 98/83/EC [31].

The high bicarbonate content was generally correlated with calcium contents above 150 mg/l. The high Ca content found in samples 1, 3, 5 and 9 acts as a buffer against the pH lowering in these waters. The Mg contents were below 50 mg/l, except for two samples. No samples were found with Na contents higher than 200 mg/l, thus none of the samples had salty taste. In one of the samples, the F^- content was higher than 1, without exceeding the threshold (1.5 mg/l) that requests mentioning on the label. The nitrites and phosphates were below 0.02 mg/l.

Besides the mineralogical sources, some metals may originate in the packaging material, storage tanks or pipelines [16, 32]. However, our data indicates that toxic metals (Al, As, Cd, Cr, Cu and Pb) were below the

detection limits in all samples. Generally, B is present in trace concentration in groundwater, thus higher concentrations indicate a possible anthropogenic contamination as boron compounds are used in detergents, bleaches, wood preservatives, fertilizers, herbicides, astringents, antiseptics [25], however no limit has been set for B in bottled water. In our study the highest B concentration was found both in the carbonated and in the partially degassed sorts of Roua Muntilor mineral water (8.9 and 5.8 mg/l), while values above 0.5 mg/l were found in six other samples. The low Mn contents found in the majority of samples are probably due to the Mn elimination by aeration, as increased Mn contents may cause undesirable taste and odor.

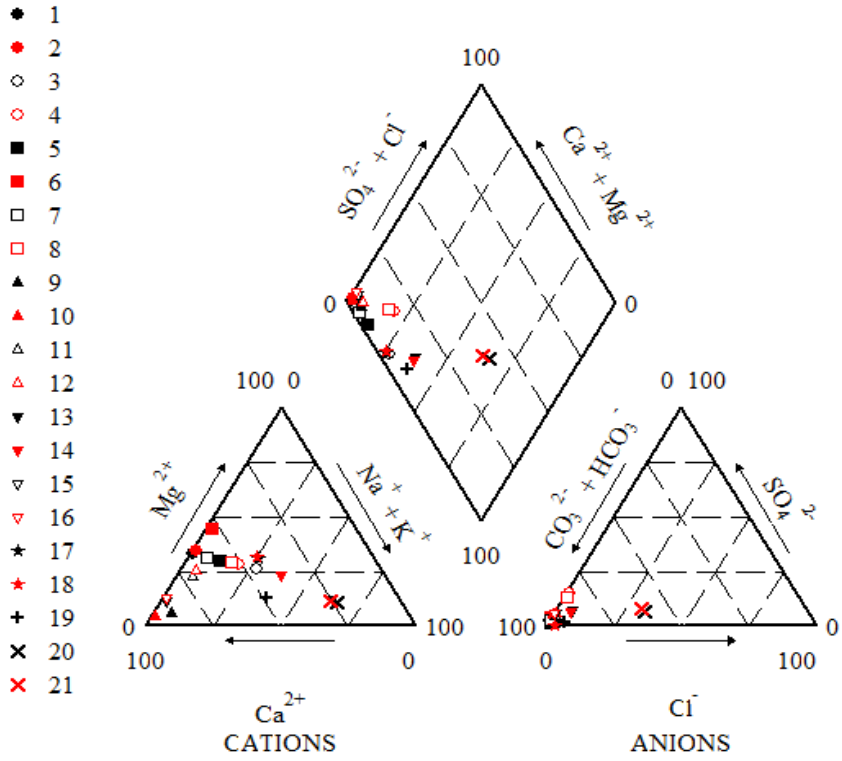


Figure 1. Piper diagram of the studied natural mineral waters

In order to identify the different water types, the main components (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-} and HCO_3^-) expressed in mEq/l were plotted on the Piper diagram (fig.1). The diagram shows that all waters are Ca-HCO_3 types, except for samples 20 and 21 which were found to be of Ca-Na-HCO_3 type.

Compared to the threshold limits, all analyzed samples comply with the legislated limits for natural mineral [30] and for drinking waters [31], except Mn that exceeded the threshold for drinking water in 4 samples.

The measured values were found to be in good agreement with those reported on the label, indicating a stable chemical composition of these waters. Values regarding trace metal contents were not provided on the label of the studied mineral waters.

CONCLUSIONS

The chemical composition of 21 bottled natural mineral waters available on the Romanian market was studied. All mineral waters have low mineral contents except for one that was found to be rich in minerals. Generally, the carbonated and partially degassed mineral waters had high bicarbonate contents. No samples were found to be with Na contents higher than 200 mg/l, thus none of the samples had salty taste. In one of the samples, the F^- content was higher than 1, without exceeding the threshold (1.5 mg/l) that requests mentioning on the label. The Piper diagram revealed that most of the waters are Ca-HCO_3 type and two are Ca-Na-HCO_3 type. The majority of waters comply with the legislated limits for natural mineral and for drinking waters, except 4 samples that exceed the Mn threshold for water intended for human consumption. The measured values were in good agreement with those reported on the label.

EXPERIMENTAL SECTION

During 2015, twenty one bottled natural mineral waters (Table 4) were bought from the Romanian varieties available on the market in Cluj-Napoca town.

Table 4. General information of the studied bottled natural mineral waters

Sample	Brand name	Source	Type*
1	Aqua Carpatica	F2 Paltinis	C
2	Aqua Carpatica	Blajenaru	S
3	Bilbor	F1	C
4	Bilbor	Q1	S
5	Borsec	Borsec	NC
6	Borsec	Borsec Faget	S
7	Bucovina	F2-Rosu, C7 Secu	C

Sample	Brand name	Source	Type*
8	Bucovina	F2-Rosu, C7 Secu	S
9	Dorna	Poiana Vinului	C
10	Dorna	Izvorul Alb	S
11	Izvorul Minunilor	Stana de vale	C
12	Izvorul Minunilor	Stana de vale	S
13	Lipova	F9bis, 8E	C
14	Lipova	F9bis, 8E	D
15	Perenna Premier	Calina-Muntii Dognecei	C
16	Perenna Premier	Calina-Muntii Dognecei	S
17	Perla Harghitei	Sancraieni	C
18	Perla Harghitei	Sancraieni	D
19	Poiana Negrii	Foraj FD	C
20	Roua Muntilor	S1, S2 Covasna	C
21	Roua Muntilor	S1, S2 Covasna	D

*NC=natural carbonated natural mineral water; C=carbonated natural mineral water; S=still natural mineral water; D=partial degassed natural mineral water.

Certified standard solutions, high purity reagents (Merck) and ultrapure water (Millipore, Milli-Q) were used for sample preparation and analyses. Certified reference materials SRM 1643e: trace elements in water (NIST) and ERM-CA015 Hard drinking water–anions (LGC) were used for the quality control of the determination of metals and anions, respectively.

The pH and EC were determined immediately after opening the bottles using a WTW 350i multiparameter. For the determination of dry residue, bicarbonates and anions, the samples were degassed by ultrasonication. For the determination of major and trace elements the samples were degassed by ultrasonication and acidified by adding 1 ml 63 % HNO_3. The HCO_3^- content was determined by titration with HCl, against methyl orange indicator, the dry residue (R) by gravimetric method while the anions (Cl^-, F^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) using a 761 ion chromatograph (Methrom). The major elements (Ca, Mg, Na, K) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), using an Optima 3500 DV (Perkin Elmer) spectrometer and the trace metals (Al, As, B, Ba, Cd, Cr, Co, Cu, Li, Mn, Ni, Pb, Se, Sr, Zn) by inductively coupled plasma mass spectrometry (ICP-MS) using an ELAN DRC II (Perkin Elmer) spectrometer. To avoid polyatomic interferences methane was used as reaction gas for of Ba, Cd, Co, Mn, Ni, Pb, Se, Sr and Zn, ammonia for Cr and Cu, and oxygen for As determination. No reaction gas was used for the determination of Al, B and Li [33].

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01 and by Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, project no. 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

1. D. Varrica, E. Tamburo, G. Dongarra, *Applied Geochemistry*, **2013**, *34*, 102.
2. M. C. Albertini, M. Dacha, L. Teodori, M. E. Conti, *International Journal of Environmental Health*, **2007**, *1*, 153.
3. C. Guler, *Chemometrics and Intelligent Laboratory Systems*, **2007**, *86*, 86.
4. R. Cidu, F. Frau, P. Tore, *Journal of Food Composition and Analysis*, **2011**, *24*, 184.
5. V. Naddeo, T. Zarra, V. Belgiorno, *Journal of Food Composition and Analysis*, **2008**, *21*, 505.
6. M. Brencic, T. Ferjan, M. Gosar, *Journal of Geochemical Exploration*, **2010**, *107*, 400.
7. M. Guadayol, M. Cortina, J. M. Guadayol, J. Caixach, *Water Research*, **2016**, *92*, 149.
8. M. J. Dumitrescu, "Mineral waters", Romanian Trade and Invest, **2012**, Available online at: http://ukrexport.gov.ua/i/imgsupload/file/Romania%20-%20Mineral%20Waters%202012r.pdf
9. The European Federation of Bottled Waters (EFBW), Available online at: http://efbw.eu/index.php
10. Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters, *The Official Journal of the European Union,* **2009**, *L164*, 45.
11. B. M. Kis, C. Baciu, *Ecoterra, Journal of Environmental Research and Protection*, **2013**, *36*, 9.
12. List of natural mineral waters recognised by member states, *The Official Journal of the European Union,* **2013**, *C315*, 66.
13. A. Feru, *Environmental Geology*, **2004**, *46*, 670.
14. A. Misund, B. Frengstad, U. Siewers, C. Reimann, *Science of The Total Environment*, **1999**, *243–244*, 21.
15. C. Reimann, M. Birke, P. Filzmoser, *Applied Geochemistry*, **2010**, *25*, 1030.
16. A. Guart, F. Bono-Blay, A. Borrell, S. Lacorte, *Food Chemistry*, **2014**, *156*, 73.
17. Z. Peh, A. Sorsa, J. Halamic, *Journal of Geochemical Exploration*, **2010**, *107*, 227.
18. E. Dinelli, A. Lima, B. De Vivo, S. Albanese, D. Cicchella, P. Valera, *Journal of Geochemical Exploration*, **2010**, *107*, 317.
19. B.S. Frengstad, K. Lax, T. Tarvainen, O. Jeager, B.J. Wigum, *Journal of Geochemical Exploration*, **2010**, *107*, 350.
20. L. Bityukova, V. Petersell, *Journal of Geochemical Exploration*, **2010**, *107*, 238.

21. M. A. Saleh, F. H. Abdel-Rahman, B. B. Woodard, S. Clark, C. Wallace, A. Aboaba, W. Zhang, J. H. Nance, *Journal of Environmental Science and Health Part A*, **2008**, *43*, 335.
22. R. W. Dabeka, H. B. Conacher, J. F. Lawrence, W. H. Newsome, A. Mckenzie, H. P. Wagner, R. K. Chadha, K. Pepper, *Food Additives & Contaminants*, **2002**, *19*, 721.
23. D. Bertoldi, L. Bontempo, L. Roberto, G. Nicolini, S. Voerkelius, G.D. Lorenz, H. Ueckermann, H. Froeschl, M. J. Baxter, J. Hoogewerff, P. Brereton, *Journal of Food Composition and Analysis*, **2011**, *24*, 376.
24. U. Fugedi, L. Kuti, G. Jordan, B. Kerek, *Journal of Geochemical Exploration*, **2010**, *107*, 305.
25. M. Birke, U. Rauch, B. Harazim, H. Lorenz, W. Glatte, *Journal of Geochemical Exploration,* **2010***, 107*, 245.
26. A. Astel, R. Michalski, A. Lyko, M. Jablonska-Czapla, K. Bigus, S. Szopa, A. Kwiecinska, *Journal of Geochemical Exploration*, **2014**, *143*,136.
27. M. Felipe-Sotelo, E. R. Henshall-Bell, N.D.M. Evans, D. Read, *Journal of Food Composition and Analysis*, **2015**, *39*, 33.
28. D. T. Udagedara, D. T. Jayawardana, *Environmental Earth Sciences*, **2015**, *73*, 7957.
29. Romanian Government Decision No. 1020/2005 for the approval of technical norms for natural mineral waters exploitation and commercialization, *The Official Gazette of Romania*, **2005**, 854 [in Romanian].
30. Commission Directive 2003/40/EC of 16 May 2003 establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters, *The Official Journal of the European Union,* **2003***, L 126*, 34.
31. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, **1998**, *Official Journal of the European Communities*, *L330*, 32.
32. P. L. Smedley, *Applied Geochemistry*, **2010**, *25*, 1872.
33. T. W. May, R .H. Wiedmeyer, *Atomic Spectroscopy*, **1998**, *19*, 150.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 401-406)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

REVISED RARE EARTH ELEMENTS COMPOSITION OF MOCS METEORITE USING HR-ICP-MS AND ICP-QMS ANALYSIS

CLAUDIU TĂNĂSELIA[a*], OANA CADAR[a], MARA CÂMPEANU[b], CONSTANTIN BALICA[b], THOMAS DIPPONG[c]

ABSTRACT. Rare earth elements composition of Mocs meteorite was analyzed using two inductively coupled plasma mass spectrometry methods: a quadrupole and a single-detector sector-field high-resolution instrument. The obtained results are in good agreement with previous studies found in literature for both Mocs and chondritic materials analysis regarding REE composition. Allende meteorite reference material was used for calibration. Sample preparation for ICP-MS analysis was carried out following a custom protocol derived from the literature: the samples were finely ground, then treated in a multi-step procedure [1]. A mix of hydrofluoric acid, nitric acid and perchloric acid was used to digest the sample, which was finally dissolved in nitric acid and diluted accordingly, before being measured directly by ICP-MS instruments. Mocs samples were made available from Museum of Mineralogy, Babeș-Bolyai University Cluj-Napoca and Allende meteorite reference sample was provided by Smithsonian Institution (split 7, position 17) [2].

Keywords: *HR-ICP-MS, ICP-QMS, meteorite, chondrite, Mocs, REE*

INTRODUCTION

Mocs meteorite, classified as L5-6 chondrite [3], fell as a shower of stones on 03 February 1882, over an area of several dozen squared kilometers, near Mocs village (now called Mociu, Cluj County, Romania, coordinates 46°48'N, 24°2'E; meteorites are usually named after the settlement closest to their recovery area and their name [4] doesn't change afterwards to reflect any future

[a] *INCDO INOE 2000 ICIA, 67 Donath, 400293 Cluj-Napoca, Romania*
[b] *Department of Geology, Faculty of Biology and Geology, Babeș-Bolyai University, 1 Kogălniceanu, 400084 Cluj-Napoca, Romania*
[c] *Department of Chemistry and Biology, Technical University of Cluj-Napoca, North University Center of Baia Mare, 76 Victoriei, 430122 Baia Mare, Romania*
** Corresponding author: claudiu.tanaselia@icia.ro*

change of settlement's name). The number of recovered fragments was estimated at 3000, with a total weight of about 300 kg [5]. According to the Meteoritical Bulletin Database, Mocs is one of the 48 approved meteorites classified as L5-6 and one of the 9 approved meteorite recovered from current Romania territory [6]. About 86% of the total number of meteorites falling on Earth are ordinary chondrites, divided into the H, L and LL groups, based on iron, nickel and other metals content [7]. From a total number of over 53000 recorded meteorites, over 46000 are classified as ordinary chondrites [8]. Our experiments concerned whole rock fragments (no crust), from the collection of the Museum of Mineralogy, Babeș-Bolyai University, Cluj-Napoca. Rare earth elements composition offers clues for a variety of geochemical and cosmochemical processes and meteorites display variations in REE concentration and abundances patterns that offers clues about their origin and past history [9].

RESULTS AND DISCUSSION

Due to sample matrix difference between samples and standard, a multiple point calibration curve was found unsuitable for these analyses. Thus, a bracketing calibration, involving a sample from Allende reference material was used. A standard sample was always run before and after each Mocs sample and sample concentration was calculated from standard know concentration and signal counts. In this way, the matrix for the samples and the standards matched, and issues like signal drifting and recovery were avoided.

Table 1. Rare earth elements composition of Mocs meteorite, using data from both high-resolution (HR-ICP-MS) and quadrupole (ICP-QMS) and methods inductively coupled plasma mass spectrometry methods

mg/kg	HR-ICP-MS	*3σ*	ICP-QMS	*3σ*
La	0.41	*0.05*	0.47	*0.05*
Ce	1.31	*0.17*	1.52	*0.17*
Pr	0.17	*0.01*	0.20	*0.02*
Nd	0.86	*0.08*	0.97	*0.11*
Sm	0.27	*0.01*	0.28	*0.03*
Eu	0.04	*0.01*	0.03	*0.01*
Gd	0.34	*0.01*	0.38	*0.04*
Tb	0.07	*0.01*	0.07	*0.01*
Dy	0.36	*0.03*	0.37	*0.03*
Ho	0.09	*0.01*	0.10	*0.01*
Er	0.26	*0.01*	0.27	*0.03*
Tm	0.04	*0.01*	0.04	*0.01*
Yb	0.26	*0.01*	0.26	*0.03*
Lu	0.04	*0.01*	0.05	*0.01*

Previous studies for rare earth content of Mocs [10] used the same meteorite as source of sample material, but different sample preparation method was used. Current method implies two spectrometric methods and a different calibration method, using bracketing and a suitable reference standard material. Data are listed in Table 1 and patterns for rare earth elements composition from Figure 1 are normalized to Solar System abundances [11]. Mocs samples appear slightly enriched than the other CI chondrites (a group of carbonaceous chondrites) reported and respectively than the reported mean for L chondrites [6]. The pattern is similar with Aumiers and Milena L-type chondrites [13], being slight enrichment in low mass rare earth elements in respect to high mass rare earth elements and negative Europium anomaly. However, the Europium anomaly of Mocs samples is more pronounced showing a strong depletion in Europium relative to the CI line, while the Dysprosium slight depletion is similar with other chondrites.

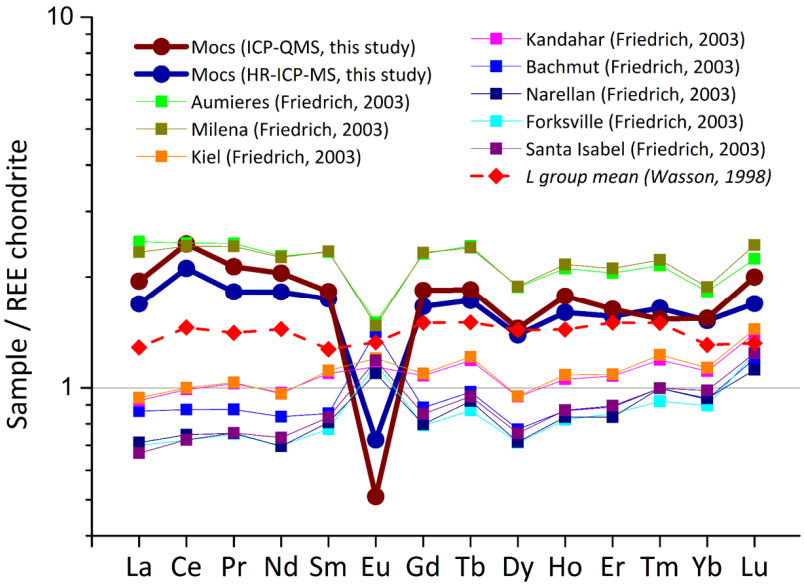


Figure 1. Rare earth elements patterns for Mocs chondrite and other chondrites [6,7], normalized to Solar System abundances

CONCLUSIONS

Despite having significant lower sensitivy than HR-ICP-MS, quadrupole ICP-MS can successfully be used for direct rare earth elements determination (microwave acid digestion of samples, without intermediary pre-concentration

steps), if a suited certified reference material is provided for calibration. Rare earth elements patterns for Mocs meteorite, normalized to solar system abundances, show a general enrichment for almost all the rare elements concentration, with the exception of Europium. Data for both HR-ICP-MS and ICP-QMS are in good agreement for all the measured elements, even if HR-ICP-MS provided up to two orders of magnitude higher sensitivity.

EXPERIMENTAL SECTION

Nu Instruments AttoM high-resolution ICP-MS (HR-ICP-MS) from Department of Geology, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, and Perkin-Elmer Elan DRC II quadrupole inductive coupled mass spectrometer (ICP-QMS) from INCDO-INOE 2000 Research Institute for Analytical Instrumentation Cluj-Napoca were used. Both instruments were carefully optimized before each sample batch, using routine procedures, providing best signal/noise ratio during analysis, thus ensuring best sensitivy. Elan DRC II was used in DRC *rf-only* mode (no gas) and pulse detector mode, providing maximum sensitivity; for reading, peak-hoping mode was selected. AttoM instrument was used on low-resolution mode and for the detector no attenuation was necessary, since the concentration were low enough not to generate high counts and trigger mode change; deflector jump mode was used for data acquisition.

Mocs samples were ground to very fine powder and 100 mg for Mocs and Allende meteorites were used for analysis. Two digested methods were performed, both involving microwaves. In the first stage, 3 mL HNO_3, 3 ml HF and 2 mL H_2O were added to the sample and then placed in the microwave oven for 30 minutes (up to 22°C in the first 10 minutes, no change for another 10 minutes, then up to 240°C for the remaining time). After cooling, the digested solutions were evaporated and dried down on a hot plate. The second stage of acid treatment consisted on 1 mL HNO_3, 2 mL HCL and 5 mL H_2O and placed again in the microwave oven for 35 minutes (up to 200°C in the first 15 minutes, then the temperature was kept constant). After cooling, the final solution was brought to a total volume of 50 mL with deionized water in a volumetric flask, ready for direct ICP-MS analysis. A blank solution was prepared and its contribution was extracted from samples detector counts (cps level). No intermediary pre-concentration steps were involved, both instruments used being able to directly measure the samples.

While for HR-ICP-MS instrument the sensitivy varied from 4079 cps/ppb for Dysprosium to 289955 cps/ppb for Lanthanum, for the quadrupole system it varied less, with only one order of magnitude, from 559 cps/ppb for

Neodymium to 3354 cps/ppb for Lutetium, but we still had measurable signal for all the elements considered for this study. Allende meteorite sample was used as a calibration standard and its counts per second values for every element was used to quantify the Mocs samples, after applying a mass correction coefficient, due to different weighted masses considered.

Results of rare earth elements analysis with both HR-ICP-MS and ICP-QMS are listed in Table 1. Results are expressed in ppm (mg/kg), with 3σ level standard deviation. In both cases, Allende certified reference material was used for calibration, using the bracketing technique, since it was the best reference material found that matched the matrix of chondritic samples. Figure 1 shows the values normalized to solar system abundances [5], together with other chondrites from L5-6 group [6] and also an average [7] for group L chondrites.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCS, project OPTRONICA IV number PN 16.40, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164 and Sectorial Operational Program for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU/159/1.5/S/132400 with the title „Young successful researchers – professional development in an international and interdisciplinary environment". Access to HR-ICP-MS instrument was possible trough RICI infrastructure project.

REFERENCES

1. J.A. Barrat, B. Zanda, F. Moynier, C. Bollinger, C. Liorzou, G. Bayon; *Geochimica et Cosmochimica Acta*, **2012**, *83*, 79.
2. E. Jarosewich, R.S. Clarke, J.N. Barrows, *Smithsonian Contributions to the Earth Sciences,* **1987**, *27*.
3. Y. Miura,G. Iancu,K. Yanai, H. Haramura, *Proceedings NIPR Symposium of Antarctic Meteorites*, **1995**, *8*, 153.
4. M.M. Grady, R. Hutchinson, A.L. Graham AL, "Catalogue of Meteorites; with special reference to those represented in the collection of the British Museum (Natural History)", 5th ed., British Museum (Natural History) UK, **2000**, pp 689.
5. K. Lodders, H. Palme, H.P. Gail, "Abundances of the elements in the solar system", Trumper, Landolt-Bornstein, New Series, VI/4B, Springer, Berlin, 2009, pp. 560–598.
6. J.M. Friendrich, M.S. Wang, M. Lipschutz, *Geochimica et Cosmochimica Acta*, **2003**, *67*, 2467.

7. J.T. Wasson, G.W. Kallemeyn, *Philosophical Transactions of The Royal Society A*, **1998**, *325*, 535.
8. C. Tănăselia, M. Miclean, C. Roman, D. Pop, *Studia UBB Chemia*, **2012**, *58*, 145.
9. Meteoritical Bulletin Database: http://www.lpi.usra.edu/meteor/index.php?sea=L5-6&sfor=types&stype=exact&lrec=50&srt=name, *retrieved 02 March 2016.*
10. B. Schmitz, G.R. Huss, M.M.M. Meier, B. Peucker-Ehrenbrink, R.P. Church, A. Cronholm, M.B. Davies, P.R. Heck, A. Johansen, K. Keil, P. Kristiansson, G. Ravizza, M. Tassinari, F. Terfelt, *Earth and PLanetary Science Letters*, **2014**, *400*, 145.
11. Meteoritical Bulletin Database: http://www.lpi.usra.edu/meteor, *retrieved 02 March 2016*.
12. N. Bauphas, A. Pourmand, *Geochimica et Cosmochimica Acta*, **2015**, *163*, 234.
13. D. Luttge-Pop, R.A. Andrei, N. Har, *Studia UBB Geologica*, **2013**, *2*, 41.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 407-414)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

LC-MS/MS METHOD FOR DETERMINATION OF L-A-PHOSPHATIDYLCHOLINE FROM SOYBEAN

DORINA SIMEDRU[a], ANCA NAGHIU[a], OANA CADAR[a], MARIUS DORDAI[a,b*], EMIL LUCA[b], IOAN SIMON[c]

ABSTRACT. The purpose of this study was to develop a quick procedure for the determination of α-phosphatidylcholine from soybean. The procedure is based on a simple solid-liquid extraction followed by a quick analytical method carried out with an LC-ESI-MS/MS system. A chromatographic column of 2×50 mm with a particle size of 2.5 µm was used. The LC-ESI-MS/MS method was developed in positive ionization mode. The analytical method was described in terms of: linearity, detection and quantification limits, accuracy and precision and matrix effect. The calibration curve was developed in the range of 5 to 200 ng/ml with a correlation coefficient r^2 of 0.995 and detection limit of 0.5 ng/ml. The extraction method was tested for the recovery degree. The recovery obtained was 97.2±1.2%. The method was used to determine the content of α-phosphatidylcholine from five soybean varieties from Romanian market. No significant differences were obtained between the five varieties regarding the α-phosphatidylcholine content.

Keywords: *lecithin, α-phosphatidylcholine, soybean, solid-liquid extraction, detection limits, accuracy.*

INTRODUCTION

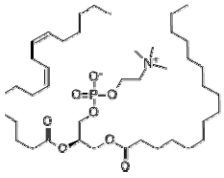
The soybean (Glycine max (L.) Merrill) is economically the most important bean in the world [1]. In February 2016, the United States Department of Agriculture (USDA) estimated that the Global Soybean Production 2015/2016

[a] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca Donath Street, no. 67, RO- 400293 Cluj-Napoca, Romania*
[b] *University of Agricultural Sciences and Veterinary Medicine, USAMV, Calea Manastur 3-5, 400372 Cluj-Napoca, Romania*
[c] *University of Medicine and Pharmacy "Iuliu Hatieganu", UMF Cluj-Napoca, 18 Republicii Street, 400015 Cluj-Napoca, Romania*
** Corresponding author: lucian.dordai@icia.ro*

will be 320.51 million metric tons [2]. 10% of this production is directed to human consumption [3, 4]. When talking about human consumption, two important aspects are taken in consideration, the nutritional value and the impact on human health. The soybean contains all eight essential amino acids and it's a good source of fiber, iron, calcium, zinc and vitamins, has no cholesterol and is low in saturated fat [5]. It also contains bioactive components such as saponins, protease inhibitors, phytic acid and isoflavones [6].

One of the important products from soybeans is the commercial lecithin, a complex mixture of phospholipids (PL) containing phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylinositol (PI), sphingolipids, triglycerides, free fatty acids and glycolipids which are widely used as natural emulsifiers, wetting agents and baking improvers [7] as well as dietetics, cosmetics and pharmaceuticals [8]. From these phospholipids, PC is more important (Table 1).

Table 1. Basic information about L-α-phosphatidylcholine [9]

Chemical name	General data		Structural formula
L-α-phosphatidylcholine	Synonyms	L-α-Lecithin, 3-sn-Phosphatidylcholine, L-α-Phosphatidylcholine	
	Chemical class	Phospholipids	
	Chemical formula	$C_{42}H_{80}NO_8P$	
	Molecular weight ($g \cdot mol^{-1}$)	758.06	

L-α-phosphatidylcholine is in the largest concentration in the membrane; it supports all metabolism and is often used under the name of lecithin [10]. Due to the importance of PC, the detection method is very important. The use of analytical methods is critical in order to certify the composition of PC containing products. Analytical method such as: Fourier transform infrared (FTIR) spectrometry [8, 11], high performance liquid chromatography (HPLC) methods with UV detection [12, 13] and evaporative light scattering detector (ELSD) detector [12, 14], matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) [15] and thin-layer chromatography and 1H, 13C, 31P NMR [8] were used intensively in past years.

The purpose of this study is to obtain a quick LC-MS/MS analytical method for determining the L-α-phosphatidylcholine from soybeans. In order to obtain good analytical data, a reliable LC-MS/MS method was

developed. The method is described in terms of retention time, linear range, linear equation, correlation coefficient, detection and quantification limits.

RESULTS AND DISCUSSION

LC-MS/MS profile

Several experiments were performed in order to establish the ionization mode, the precursor and product ions (MRM transition) and the parameters specific to the MS/MS method. For L-α-phosphatidylcholine the protonated molecule $[M+H]^+$ was proved to be more abundant and, therefore selected for further investigation. The compound dependent parameters, specific to the investigated compound and with an important contribution to the sensitivity of the method, are presented in Table 2. The source dependent parameters, with an important contribution in optimizing the compound signal in HPLC conditions, and the HPLC parameters are presented in Table 2.

The MRM (multiple reaction monitoring) transition of the pair 760.5→184.1 for 100 ng/ml of L-α-phosphatidylcholine is presented in Figure 1.

Table 2. LC-MS/MS parameters for determining L-α-phosphatidylcholine

Compound	**L-α-phosphatidylcholine**
MS/MS parameters	
Ionization mode	Pozitive
Compound dependent parameters	DP (V):86.00; EP (V):4.50; CEP (V): 30.00; CE (V):39.00; CXP (V):4.00
Source dependent parameters	CUR:15.00 psi; CAD:Medium; IS:4500.00V; TEM:600°C; GS1:25.00 psi; GS2:35.00 psi
MRM transition	760.5→184.1
HPLC parameters	
Chromatographic column	Phenomenex Synergi Fusion 2.5µm, 2×50 mm
Flow rate	0.5 ml/min
Column temperature	40°C;
Injection volume	40 µl
Mobile phase:	H_2O:CH_3CN (10:90 v/v)

Method evaluation

The LC/ESI(+)-MS/MS was evaluated in terms of retention time, linear range, linear equation, correlation coefficient, detection and quantification limits and accuracy. The obtained values are presented in Table 3. The matrix effect was -8% and was not taken in consideration for further investigations. The recovery of the extraction method was evaluated and obtained result was 97.2±1.2%.

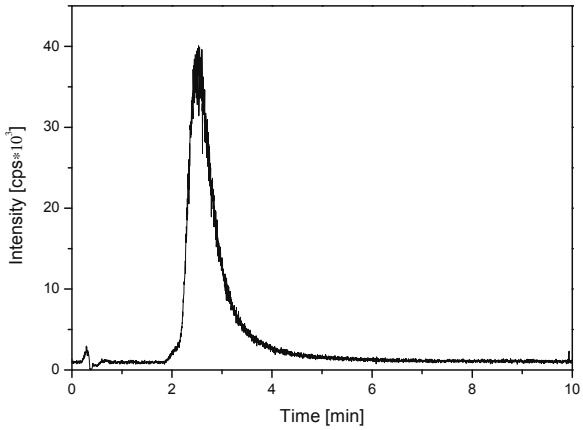


Figure 1. MRM chromatogram for 100 ng/ml of L-α-phosphatidylcholine

Table 3. Experimental and statistical parameters of LC-ESI-MS/MS method for L-α-phosphatidylcholine determination

Experimental parameters			
Retention time t_R (min)	2.54		
Linear range (ng/ml)	5-200		
Linear equation	$1.85x*10^4+976$		
Correlation coefficient r^2	0.995		
Detection limit LOD (ng/ml)	0.5		
Quantification limit LOQ (ng/ml)	1.5		
Statistical parameters			
	Concentration (ng/ml)		
	20	40	60
Intra-day			
Mean ±SD (ng/ml)	19.64±0.21	39.82±0.22	59.84±0.42
RSD (%)	1.07	0.55	0.70
Inter-day			
Mean ±SD (ng/ml)	19.51±0.25	39.77±0.31	59.79±0.45
RSD (%)	1.28	0.78	0.75

Real sample experiments

Five varieties of soybean were acquired from a Romanian market. The soybean varieties were tested with the developed LC-MS/MS method in order to evaluate the content of L-α-phosphatidylcholine. Three samples from every variety of soybeans were investigated. The results are presented in Table 4.

Table 4. Concentrations of L-α-phosphatidylcholine in the five investigated soybean varieties

Soybean variety	**L-α-phosphatidylcholine (mg/kg)**				
	1	**2**	**3**	**4**	**5**
Experimental values	2804	3351	2978	3002	3411
	2815	3354	2971	3015	3415
	2809	3348	2988	3019	3418
MEAN	**2809**	**3351**	**2978**	**3015**	**3415**

A rough analysis of the results shows that L-α-phosphatidylcholine represents ~0.3% of the soybean content. In this situation, it can be stated that the differences between the soybean varieties when spoken about L-α-phosphatidylcholine content are insignificant.

CONCLUSIONS

A LC-MS/MS method was developed for identification and quantification of L-α-phosphatidylcholine from soybean. An HPLC Agilent 1200 series coupled with an ABI Sciex 3200 QTRAP mass spectrometer with a TurboV ionization source was used in ESI positive ion mode. The chromatographic column Phenomenex Synergi Fusion 2.5 µm, 2×50 mm showed a good chromatographic peak. The specific parameters for mass spectrometer and also HPLC were identified and selected to assure the most sensitive response of the equipment. The calibration curve was developed in the range of 5 to 200 ng/ml with a correlation coefficient r^2 of 0.995 and detection limit of 0.5 ng/ml. An extraction procedure was tested and the recovery was 97.2±1.2%.

The developed method was used to test the content of L-α-phosphatidylcholine from five varieties of soybean acquired from a Romanian market. There were no significant differences between the content of the investigated soybean varieties, the content of L-α-phosphatidylcholine being ~0.3% of the soybean content in all soybean varieties.

EXPERIMENTAL SECTION

Standards and reagents

Lyophilized powder of L-α-phosphatidylcholine from egg yolk (≥99%) used to prepare the standard solutions was purchased from Sigma-Aldrich (Steinheim, Germany). Methanol LC-MS Optigrade (≥99.8%) used to prepare the stock solution and Acetonitrile LC-MS Optigrade (≥99.8%) used for mobile phase were purchased from LGC Standards. Chloroform anhydrous (≥99%) used for extraction was purchased from Sigma-Aldrich (Steinheim, Germany). Ultra pure water was obtained by using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA).

Standard solution preparation

The stock solution (1mg/ml) was obtained by dissolving 1 mg of l-α-phosphatidylcholine in 1 ml of CH_3OH. Two solutions, one of 100 ng/ml l-α-phosphatidylcholine in CH_3OH and the other of 100 ng/ml l-α-phosphatidylcholine in CH_3CN were prepared from the stock solution and used for method development and optimization. Six concentration levels of 5, 25, 50, 75, 100 and 200 ng/ml were prepared by diluting the stock solution with a mixture of H_2O/CH_3CN (10:90 v/v). These standard solutions were used for obtaining the calibration curve. Three concentration levels of 20, 40 and 60 ng/ml were prepared by diluting the stock solution with a mixture of H_2O/CH_3CN (10:90 v/v) and were used for accuracy and precision studies.

Sample extraction

The soybeans were chopped using a special grinding mill. 1ml of chloroform was added on 0.5 mg of soy. The mix was centrifuge at 10°C for 20 min using a speed rotation of 15000 RPM. The supernatant was recovered and then dried using a rotary evaporator. The solution was reconstituted with 1ml of H_2O/CH_3CN (10:90 v/v).

Analytical equipment

A high performance liquid chromatograph HPLC Agilent 1200 Series coupled with an ABI Sciex 3200 QTRAP mass spectrometer was used for this study.

LC-MS/MS profile development

The MS/MS profile is developed in three steps. First step, a solution of 100 ng/ml l-α-phosphatidylcholine in CH_3OH was injected directly in MS

in order to establish the ionization mode. Also, the parent and the product ions were selected at this step. Second step, a solution of 100 ng/ml l-α-phosphatidylcholine in CH_3OH was injected directly in MS in order to establish the compound dependent parameters (DP (declustering potential), EP (entrance potential), CE (collision energy) and CXP (collision cell exit potential)). Third step, after connecting the HPLC, a solution of 100 ng/ml l-α-phosphatidylcholine in CH_3CN was injected into the LC-MS/MS system and the source dependent parameters (CUR (curtain gas), CAD (collision gas), IS (ionspray voltage), TEM (temperature), GS1 (gas 1) and GS2 (gas 2)) were established.

The HPLC parameters: chromatographic column, flow rate, column temperature, injection volume and mobile phase were established after several experiments and finalize the LC-MS/MS method.

Method evaluation

The LC/ESI(+)-MS/MS was evaluated in terms of retention time, linear range, linear equation, correlation coefficient, detection and quantification limits, accuracy and precision.

Linearity. Six levels of concentration ranging from 5 to 200ng/ml were prepared by successive dilution with mobile phase from the stock solution. The calibration curve was obtained by plotting the peak area to corresponding concentrations. Useful information such as: linear equation and correlation coefficient were obtained.

Detection and quantification limits. Detection (LOD) and quantification (LOQ) limits were estimated by analyzing standard solutions at levels producing signals at signal-to-noise ratios of 3 and 10 respectively.

Accuracy. The intra- and inter- day accuracies were estimated by preparing three concentration levels which were used in both experiments. For intra-day study three replicas of three concentration levels were analyzed. For inter-day study three concentration levels were analyzed once per day for three consecutive days.

Matrix effect. The matrix effect was evaluated as follows. The value obtained for the analyte of interest after extraction was compared with pure solutions prepared in mobile phase containing equivalent amounts of the analyte of interest. The difference in response between the extracted sample and the pure solution multiplied by 100 and divided by the pure solution response determines the degree of matrix effect occurring to the analyte in question under chromatographic conditions [16]. The target for the matrix effect value was chose to be between -20% and +20%.

Recovery. The recovery (R) of the extraction method was determined by using the standard addition method. A sample of androsterone was

extracted and then measured (*initial amount*). Then, other sample of the same celery was spiked with a known concentration of analyte (*spiked amount*), extracted and then measured (*final amount*).

The recovery was calculated using the following equation:

R (%)=100×(final amount - initial amount)/spiked amount.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

1. A. L. Capriotti, G. Caruso, C. Cavaliere, R. Samperi, S. Stampachiacchiere, R. Z. Chiozzi, A. Lagana, *Journal of Agricultural and Food Chemistry,* **2014**, *62* (40), 9893.
2. http://www.globalsoybeanproduction.com/
3. A. P. Gandhi, I*nternational Food Research Journal*, **2009**, *16*, 11.
4. A. P. Gandhi, *World Grain (USA)*, **2006**, *February issue*, 59.
5. Q. Xu, M. Nakajima, Z. Liu, T. Shiina, "Soybean-based Surfactants and Their Applications", Soybean - Applications and Technology, Prof. Tzi-Bun Ng (Ed.), Shanghai, **2011**, chapter 20.
6. J. R. Barrett, *Environmental Health Perspectives*, **2006**, *6(114),* 353.
7. V.V. Patil, R. V. Galge, B. N. Thorat, *Separation and Purification Technology*, **2010**, *75,* 138.
8. J. V. John, H. Park, H. Lee, H. Suh, Il. Kim, *European Journal of Lipid Science and Technology*, **2015**, *117*, 1647.
9. http://www.chemicalbook.com/ChemicalProductProperty_EN_CB1242041.htm
10. http://www.bodybio.com/BodyBio/docs/BodyBioBulletin-Phosphatidylcholine.pdf
11. J. Kuligowski, G. Quintás, S. Garrigues, M. de la Guardia, *Talanta*, **2008**, *77*, 229.
12. J. Zhou, X. Hu, T. Wang, H. Liang, Q. Yuan, *Chinese Journal of Chemical Engineering*, **2012**, *20(4),* 665.
13. A.A. Carelli, M.I.V. Brevedan, G.H. Crapiste, G.H., *Journal of the American Oil Chemists' Society*, **1997**, *74 (5)*, 511.
14. P.E. Balazs, P.L. Schimit, B.F. Szuhaj, *Journal of the American Oil Chemists' Society*, **1996**, *73 (2),* 193.
15. C. D. Calvano, O. N. Jensen, C. G. Zambonin, *Analytical and Bioanalytical Chemistry*, **2009**, *394*, 1453.
16. P. J. Taylor, *Clinical Biochemistry*, **2005**, *38*, 328.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 415-422)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

DETERMINATION OF ANDROSTERONE FROM CELERY BY A NEW VALIDATED LC-MS/MS METHOD

DORINA SIMEDRU[a], ANCA NAGHIU[a], OANA CADAR[a], MARIUS DORDAI[a,b*], EMIL LUCA[b], IOAN SIMON[c]

ABSTRACT. Celery is recently the subject of various studies due to its role in human nutrition and for medicine purposes. One of the most speculated ideas is that celery contains high quantities of androsterone which makes it suitable for infertility treatments. Due to this trend, the purpose of this study was to develop an analytical method suitable to confirm and measure the quantity of androsterone from celery root. A LC-MS/MS method was developed using a Turbo V source in positive ionization mode. Analytical parameters such as: linearity, detection and quantification limits, accuracy and precision and matrix effect were evaluated. The calibration curve was developed in the range of 100 to 400 ng/ml with a correlation coefficient r^2 of 0.9968 and detection limit of 10 ng/ml. The extraction method was tested for the recovery degree. The recovery obtained was 92.1±2.2%. The method was used to determine the content of androsterone from three celery varieties from Romanian market.

Keywords: *androsterone, celery, solid-liquid extraction, detection limits, accuracy.*

INTRODUCTION

During ancient times, celery (*Apium graveolens, Apiaceae family*) was used only for medical purposes such as: treatments for colds, flu, water retention, poor digestion, different types of arthritis, and certain diseases of

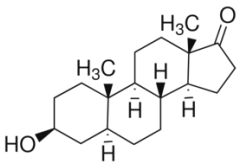
[a] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca Donath Street, no. 67, RO- 400293 Cluj-Napoca, Romania*
[b] *University of Agricultural Sciences and Veterinary Medicine, USAMV Calea Manastur 3-5, 400372 Cluj-Napoca, Romania*
[*] *Corresponding author: lucian.dordai@icia.ro*
[c] *University of Medicine and Pharmacy "Iuliu Hatieganu", UMF Cluj-Napoca, 18 Republicii Street, 400015 Cluj-Napoca, Romania*

the liver and spleen [1]. Celery begins to be used as a vegetable in Italy in the fourth century [2] and Britain by sixteenth century [3]. Nowadays, all parts of celery are used for both nutrition and medicine purposes.

It was proven so far that celery contains compounds such as: phalides, coumarrins [4], fatty acids [5], folate, potassium, molybdenum, small amounts of vitamin C, vitamin A and some B vitamins, flavonols and flavone antioxidants, androsterone [6, 7] and androstenol [6], which have many health benefits. Some of these benefits are: anti-rheumatic, hypoglycemic, sedative, antiseptic for urinary tract, blood pressure lowering, diuretic, analgesic, anti-inflammatory, detoxification, anti-spasmodic, anti-bacteria and stomach tonic [4-6]. A special attention is given to the potential of celery to have aphrodisiac effects [7] especially nowadays, when infertility is a major health problem considered to be directly related to other health problem such as coronary heart diseases and diabetes or caused by exposure to different toxic factors, chronic smoking, alcohol intake and prolonged exposure to contaminants and air pollutants [7]. Until now, few studies are available on this subject [8-10]. They tried to quantify the androsterone content by chromatographic means such as: GC-MS [8, 9] and LC-MS/MS techniques [8, 10].

The present study aims to develop an easy and reliable method for determining the androsterone (Table 1) content in celery root by means of LC-MS/MS.

Table 1. Basic information about Androsterone [11]

Chemical name	General data		Structural formula
Androsterone	Synonyms	3α-hydroxy-5α-androstan-17-one	
	Chemical class	Steroid hormone	
	Chemical formula	$C_{19}H_{30}O_2$	
	Molecular weight ($g \cdot mol^{-1}$)	290.440	

RESULTS AND DISCUSSION

LC-MS/MS profile

Several experiments were performed in order to establish the MS/MS parameters: the ionization mode, the precursor and product ions (MRM transition), the parameters specific to the MS/MS method and HPLC parameters. These parameters are presented in Table 2.

The MRM (multiple reaction monitoring) transition of the pair 291.4→273.4 for 400 ng/ml of Androsterone is presented in Figure 1.

Table 2. LC-MS/MS parameters for determining Androsterone

Compound	**Androsterone**
MS/MS parameters	
Ionization mode	Pozitive
Compound dependent parameters	DP (V):51.00; EP (V):5.50; CEP (V): 12.00; CE (V): 13.00; CXP (V):4.00
Source dependent parameters	CUR:10.00 psi; CAD:Medium; IS:5500.00V; TEM:450°C; GS1:35.00 psi; GS2:20.00 psi
MRM transition	291.4→273.4
HPLC parameters	
Chromatographic column	Phenomenex Synergi Fusion 2.5µm, 2×50 mm
Flow rate	0.5 ml/min
Column temperature	20°C;
Injection volume	40 µl
Mobile phase:	A:B (90:10 v/v); where A: CH_3CN+ 0.1% HCO_2H and B: H_2O + 0.1% HCO_2H

Method evaluation

In order to validate the developed LC/ESI(+)-MS/MS method the following parameters were evaluated: retention time, linear range, linear equation, correlation coefficient, detection and quantification limits and accuracy. The obtained values are presented in Tables 3 and 4. The matrix effect was 6% and was not taken in consideration for further investigations. The recovery of the extraction method was evaluated and obtained result was 92.1±2.2%.

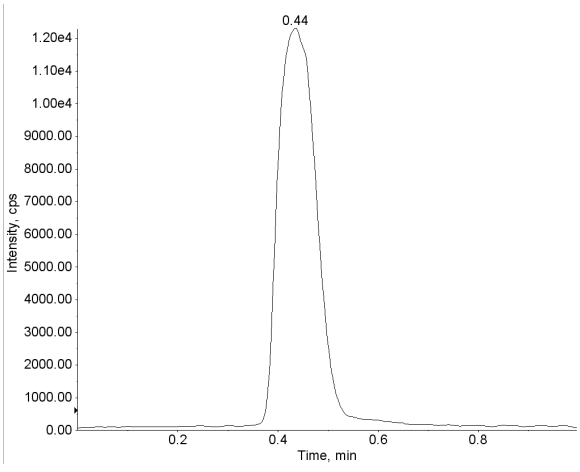


Figure 1. MRM chromatogram obtained for 400 ng/ml of Androsterone

Table 3. Experimental parameters of LC-ESI-MS/MS method for L-α-phosphatidylcholine determination

Analyte	**L-α-phosphatidylcholine**
Retention time t_R (min)	0.44
Linear range (ng/ml)	100-400
Linear equation	$1.44x+1.38*10^3$
Correlation coefficient r^2	0.9968
Detection limit LOD (ng/ml)	10
Quantification limit LOQ (ng/ml)	30

Table 4. Statistical parameters of LC-MS/MS method for Androsterone determination

Statistical parameters	**Concentration (ng/ml)**		
	140	240	340
Intra-day			
Mean ±SD (ng/ml)	135.01±0.22	238.04±0.14	337.12±0.14
RSD (%)	0.16	0.06	0.04
Inter-day			
Mean ±SD (ng/ml)	132.22±0.26	235.88±0.28	335.12±0.41
RSD (%)	0.19	0.12	0.12

Real sample experiments

Three varieties of celery root from Romanian market were tested for the androsterone content using the extraction and the LC-MS/MS methods described. The results varied from 7.44 – 12.03 mg/kg in wet celery. These results offer a first view on the Romanian celery market when referring to androsterone.

Table 5. The values obtained for androsterone in the investigated celery varieties

Celery variety	**Androsterone (mg/kg)**		
	1	**2**	**3**
Experimental values	7.44	12.05	9.81
	7.61	12.18	9.74
	7.72	12.31	9.61
MEAN	**7.61**	**12.18**	**9.74**

A rough analysis of the results shows that Androsterone represents between 0.0007% and 0.0012% from total celery which can be a considered a very low quantity but also one must have in mind that the percent of water in celery is very high.

CONCLUSIONS

A LC-MS/MS method was developed for identification and quantification of Androsterone from celery. An HPLC Agilent 1200 series coupled with an ABI Sciex 3200 QTRAP mass spectrometer with a TurboV ionization source was used in ESI positive ion mode. The chromatographic column Phenomenex Synergi Fusion 2.5µm, 2×50 mm showed a good chromatographic peak. The specific parameters for mass spectrometer and also HPLC were identify and selected to assure the most sensitive response of the equipment. The calibration curve was developed in the range of 100 to 400 ng/ml with a correlation coefficient r^2 of 0.9968 and detection limit of 10 ng/ml. An extraction procedure was tested and the recovery was of value 92.1±2.2%.

The developed method was used to test the content of androsterone from three varieties of celery acquired from a Romanian market. The results show that the investigated celery varieties have very low content of androsterone.

EXPERIMENTAL SECTION

Standards and reagents

Androsterone was purchased from Dr. Ehrenstorfer. Methanol LC-MS Optigrade (≥99.8%), acetonitrile LC-MS Optigrade (≥99.8%) and formic acid (≥99.8%) were purchased from LGC Standards. Ultra pure water was obtained by using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA).

Standard solution preparation

The stock solution (1mg/ml) was obtained by dissolving 1 mg of androsterone in 1 mL of CH_3OH. Six concentration levels of 100, 150, 200, 250, 350 and 400 ng/ml were prepared by diluting the stock solution with a mixture of CH_3CN/ H_2O (90:10 v/v). These standard solutions were used for obtaining the calibration curve. Three concentration levels of 140, 240 and 340 ng/ml were prepared by diluting the stock solution with a mixture of H_2O/CH_3CN (10:90 v/v) and were used for accuracy and precision studies.

Sample extraction

The celery root was chopped and slowly dried in an oven at 35°C. 6mL of $(CH3)_2CO$:CH_3OH (50:50, v/v) were added on 3 mg of chopped dried celery. The mixture was centrifuge for 10 min at 22°C with a speed of 4000 RPM. The supernatant was dried with a rotary evaporator and the content was reconstituted with CH_3CN:H_2O (10:90 v/v).

Analytical equipment

A high performance liquid chromatograph HPLC Agilent 1200 Series coupled with an ABI Sciex 3200 QTRAP mass spectrometer was used for this study.

LC-MS/MS profile development

The development of LC-MS/MS profile has to follow several basic steps: identifying the ionization mode, establishing the compound dependent parameters (DP (declustering potential), EP (entrance potential), CE (collision energy) and CXP (collision cell exit potential)), establishing the source dependent parameters (CUR (curtain gas), CAD (collision gas), IS (ionspray voltage), TEM (temperature), GS1 (gas 1) and GS2 (gas 2)), choosing the optimal MRM (multiple reaction monitoring) transition, identifying the appropriate chromatographic column (the dimension of the particles, its length and diameter) and establishing the HPLC parameters. Only C18 columns were tested.

All these parameters have important roles in obtaining a strong and sensitive analytical method.

Method validation

In order to validate the developed LC/ESI(+)-MS/MS the following parameters were evaluated: retention time, linear range, linear equation, correlation coefficient, detection and quantification limits, accuracy and precision.

Linearity. Six levels of concentration ranging from 100 to 400ng/ml were prepared by successive dilution with mobile phase from the stock solution. The calibration curve was obtained by plotting the peak area to corresponding concentrations. Useful information such as: linear equation and correlation coefficient were obtained.

Detection and quantification limits. Limit of detection (LOD) and limit of quantification (LOQ) were estimated by analyzing standard solutions at levels producing signals at signal-to-noise ratios of 3 and 10 respectively.

Accuracy. The intra- and inter- day accuracies by preparing three concentration levels which were used in both experiments. For intra-day study three replicas of three concentration levels were analyzed. For inter-day study three concentration levels were analyzed once per day for three consecutive days.

Matrix effect. The evaluation of matrix effect is very important in chromatography because it can cause signal suppression. In this study the target for matrix effect was proposed to be between -20% and +20. The value obtained for the analyte of interest after extraction was compared with pure solutions prepared in mobile phase containing equivalent amounts of the analyte of interest. The difference in response between the extracted sample and the pure solution multiplied by 100 and divided by the pure solution response determines the degree of matrix effect occurring to the analyte in question under chromatographic conditions [11]. For this method, the value obtain was 6%.

Recovery. The recovery (R) of the extraction method was determined by using the standard addition method. A sample of celery was extracted and then measured (*initial amount*). Then, other sample of the same celery was spiked with a known concentration of analyte (*spiked amount*), extracted and then measured (*final amount*).

The recovery was calculated using the following equation:

R (%)=100×(final amount - initial amount)/spiked amount.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme “Increase of Economic Competitiveness”, Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

[1]. http://umm.edu/health/medical/altmed/herb/celery-seed;
[2]. A. Kokotkiewicz, M. Luczkiewicz, “Essential Oils in Food Preservation, Flavor and Safety, Flavor and Safety”, Elsevier B.V., London, **2016**, chapter 37.
[3]. G. Tobyn, A. Denham, M. Whitelegg, “The Western Herbal Tradition: 2000 years of medicinal plant knowledge”, Elsevier B.V., Edinburgh, **2011**, chapter 9.
[4]. M. Modaresi, G. Ghalamkari, A. Jalalizand, *APCBEE Procedia*, **2012**, *4*, 99.
[5]. H. B. Sowbhagya**,** *Critical Reviews in Food Science and Nutrition*, **2014**, *54(3),* 389
[6]. http://www.livescience.com/50640-celery-nutrition.html
[7]. M. A. Shalaby, H. Y. El Zorba, *Global Veterinaria*, **2010**, *5(2),* 122.
[8]. N. Krone, B. A. Hughes, G. G. Lavery, P. M. Stewart, W. Arlt, C. H.L. Shackleton, *The Journal of Steroid Biochemistry and Molecular Biology*, **2010**, *121(3-5)*, 496.
[9]. T. Trinh, N. B. Harden, H. M. Coleman, S. J. Khan, *Journal of Chromatography A*, **2011**, *1218*, 1668.

[10]. Y. C. Ma, H. Y. Kim, *Journal of the American Society for Mass Spectrometry,* **1997**, *8(9)*, 1010.
[11]. https://en.wikipedia.org/wiki/Androsterone
[12]. P. J. Taylor, *Clinical Biochemistry*, **2005**, *38*, 328.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 423-429)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

COMPARATIVE STUDIES BETWEEN CLASSICAL AND MODERN SAMPLING TECHNIQUES TO IDENTIFY THE CONTAMINANTS FROM ENTOMOLOGY ITEMS

DORINA SIMEDRU, ANCA NAGHIU, MARIUS ROMAN[a*], MIRELA MICLEAN, ANA BORGOVAN

ABSTRACT. Preservation of old entomology items, although very important, is very difficult due to the contamination which they were subjected. In order to test the items contamination level with the classical sampling method, one must be very careful because they are very easily to break and can develop mold spores due to the moistening. The subject of this study is to recreate in the laboratory the stages of preserving the entomology samples by using petroleum products and naphthalene. Then the samples are subjected to two types of sampling, the classical sampling and a new sampling using a special pump for air sampling. After a month in which the items were kept in controlled environment, the sampling procedure was performed and the sample were analyzed. The results showed differences in the results obtained by two sampling techniques. The classical method proved to be more efficient but the items which were studied presents several defects.

Keywords: *sampling procedure, museum, entomology items, naphthalene, total petroleum hydrocarbons*

INTRODUCTION

Many museums and academic institutions maintain first-rate collections of biological materials [1]. These biological collections make innumerable contributions to science and society in areas such as: homeland security, public health and safety, monitoring of environmental change, and

[a] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca Donath Street, no. 67, RO- 400293 Cluj-Napoca, Romania*
[*] *Corresponding author: marius.roman@icia.ro*

traditional and systematic taxonomy [1]. For these reasons, the preservation of these collections is very important. The key to long-term preventive conservation in natural history collections is to control the collection environment [2]. Although, nowadays, the biggest museum have implemented integrated pest management techniques or have created new museum spaces with very strict environmental conditions, there are a large number of museum which, despite the current evidence that the use of chemical is declining, are still using pesticides for collection vulnerable to pest activity [3]. “Pesticides” such as: arsenic, mercury, naphthalene, paradichlorobenze and dichloro-diphenyl-trichloroethane (DDT) [3], para-dichlorobenzene, ‘Vapona’ and naphthalene are used since the late 18th century and are still used in some museum [4].

Generally, when talking about museum pollution, museum restorers are thinking about impurities in the environment which may come from natural or man-made source [5]. Their studies are generally focused on airborne sources of pollution, both gaseous and particulate and they are considered to be the primary agents destructive of work of arts [5]. Mainly, the restorers are trying to identify the sources of VOCs, NO_2, SO_2 and O_3 neglecting the pollutants with high impact on human health such as petroleum products (TPH) or naphthalene.

In order to correctly quantify these pollutants a proper sampling is required. In earlier times, the most used procedure for sampling the museum contaminants consists in wiping the surfaces with cotton gauzes wet with distillate water [6, 7]. The procedure described is nowadays generally used for sampling metals such as zinc [8] or for arsenic and mercury based pesticides [9]. In other study, the authors split the pesticides into volatile and non-volatile compounds and the sampling is made accordingly: for volatile compounds they are using solid phase microextraction (SPME) sampling apparatus and for non-volatile compounds they are still wiping the surface of the museum items with cotton swabs [10]. Besides these procedures (active sampling), there are several others procedures which can allow to investigate the accumulation of the pollutants in longer period of time (passive sampling) [11]. The traditional procedure, the wiping procedure, can damage a significant part of museum items comparing to the newer procedures which are safer but more expensive.

This study tried to re-create in laboratory the stages of preserving the entomology samples by using petroleum products and naphthalene. The purpose was to identify a simple sampling method more suitable for entomology items comparing with the traditional one. This sampling method should allow the obtaining of good analytical results without destroying the sample or put it under any risk conditions and, in the same time, to be cheaper than sophisticated methods presented above.

RESULTS AND DISCUSSION

The results obtained for both experiments are presented in Table 1. It can be observed from all data that the value of standard deviation is low. That means that the distribution of the results for every sampling is close to the mean.

The following notation will be done: S1 refers to pump sampling technique and S2 refers to classical sampling technique.

Observing the data, there are several relations that can be followed:

1. The relation between the values of TPH obtained with both sampling techniques, for fresh leaves;
2. The relation between the values of TPH obtained with both sampling techniques for dried leaves;
3. The relation between the values of TPH obtained with first sampling technique, for fresh and dried leaves;
4. The relation between the values of TPH obtained with second sampling technique, for fresh and dried leaves;
5. The relation between the values of naphthalene obtained with both sampling techniques, for fresh leaves;
6. The relation between the values of naphthalene obtained with both sampling techniques, for dried leaves;
7. The relation between the values of naphthalene obtained with first sampling technique, for fresh and dried leaves;
8. The relation between the values of naphthalene obtained with second sampling technique, for fresh and dried leaves;

As it can be observed, for both contaminants TPH and Naphthalene, when comparing the same type of leaves (fresh or dried) the values are much lower for S1 comparing with S2. This result can be due to both: the sampling time using S1 is too short, or an interaction between TPH particles and the surface of the leaves is too strong. When comparing different types of leaves, fresh with dried, it can be observed that both S1 and S2 are higher for dried leaves. This could be due to the fact that the contaminants didn't connect with the structure of the leaves as when they were fresh. These results can suggest that the condition (fresh or dried) of the leaves in the moment they are treated is very important for the step of identifying the contaminants. It is supposed that in time, in function of the initial condition of the leaves in the moment of treatment, other chemical interactions can appear such as a possible crystallization of naphthalene which will send to the surface of the leaves also other contaminants.

The main idea which can be drawn until know is that the classical sampling technique is more efficient especially when the treatment was performed on dried leaves, although this sampling method presents a real danger for entomology items.

Table 1. The results obtained for both experiments, according to the sampling technique used on fresh or dried leaves.

No.	TPH				Naphtalene			
	Fresh leaves		Dried leaves		Fresh leaves		Dried leaves	
	S1	S2	S1	S2	S1	S2	S1	S2
1.	8.34	35.7	13.7	140.03	110.25	449.02	129.32	468.38
2.	8.65	34.8	12.98	139.25	115.68	451.06	125.77	471.22
3.	8.02	35.02	13.04	139.36	118.52	449.22	130.21	469.02
4.	7.89	36.03	13.18	140.21	117.69	452.04	124.54	473.51
5.	8.64	35.22	12.88	140.15	112.45	455.88	129.05	465.85
6.	8.54	35.35	13.56	138.26	122.01	450.02	122.01	471.11
7.	7.93	34.97	13.87	140.03	119.38	452.42	128.44	472.32
8.	9.00	35.25	13.22	140.64	114.22	449.83	127.54	469.88
9.	8.12	35.74	13.05	140.32	119.05	447.65	122.92	471.53
10.	7.81	34.88	13.72	139.99	117.89	450.21	122.31	473.22
Mean	**8.23**	**35.23**	**13.20**	**140.03**	**117.79**	**450.11**	**126.65**	**471.16**
StDev	**0.40**	**0.41**	**0.36**	**0.69**	**3.55**	**2.29**	**3.11**	**2.36**

One has been observed, during the performed experiments, that the dried leaves are very sensitive and very easily to break with the traditional method. Also, once dried, the sample is subject to the possibility of developing mold spores.

Several other experiments must be performed to change the traditional method with the pump sampling. For the beginning we are planning to investigate the effect of different time of sampling for S1.

CONCLUSIONS

The study presents the results obtained by two experiments developed with the purpose to find a safer way to determine the contamination levels on museum entomology items comparative to the traditional method.

In order to reduce the possibility of damaging the museum items to be investigated, two experiments were performed. The difference between the two experiments was given by the sampling procedures which were: the classical procedure, consisting in cleaning the items with sterile gauze pad inserted before in distilled water, and the second, consisting in aspirating the items with the sampling pump.

The experiments were performed on fresh and dried leaves contaminated with TPH and naphthalene. The classical sampling method was proved to be more efficient with the known disadvantage, the danger to destroy the items. Also, both sampling techniques were more efficient on dried samples.

The final conclusion to be drawn from this study is that more experiments should be performed, including on real museum items to improve the pump sampling techniques or to find a correlation factor between the two sampling techniques so as to in the future, the classical method to be replaced.

EXPERIMENTAL SECTION

Materials, standards and reagents

Sterile gauze pad acquired from a local drug store and 0.45 µm paper filters acquired from Sensidyne were used for sampling purposes.

BAM-K009 Lubricating oil (type B) was acquired from LGC Standards, Germany. N-heptane Picograde® for residue analysis, n-decane and n-tetracontane were acquired from LGC Standards, Germany. Florisil (60-100 mesh) was acquired from Meck, Darmstadt, Germany. Crude oil was acquired from local market.

PAH Calibration Mix in Acetonitrile was acquired from Supelco. Cyclohexane for HPLC (purity ≥99.9%), Acetonitrile Chromasolv gradient grade for HPLC (purity ≥99.9%) was acquired from Sigma – Aldrich. Naphthalene was acquired from local market. The ultra-pure water was obtained with a Milli-Q water purification system from Millipore.

Extraction methods

In order to extract the Total Petroleum Hydrocarbons (TPH), the paper filters and sterile gauze pads used for sampling were ultrasonically extracted in n-heptane then the extract was purified with Florisil, 60-100 mesh. A volume of 1 µl aliquot of the final solution was injected in the gas chromatograph in splitless mode, using He as carrier gas.

In order to extract the naphthalene, the paper filters and sterile gauze pads which were used for sampling were left for 24 hours in cyclohexane then the extract was purified with Florisil, 60-100 mesh. The remaining solution was dried using a rotary evaporator and then reconstituted with 1mL of Acetonitrile.

Analytical equipment

The sampling was performed using a Gilian GilAir Plus by Sensidyne pump with a flow of 2 ml/min for 2 min.

The TPH analysis was performed with a gas chromatograph (Agilent 7890N) coupled with a flame ionization detector (FID) and equipped with automatic liquid sampler (HP Model 7673) using He as carrier gas and a HP-5 fused silica capillary column from J&W Scientific.

The naphthalene analysis was performed with Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with FLD detector, using a ZORBAX Eclipse PAH 5µm, 4.6×150 mm chromatographic column from Agilent and a gradient mobile phase of Acetonitril and Water.

Experimental procedure

In order to reduce the possibility of damaging the museum items to be investigated, two experiments were performed. The difference between the two experiments was given by the sampling procedures which were: the classical procedure consisting in cleaning the items with sterile gauze pad inserted before in distilled water, and the second consisting in aspirating the items with the sampling pump.

Experiment no. 1. 20 fresh leaves and 20 dried leaves contaminated on purpose with the same quantity of commercial crude oil were inserted in desiccators for 1 month. 10 fresh leaves and 10 dried leaves were easily cleaned with sterile gauze pad inserted before in distilled water and 10 fresh leaves and 10 dried leaves were aspirated with the sampling pump. The pump was preset to a flow of 2 ml/min. The sampling time (2 min) was given by the capacity of the operator to cover the entire leave. The samples were prepared further for TPH analysis and analyzed according to a previous developed method [12].

Experiment no. 2. 20 fresh leaves and 20 dried leaves were inserted in a controlled environment which was contaminated on purpose with commercial naphthalene were inserted in desiccators for 1 month. 10 fresh leaves and 10 dried leaves were easily cleaned with sterile gauze pad inserted before in distilled water and 10 fresh leaves and 10 dried leaves were aspirated on a filter with the sampling pump. The pump was preset to a flow of 2 ml/min. The sampling time (2 min) was given by the capacity of the operator to cover the entire leave. The samples were prepared further for Naphthalene analysis. The sterile gauze pads and the filters were inserted in distillated water for 24 hours and then the waters were analyzed according to a previous developed method [13].

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

1. A. V. Suarez, N. D. Tsutsui, *BioScience,* **2004,** *54(1),* 66.
2. D. J. Carter, A. K. Walker, "Collection environment", Oxford: Butterwoth Heinemann, **1999,** chapter 7.
3. http://www.amnh.org/our-research/natural-science-collections-conservation/general-conservation/health-safety/residual-pesticides.
4. M. J. Linnie, M. J. Keatinge, *International Biodeterioration & Biodegradation,* **2000,** *45(1/2),* 1.
5. P.B. Hatchfield, "Pollutants in the Museum Environment", Archetype Publications Ltd., London, **2002**, chapter 1.
6. https://www.bnl.gov/esh/shsd/sop/pdf/ih_sops/ih75190.pdf
7. http://collections.rmsc.org/LibCat/links/Surface_wipe_procedure.pdf
8. N. Odegaaed, A. Sadongei, "Old Poisons new problems – A museum resource for managing contaminate cultural materials", Walnut Creek: Altamira Press, 2005.
9. P. Reuben, *Collection Forum*, **2006**, *20(1-2),* 33.
10. P. Jane Sirois, J. S. Johnson, A. Shugar, J. Poulin, O. Madden, "Preserving Aboriginal Heritage: Technical and Traditional Approaches", Minister of Public Works and Government Services, Canada, **2008**.
11. I. D. Rushworth, C. Higgitt, M. Smith, L. T. Gibson, *Heritage Science*, **2014**, *2*, 3.
12. M. Şenilă, E. Levei, L. Şenilă, O. Cadar, M. Roman, M. Miclean, *Studia UBB Chemia*, **2015**, *LX, 2, Tom I,* 137.
13. A. Naghiu, D. Simedru, M. Miclean, L. Senila, M. Roman, C. Laslo, "The 17th international symposium on analytical and environmental problems", Szeged, Ungaria, **2011**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 431-440)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

DETERMINATION OF THE ORGANOCHLORINE PESTICIDE RESIDUES CONTENTS IN GRAPES BY SBSE-TD-GC-ECD ANALYSIS

IOAN SIMON[a], MIRELA MICLEAN[b*], OANA CADAR[b], LĂCRIMIOARA SENILA[b]

ABSTRACT. Stir bar sorptive extraction (SBSE)-thermal desorption (TD) procedure combined with gas chromatography electron capture detection (GC–ECD) was applied to the determination of 20 organochlorine pesticides (OCPs) in six white Romanian grape varieties. Analyses were performed using stir bars coated with 1.0 mm polydimethylsiloxane. The method provided satisfactory analytical performance to monitor OCPs in grape matrices at the trace level. By using the standard addition methodology, good linearity (r^2>0.99) was found for all cases, depending on the particular OCP and also good sensitivity was achieved for all the investigated OCPs in agreements with the European Union regulations for the maximum residue limits (MRLs) of pesticides in agricultural vegetables. The method has multiple advantages, such as: simplicity, almost solventless and requires low sample amount, in comparison with conventional methods of sample preparation to analyse pesticides in vegetable matrices. The obtained results showed that OCPs were detected in all the investigated grape samples, with total contents varied between 0.32 µg/kg and 3.48 µg/kg, the concentrations were much lower than their specific MRLs.

Keywords: *organochlorine pesticides, stir bar sorptive extraction, GC-ECD, grapes*

INTRODUCTION

Pesticides are widely used in fruit and vegetables growing, because of their susceptibility to insect and diseases attacks. Most of organochlorine pesticides have been banned in many countries because their toxicity to

[a] *University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Department of Surgery, 18 Republicii Street, 400015 Cluj-Napoca, Romania, isimon@umfcluj.ro*
[b] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, 400293 Cluj-Napoca, Romania.*
[*] *Corresponding author: mirela.miclean@icia.ro*

humans, but because of their considerable stability in the environment (as long as 30 years), their residues still appear as contaminants in food, as well as in the environment [1, 2].

Organochlorine pesticides (OCPs) are the most persistent organic contaminants in the environment, being classified as persistent organic pollutants (POPs) due to their ubiquity, persistence and bioaccumulation in the environment [3]. The high toxicity of OCPs poses significant threats to human health and biodiversity [4]. Recent reports identified an association between exposure to pesticides and different types of human cancer [5]. Many of the OCPs are known as endocrine disruptors, also they cause immune suppression and inhibit various enzymes. DDT has been reported to affect neurobehavioral functions and to be associated with premature births [6].

Agricultural soils are important reservoirs for OCPs due to their tremendous retention capabilities for these compounds and they can enter the food chain directly through absorption into vegetation [4]. Also, OCPs can be transferred from air and atmospheric particulates into the vegetables [7]. Therefore, residues of pesticide could affect the consumers especially when these commodities are freshly consumed [1].

Grape production is an important activity due to the high nutritional properties of grapes, being consumed both as fresh and as processed products [8]. The increase of fruit intake contributes to the prevention of chronic diseases, but could also significantly increase pesticide exposure and may thus be of health concern [9].

In order to measure the low concentrations of OCPs residues in fruit samples, highly selective, sensitive and accurate analytical methods are needed [8]. Sample preparation still remains a critical step, being complex, laborious and time-consuming, especially for the biological matrices. Initially, classical techniques of sample preparation, such as Soxhlet and (solid)liquid-liquid extraction, that employed large amounts of toxic organic solvents and generate environmentally hazardous waste were used [10, 11]. In the last decades, various microextraction methods have been innovatively employed for effective concentration of OCPs in liquid samples, before instrumental analysis. These include, among others, single drop microextraction, dispersive liquid-liquid microextraction, solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE) followed by thermal desorption [12]. These methods are more environmental friendly, in agreement with modern green chemistry and analytical principles [13]. In European Union, there are several standardized methods for pesticides residues determination in foods of plant origin by GC or LC-MS/MS, after extraction with organic solvents and clean-up with different techniques [14, 15, 16].

SBSE is a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices using a thick

film of polydimethylsiloxane (PDMS). This technique is based on the same mechanisms of SPME, but SBSE enables a much higher capacity because of the larger amount of polymeric phase compared to SPME [17, 18].

For quantitative analysis, most determinations of OCPs have been developed using chromatographic techniques due to their high resolution capacity and the availability of selective detectors, such as gas chromatography (GC) with electron capture detector (ECD) because of the halogen atoms in their chemical structure [1, 19]. Also, the use of multi-residue methods capable of analysing large numbers of pesticides in one single run is efficient approach [8].

The purpose of this study was to determine the levels of 20 OCPs (α-, β-, γ-, δ-, ε-isomers of hexachlorocyclohexane, 1,1,1-trichloro-2,2-bischlorophenylethane (DDT), 1,1-dichloro-2,2,-bischlorophenylethane (DDD), and dichlorodiphenylchloroethylene (DDE), each with their isomers 4,4′- and 2,4′-, also aldrin, dieldrin, heptachlor, heptachlor epoxide (isomer A), heptachlor epoxide (isomer B), alfa-endosulfan, beta-endosulfan, hexachlorobenzene (HCB) in six white grape varieties samples collected in a vineyard situated in the central part of Romania, using by SBSE, followed by thermal desorption (TD)-GC-ECD methodology.

RESULTS AND DISCUSSION

The method was validated by assessing linearity and precision. The accuracy of the method was calculated in terms of recoveries, using fresh grape samples (Sauvignon Blanc variety) fortified with standard pesticides mixture at 100 µg/kg. Limits of detection (LODs) and quantification (LOQs) were calculated as the concentration of OCPs in low level spiked matrix giving the response with a signal/noise ratio of 3 and 10, respectively.

The linearity of OCPs calibration plot was investigated over a concentration range of 0.1–100 µg/kg. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte / peak area of IS) to the relative concentration of analytes (concentration of analyte / concentration of IS). The matrix-matched standards were used for all quantification purposes to avoid any ambiguity.

The correlation coefficient (r^2) for each pesticide was greater than 0.99, indicating good linearity, as listed in Table 1.

The recovery was evaluated by spiking pesticides standards in grape sample at level of 100 µg/kg. The non-spiked and spiked samples were analyzed by SBSE, followed by TD–GC–ECD. The recoveries were calculated by subtracting the results for the non-spiked samples from those for the spiked samples. These QC samples were quantified against the matrix spiked calibration curve. The recovery rate was replicated three times and the obtained data are presented in Table 1.

Table 1. Mean recoveries (%) and relative standard deviations (RSDs) of GC–ECD determination of 20 OCPs spiked (100 µg/kg) in grape samples

OCPs	Recovery (% ± RSD)	r^2	LOD (µg/kg)	LOQ (µg/kg)
Hexachlorobenzene (HCB)	82.5 ± 4.6	0.9965	0.08	0.28
α-HCH	86.2 ± 5.1	0.9918	0.37	1.36
Pentachloronitrobenzene	88.4 ± 6.7	0.9930	0.08	0.25
γ-HCH	80.5 ± 11.0	0.9884	0.10	0.36
β-HCH	83.4 ± 4.9	0.9924	0.10	0.38
Heptachlor	73.4 ±13.0	0.9901	0.08	0.28
δ-HCH	61.1 ±20.9	0.9954	0.35	1.30
ε-HCH	76.2 ± 15.7	0.9944	0.38	1.30
Aldrin	71.8 ± 14.5	0.9953	0.04	0.15
Heptachlor epoxide β	90.5 ± 9.2	0.9941	0.03	0.10
Heptachlor epoxide α	86.2 ± 8.4	0.9912	0.05	0.18
α-Endosulfan	93.6 ± 5.0	0.9944	0.43	1.50
2,4'-DDE	51.7 ± 13.8	0.9884	0.20	0.66
4,4'-DDE	60.1 ± 18.3	0.9946	0.08	0.30
Dieldrin	98.5 ± 12.5	0.9977	0.08	0.26
2,4'-DDD	72.9 ± 19.0	0.9928	0.05	0.18
4,4'-DDD	79.0 ± 16.7	0.9945	0.08	0.28
2,4'-DDT	70.8 ± 18.4	0.9964	0.90	3.10
β-Endosulfan	89.1 ± 8.1	0.9948	0.48	1.60
4,4'- DDT	67.9 ± 24.0	0.9980	0.85	2.81

Figure 1 presents the SBSE-TD-GC-ECD chromatogram of OCPs in FA grape sample.

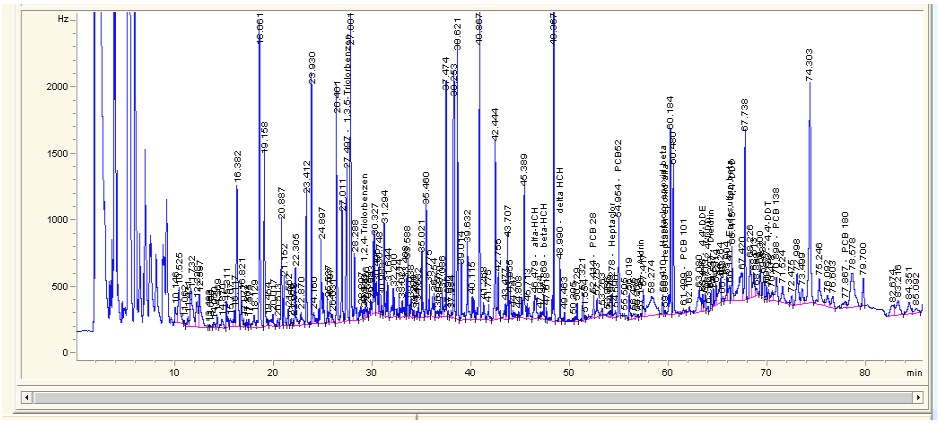


Figure 1. SBSE-TD-GC-ECD chromatogram of OCPs in FA grape sample

The limits of quantification varied between 0.10 μg/kg g (heptachlor epoxide β) and 3.10 μg/kg (2,4'-DDT) and are in compliance with the European Union regulations for the maximum residue limits (MRLs) of OCPs in agricultural vegetables [20, 21].

The recovery rate for the 20 OCPs were within acceptable range [22], with values between 60.1% (4,4'-DDE) and 98.5% (dieldrin), except for 2,4'-DDE with value of 51.7%.

The obtained results shown in Table 1 indicated that the method SBSE-TD-GC-ECD applied for grape matrix gave satisfactory performance for multiresidue analysis of 20 OCPs.

The SBSE-TD-GC-ECD method was used to determine the concentration of 20 OCPs in six white Romanian grape varieties samples and the obtained results are shown in Table 2.

Table 2. Concentrations of OCPs in different grape varieties (μg/kg)

OCPs	RI	MO	SB	FR	FA	CH
Hexachloro benzene (HCB)	1.33	1.05	<LOQ	0.61	0.42	0.60
Pentachloronitro benzene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
α-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
γ-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
β-HCH	0.41	0.40	<LOQ	<LOQ	<LOQ	0.39
Heptachlor	0.52	<LOQ	0.32	<LOQ	<LOQ	<LOQ
δ-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ε-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Aldrin	<LOQ	0.19	<LOQ	<LOQ	<LOQ	<LOQ
Heptachlor epoxide β	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Heptachlor epoxide α	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
α-Endosulfan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Dieldrin	0.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
β-Endosulfan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDT	<LOQ	<LOQ	<LOQ	2.87	<LOQ	<LOQ

Low amounts of OCPs were detected in all grape samples, the total concentrations ranged between 0.32 μg/kg (SB) and 3.48 μg/kg (FR). In the latter, the highest contribution was given by 4,4'-DDT, with 2.87 μg/kg. The

compound that occurs most often was HCB and ranged between 0.42 µg/kg (FA) and 1.33 µg/kg (RI). Among the HCH isomers, only β-HCH was recorded in 3 of the investigated samples: RI (0.41 µg/kg), MO (0.40 µg/kg) and CH (0.39 µg/kg). Heptachlor was detected in 2 samples: RI (0.52 µg/kg) and SB (0.32 µg/kg). Aldrin and dieldrin were determined in 2 samples: MO (0.19 µg/kg) and RI (0.35 µg/kg).

For some of the investigated OCPs in grapes, the European legislation [21] set the MRLs presented in Table 3. All the obtained concentrations of OCPs in the present study were much lower than their corresponding MRL.

Table 3. Maximum residue limits for OCPs in grapes, in mg/kg

OCP	MRL (mg/kg)
Aldrin and dieldrin (expressed as dieldrin)	0.01
DDT (sum of 4,4'-DDT, 2,4'-DDT, 4,4'-DDE, 4,4'-DDD), expressed as DDT	0.05
Endosulfan (sum of α-, β-endosulfan and endosulfan sulfate)	0.05
Heptachlor (sum of heptachlor and heptachlor epoxide), expressed as heptachlor	0.01
Hexachlorobenzene	0.01
γ-HCH	0.01
Sum of HCH isomers, except γ-HCH	0.01

CONCLUSIONS

The determination of 20 OCPs concentrations in grape samples using SBSE followed by TD-GC-ECD was investigated. The results showed that OCPs were detected in all the investigated grape samples, with total contents varied between 0.32 µg/kg (SB) and 3.48 µg/kg (FR). The method produced satisfactory results for linearity, recovery and limits of detection, situated well below the MRLs for all determined pesticides, in compliance with the EU directives [21, 22]. Also, the method has many advantages comparing to classical methods, such as a small sample amount, simplicity, low cost and almost solventless.

EXPERIMENTAL SECTION

Samples

Samples were collected in autumn 2015, in a vineyard situated in the central part of Romania and comprises of six white grape varieties: Feteasca Regala (FR), Feteasca Alba (FA), Riesling Italian (RI), Sauvignon Blanc (SB), Muscat Ottonel (MO), and Chardonnay (CH).

The sample size was at least one kg of fresh product. The portion of raw agricultural commodity prepared as the analytical sample for determination of pesticide residues was carried out according to the Codex Alimentarius, Volume 2A, Part 1-2000. A representative portion of the analytical sample was subjected to the further analysis [1].

Reagents and materials

The solvent methanol was gas chromatography grade of quality (LGC Standards, Germany).

Standard solution (Mix Standard solution for EN ISO 6468 CERTAN, NE7550) for organochlorine pesticides, polychlorinated biphenyls and chlorobenzenes was purchased from LGC Standards GmbH, Germany and contained 36 compounds at 10 µg/mL each analyte: α-, β-, γ-, δ-, ε-isomers of hexachlorocyclohexane (expressed as HCHs), 4,4′-DDE, 2,4′-DDE, 4,4′-TDE, 2,4′-TDE, 2,4′-DDT and 4,4′-DDT (expressed as DDTs), aldrin, dieldrin, heptachlor, heptachlor epoxide (isomer A), heptachlor epoxide (isomer B), α-endosulfan, β-endosulfan, hexachlorobenzene (HCB) (and also other 16 compounds which were not determined in this study. Internal standard, 1-bromo-2-nitrobenzene was also acquisitioned from LGC Standards GmbH, Germany was used in concentration of 100 µg/kg. An intermediate standard mixture of 1 µg/mL was prepared by diluting the stock standard solution, from which the working calibration standards were prepared by appropriate serial dilution with methanol. For quantification, a series of six matrix matched standards (0.1, 0.5, 1.0, 10, 20, 50, 100 µg/kg sample) were freshly prepared by the addition of the appropriate working pesticide standard solutions to the grape sample berries, before homogenization and ultrasonication, which were subjected to SBSE extractions, then to TD-GC-ECD analysis, using the procedure described in Section “Sample preparation”. Before preparation of the matrix matched standards, the grapes were analyzed in order to determine the concentrations of the targeted pesticide residues.

For SBSE extractions, SBSE stir bars (Twister, Gerstel, Müllheim an der Ruhr, Germany), 10 mm long, coated with a 1.0 mm polydimethylsiloxane (PDMS) layer were used. Prior to use, the stir bars were conditioned for 4 h at 300 °C in a flow of helium.

For the extraction, 20 mL headspace vials from Agilent Technologies (Palo Alto, CA, USA) were used.

Instrumentation and operating conditions

A thermo-desorption unit (TDU) (Gerstel) was installed on top of an Agilent Technologies 7890A gas chromatograph (GC) with electron-capture detector (µ-ECD) equipped with a programmed temperature vaporization (PTV) injector (Gerstel).

Splitless thermal desorption was performed by programming the TDU from 40 to 280 °C (5 min) at a rate of 60 °C/min. The analytes were cryo-focused in the PTV at -150 °C with liquid nitrogen prior to injection. An empty baffled liner was used in the PTV injector. For splitless injection, the PTV was ramped from -150 to 280 °C (2 min) at a rate of 600 °C /min. The used capillary column was a 30 m × 0.32 mm I.D. × 0.25 µm film thickness, coated with cross-linked 5% phenyl methyl polysiloxane was used (HP-5, Agilent J&W). The injection port temperature was 280 °C and the detector temperature was 300 °C. The column temperature program consisted of 4 stages: from 80°C to 196°C (rate 4°C/min, 2 min), from 196°C to 224°C (rate 4°C/min, 2 min), from 224°C to 240°C (rate 4°C/min, 2 min) and from 240°C to 275°C (rate 4°C/min, 2 min). High purity Helium was used as carrier gas. Blank runs of the stir bar were carried out before and after each analysis to verify the absence of any carry-over effect.

Ultrapure water (18.2 MΩ cm) was prepared by a Direct Q UV 3 Millipore system (Bedford, MA, USA).

The following instruments were used for homogenization, an Ultra-Turrax (Ika, Germany) mixer, for ultrasonication, an ultrasonic bath (Sonorex, Bandelin, Germany) and for centrifugation, a Universal 320 centrifuge (Hettich, Germany).

Sample preparation

The used method was previously described by Sandra et al. [17], with small modifications. Briefly, to the accurately weighted fruit samples (approximately 15 g), 30 mL methanol was added, then the mixture was homogenized and ultrasonicated for 15 min. A fraction (10 mL) was centrifuged (5 min at 5000 rpm) and then 1 mL of the supernatant methanol phase was extracted using a SBSE stir bar in a 20 mL headspace vial, after addition of 10 mL of ultrapure water, for 180 min with a stirring rate of 1000 rpm and at room temperature. After extraction, the stir bar was introduced in the liner of the thermal desorption system (TDU), followed by the GC-ECD analysis.

All assays, following the entire procedure, SBSE-TD-GC-ECD were performed in triplicate and blank assays were also performed using the same procedure and grape samples without spiking.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCS, project no. PN 16.40.02.01, Sectorial Operational Programme “Increase of Economic Competitiveness”, Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

1. Z. Sharif, Y.B. C. Man, S.A.H. Nazimah, C.K. Chin, I. Tsukamoto, A.H. Hamanyza, I. Norhaizan, *Food Chemistry*, **2007**, *102*, 98.
2. J. Fenik, M. Tankiewicz, M. Biziuk, *Trends in Analytical Chemistry*, **2011**, *30* (6), 814.
3. Z. Sharif, Y. B. C. Man, N. S. A. Hamid, C. C. Keat, *Journal of Chromatography A*, **2006**, *1127*, 254.
4. C. Qu, S. Qi, D. Yang, H. Huang, J. Zhang, W. Chen, H. Keleta Y., E. H. Sandy, J. Yang, X. Xing, *Journal of Geochemical Exploration*, **2015**, *149*, 43.
5. A.F.A. Mahmoud, Y. Ikenaka, Y.B. Yohannes, W.S. Darwish, E.A. Eldaly, A.E.M.A. Morshdy, S.M.M. Nakayama, H. Mizukawa, M. Ishizuka, *Chemosphere*, **2016**, *144*, 1365.
6. S. Ennaceur, N. Gandoura, M. R. Driss, *Bulletin of Environmental Contamination and Toxicology*, **2007**, *78*, 325.
7. A. Zhang, W. Luo, J. Sun, H. Xiao, W. Liu, *Science of the Total Environment*, **2015**, *505*, 1142.
8. S. Grimalt, P. Dehouck, *Journal of Chromatography A*, **2016**, pii: S0021-9673(15)01874-9, http://dx.doi.org/10.1016/j.chroma.2015.12.076.
9. S. N. Sinha, M. V. Vardhana Rao, K. Vasudev, M. Odetokun, *Food Control*, **2012**, *25*, 636.
10. A. Neves Dias, V. Simão, J. Merib, E. Carasek, *Talanta*, **2015**, *134*, 409.
11. J. Cai, G. Chen, J. Qiu, R. Jiang, F. Zeng, F. Zhu, G. Ouyang, *Talanta*, **2016**, *146*, 375.
12. Z. Huang, H. K. Lee, *Journal of Chromatography A*, **2015**, *1401*, 9.
13. A. Masiá, K. Vásquez, J. Campo, Y. Picó, *Journal of Chromatography A*, **2015**, *1378*, 19.
14. EN 12393:2013. Foods of plant origin. Multiresidue methds for the determination of pesticide residues by GC or LC-MS/MS. Part 1, 2, 3.
15. EN 15662:2009. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method.
16. EN 15637:2009. Foods of plant origin - Determination of pesticide residues using LC-MS/MS following methanol extraction and clean-up using diatomaceous earth.
17. P. Sandra, B. Tienpont, F. David, *Journal of Chromatography A*, **2003**, *1000*, 299.
18. M. Barriada-Pereira, P. Serôdio, M.J. González-Castro, J.M.F. Nogueira, *Journal of Chromatography A*, **2010**, *1217*, 119.
19. B. Jin, L. Xie, Y. Guo, G. Pang, *Food Research International*, **2012**, *46*, 399.
20. Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I.

21. European Commission website. EU Pesticides Database. Available at: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN
22. European Commission. Directorate-General for Health and Food Safety. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11945/2015.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 441-449)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

INFLUENCE OF TABLET FORMULATION ON *IN VITRO* RELEASE OF MAGNESIUM

CORINA MOISA[a], MARIA-ALEXANDRA HOAGHIA[b], DORINA SIMEDRU[b], OANA CADAR[b,*]

ABSTRACT. The transfer of active substances of pharmaceutical forms is influenced by several factors, among which the nature of the excipients used in compression. The objective of this study was to investigate the influence of cellulose and its derivatives (hydroxypropylcellulose, hydroxylpropylmethylcellulose, carboxymethylcellulose) on the release profile of magnesium from marketed tablets (T1-T4). *In vitro* release experiments were carried out using a dissolution apparatus, in ultrapure water and simulated intestinal fluid (pH=6.8). In selected formulations, the amount of magnesium released was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). The magnesium dissolution profiles demonstrated complete dissolution for T1, even just after 5 min. Magnesium release rate decreased with the increase in cellulose and/or its derivatives proportion: T1<T3<T2<T4. An agitation speed of 50 rpm showed a more satisfactory magnesium release profile than 100 rpm.

Keywords: *magnesium release, in vitro, pH, matrix tablet*

INTRODUCTION

Magnesium is a macroelement mineral, the fourth most abundant cation in the human body and the second most abundant intracellular cation, after potassium [1, 2]. It takes part, as a cofactor, in more than 300

[a] *University of Oradea, Faculty of Medicine and Pharmacy, 29 Nicolae Jiga Street, RO-410028, Oradea, Romania*
[b] *Research Institute for Analytical Instrumentation, 67 Donath Street, RO-400293, Cluj-Napoca, Romania*
[*] *Corresponding author: oana.cadar@icia.ro*

enzymatic reactions included in the spectrum of the metabolic activity [3, 4]. Magnesium is essential both for the absorption and metabolism of calcium and vitamin D, and the calcium transport. Magnesium is important for muscle contraction by acting as a calcium antagonist, cellular proliferation, apoptosis, immune response, etc. Magnesium deficiency mobilizes calcium from bones, leading to abnormal calcifications in arteries and kidneys [5-7].

A low level of magnesium in the body is associated with significant cardiovascular conditions, including hypertension, heart rhythm disorders (arrhythmias), coronary artery disease (angina pectoris, myocardial infarction), hypercholesterolemia, hypertriglyceridemia, congestive heart failure and aggravation of pulmonary diseases [8]. Accidentally or not, the increasing incidence of cardiovascular disease in recent years corresponds to an increasingly lower magnesium intake [9-12]. Magnesium deficiency is associated with high levels of total cholesterol and triglyceride and low level of HDL cholesterol, respectively. In terms of insufficient dietary intake, administration of pharmaceutical forms of magnesium is increasingly used [9].

More than 90 % of drugs are orally administered. Solubility is one of the significant parameters to attain desired concentration of a drug in systemic circulation for pharmacological response to be shown [13]. Therapeutic effectiveness of a drug is directly related to the absorption and bioavailability of the drug after oral administration [14]. In the gastro intestinal tract, most absorption of oral drugs occurs in the small intestine due to its large surface area and high blood perfusion rate [15]. It is desirable that the oral tablet displays minimal drug release in the stomach and the small intestine (duodenum) and starts drug release in the tracts of the lower small intestine and colon.

The successful treatment of hypomagnesaemia depends on both the type of magnesium administered and the duration of the therapy. Magnesium efficient formulas are those in which magnesium is organically bound (acetate, bicitrate, methionate, ascorbate, gluconate, propionate or orotate). Magnesium mineral salts (chloride, sulphate) can't be absorbed, often having a laxative effect and thus reducing the therapeutic efficacy [16, 17]. *In vitro* dissolution study is an important aspect in the evaluation of the best formulation and also for understanding the possible risks like dose dumping, chemical contamination and drug interactions with food or other drugs [18-20].

The rate of drug release is dependent on various factors such as the material matrix (composition, structure, swelling, degradation), the release medium (pH, temperature, ionic strength, enzymes) and the drug composition (solubility, stability, interaction with matrix) [21]. Cellulose and cellulose derivatives are commonly used in the formulation of dosage forms and healthcare products. Polymer content, molecular weight, concentration, degree of substitution and particle size have been shown to have an important effect

on drug release. However, the most significant factors that affect the drug release rate from cellulose matrices are the polymer concentration and drug/polymer ratio [22].

The aim of this study was to evaluate the magnesium release from marketed tablets containing cellulose and/or its derivatives, in two dissolution media: ultrapure water (UW) and simulated intestinal fluid (SIF, phosphate buffer, pH 6.8, enzyme free) at different time intervals.

RESULTS AND DISCUSSIONS

Magnesium supplements come in a variety of salts (e.g. citrate, oxide, gluconate, acetate, orotate) [23], however their bioavailability differs. Some studies reported the use of organic over inorganic forms: investigations of magnesium orotate, citrate and gluconate which demonstrated high solubility and bioavailability [24-26].

In vitro dissolution studies are important quality and stability tools for predicting *in vivo* performance. In this study, four marketed tablet brands containing the same active ingredient and different excipients, were studied for their *in vitro* magnesium dissolution behaviour in ultrapure water and simulated intestinal fluid (pH=6.8) for 5, 10, 30, 60 and 120 min time period, using USP reference dissolution apparatus. The content of cellulose and/or its derivatives in studied tablets was increasing in the following order: T1<T3<T2<T4. The magnesium release from the active ingredient itself was completely after 5 min. The obtained results for marketed tablets (T1-T4) are shown in Figures 1-4. The magnesium dissolution profiles demonstrated complete dissolution for T1, even just after 5 min.

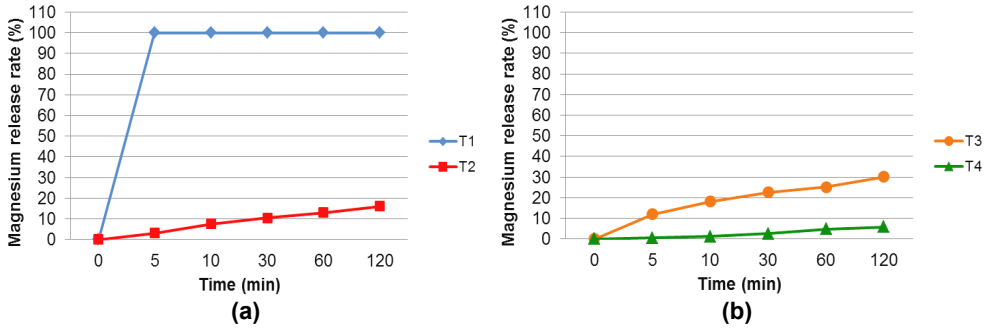


Figure 1. Release profile of magnesium from tablets of different excipients in UW, 50 rpm: a) T1, T2 and b) T3, T4.

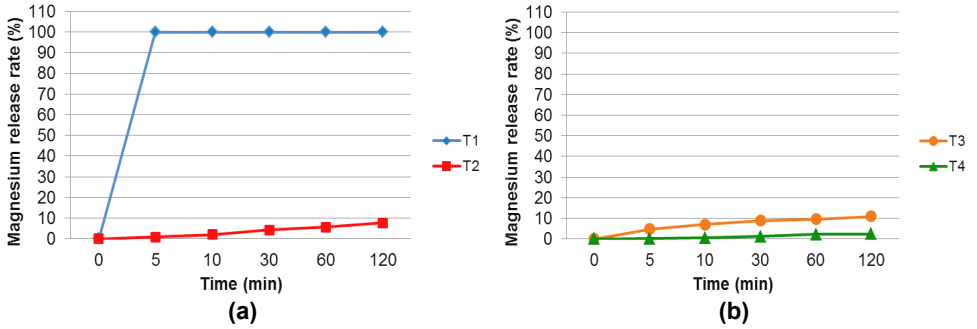


Figure 2. Release profile of magnesium from tablets of different excipients in SIF, 50 rpm: a) T1, T2 and b) T3, T4.

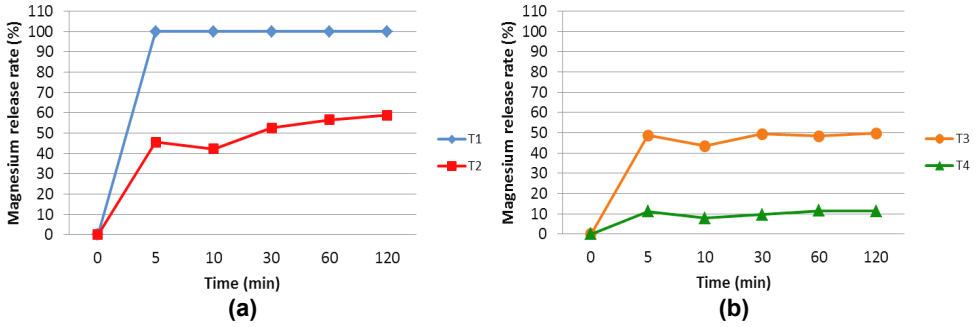


Figure 3. Release profile of magnesium from tablets of different excipients in UW, 100 rpm: a) T1, T2 and b) T3, T4.

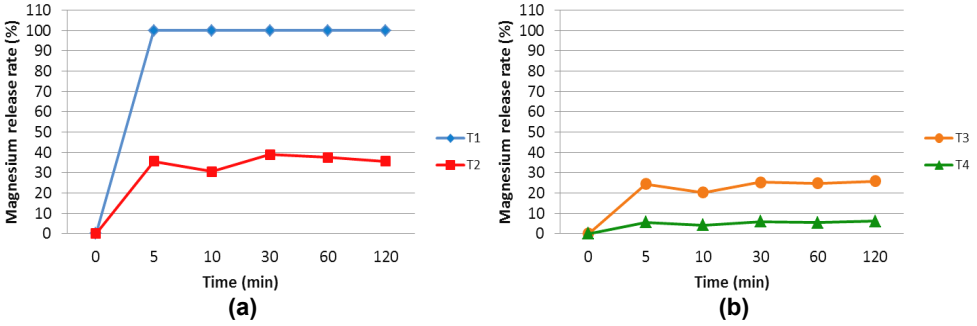


Figure 4. Release profile of magnesium from tablets of different excipients in SIF, 100 rpm: a) T1, T2 and b) T3, T4.

In vitro magnesium release behavior of investigated tablets was not affected by pH and dissolution media composition. The presence of cellulose derivatives in the composition of the tablets leaded to a delay in the release of

the magnesium from the tablet both in ultrapure water and simulated intestinal medium (phosphate buffer solution, pH 6.8). The tablet T1, containing no cellulose and/or its derivatives, exhibited better dissolution profile than tablets formulated with cellulose and/or its derivatives (T2-T4).

In the presence of water, the cellulose derivatives modify their properties, their main property being swelling, which leads to the formation of a gel that prevents the release of magnesium. The obtained results indicate that cellulose and/or its derivatives could retard the magnesium release in both simulated intestinal fluid and ultrapure water, but at different levels. Results revealed a significant difference in magnesium release behavior between the tablets with lactose and those with cellulose and its derivatives. A very rapid release of magnesium from lactose tablets was found (5 min) in both UW and SIF, whereas tablets containing cellulose and/or its derivatives showed the retardation of magnesium release. This was due to the high water solubility of lactose; therefore, water easily penetrates into tablets and magnesium ions are rapidly released. Cellulose and its derivatives are less soluble in water than lactose; consequently, the water penetration into the tablet matrix is more difficult. This leads to the delay of magnesium release. The amount of cellulose derivatives in the composition of the tablets influences the magnesium's time release from the tablet. Therefore, in tablets containing low quantity of cellulose derivatives, the magnesium dissolves faster in the dissolution medium compared to tablets containing cellulose derivatives. Similar results were obtained by Enayatifard [27], which showed that the release rate decreased as the concentration of hydroxypropylmethylcellulose increased.

The immediate-release tablets (T1) are entirely available immediately for absorption following oral ingestion, providing a faster therapeutic effect. In contrast, the market tablets (T2-T4), with slower magnesium release, are appropriate for maintenance treatment.

Mild agitation conditions should be maintained during dissolution experiments to allow maximum discriminatory power. Generally, the dissolution apparatus tends to become less discriminating when operates at faster speeds, resulting in a flatter drug release profile [28]. For our study, it can be concluded that the magnesium release profile at 50 rpm detected small changes in tablet composition (Figure 1 and 2), while, at 100 rpm, dissolution proceeded to quickly (Figure 3 and 4). Therefore, the satisfactory magnesium release profile was observed at 50 rpm. This result was in agreement with the finding of Soni et *al.* [29].

The pH of the dissolution medium was measured before and after the dissolution test (Figure 5 and 6). The pH slowly changes during the dissolution test due to the solubility of the drug substance or the excipients [30].

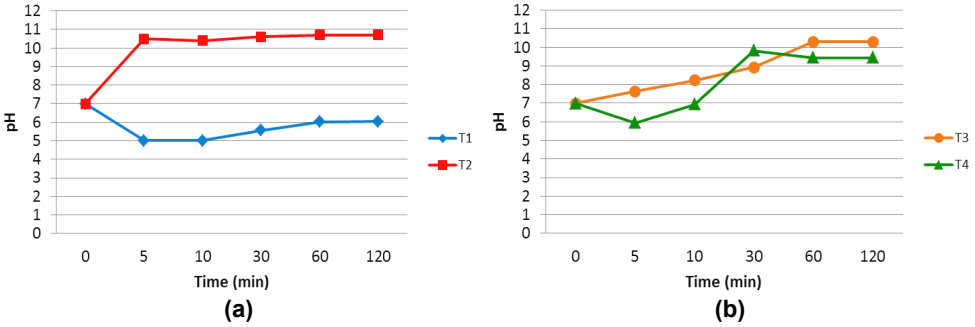


Figure 5. Changes in pH after immersion of different tablet brands in UW, 50 rpm: a) T1, T2 and b) T3, T4.

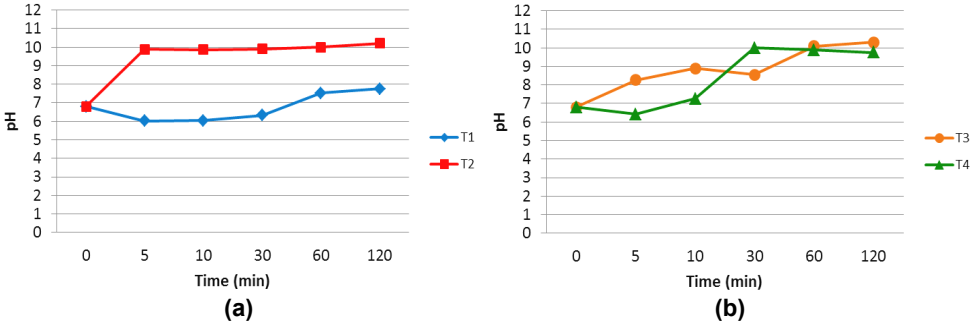


Figure 6. Changes in pH after immersion of different tablet brands in SIF, 50 rpm: a) T1, T2 and b) T3, T4.

CONCLUSIONS

In this study, *in vitro* magnesium dissolution behaviour from the same drug, belonging to different brands, in ultrapure water and simulated intestinal medium was tested at periodic time intervals. The magnesium release in buffer solution and ultrapure water was independent of the pH and dissolution media composition. Furthermore, it was shown that higher amount of cellulose and/or its derivatives in formulations caused a decrease in magnesium release rates. Increase in agitation speed from 50 to 100 rpm increased "*in vitro*" magnesium release rate from tablets. pH of the dissolution medium changes as the dissolution of active ingredient and drug components occurs.

EXPERIMENTAL SECTION

Materials

To perform the study, both magnesium and magnesium/calcium tablets of four different manufacturers, with a range of nine commonly used excipients in pharmaceutical formulations: microcrystalline cellulose, hydroxypropyl-methylcellulose, carboxymethylcellulose, hydroxypropyl-methylcellulose, lactose monohydrate, magnesium stearate, gelatin, titanium dioxide and talcum were used. Five blister packs were purchased from a local pharmacy shop. The market tablets belong to four different brands. The labelled shelf life of all tablets was 2-4 years after production date and was taken for evaluation before one year of the labelled expiry date. After purchasing, the tablets were coded separated as pair of magnesium and magnesium/calcium: T1 and T2 for magnesium tablets and T3 and T4 for magnesium/calcium tablets (Table 1). The total cellulose (derivatives) content of the tablet weight was: T1 (0%) < T3 (10%) < T2 (25%) < T4 (35%). All reagents were of analytical grade, purchased from Merck (Germany) and used without further purification.

Table 1. Details of magnesium and calcium/magnesium market tablet formulations.

Crt. No.	Formulation code	Type	Cellulose and its derivatives
1	R (reference/active ingredient)	Mg	-
2	T1	Mg	-
3	T2	Mg	Microcrystalline Cellulose Hydroxypropylcellulose Hydroxypropylmethylcellulose Carboxymethylcellulose
4	T3	Ca/Mg	Microcrystalline Cellulose Hydroxypropylmethylcellulose
5	T4	Ca/Mg	Hydroxypropylmethylcellulose

Magnesium ion release study

The dissolution tests were carried out to determine magnesium release pattern in a dissolution apparatus (Electrolab, TDT-08L Plus) following the USP paddle method, at 37 ± 0.5º C, 50 and 100 rpm rotation speed. The experiments were performed in 900 ml dissolution media: (*i*) ultrapure water (pH=6.998) and (*ii*) simulated intestinal fluid (pH 6.8, buffer solution containing K_2HPO_4-NaOH), respectively, during a specific period of time (5, 10, 30, 60 and 120 min). The amount of magnesium was equivalent to 200 mg. Also, the magnesium release from the active substance was studied. A 5 ml sample

was collected at predetermined time point, filtered through a 0.45 µm pore size filter and analyzed for magnesium content using inductively coupled plasma optical emission spectrometer (ICP-OES) Optima 5300 DV (Perkin Elmer, USA). The limit of detection (LOD=0.01 mg/L) was calculated on the basis of the equation LOD=3s_b/m, where s_b was the standard deviation of 10 successive measurements of blank and m was the slope of calibration curve [7]. The possible interference with phosphorous, potassium and sodium related interference were not observed at the magnesium emission line (285 nm). The pH was measured with a 350i multiparameter (WTW, Weilheim, Germany). Each experiment was repeated in triplicate. All dilutions were prepared using deionized water (18.2 MΩ/cm) obtained from a Millipore Direct-Q3 UV Ultrapure water system (Millipore, Molsheim, France). All PTFE and glass vessels were soaked in 10% (v/v) HNO_3 overnight and rinsed with Milli-Q water prior to use.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01 and Sectoral Operational Programme “Increase of Economic Competitiveness”, Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164.

REFERENCES

1. R. Swaminathan, *Clinical Biochemist Reviews*, **2003**, *24*, 47.
2. B.M. Altura, Magnesium and trace elements, **1991-1992**, *10*, 167.
3. W. Jahnen-Dechent, M. Ketteler, *Clinical Kidney Journal*, **2012**, *5*, i3.
4. L.E. Bailey, K. Wrogeman, “Orotic Acid in Cardiology: International Symposium on Orotic Acid and Magnesiumorotate, Rudesheim/Rhine”, Thieme Medical Publishers, Inc., New York, **1992**, 33.
5. R.J. Elin, *Magnesium Research*, **2010**, *23*, 194.
6. V.V. Ranade, J.C. Somber, *American Journal of Therapeutics*, **2001**, *8*, 345.
7. O. Cadar, M. Miclean, M. Senila, A. Hoaghia, M. Sager, *Studia Universitatis Babes-Bolyai, Chemia*, **2015**, *4*, 77.
8. A.C. Coman, D. A. Todea, F. Popa, T. Radu, O. Cadar, C. Borzan, *Journal of Optoelectronics and Advanced Materials*, **2015**, *17*, 1563.
9. A. Mazur, J.A.M. Maier, *Reference Module in Food Science, Encyclopedia of Food and Health, Magnesium*, **2016**, 587.
10. E.S. Ford, C. Li, L.C. McGuire, A.H. Mokdad, S. Liu, *Obesity (Silver Spring)*, **2007**; *15*, 1139.

11. S.H. Jee, E.R. Miller 3rd, E. Guallar, V.K. Singh, L.J. Appel, M.J. Klag, *American Journal of Hypertension*, **2002**, *15*, 691.
12. R. Swaminathan, *Clinical Biochemistry Review*, **2003**, *24*, 47.
13. P.S Mohanachandran, P.G Sindhumol, T.S Kiran, *International Journal of Comprehensive Pharmacy*, **2010**, *1*, 1.
14. P. Khadka, J. Ro, H. Kim, I. Kim, J.T. Kim, J.M. Cho, G. Yun, J. Lee, *Asian Journal of Pharmaceutical Sciences*, **2014**, *9*, 304.
15. K.S. Pang, *Drug Metabolism and Disposition*, **2003**, *31*, 1507.
16. M.E. Maguire, J.A. Cowan, *Biometals*, **2002,** *15*, 203.
17. W. Wacker, "Magnesium and man", Harvard Univ. Press, Cambridge, **1980**, 160.
18. L. Stefanescu, B.M. Robu, A. Ozunu, *Environmental Science and Pollution Research*, **2013**, *20*, 7719.
19. M.A. Hoaghia, C. Roman, E.A. Levei, D. Ristoiu, *Studia Universitatis Babes-Bolyai, Chemia*, **2015**, *1*, 109.
20. O. Anand, L.X. Yu, D.P. Conner, B.M. Davit, *American Association of Pharmaceutical Scientists*, **2011**, *13*, 328.
21. Y. Fu, W.J. Kao, *Expert opinion on drug delivery*, **2010**, *7*, 429.
22. S. Kamel, N. Ali, K. Jahangir, S.M. Shah, A.A. El-Gendy, *Express polymer letters*, **2008**, *11*, 758.
23. L. Braun, M. Cohen, "Herbs and Natural Supplements", Marrickville, NSW, Australia, **2015**, Volume 2, 4th Edition.
24. J.S. Lindeberg, M.M. Zobitz, J.R. Poindexter, C.Y. Pak, *Journal of the American College of Nutrition*, **1990**, *9*, 48.
25. A. Walker, G. Marakis, S. Christie, M. Byng, *Magnesium Research*, **2003**, *16*, 183.
26. C. Coudray, M. Rambeau, C. Feillet-Coudray, E. Gueux, J.C. Tressol, *Magnesium Research*, **2005**, *18*, 215.
27. R Enayatifard, M Saeedi, J Akbari, Y.H. Tabatabaee, *Tropical Journal of Pharmaceutical Research*, **2009**; *8*, 425.
28. L. Giorgetti, M.G. Issa, H.G. Ferraz, *Brazilian Journal of Pharmaceutical Sciences*, **2014**, *50*, 513.
29. T. Soni, C. Nagda, T. Gandhi, N.P. Chotai, *Dissolution Technologies*, **2008**, *15*, 31.
30. L.R. Shaw, W.J. Irwin, T.J. Grattan, B.R. Conway, *Drug Development and Industrial Pharmacy*, **2005**, *31*, 515.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 451-460)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

HEALTH RISK ASSESSMENT ASSOCIATED WITH NITROGEN COMPOUNDS CONTAMINATED DRINKING WATER IN MEDIAS REGION

MARIA-ALEXANDRA HOAGHIA[a,b,*], MARIANA LUCIA ANDREI[b], OANA CADAR[a], LACRIMIOARA SENILA[a], ERIKA LEVEI[a], DUMITRU RISTOIU[b]

ABSTRACT. High concentrations of nitrogen compounds in drinking water may cause negative health effects. The aim of the present study was to assess the content of nitrogen compounds namely, nitrates, nitrites and ammonium in drinking waters from Medias region (Medias, Copsa Mica towns and Tarnava village) and to investigate the health risk associated with the consumption of drinking water contaminated with these compounds. The health risk was calculated using chronic daily intake, hazard quotient and total hazard quotient. High concentrations of nitrite, nitrate and ammonium were found, at least one of the nitrogen compounds exceeding the maximum allowable concentrations (0.5 mg/L NO_2^-, 0.5 mg/L NH_4^+, 50 mg/L NO_3^-) in about half of the analysed samples. Generally, the chronic daily intake values were lower for nitrite and ammonium than for nitrate. The hazard quotients for nitrate were higher than the critical unity value, indicating that the consumption of contaminated waters from Tarnava village and Medias town may cause potential non-carcinogenic risk. Moreover, for the same samples, the total hazard quotients were higher than the critical unity value, suggesting that potential adverse health effects may appear after the consumption of drinking water.

Keywords: *drinking water source, nitrite, nitrate, ammonium, chronic daily intake, hazard quotient, total hazard quotient*

[a] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, RO-400293 Cluj-Napoca, Romania*
[b] *Babes-Bolyai University, Faculty of Environmental Science and Engineering, 30 Fantanele, RO-400294 Cluj-Napoca, Romania*
[*] *Corresponding author: alexandra.hoaghia@icia.ro*

INTRODUCTION

Ensuring access to adequate supply of safe drinking water is a major challenge all around the world. Groundwater is an important drinking water source, thus its quality protection is mandatory.

The presence of undesirable contaminants, such as nitrate (NO_3^-), nitrite (NO_2^-) or ammonium (NH_4^+) may decrease the drinking waters quality by the unpleasant odour and taste and possible adverse health effects. The main exposure pathway to nitrogen compounds is the ingestion of water or foodstuffs that contain high levels of nitrates, nitrites and ammonium [1-5]. In the human body, NH_4^+ is oxidised to NO_3^-, while NO_3^- under the action of specific enzymes is converted into NO_2^- that further reduces into N-nitroso compounds (nitrosamides, nitrosamines), with potential carcinogenic effects. Furthermore, the gastro intestinal tract and saliva are favourable environments for the conversion of nitrogenous compounds into NO_2^- and NO_3^- [6, 7, 8]. The NO_2^- reacts with the haemoglobin which is converted into methemoglobin, which stopes carrying oxygen to all cell tissues [5, 9-10]. The most common effects that appear in case of bottled-fed infants are “blue-baby syndrome” or methemoglobinemia and developmental toxicity, which represent the main health concern regarding the NO_2^- and NH_4^+ concentrations [2, 6, 7, 11]. Moreover the high concentrations of NO_3^- in the drinking water may increase the risk for bladder cancer [12].

Although the occurrence of NO_3^-, NO_2^- and NH_4^+ is part of the nitrogen cycle, the highest concentrations are generated by the anthropogenic activities. The agriculture (livestock, use of fertilizers) and the inadequate sewage management in industrial and household activities are considered the most important diffuse or non-point sources of NO_3^-, NO_2^- and NH_4^+ contamination [1-4, 8]. The main natural source of nitrogen compounds is the anaerobic organic material decomposition [8, 9, 13]. Several studies showed that the key source of high NO_3^-, NO_2^- and NH_4^+ levels in drinking-water are the sewage effluents runoff from household and agricultural activities [2, 13-16]. In Romania, high concentrations of NO_3^- and NO_2^- in drinking waters, which exceed the corresponding maximum allowable concentrations (*MACs*) were also reported [16-19]. Studies showed possible connection between the high concentrations of nitrogen compounds and the location of the well waters close to domestic and agricultural sources [19].

To prevent these health threats, European legislation and World Health Organization guidelines set maximum allowable concentrations for the NO_2^- (0.5 mg/L), NH_4^+ (0.5 mg/L) and NO_3^- (50 mg/L) in drinking water [2, 20-22]. The health risks associated with contaminated drinking water consumption can be assessed using mathematical indices, such as chronic daily intake (*CDI*), hazard quotient (*HQ*) and total hazard quotient (*THQ*).

The aim of the current study was to assess the NO_3^-, NO_2^- and NH_4^+ levels in the drinking waters from Tarnava village, Copsa Mica and Medias towns. Furthermore, the obtained data was used to assess the health risk associated with the consumption of water contaminated with NO_3^-, NO_2^- and NH_4^+. As the reference doses were established only for nitrate and nitrite, these two parameters were used to calculate the *HQ* and the *THQ*.

RESULTS AND DISCUSSION

The results show high concentrations of NO_3^-, NO_2^- and NH_4^+ in the studied well and spring waters (figure 1).

The NO_3^- concentrations in well water samples from Tarnava village (W6, W7) and Medias town (W1, W4, W 5) exceeded almost three times the *MAC* (50 mg/L), while the NO_2^- concentrations exceeded the *MAC* (0.5 mg/L) in water samples collected from all studied localities (W1, W6-W9, S) [16-18]. The measured NO_3^- concentration in spring sample S were lower than the *MA*C, while the NO_2^- concentration exceeded four times the *MAC* and the NH_4^+ level is slightly exceeded [16-18]. Samples W7, W8 and W9 presented the highest NH_4^+ concentrations, exceeding the *MAC* [16-18].

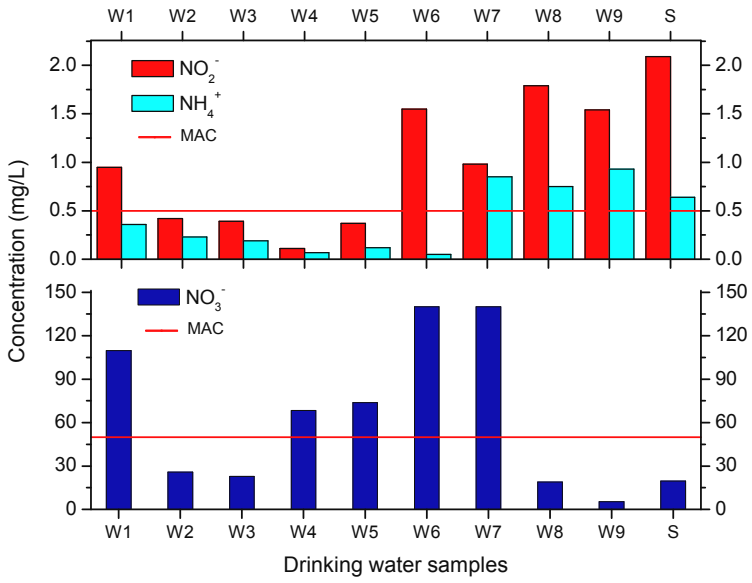


Figure 1. Concentrations of NO_2^-, $NO_3^-$and NH_4^+, in drinking waters

The *HQ* and *CDI* approaches were used to assess the non-carcinogenic risks, specifically for methemoglobinemia, associated with the ingestion of NH_4^+, NO_3^- and NO_2^- from drinking water sources. The *THQ* was used to summarize the total amount of chemicals ingested by drinking water. The *HQ* and *THQ* indices were applied only for NO_3^- and NO_2^-, since reference doses were established, by the IRIS (Integrated Risk Information System), U.S. E.P.A. (United States Environmental Protection Agency) for NO_2^- and NO_3^-, but not for NH_4^+.

Results indicate high values for the *HQ* for NO_3^-, exceeding the critical unity value in Tarnava village (W7) and Medias town (W1, W4, W5), which suggest potential non-carcinogenic effects for consumers [23, 24]. Rest of the studied well waters (W2, W3, W6, W8, W9) and spring (S) have *HQ* values below 1.00. The *HQ* results calculated for the NO_3^- concentrations range from 0.102 to 2.692 (Figure 2). Drinking water sources presented high NO_2^- levels, but low values for the *HQ*. The highest value were obtained for the spring sample (S) and the lowest value for W4 (Table 1). Possible sources that can contribute to the high concentrations NO_2^- and NO_3^- are the agricultural and the domestic activities.

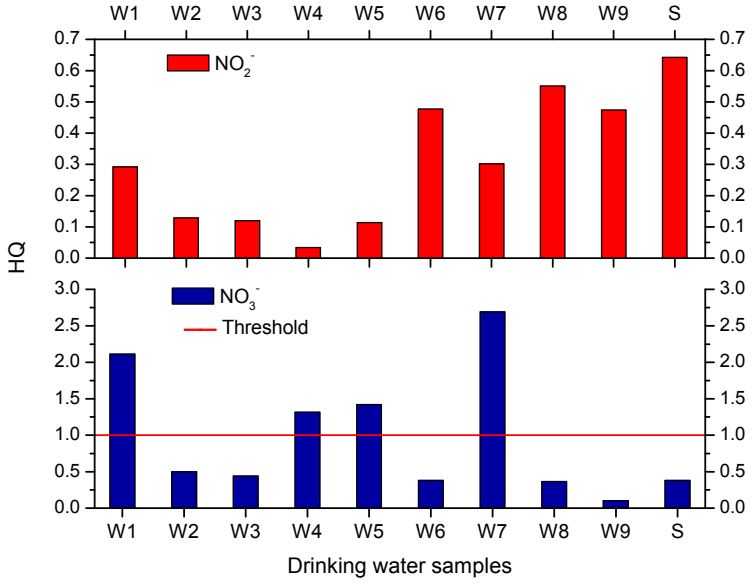


Figure 2. The hazard quotients for NO_3^- and NO_2^- in drinking waters

Table 1. The *CDI*, *HQ* and *THQ* for the drinking waters

	W1		W2		W3		W4		W5	
	CDI mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***
NO_2^-	0.029	0.292	0.013	0.129	0.012	0.120	0.003	0.034	0.011	0.114
NO_3^-	3.378	2.112	0.797	0.498	0.701	0.438	2.101	1.313	2.270	1.419
NH_4^+	0.011		0.007		0.006		0.002		0.004	
THQ	2.404		0.628		0.558		1.347		1.533	
	S		**W6**		**W7**		**W8**		**W9**	
	CDI mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***	***CDI*** mg/kg/day	***HQ***
NO_2^-	0.064	0.643	0.048	0.477	0.030	0.302	0.055	0.551	0.047	0.474
NO_3^-	0.606	0.379	4.308	2.692	4.308	2.692	0.585	0.365	0.163	0.102
NH_4^+	0.020		0.002		0.026		0.023		0.029	
THQ	1.022		3.169		2.994		0.916		0.576	

The *CDI* for NO_3^- and NO_2^- showed low values for samples from Copsa Mica and Medias towns (Figure 3) and high values for water samples collected from Tarnava village. The *CDI* ranged between 0.003 and 0.064 mg/kg/day for NO_2^- and 0.163 and 4.308 mg/kg/day for NO_3^- with a mean of 0.031 mg/kg/day and 1.922 mg/kg/day, respectively.

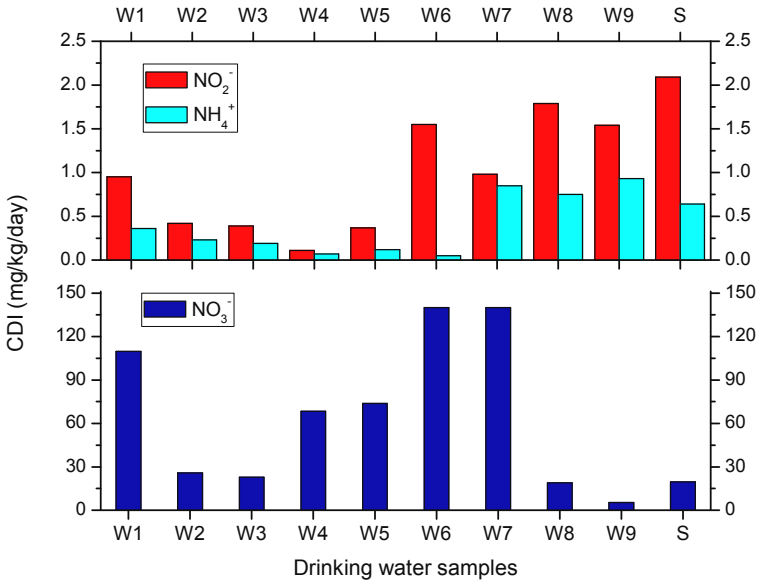


Figure 3. The chronic daily intake for NO_3^-, NO_2^- and NH_4^+ in drinking waters

The *CDI* calculated for the samples from Tarnava village (W6, W7) and Medias town (W1, W4, W5) exceeds 1.00. Obtained *CDI* values for NH_4^+ ranged from 0.002 to 0.029 mg/kg/day, with a mean of 0.013 mg/kg/day. The lowest values were obtained for W4 and W6, while the highest for W9. According to Buss et al. (2014), NH_4^+ is typically present in the wastewater discharges and landfill leachates, which could represent a possible NH_4^+ source for well waters [25]. Low *CDI* values for NO_2^- and NO_3^- (< 0.2 mg/kg/day) and *HQ* values for NO_2^- and NO_3^- (< 0.2) were found in river water samples used as drinking water sources in Poland [25]. Respective values give no cause for concern regarding the non-carcinogenic risk at NO_3^- and NO_2^- [26].

The *THQ* values were higher than 1.00 for the spring sample S and for the well waters from Tarnava village (W6, W7) and Medias (W1, W4, W5). In the case of well waters from Copsa Mica (W8, W9) and from Medias town (W2, W3) the THQ values were lower than 1.00 (Table 1).

CONCLUSIONS

The studied well water samples from Tarnava village are contaminated with NO_3^-, NO_2^- and NH_4^+. The *HQ* for NO_3^- indicated values above the critical unity value in case of one water sample, while the *HQ* for NO_2^- were below the critical unity value. Obtained *THQ* values for the two well water samples from Tarnava village were 3.169 and 2.994. Chronic daily intake of NO_2^- and NH_4^+ were below 0.100 mg/kg/day, while the CDI of NO_3^- was 4.308 mg/kg/day.

Well water and spring samples from Medias town are characterized by low NO_2^- and NH_4^+ concentrations, except one sample, which exceeds the *MAC*. Three water samples exceeded the *MAC* for NO_3^-. Three waters from Medias town showed possible non-charcinogenic risk, according to the *HQ* values, which give a cause of concern. While *HQ* calculated for NO_3^- showed values below 1.00. Results for the *THQ* ranged between 0.558 and 2.404. The obtained values for the *CDI* of NO_2^- ranged between 0.003 and 0.029 mg/kg/day, the calculated values for the *CDI* of NO_3^- ranged from 0.606 to 3.378 mg/kg/day, while the *CD*I of NH_4^+ ranged from 0.002 to 0.011 mg/kg/day.

The NO_3^- concentrations measured for well water samples collected from Copsa Mica town are lower than *MAC*. While the NO_2^- and the NH_4^+ exceeded the *MACs.* The *HQ* for NO_2^- and NO_3^- were lower as the critical unity value, indicating that the water samples present no potential non-carcinogenic

risks. The *THQ* results were below 1.00, suggesting that potential adverse health effects may not appear after the consumption of waters from the studied water sources. Chronic daily intake for NO_2^- and NO_3^- were below 0.200 mg/kg/day, while for NH_4^+ below 0.100. The domestic and agricultural activities were taken into consideration as possible sources for the high nitrogen compounds concentrations. Under these circumstances it is recommended a filtration process for the drinking water before consumption and if it is possible a disinfection of the well waters, for the consumer which have no alternative drinking water sources.

EXPERIMENTAL SECTION

Study area, sampling and chemical analysis

The study region is part of Transylvania, localized in central Romania (Figure 4). This part of the country is characterized by an average annual temperature of 8.6 °C and an annual precipitation range of 700 mm [24]. Population from rural and small urban areas in the study area (Tarnava village, Copsa Mica and Medias towns) practice agricultural activities as livestock growing and crop cultivation. Private well waters and public natural springs are used as drinking water sources [17, 18].

One spring sample (S1) from Medias, two well water samples (W1, W2) from Tarnava village and two well water samples (W3, W4) from Copsa Mica were collected [17, 18]. The drinking water samples were collected during summer of 2015, one from each sampling point (Figure 4) in polyethylene bottles and kept at 4 °C in a refrigerator until the chemical analysis. Water samples were filtered using cellulose acetate membrane filters with pore-size of 0.45 μm [17, 18]. The NO_2^-, NO_3^- and NH_4^+ concentrations were measured by ion-liquid chromatography, according to ISO 10304-1:2007, using the 761 Compact IC (Methrom, Herisau, Switzerland) and the NH_4^+ as indophenol blue complex, according to SR ISO 7150-1:2001 by spectrophotometer Lambda 25, (Perkin-Elmer, Beaconsfield, UK).

The accuracy of NO_2^-, NO_3^- and NH_4^+ determinations was tested by analysing Nitrite standard solution (40 mg/L), Nitrate standard solution (15.0 mg/L), and Ammonium standard solution (1.0 mg/L) purchased from Merck. The found results were in good agreement with the certified values for all parameters. The recovery expressed as relative standard deviation degree ranged between 89 % and 100 %.

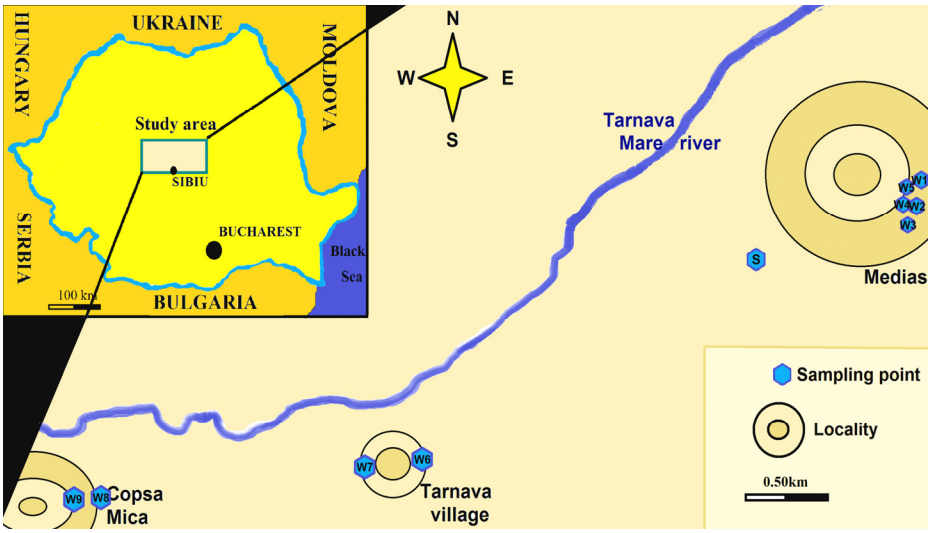


Figure 4. Study area and the location of the sampling points

Human health risk assessment

In order to estimate health risk associated with NO_2^- and NO_3^- in drinking water, the *CDI* was calculated using the following equation [27]:

$$CDI = \frac{C \times DI}{BW} \quad (1)$$

Where *C* represents the NO_3^- and NO_2^- concentrations in drinking water (mg/L), *DI* is the daily intake rate (2 l/day) and *BW* represents the body weight (72 kg) [27].

The hazard quotient (*HQ*) estimates the non-carcinogenic risk posed by NO_3^- and NO_2^- in drinking water, and was calculated using the following equation [27]:

$$HQ = \frac{CDI}{RfD} \quad (2)$$

The *RfD* represents the reference dose with values of 0.10 mg/kg/day for NO_2^- and 1.6 mg/kg/day for NO_3^-. The reference doses were set by EPA's IRIS (Integrated Risk Information System) Program [28, 29]. There are no potential non-carcinogenic risks for the exposed population in cases when the *HQ* does not exceed unity (*HQ* < 1.00) [27, 29]. In the current study the oral exposure was taken into consideration for the count of the *HQ*.

THQ represents a summation of the ingestion of NO_3^- and NO_2^- through drinking water [30].

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164 and by Romanian financial authority CNCS-UEFISCDI, Partnership project VULMIN, Contract No, 52/2012.

REFERENCES

1. J. Czekaj, S. Jakobczyk-Karpiez, H. Rubin, S. Sitek, A.J. Witkowski, Physics and Chemistry of the Earth, Parts A/B/C, 2015, doi:10.1016/j.pce.2015.11.005.
2. ***, Guidelines for Drinking-water quality, Fourth edition, World Health Organisation, **2011**. Online source: *http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf*
3. A. Pisciotta, G. Cusimano, R. Favara, *Journal of Geochemical Exploration*, **2015**,*156*, 89.
4. C.M. Benkovitz, M.T. Scholtz, J. Pacyna, L. Tarrason, J. Dignon, E.C. Voldner, P.A. Spiro, J.A. Logan, T.E. Graedel, *Journal of Geophysical Resources*, **1996**, *101*, 29239.
5. S.A.R. Mousavu, S. Ibrahim, M.K. Aroua, S. Ghafari, *International Journal of Chemical and Environmental Engineering*, **2010**, *1 (1)*, 1.
6. A.M. Fan, V.E. Steinberg, *Regulatory Toxicity and Pharmacology*, **1996**, *23*, 35.
7. K. A. Garba, *International Journal of Research in Ayurveda and Pharmacy*, **2015**, *6*, 348.
8. N. Kresic, "Hydrology and groundwater modelling", CRC Press, Taylor & Francis Group, Boca Raton, **2007**, 828.
9. P.J. Weyer, F.R. Cerhan, C.K. Burton, G.R. Hallberg, J. Kantamneni, G. Breuer, M.P. Jones, W. Zheng, C.F. Lynch, *Epidemiology*, **2001**, *11*, 327.
10. Shrimali, M. and K.P. Singh, *Environmental Pollution*, **2001**, *112 (3)*, 351.
11. WHO, *Guidelines for drinking - water quality,* **2008**, *1*.
12. W. Wang, Y. Fan, G. Xiong, J. Wu, *Journal of Huazhong University of Science and Technology [Medical Science]*, **2012**, *32*, 912.
13. J.K. Bohlke, R.L. Smith, D.N. Miller, *Water Resources Research - Water resources* , **2006**, *42 (5)*, 1.
14. G.W. Randall, D.J. Mulla, *Journal of Environmental Quality*, **2001**, *30*, 337.
15. S. Chaudhuri, S. Ale, *Journal of Hydrology*, **2014**, *513*, 376.
16. M.A. Hoaghia, C. Roman, C. Tanaselia, D. Ristoiu, *Studia Universitatis Babes-Bolyai, Chemia*, **2015**, *60*, 161.
17. M.A. Hoaghia, M. Kovacs, E. Levei, M. Miclean, M. Roman, M. Senila, C.Tanaselia, D. Ristoiu, *Agriculture Science and Practice Journal*, 2015, *1*, 93.

18. M.A. Hoaghia, E. Levei, C. Roman, M. Senila, C. Tanaselia, D. Ristoiu, *Food and Environment Safety*, **2015**, *14*, 138.
19. M. Pele, G. Vasile, M. Artimon, *Scientific Papers*, *UASVM Bucharest*, **2010**, *53*, 145.
20. ***, Council Directive 91/676/EEC, concerning the Protection of the water against pollution caused by nitrates from agricultural sources". *Official Journal of European Communities* L. 375, 1/5, **1991**.
21. ***, Council Directive 98/83/EEC, on the quality of water intended for human consumption". *Official Journal of European Communities L*. 330, 32, **1998**.
22. ***, 311 Low from 6rd June **2004** that improves and complement Low 458 from 29 July **2002** regarding the quality of drinking water. *Official Gazette* 2004, Part I, no. 582/30.06.2004 [In Romanian].
23. M.S. Qaiyum, M.S. Shaharudi, A.I. Syazwan, A. Muhaimin, *Journal of Water Resource and Protection*, **2011**, *3,* 268.
24. L. Badea, P. Gastescu, V.A. Velcea, "Romanian Geography", Academia Republicii Socialiste Romania Publishing House, Bucuresti, **1983**, 663 p.
25. Buss S.R., Herbert A.W., Morgan P., Thornton S.F., Smith J.W.N., *Quarterly Journal of Engineering Geology and Hydrogeology*, **2014**, *37*, 347.
26. Pawelczyk A., *Water Science Technology*, **2012**, *66*, 666.
27. S. Muhammad, M.T. Shah, S. Khan, *Microchemical Journal*, **2011**, *98*, 334.
28. EPA, **1991**, IRIS (Integrated Risk Information System), "A-Z List of substances". Online source: *http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0078.*
29. EPA, **1997**, IRIS (Integrated Risk Information System), "A-Z List of substances". Online source: *http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showQuickView&substance_nmbr=0076.*
30. S. Ferson, "RAMAS Risk Calc 4.0 Software: Risk Assessment with Uncertain Numbers", CRC Press, Boca Raton, Florida, U.S.A., **2002**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 461-471)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

THE EVALUATION OF THE METAL CONTAMINATION OF DRINKING WATER SOURCES FROM MEDIAS TOWN, ROMANIA USING THE METAL POLLUTION INDICES

MARIA-ALEXANDRA HOAGHIA[a,b,*], CECILIA ROMAN[a], EMOKE DALMA KOVACS[a], CLAUDIU TANASELIA[a], DUMITRU RISTOIU[b]

ABSTRACT. Drinking water represents a direct source for the toxic and persistent metals entrance in the organisms. Their bioaccumulation makes them harmful for living organisms, including humans. Assessing the quality of the drinking water sources represents a significant prevention and protection measure. Quality assessment methods such as heavy metal pollution index, degree of contamination and heavy metal evaluation index are applied worldwide. The aim of current study was to evaluate the metal contamination of drinking water sources from Medias town using the pollution and quality assessment indices. The concentrations of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were assessed in 21 drinking water sources (19 well waters and 2 public springs). Results indicate Mn and Cd concentrations exceeding the threshold limits of the drinking water quality guidelines, only in summer season, in three samples. In the spring season the regulatory limits were not exceeded. The quality assessment indices show low contamination degree, all values being lower than the critical ones. Positive correlations were observed between pollution indices, As, Cd, Mn, Zn, Ni and Cu concentrations.

Keywords*: drinking water sources, contamination index (C_d), heavy metal evaluation index (HEI), heavy metal pollution index (HPI)*

INTRODUCTION

Today, throughout the world, the quality of drinking water sources is declining especially due to the population and economic growth. Countless and diverse wastes, hazardous contaminants and emissions are released

[a] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, RO-400293 Cluj-Napoca, Romania.*
[b] *Babeş-Bolyai University, Faculty of Environmental Science and Engineering, 30 Fantanele, RO-400294 Cluj-Napoca, Romania*
[*] *Corresponding author: alexandra.hoaghia@icia.ro*

directly and indirectly into the environment [1-3]. Metals are among the most frequent contaminants that pose a hazard for the environment due to their persistence, toxicity, bioaccumulation and non-biodegradability [4, 5]. High concentrations of metals in aqueous environments led to serious problems concerning the human, animal and plant health [6, 7]. Accumulated metals in the human body may cause damage of internal organs, neurological disorders, inhibition of embryo and children development, and even death [8, 9]. Bioaccumulation of metals occurs in plants also, therewith negative effects and affection of the photosynthesis, absorption and exchange of gases [10, 11]. Anthropogenic sources (metallurgical industry, smelting, mining, galvanic processes, traffic) and geological background are reflected by the metals spatial variability [12-14]. As a worldwide concern, water quality studies are carried out to monitor and to prevent the chemical pollution [15, 16].

Metal pollution indices are significant tools for drinking water quality assessment and have been successfully applied all over the world [17-20]. Assessment methods, such as the quality index are effective in gathering a composite influence of indicators on the overall contamination [21-23]. The C_d (degree of contamination) represents an approach to evaluate the areas characterized by unsafe concentrations of defined metals [24]. The applied method considers the combined effects of the studied elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) with harmful effects of a consumed contaminated drinking water [25]. The *HPI* (heavy metal pollution index) is based on the weighted arithmetic quality mean method generated in two basic steps: selection of a rating scale (an arbitrary value between 0 and 1) for all quality characteristics, giving weightage to the selected elements followed by the selection of the pollution indicators, which represents the essential part of the index count [17]. For drinking water, the critical *HPI* value of 100 is considered [17].

Study results indicate exceedances of the metal maximum admissible concentrations (*MACs*) for the As, Fe, Mn, Pb and Cr concentrations, and high values for the metal pollution indices. Results present values higher as the critical ones, which are 3 for the C_d (degree of contamination), 100 for the *HPI* (heavy metal pollution index) and 400 for the *HEI* (heavy metal evaluation index) [19].

The aim of the current research was to assess the quality of the groundwater used as drinking water source, from Medias town, starting from the study carried out in 2014, in which the chemical status and inorganic contamination of well waters from urban area (Medias town) and mining areas (Tarna Mare, Trut and Turulung) were investigated and compared [26]. Considering the high values of metals (Cd and Mn exceeding the maximum admissible limits) present in the well waters from Medias town, studying the pollution status from a different view, using different methods represents a necessity due to the use of well waters as drinking water source. Hereby, in

the present research metal pollution indices methods (C_d, *HPI* and *HEI*) were used as water pollution evaluation tools, summarizing the combined effects of metals concentrations (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn). High concentrations of metals are considered toxic and harmful to the water quality and ecosystems and unassailably to animals and human health [24].

RESULTS AND DISCUSSION

The descriptive statistics of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the studied drinking water samples are given in table 1. The studied metals, except Mn and Cd, are below the regulatory limits of the drinking water standards. Water samples W9 and W10 have Mn concentrations (66 µg/l and 82 µg/l) higher than *MAC* in the summer season with 33 % and 41 %, respectively [26]. In the spring season, Mn level is lower as 50 µg/l [26]. Still, average values of Mn concentrations in both seasons for W9 and W10 are 49 µg/l and 50 µg/l, second value reaching the regulatory limit [26]. In the summer season, 29 % of the samples present higher Mn concentrations as in the spring season [26]. In the spring season the maximum concentration was 33 µg/l, while in the summer season was 82 µg/l, and the lowest level was 0.12 µg/l, respectively 0.20 µg/l [26]. According to Zaporozec (1981), the high concentrations of Mn have naturally occurrence in the groundwater system from the soil matrix [27].

The Cd threshold limit (5 µg/l) is exceeded for sample W12 almost two times (average of 9.6 µg/l); 71 % of the samples present higher Cd levels in summer than in spring while 67 % of the samples have concentrations, below the quantification limit (0.10 µg/l). Possible source for the high Cd concentrations is a former non-ferrous metallurgical plant, localized at a relatively short distance (almost 15 km).

Table 1. Descriptive statistics for the studied elements [26, 28]

Element (µg/l)	Minimum	Maximum	Mean	Median	Standard Deviation	MAC*
As	0.08	4.3	0.77	0.33	1.0	10
Cd	<0.10**	9.6	0.55	0.04	2.1	5
Cr	0.47	10	2.8	2.2	2.0	50
Cu	0.84	8	3.9	3.7	2.2	100
Fe	32	99	58	52	19	200
Mn	0.30	50	9.7	3.3	15	50
Ni	1.0	5.2	2.2	1.8	1.1	20
Pb	0.10	6.2	1.02	0.40	1.61	10
Zn	10	970	132	48	228	5000

*According to Romanian legislation regarding the drinking water quality [28]
**Below the quantification limit (0.10 µg/l)

The calculated pollution indices for the studied water samples are given in table 3. According to the C_d, the lowest degree of contamination was obtained for water sample W2 (-8.7), while the highest degree of contamination was determined for water samples W12 (-5.2). The studied region was found to have low degree of contamination, as the C_d average value (-8.0) indicates. Similar results were obtained by Backman et al. (1997), using the C_d for groundwater samples from Slovakia and Finland, but also values of C_d = *1*-3 and C_d >3 for studied elements (Al, As, B, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Rb, Sb, Se and Zn) which indicates groundwater contamination, having as contamination sources: mining activities, natural occurrence and geogenic processes of metals [24]. In a different study conducted in Bangladesh, on drinking water sources, the C_d was used; values between 3.45 and 85 were obtained, results suggesting a high contamination status of the water samples [20].

Table 2 presents the indices used for the calculation of *HPI* count for water samples. The mean concentrations of the two data sets obtained in both sampling campaigns were used. The Romanian threshold limits (Law 311/2004 on drinking water quality were used as the standard permissible values [28].

According to the *HPI* results, the highest obtained value is 100.3 for sample W12, while spring sample S2 is characterized by the lowest metal pollution (1.8). Thought the *HPI* mean value indicates low metal pollution (10). The highest concentrations for Pb, Cd and As were measured in sample W12, which implies the highest value for *HPI*. Higher *HPI* values than the critical value of 100, for studied metals (As, B, Cd, Co, Cr, Fe, Mn, Pb, Se, Sr and Zn) were obtained by Ehya and Marbouti (2016) for groundwater samples collected from Iran, due to the leaking of industrial and urban sewages into the aquifers, as well as fossil fuels combustion [29]. Lower *HPI* values than 100, for studied metals (Cd, Pb, and Zn) were obtained in Tirupati, Indian groundwater samples used as drinking water source [17].

Based on the average *HEI* value (1.0) and the metal indices calculation, the studied water samples are assessed as having low metal level. Sample W2 has the lowest *HEI* value (0.34), while sample W12 has the highest *HEI* value (3.8), indicating low metal pollution level. Bhuyan et al. (2010) used as well the *HEI* for drinking water samples and obtained results between 10.26 to 367 [20].

Correlations between the measured metal concentrations and metal pollution indices were calculated using the correlation matrix.

Table 2. *HPI* calculation for drinking water sources of Medias town, $\sum W_i$=0.51 $\sum W_i$ × Q_i=5.2; *HPI*=10

Metals	Mean concentration -M_i-(µg/l)	Standard permissible value -S_i-(µg/l)	Unit weightage -W_i-	Sub index -Q_i-	$W_i \times Q_i$
As	0.77	10	0.10	7.7	0.77
Cd	0.55	5	0.20	11	2.2
Cr	2.8	50	0.02	5.5	0.11
Cu	3.9	100	0.01	3.9	0.04
Fe	58	200	0.01	29	0.14
Mn	9.7	50	0.02	19	0.39
Ni	2.2	20	0.05	10.9	0.54
Pb	1.0	10	0.10	10.2	1.0
Zn	132	5000	0.0002	2.6	0.001

Table 3. Calculation of the metal pollution evaluation indices

Sample no.	C_d	Mean deviation	*HPI*	Mean deviation	*HEI*	Mean deviation
W1	-7.9	-0.07	8.6	1.7	1.1	-0.07
W2	-8.7	0.66	2.1	8.2	0.34	0.66
W3	-8.5	0.47	3.5	6.8	0.53	0.47
W4	-8.2	0.20	7.8	2.9	0.80	0.20
W5	-8.3	0.26	4.1	6.2	0.74	0.26
W6	-8.5	0.45	3.5	6.8	0.55	0.45
W7	-8.5	0.53	2.8	7.5	0.47	0.53
W8	-7.8	-0.21	5.2	5.1	1.2	-0.21
W9	-7.3	-0.68	6.6	3.7	1.7	-0.68
W10	-7.5	-0.45	6.3	4.0	1.5	-0.45
W11	-8.3	0.26	3.8	6.5	0.75	0.26
W12	-5.2	-2.8	**100.3**	-90	3.8	-2.7
W13	-8.2	0.22	7.2	3.1	0.78	0.22
W14	-7.3	-0.67	19	-8.9	1.7	-0.67
W15	-8.2	0.21	4.8	5.5	0.79	0.21
W16	-8.4	0.38	3.5	6.8	0.62	0.38
W17	-8.4	0.41	3.6	6.7	0.59	0.41
W18	-7.9	-0.05	5.9	4.4	1.1	-0.05
W19	-8.1	0.13	7.3	3.0	0.87	0.13
S1	-8.1	0.15	8.6	1.6	0.86	0.15
S2	-8.6	0.56	1.8	8.5	0.44	0.56
Minimum	-8.7		1.8		0.34	
Maximum	-5.2		100.3		3.8	
Mean	-8.0		10		1.0	

Thus, positive relationships between metal concentrations can be observed, such as Zn and Mn and Zn and Cu. Also, there is a positive and direct correlation between As, Cd and Pb. The level of Cd is as well directly related to the Ni concentration (Tabel 4).

Table 4. Correlation matrix for the obtained metal concentrations and metal pollution indices values (n=21)

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Cd	HPI	HEI
As	**1**											
Cd	**0,797***	**1**										
Cr	0,081	0,176	**1**									
Cu	0,184	0,190	0,339	**1**								
Fe	-0,038	-0,116	0,421	0,125	**1**							
Mn	-0,089	-0,091	-0,040	0,332	-0,198	**1**						
Ni	0,425	**0,534**	0,012	-0,046	0,136	-0,218	**1**					
Pb	**0,838**	**0,720**	0,233	0,047	-0,067	0,018	0,282	**1**				
Zn	0,161	0,352	0,093	**0,580**	-0,210	**0,579**	0,017	0,147	**1**			
Cd	**0,785**	**0,861**	0,272	0,375	-0,015	0,347	0,426	**0,788**	**0,547**	**1**		
HPI	**0,628**	0,285	0,306	0,307	0,002	0,275	0,154	**0,744**	0,194	**0,573**	**1**	
HEI	**0,785**	**0,861**	0,272	0,375	-0,015	0,347	0,426	**0,788**	**0,547**	**1,000**	**0,573**	**1**

*Values in bold are different from 0 with a significance level alpha=0.05

The Pearson correlation matrix revealed that the metal pollution indices (C_d, *HPI* and *HEI*) are directly affected by As, Cd, Pb and Zn concentrations. Additionally, the comparison between the used assessment methods indicates a significant correlation, especially between C_d and *HEI*, positively correlated (table 4). For example, the highest As, Pb and Cd concentrations in sample W12, is directly correlated with the highest *HPI*.

CONCLUSIONS

The studied drinking water sources from Medias town present high concentrations of Cd and Mn, exceeding the *MAC*s, while As, Fe, Cr, Cu, Zn, Pb, Ni do not exceed the threshold limits. The Mn level exceeds the *MAC* in the summer season with 33 % (W9) and 41 % (W10), but in the spring season does not exceed the corresponding *MAC*. In addition, in summer season Cd *MAC* is surpassed in 71 % of the studied samples. There is a positive correlation between Pb, Cd and As, similarly between Zn and Cu and Zn and Mn concentrations. Metal pollution indices (C_d, *HPI* and *HEI*) showed that water samples are characterized with a low contamination degree ($C_d < 0$) and the calculated *HPI* was below the critical value of 100, except sample W12, which exceeds the threshold with 0.003 %. Sample W12 presents the

highest As, Cd and Pb concentrations for which the highest *HPI* was obtained, which indicates a positive correlation between the metal concentrations and the HPI results. The *HEI* classifies the water samples as low contamination. Methods C_d and *HEI* present a significant correlation and positive correlation between C_d and *HPI*, *HEI* and C_d.

EXPERIMENTAL SECTION

General description of the study area

Study area is located in Medias, Sibiu County, Transylvania, Romania (figure 1). At 15 km South-West from Medias, in Copsa Mica town, one of the biggest Romanian non-ferrous metallurgical industry unities was localized. Copsa Mica area is known as a metal pollution *hot spot* in Romania and in Europe [30, 31]. The main activity of the company was the production of Pb, Sn, Cu and Zn, until 2005 [30, 31]. The area was the subject of numerous studies regarding the environment pollution, indicating high level of metals, which exceed the *MAC* and deteriorate the health condition after the exposure. As a result, mortality and morbidity among the inhabitants of Copsa Mica area increased [32].

Medias area is characterized by hill formations localized on the Tarnava Mare terraces [33]. Smooth cliffs and horizontal fragmentation characterize Medias town surroundings [34]. The climate is continental temperate and it is governed by the average annual temperature of 8 ºC, 600-800 mm for the mean annual precipitations and average annual humidity of 87 % [34].

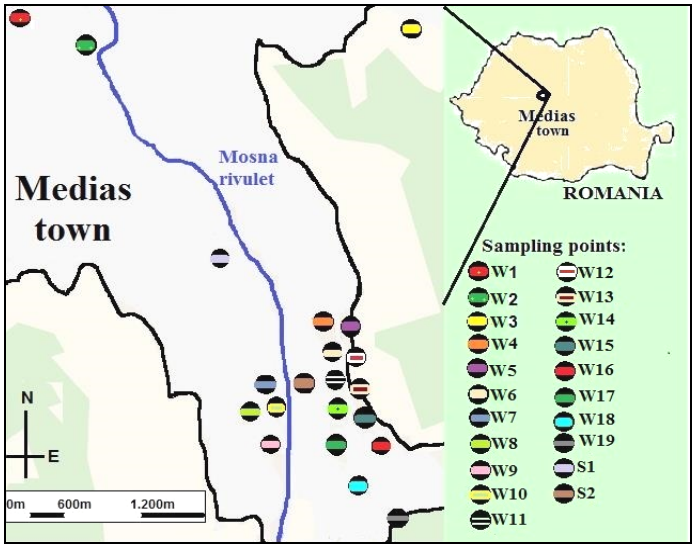


Figure 1. Study area including sample location map

Town is crossed from Vest to East by Tarnava Mare River and from South to North by its right side tributary, Mosna Rivulet [35]. Tarnava Mare's water is used as drinking water source as well as the natural springs. The groundwater is directly used from private well waters and public natural springs by the inhabitants.

The main objectives of the current research are to determine the metal content of the groundwater used as drinking water sources and to assess the water quality using the metal pollution indices.

Sampling, sample preparation and instrumentation

A number of 21 groundwater samples were collected from drinking water sources (19 well waters, W1-W19 and 2 public springs, S1-S2) localized in Medias town [26]. Samples were collected in two sampling campaigns (spring and summer 2014). Polyethylene bottles (1000 ml) were used for the collection of the water samples, which were preserved with HNO_3 (for acidification at pH 2) and until the chemical analysis kept at 4 ºC; 0.45 µm acetate cellulose filter membrane were used for sample filtration. The metal content (As, Cd, Cr, Cu, Fe, Mn, Pb and Zn) was measured by inductively coupled plasma mass spectrometry (ICP-MS) using an ELAN DRC II (Perkin-Elmer, Canada) instrument.

Evaluation methods

Three quality evaluation methods were used in the study, namely the degree of contamination (C_d), the heavy metal pollution index (*HPI*) and the heavy metal evaluation index (*HEI*).

The degree of Contamination (C_d)

The C_d is calculated using according to equation 1 and 2 [24] each water sample separately, as a sum of contamination factors of the studied metals exceeding the upper permissible values [19, 24].

$$C_d = \sum_{i=1}^{n} C_{fi} \tag{1}$$

$$C_{fi} = \frac{C_{Ai}}{C_{Ni}} - 1 \tag{2}$$

Where, C_{fi} represents the contamination factor for the *i*-th element (i = As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn), C_{Ai} is the analytical value for the *i*-th element and C_{Ni} is considered to be the upper permissible concentration of the *i*-th element; with *Ni* as the normative value regularized by the Romanian

legislation [19, 24]. Studied metals maximum admissible concentrations presented by Law 311 from 2004, regarding the quality of the drinking water were used [28]. The C_d values are classified in three categories, reflecting the contamination level (*CL*): low *CL* (C_d < 1), medium *CL* (1 < C_d < 3) and high *CL* (C_d > 3) [19, 24].

Heavy metal pollution index (HPI)

The quality of water with respect to metals (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) is given by *HPI*. The *HPI* was calculated according to equations 3 and 4, [17]:

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \tag{3}$$

$$Q_i = \sum_{i=1}^{n} \frac{\{M_i(-)I_i\}}{(S_i - I_i)} \times 100 \tag{4}$$

The unit weightage of each *i*-th parameter is represented by W_i with *n* as the total number of the indicators considered [17]. The Q_i and the M_i are the sub-index and the monitored value of the *i*-th parameter, while the standard value and the desirable value of the *i*-th parameter are S_i *and* I_i, respectively [17]. The W_i was defined as inversely proportional to S_i, which was taken according to the Romanian legislation [28]. In current study the desirable value, I_i is considered 0, because in the Romanian legislation no ideal value was established.

Heavy metal evaluation index (HEI)

The *HEI* represents a quality assessment tool with respect to metals. The method is expressed using equation (5) [19]:

$$HEI = \sum_{i=1}^{n} \frac{H_c}{H_{mac}} \tag{5}$$

The technique uses H_c as the monitored value and H_{mac} as the MAC of the *i*-th parameter [19]. In the current research, the considered MAC, is used according to Romanian legislation, Law 311, from 2004 [28]. Bhuyan et al. (2010) suggested *HEI* criteria for groundwater, with a baseline established by Edet and Offiong, but for surface water (2001): low pollution (*HEI* < 40), medium (*HEI* = 40-80) and high pollution (*HEI* > 80) [19, 20].

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme "Increase of Economic Competitiveness", Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164 and by Romanian financial authority CNCS-UEFISCDI, Partnership project VULMIN, Contract No, 52/2012.

REFERENCES

1. L.O. Nriagu, *Science*, **1996**, *272*, 223.
2. M. Senila, E.A. Levei, L.R. Senila, *Chemistry Central Journal*, **2012**, *6*, 119.
3. M.S. Islam, M.K. Ahmed, M. Raknuzzaman, M. Habibullah-Al-Mamun, M.K. Islam, *Ecological Indicators*, **2015**, *45*, 282-291.
4. J. O. Duruibe, M.D.C. Ogwuegbu, J.N. Egwurugwu, *International Journal of Physical Sciences*, **2007**, *2*, 112.
5. O. Cadar, M. Miclean, S. Cadar, C. Tanaselia, L. Senila, M. Senila, *Environmental Engineering and Management Journal*, **2015**, *14,* 2523.
6. C.M. Zvinowanda, J.O. Okonkwo, P.N. Shabalala, N.M. Agyei, International Journal of Environmental Science and Technology, **2009**, *6*, 425.
7. M. Senila, E.A. Levei, L.R. Senila, M. Roman, Journal of Chemistry, **2015**, article ID 762121, 1.
8. D. V. Dumitrescu, E.S. Gurzău, A.E. Gurzău, D. Marchean, I. Chera, P. Motoiu, A. Muică, Copşa Mică area and heavy metals pollution, Proc, of PRE9: Protection and Restoration of the Environment IX, Kefalonia, Greece, **2012**, 186.
9. E. Muntean, N. Muntean, M. Duda, *ProEnvironment*, **2013**, *6*, 469.
10. M. Senila, C. Tanaselia, E. Rimba, *Carpathian Journal of Earth and Environmental Sciences*, **2013**, *8*, 145.
11. N. Alia, K. Sardar, M. Said, K. Salma, A. Sadia, S. Sadaf, A. Toqeer, S. Miklas, *International Journal of Environmental Research and Public Health,* **2015**, *12*, 7400.
12. M. Imperato, P. Adamo, D. Naimo, M. Arienzo, D. Stanzione, P. Violante, *Environmental Pollution*, **2003**, *124*, 247.
13. T. Frentiu, M. Ponta, E. Levei, E. Gheorghiu, M. Benea, E. Cordos, *Chemical Speciation and Bioavailability,* **2008**, *20*, 99.
14. A. E Levei, T. Frentiu, M. Ponta, M. Senila, O. Moldovan, *Environmental Engineering and Management Journal*, **2015**, *14*, 2567. – marime 10
15. A. Maher, M. Sadeghi, A. Moheb, *Desalinization*, **2014**, *352*,166.
16. C. Wang, N. Yuan, Y. Pei, *Journal of Hazardous Materials*, **2014**, *274*, 342.
17. S.V. Mohan, P. Nithila, S. Jayarama Reddy, *Journal of Environmental Science and Health*, **1993**, *31 (2)*, 283-289.
18. A. Rapant, M. Raposova, D. Bodis, K. Marsina, I. Slaninka, *Journal of Geochemical Exploration*, **1999**, *66*, 151.

19. A.E. Edet, O.E. Offiong, *GeoJournal*, **2002**, *57*, 295.
20. M.A.H. Bhuiyan, M.A. Islam, S.B. Dampare, L. Parvez, S. Suzuki, *Journal of Hazardous Materials*, **2010**, *179*, 1065.
21. R. K. Horton, *Journal of Water Pollution, Control Federation*, **1965**, *37*, 300.
22. H.M. Joung, W.W. Miller, C.N. Mahammah, J.C.A. Gultjens, *Journal of the Environmental Quality*, **1979**, *8*, 95.
23. B. Prasad, J.M. Bose, *Environmental Geology*, **2001**, *41*,183.
24. B. Backman, D. Bodis, P. Lahermo, S. Rapant, T. Tarvainen, *Environmental Geology*, **1997**, *36*, 55.
25. M.V. Prasanna, S.M. Praveena, S. Chidambaram, R. Nagarajan, A. Elayaraja, *Environmental Earth Science*, **2012**, *67*, 1978.
26. M.A. Hoaghia, E. Levei, C. Roman, M. Senila, C. Tanaselia, D. Ristoiu, *Journal of Faculty of Food Engineering, Food and Environmental Safety*, **2015**, *14 (2)*, 138.
27. A. Zaporozec, *GeoJournal*, **1981,** *5*, 457.
28. Law 311 from 6rd June 2004 that improves and complement 458 Law from 29 July 2002 regarding the quality of drinking water, Official Gazette 2004, Part I, no. 582/30.06.2004 [In Romanian].
29. F. Ehya, Z. Marbouti, *Environmental Earth Science*, **2016**, *75* (*455)*, 1.
30. Primăria municipiului Mediaş, *Raport municipal Mediaş 2014*. Online source accessed in 18.02.2016 from:
http://www.primariamedias.ro/portal/portal.nsf/All/FFC09C0BDE05DC6DC22579C20043FC40/$FILE/RAPORT%20MUNICIPIUL%20MEDIAS%202014.pdf
31. D.V. Nica, M. Bura, I. Gergen, M. Harmanescu, D.M. Bordean, *Chemistry Central Journal*, **2012**, *6 (1)*, 55.
32. D. Curseu, M. Popa, D. Sirbu, Z. Moldovan, E. Czuczi, "Tenth International Water Technology Conference", IWTC10, Alexadria, Egypt, **2006**, 1185. Acceced online in 05 January 2016 form:
https://www.researchgate.net/publication/267851184_Water_pollution_in_the_Copsa_Mica_region_after_Romanian_revolution
33. K.O, Reti, G. Roşian, *Environment & Progress*, **2007**, *11*, 412.
34. E.D. Horhoi, Tarnava Mare channel environmental quality: a geoecological study, Edition, Logos 1994, Oradea, Romania, **2001**.
35. L. Badea, M. Buza, G. Niculescu, M. Sandu, W. Schreiber, M. Serban, A. Kadar, "*Relief units from Romania, Apuseni Mountains and Transylvania Plateau*", Edition by Ars Docendi, Bucureşti, Romania, **2006**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 473-483)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

CONSTRUCTION AND CHARACTERISATION OF A MICROBIAL FUEL CELL WITH SOIL MICROORGANISMS

STEFANA MADALINA GROZA, MIHAI POZNA, MIRCEA ANTON[a,*]

ABSTRACT. Microbial fuel cells are based on the ability of microorganisms to produce energy through biomass degradation. This study presents the construction, electrical characterization and possible applications of a type of MFC with microorganisms from soil. Although the obtained power density is low compared to other authors (1W/m^3, respectively 500mA/m^2), the proposed construction is very simple, without moving parts, chemical substances or external electrical energy consumption. Possible applications of this type of MFC are presented: sodium acetate aqueous solution sensor and electrical energy source in isolated areas.

Keywords*: microbial fuel cell, soil microorganisms, power density, miniaturised MFC*

INTRODUCTION

Microbial fuel cells (MFCs) represent a promising technology, based on the conversion of chemical energy into electrical energy *via* microbial catalysis [1-3]. This process results when bacteria switches from natural electron acceptor (oxygen or nitrate) to an insoluble acceptor (the MFC anode) [4]. Thus, an oxidation reaction occurs at the anode and electrons are released to respiratory enzymes [5-6]. The electrons are then conducted over a resistance towards the cathode, where a reduction reaction develops. In order for the electroneutrality to be preserved, an equal number of protons must be exchanged between the electrodes [6].

Due to the potential of microbial fuel cell systems, research has been made in every aspect regarding the power density – electrodes with

[a] *Babeş-Bolyai University, Faculty of Environmental Sciences and Engineering, 30 Fantanele str., RO-400327, Cluj-Napoca, Romania*
[*] *Corresponding author: mircea.anton@ubbcluj.ro*

different catalysts (platinum, Fe (III), Mn (IV)), electrogenic bacterial strains (*Brevibacillus* sp. PTH1 and *Pseudomonas* sp., *Saccharomyces cerevisiae* and *Hansenula anomala, Rhodoferax ferrireducens, Geobacter* species), various mixed cultures of bacteria (activated sludge, wastewater) and MFC configurations (two-chambered, membrane-less, stacked MFC, upflow MFC, etc.) [7-8].

Despite all of the efforts that have been made, the performance for large scale MFCs is limited by bottlenecks such as transfer resistances [9], concentration polarization [10], ohmic losses and the type of membrane that is being used [11][12]. As a solution to some of MFCs technical disadvantages, the current development implied miniaturised cells with faster mass transfer and reaction kinetics, better results for power density and internal resistance and a better start-up time [13].

The technologies developed for miniaturised microbial fuel cells vary from polydimethylsiloxane (PDMS) chambers [14] to microfluidic fuel cells, from a wide range of electrodes to different strains of algae [15] and bacteria [16-19].

Microbial fuel cells with soil microorganisms represent the latest topic in the research and development of MFCs. Easy to build and manage, these types of cells are based solely on the conversion of chemical energy from the soil into electricity.

Rich in complex sugars and nutrients, soils also contain electrogenic microbes [20] and aerobic bacteria that act as oxygen filters, the same as the most expensive proton exchange membrane materials used in laboratory MFC system, and that decrease the redox potential in soil according to depth [21].

Shewanella and *Geobacter* species, both present in soils and sediments have been the most successfully researched [22].

The power in the case of this type of MFCs is given by the difference in the potential of the two electrode areas. The metabolic compounds determine a decrease in the electric potential at the anode and the dissolved oxygen determines an increase of the potential at the cathode.

So far soil MFCs have been effectively used in pollution degradation and waste treatment. From marine sediment to garden compost, it is clear that organic matter and microorganisms from soil can be used as a resource for electrical energy [23].

The carbon, nitrogen and bacteria found in soils play an extremely important role in determining the operation of the microbial fuel cell with soil-based microorganisms. Agricultural soil is richer in carbon, nitrogen and different minerals, and thus it is used more often compared to forest soil or other types of soil, due to the higher rate of electricity production [24].

RESULTS AND DISCUSSION

Determining the electromotive force and the internal electrical resistance

The electromotive force (emf) and internal resistance of the batteries have been determined through the polarization curve method. Figure 1 shows a typical polarization curve for the μ103 battery at a 0.07 mol/L sodium acetate concentration.

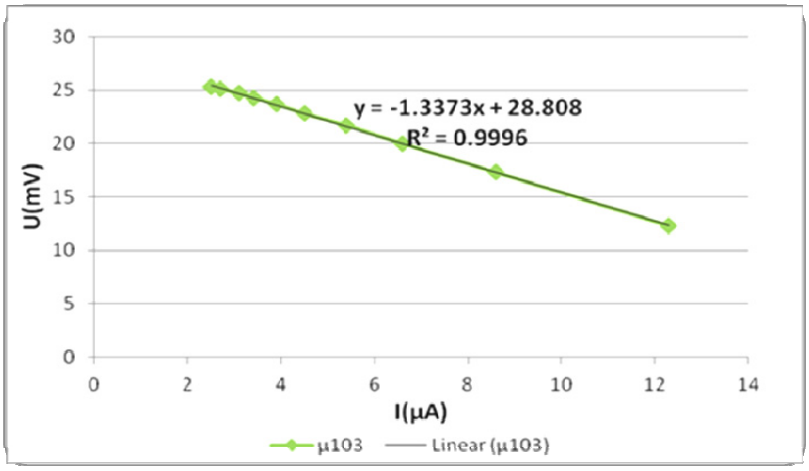


Figure 1. Polarization curve of μ103 battery for a 0.07 mol/L sodium acetate concentration

In this case the value of the emf is 29 mV and the internal resistance is 1.3 kΩ. The influence that the nutrient concentration has on the internal resistance has been studied. Figure 2 shows the influence of the concentration of the acetate on the internal resistance.

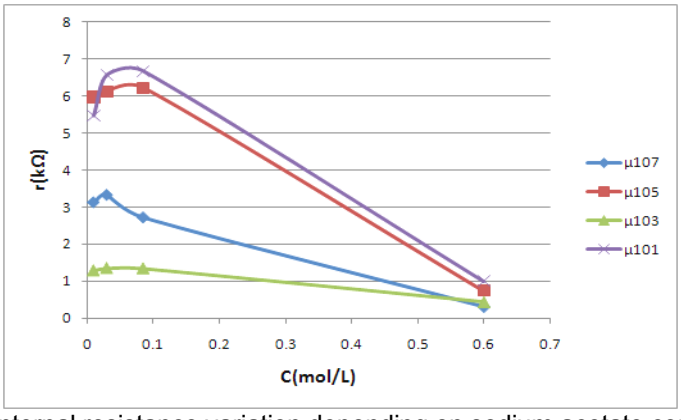


Figure 2. Internal resistance variation depending on sodium acetate concentration

A sharp increase in the internal resistance value is observed to be related to a slow increase of the sodium acetate concentration (0-0.03 mol/L).

When a maximum value is reached (at concentration between 0.03-0.085 mol/L), the internal resistance begins to drop. This decrease is supposed to appear due to the fact that the microorganism population reaches its maximum development, and the increase in sodium acetate concentration creates an excess of it in the cell material, reported to the population needs. This excess leads to an increase of the electrical conductivity in the liquid environment inside the cells, therefore reducing the internal resistance of the cells.

Power density dependence on acetate concentration

The power density generated by the microbial battery has been determined by the relation:

$$P_D = \frac{U \times I}{10 \times V}$$

where *U* is the voltage measured on the load resistance, *I* is the current through the circuit, *V* (cm^3) is the volume of one cell.

The current density has been determined by the relation:

$$I_D = \frac{I}{10 \times A}$$

where A (cm^2) is the projected area of the anode of one cell. Figure 3 shows the influence of the nutrient concentration on the power density.

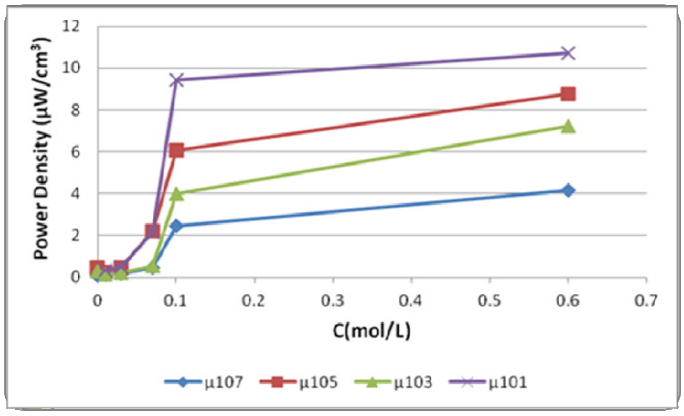


Figure 3. Power density variation depending on sodium acetate concentration

A significant power density increase in the 0.03-0.1 mol/L range has been found. It is followed by saturation at higher concentrations. This is believed to be due to the microorganism culture reaching a maximum development level, which is stationary as the nutrient concentration is increased, meaning that the generated power is limited at that level.

The majority of the miniaturised MFCs reported so far are mainly composed of two chambers, Nafion membrane, ferrycianide catholyte, pumps to circulate the electrolytes and pure bacterial strain [25][26].The model of miniature MFC proposed in this study has certain advantages: it has one chamber with no separation membrane, no artificial chemicals needed, no moving parts (and hence no electrical consumption) and contains a mix of natural microorganisms from soil.

Table 1 presents a comparison of this work with the best results from mL- scale MFCs papers.

Table 1. Comparison of the current paper with the best results from mL scale MFCs

	F.Qian et. all (2011)	**F.Qian et. all (2011)**	**Fan et. all (2007)**[27]	**Ringeisen et. all (2006)**	**Current paper**
Chamber volume (mL)	10	10	2.5	1.2	20
Projected anode area (cm^2)	5	2.25	7	2	1.5
Anode material	Carbon cloth	Gold	Carbon cloth	Graphite felt	Carbon cloth
Catholyte	Ferricyanide	Air	Air	Ferricyanide	N/A
Substrate	Trypticase soy brot	Lactate	Acetate	Lactate	Acetate
Max. current density (mA/m^2)	80	N/A	9000	11000	500
Max. power density (W/m^3)	0.2	N/A	1010	500	1
$r_{int}(k\Omega)$	13	N/A	N/A	N/A	1

Power density dependence on time

Figure 4 and Figure 5 show the power density dependence on time.

As it has been expected, the power density decreases in time more sharply for lower concentrations of nutrient, and slower for higher concentrations. Furthermore, the generated levels of power are variable depending on the batteries.

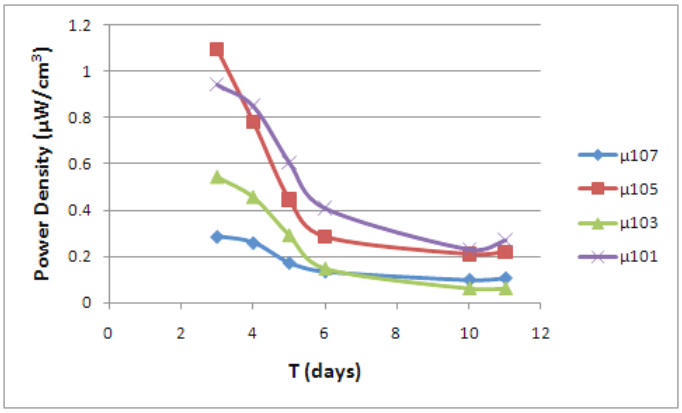


Figure 4. Power density variation depending on time in 0.03 mol/L acetate

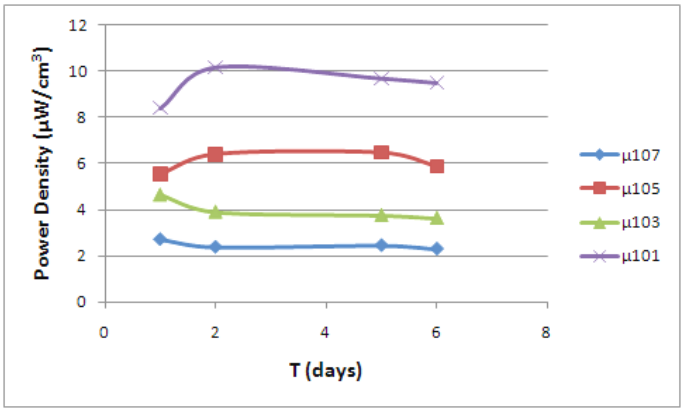


Figure 5. Power density variation depending on time in 0.1 mol/L acetate

Using the microbial fuel cell as a sodium acetate sensor

Figure 3 shows a univocal dependence of the power density regarding the sodium acetate concentration. As a consequence, the microbial battery containing microorganisms from soil can act as an acetate sensor. For the sake of simplicity, instead of the power density signal, the voltage signal has been used. The calibration curve U=f (conc) for battery µ103 has been built (Figure 6).

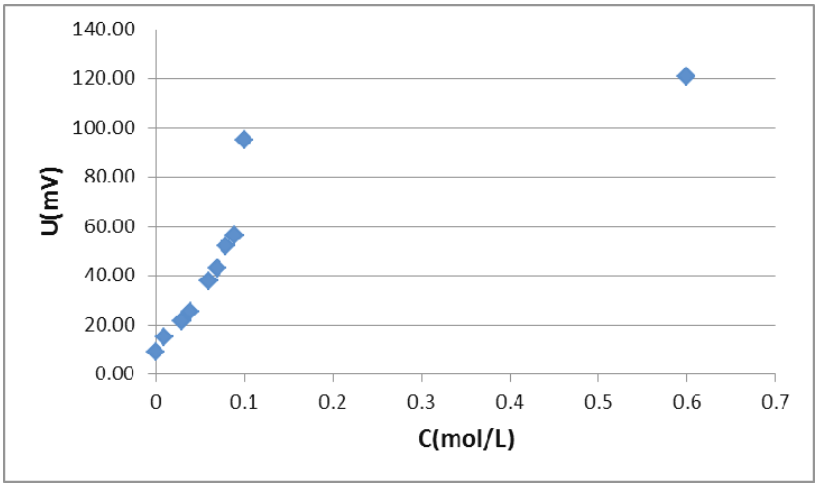


Figure 6. Voltage dependence of concentration for the µ103 battery

A steep slope of the curve is observed in the low concentration range (0-0.1 mol/L), and the saturation phenomenon at higher concentrations occurs. The MFC battery can be used as a sensor in the low concentration range, where it shows a higher sensibility. Figure 7 shows the calibration curve in the concentration domain 0-0.1 mol/L.

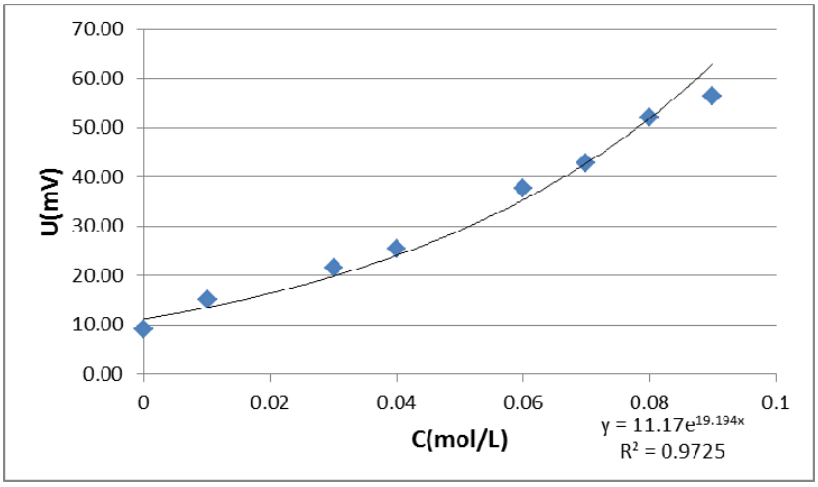


Figure 7. The calibration curve

The curve equation is $y = 11.17e^{19.194x}$, with a correlation coefficient $R^2=0.9725$.

For a standard concentration of 0.07 mol/L, by using the μ103 sensor, the concentration value c = 0.078± 0.013 mol/L has been obtained. The recovery values are presented in Table 2. Due to the fact that the obtained value is very close to the standard value, it can be assumed that microbial fuel cell batteries can be successfully used as sensors for different types of organic matter dissolved in water.

Table 2. The recovery values

Real concentration(mol/L)	Found concentration(mol/L)	Recovery values(%)
0.07	0.094	134
	0.081	116
	0.059	84

CONCLUSIONS

A miniaturised battery (made of 10 MFC cells connected in parallel) with microorganisms from soil has been built.

The maximum generated power density is 1 W/m^3 and the current density is 500 mA/m^2. Despite the fact that these values are far exceeded by other researchers' results, the current model has the following advantages: it has a simple construction, it has no membrane, no moving parts and no artificial chemical substances and nor does it consume electricity.

This type of battery could be used as a sensor for organic matter present in water.

Another application could be the production of electrical energy in crisis situations and/or isolated areas by using a plastic foil which has hundreds or thousands of cells with pre-printed electrodes. These could be filled with moist soil containing organic matter and the battery would be ready for operation.

EXPERIMENTAL SECTION

The construction of microbial fuel cells with soil microorganisms.

The 10-well array is made of plastic material characterized by a volume of approximately 2 mL/element. The electrodes have been painted on the inside of the wells using the graphite paste Electrodag 4023 ss (Acheson, Milano).

The MFC diagram is shown in Figure 8:

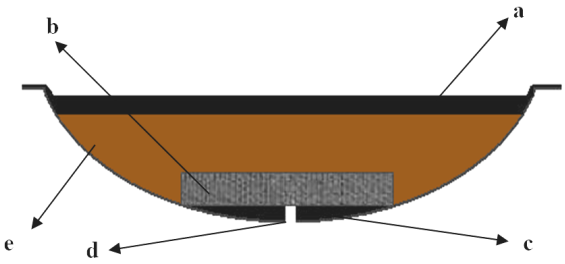


Figure 8. Scheme of MFC with microorganisms from soil
a) cathode, b) carbon cloth, c) anode, d) feed inlet, e) soil

Carbon cloth has been applied to the graphite paste to enhance the surface area of the anode, its dimensions being 1.5x1x10 mm. Ten individual cells have been connected in parallel using the graphite paste (Figure 9) and then filled with garden soil mixed saturated with water (Figure 10). The idea on which the soil MFCs were built was the presence of exoelectrogenic bacteria – most common being *Shewanella, Geobacter and Pseudomonas* [28]. It has been proven that a difference exists between forrest soil and agricultural soil, the latter containing species of *Clostridium* (important role in generating fermenting products), *Bacteroidetes*, *Geobacter* [29]. We can only speculate that the bacterial communities in the soil we used are similar with those that have been proven in previous research, bearing in mind that soil ecosystem is complex and variable.

To perform the experiments, four identical batteries denominated μ101, μ103, μ105 and μ107 have been used.

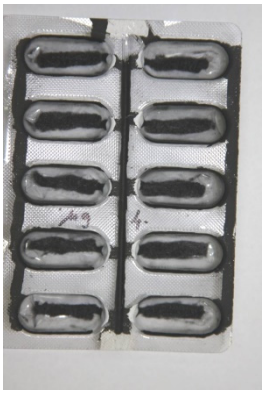


Figure 9. Blister of ten batteries ready for filling

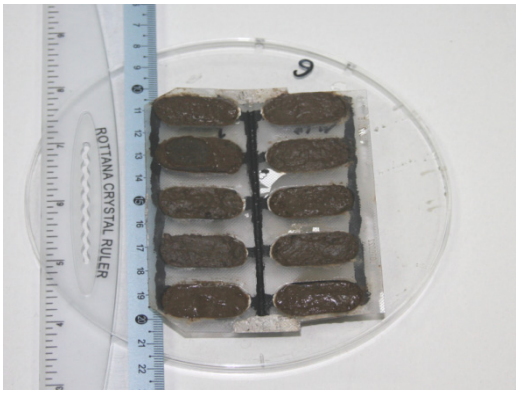


Figure 10. Battery ready for measurements

The batteries have been functioning continuously onto a 1 kΩ resistance load. They have been placed in Petri dishes containing sodium acetate solutions which have been used to feed the microorganisms. The solution enters the anodic part of the cells through an orifice drilled in the bottom. The experiment was conducted at ambient conditions.

The electrical measurements have been made using the MasTech MAS 830 (Taiwan) and PeakTech 3340 DMM (Germany) multimeters.

REFERENCES

1. B.E. Logan, "Microbial Fuel Cells", John Wiley & Sons, Inc., **2008**, pp. 4-5, pp. 13-14, pp. 44, pp. 46, pp. 70-71, pp. 76
2. D.R. Lovely, "Nature Reviews: Microbiology", Nature Publishing Group, **2006**, Volume 4, pp. 497
3. F. Qian, D.E. Morse, 2011, *Trends in Biotechnology*, **2011**, 29 (2), 62-69
4. K. Rabaey, W. Verstraete, *TRENDS in Biotechnology*, **2005**, 23, 291–8.
5. R.A. Bullen, T.C. Arnot, J.B. Lakemanc, F.C. Walsh, *Biosensors & Bioelectronics*, **2006**, 21, 2015-2045
6. B. E. Logan, J. M. Regan, *Trends in Microbiology*, **2006**, 14 (12), 512-518
7. F. Li, Y. Sharma, Y. Lei, B. Li, Q. Zhou, *Appl Biochem Biotechnol*, **2010**, 160, 168–181
8. A.E Franks, K.P. Nevin, *Energie*s, **2010**, 3, 899-919
9. J. Larminie, A. Dicks, "Fuel cell systems explained", John Wiley & Sons, Chichester, **2000**
10. K. Rabaey, N. Boon, M. Hofte, W. Verstraete, *Environ. Sci. Technol*., **2005**, 39(9), 340 1-3408.
11. S.E. Oh, B.E. Logan, *Appl Microbiol Biotechnol*, **2005**, 70, 162–169
12. A. ElMekawy, H.M. Hegab, X. Dominiguez-Benetton, D. Pant, *Biosource Technology*, **2013**, 142, 672-682
13. H. Ren, H.-S. Lee, J. Chae, *Microfluid Nanofluid*, **2012**, 13, 353-381
14. L. Wagner, J. Yang, S. Ghobadian, R. Montazami, N. Hashemi, *Open Journal of Applied Biosensor*, **2012**, 1, 21-25
15. S.B. Velasquez-Orta, T.P. Curtis, B.E. Logan, *Biotechnology and Bioengineering*, **2009**, 103 (6), 1068-1076
16. F. Qian, M. Baum, Q. Gu, D.E. Morse, *Biotechnology and Bioengineering*, **2009**, 9(21),3076-3081.
17. K. Rabaey, G. Lissens, S.D. Siciliano, W. Verstraete, *Biotechnology letters*, **2003**, 25 (18), 1531-1535.
18. Z. He, N. Wagner, S.D. Minteer, L.T. Angenent, *Environmental Science & Technology*, **2006**, 40(17), 5212-5217
19. J. Yang, S. Ghobadian, J.P.Goodrich, R. Montazami, N. Hashemi, *Phys. Chem. Chem. Phys*, **2013**, 15, 14147-14161

20. J. Li, 2013, An Experimental Study of Microbial Fuel Cells for Electricity Generating: Performance Characterization and Capacity Improvement, Kent Place School, Summit, New Jersey, USA
21. K.G. Cooke, M. O. Gay, S. E. Radachowsky, J. J. Guzman, M. A. Chiu, "Distributed Sensor Network Powered by Terrestrial Microbial Fuel Cell Technology", Trophos Energy, Inc., USA, **2012**
22. J. Niessen, J. Harnisch, F. Rosenbaum, M. Schröder, U. Scholz, *Electrochemistry Communications*, **2006**, 8, 869-873
23. H. Deng, Y.-C. Wu, F. Zhang, Z.-C. Huang, Z. Chen, H.-J., Xu, F. Zhao, *Pedosphere*, **2014**, 24(3), 330-338
24. D. Murty, M. U. F. Kirschbaum, R. E. Mcmurtrie, H. Mcgilvray, *Global Change Biology*, **2002**, 8(2), 105–23
25. F. Qian, Z. He, M.P Thelen, Y. Li, *Bioresour. Technol*., **2011**, 102(10), 5826-40
26. B.R. Ringeisen, E. Henderson, P.K. Wu, J. Pietron, R. Ray, B. Little, J.C. Biffinger,, J.M Jones-Meehan, *Environ. Sci. Technol*., **2006**, 40, 2629–2634.
27. Y.Z. Fan, H.Q. Hu, H. Liu, *J. Power Sour*. **2007**, 171, 348–354.
28. A. Wolińska, Z. Stępniewska, A. Bielecka , J. Ciepielski, *Appl. Biochem. Biotechnol.*, **2014**, 36, 635–642.
29. S.J. Dunaj, J.J. Vallino, M. E. Hines, M. Gay, C. Kobyljanec, J.N. Rooney Varga, *Environ. Sci. Technol*., **2012**, 46, 1914−1922.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 485-493)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoş on the occasion of his 80th anniversary

CLUJ AND RELATED POLYNOMIALS IN BIPARTITE HYPERCUBE HYPERTUBES

MAHBOUBEH SAHELI[a], AMIR LOGHMAN[a], MIRCEA V. DIUDEA[b*]

ABSTRACT. A novel class of counting polynomials, called Cluj polynomials was proposed on the ground of Cluj matrices. The polynomial coefficients are calculated from the above matrices or by means of orthogonal edge-cuts. In this paper Cluj polynomial in bipartite hypercube hypertubes is presented. Definitions and relations with other polynomials and topological indices are derived.

Keywords: *Cluj polynomial, vertex-Padmakar-Ivan index, Wiener index*.

INTRODUCTION

A finite sequence of some graph-theoretical categories/properties, such as the distance degree sequence or the sequence of the number of k-independent edge sets, can be described by so-called *counting polynomials*:

$$P(G,x) = \sum_k p(G,k) \cdot x^k \qquad (1)$$

where *p(G,k)* is the frequency of occurrence of the property partitions of *G*, $\cup\, p(G) = P(G)$, of length *k*, and *x* is simply a parameter to hold *k*. In the Mathematical Chemistry literature, the counting polynomials have first been introduced by Hosoya [1]. Cluj indices and polynomial have been introduced by Diudea [2-6]. In bipartite graphs, the coefficients of *CJ* polynomial can be calculated by an orthogonal edge-cut procedure [7-9]. For this, a theoretical background is needed.

[a] *Department of Mathematics, Payame Noor Universtiy, PO BOX 19395-3697 Tehran, Iran.*
[b] *Department of Chemistry, Faculty of Chemistry and Chemical Engineering, "Babes-Bolyai" University, 400028 Cluj, Romania.*
[*] *Corresponding author: diudea@chem.ubbcluj.ro*

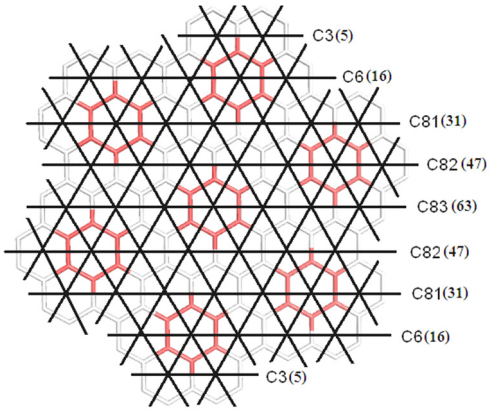
A graph *G* is a *partial cube* if it is embeddable in the *n*-cube Q_n, which is the regular graph whose vertices are all binary strings of length *n*, two strings being adjacent if they differ in exactly one position. The distance function in the *n*-cube is the Hamming distance. A hypercube can also be expressed as the Cartesian product: $Q_n = \prod_{i=1}^{n} K_2$.

For any edge $e=(u,v)$ of a connected graph *G* let n_{uv} denote the set of vertices lying closer to *u* than to *v*: $n_{uv} = \{w \in V(G) \mid d(w,u) < d(w,v)\}$. It follows that $n_{uv} = \{w \in V(G) \mid d(w,v) = d(w,u)+1\}$. The sets (and subgraphs) induced by these vertices, n_{uv} and n_{vu}, are called *semicubes* of *G*; the semicubes are called *opposite semicubes* and are disjoint [2,10].

A graph *G* is bipartite if and only if, for any edge of *G*, the opposite semicubes define a partition of *G*: $n_{uv} + n_{vu} = v = |V(G)|$. These semicubes are just the vertex proximities (see above) of (the endpoints of) edge $e=(u,v)$, which *CJ* polynomial counts. In partial cubes, the semicubes can be estimated by an orthogonal edge-cutting procedure. The orthogonal cuts form a partition of the edges in *G*:

$$E(G) = c_1 \cup c_2 \cup \ldots \cup c_k, \; c_i \cap c_j = \varnothing, i \neq j .$$

To perform an orthogonal edge-cut, take a straight line segment, orthogonal to the edge *e*, and intersect *e* and all its parallel edges (in a plane graph). The set of these intersections is called an *orthogonal cut* $c_k(e)$, $k=1,2,..,k_{max}$. An example is given in Figure 1.



$CJS(x)$ =
$3\cdot2\cdot3(x^{5}+x^{121})+3\cdot2\cdot6(x^{16}+x^{110})+$
$3\cdot2\cdot8(x^{31}+x^{95})+3\cdot2\cdot8(x^{47}+x^{79})+$
$3\cdot1\cdot8(x^{63}+x^{63})$
$CJS'(1)=21924$
$PI_v(x)$ =
$3\cdot2\cdot3(x^{5}+^{121})+$
$3\cdot2\cdot6(x^{16}+^{110})+3\cdot2\cdot8(x^{31}+^{95})+$
$3\cdot2\cdot8(x^{47}+^{79})+3\cdot1\cdot8(x^{63}+^{63})$
$PI_v'(1)=21924$
$CJP(x)$ =
$3\cdot2\cdot3(x^{5.121})+ 3\cdot2\cdot6(x^{16.110})+$
$3\cdot2\cdot8(x^{31.95})+ 3\cdot2\cdot8(x^{47.79})+$
$3\cdot1\cdot8(x^{63.63})$
$CJP'(1)=489090=CJP(G)=SZ_v'(1)$

Figure 1. Cutting procedure in the calculation of several topological descriptors

To any orthogonal cut c_k, two numbers are associated: first one represents the number of edges e_k intersected (or the cutting cardinality $|c_k|$) while the second is v_k or the number of points lying to the left hand with respect to c_k. Because in bipartite graphs the opposite semicubes define a partition of vertices, it is easily to identify the two semicubes: $n_{uv}= v_k$ and $n_{vu}= v-v_k$ or vice-versa.

By this cutting procedure, three cases have to be considered, as summarized in Table 1.

Table 1: Mathematical operations and defined three polynomials

	Operation	Polynomial name	Formula
1	Summation	Cluj-Sum	$CJS(x) = \sum_e \left(x^{v_k} + x^{v-v_k} \right)$
2	Pairwise summation	vertex-Padmakar-Ivan	$PI_v(x) = \sum_e x^{v_k + (v-v_k)}$
3	Pairwise product	Cluj-Product	$SZ(x) = \sum_e x^{v_k (v-v_k)}$

The first derivative, for x=1, of a counting polynomial provides single numbers, often called topological indices. It is easily seen that the first derivative (in x=1) of the first two polynomials gives one and the same value, but their second derivative is different and the following relations hold in any graph [11-13]

$$CJS'(1) = PI_v'(1); \quad (2)$$

$$CJS''(1) \neq PI_v''(1) \quad (3)$$

In bipartite graphs, $PI_v'(1)$ takes the maximal value, among all the graphs on the same number of vertices:

$$PI_v'(1) = e \cdot v = |E(G)| \cdot |V(G)| \quad (4)$$

This result can be used as a criterion for the “bipartivity” of a graph [7,8].

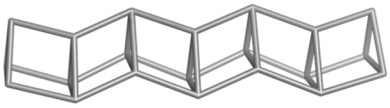
The third polynomial, $CJP(x)$, uses the pairwise product; it is precisely the (vertex) Szeged polynomial $SZ_v(x)$, defined by Ashrafi *et al* [12-14]. This comes out from the relations between the basic Cluj (Diudea [2,5]) and Szeged (Gutman [15]) indices:

$$CJP'(1) = CJDI(G) = SZ(G) = SZ_v'(1) \quad (5)$$

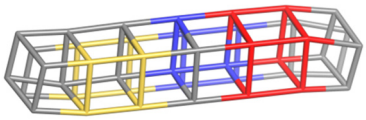
All the three polynomials (and their derived indices) do not count the equidistant vertices, an idea introduced in Chemical Graph Theory by Gutman. We call these, *polynomials of vertex proximity*.

LATTICE BUILDING

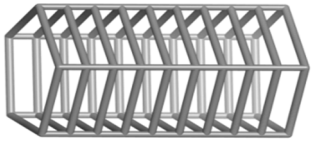
In some recent papers [16,17], Diudea *et al*. proposed the embedding of *n*-Cube in surfaces other than the sphere. In case of open tubes, some examples are given in Figure 2.



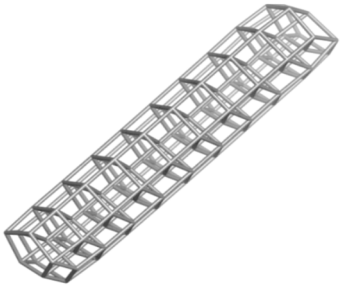
TU37_2_21



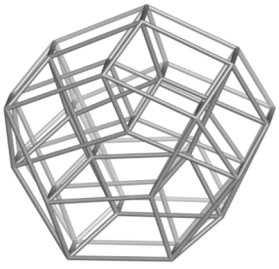
TU37_3_42



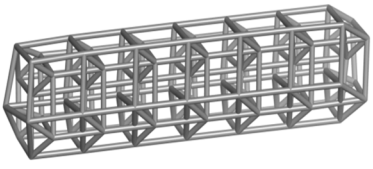
TU510_2_50



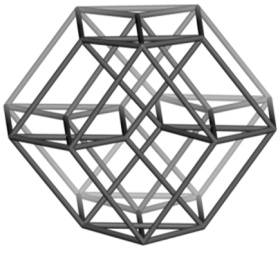
TU510_3_100



TU510_4_200; one slide



TU37_4_84



TU37_4_84; one slide

Figure 2. Examples of hypercube embedded in the (nano)tubes

RESULTS AND DISCUSSION

All the three polynomials listed in Table 1 are exemplified, for some bipartite hypercube hypertubes TU*rs* in Tables 2 to 9. The analytical formulas were derived by numerical analysis. Numerical calculations have been done by TOPOCLUJ software [18].

Table 2: Cluj and related polynomials in **TU4*s***

Number of vertices and edges	
1	$\lvert V(TU4s)\rvert = 2^{n-1}s \quad \lvert E(TU4s)\rvert = 2^{n-2}(sn+s-2)$
***PI*$_v$; *PI*$_v$`` and *PI*$_v$``**	
2	$PI_v(TU4s,x) = 2^{n-2}(sn+s-2)x^{2^{n-1}s}$
3	$PI_v'(1) = 2^{2n-3}s(sn+s-2)$
4	$PI_v''(1) = 4^{n-2}\,s\,(2^n s-2)(sn+s-2)$
Cluj polynomial and its derivatives	
5	$CJS(TU4s,x) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^n\left[x^{2^{n-1}i} + x^{2^{n-1}(s-i)}\right] + \left[2^{n-1}\left(sn-s+1+(-1)^s\right)\right]x^{2^{n-2}s}$
6	$CJS'(1) = 2^{2n-3}s(sn+s-2)$
7	$CJS''(1) = \frac{4^{n-2}}{6}s\left[2^n\left(3s^2n+5s^2-12s+4\right)-12(sn+s-2)\right]$
Szeged polynomial and index	
8	$Sz(TU4s,x) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^n x^{4^{n-1}i(s-i)} + \left[2^{n-2}\left(sn-s+1+(-1)^s\right)\right]x^{4^{n-2}s^2}$
9	$Sz(TU4s) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^{3n-2}\,i(s-i) + 2^{3n-6}s^2\left(sn-s+1+(-1)^s\right)$

Table 3: Cluj and related polynomials in **TU*rs***

Number of vertices and edges	
1	$\lvert V(TUrs)\rvert = 2^{n-2}rs \quad \lvert E(TU\,4s)\rvert = 2^{n-3}r(sn+2s-2)$
PIv; PIv`* and *PIv``	
2	$PI_v(TUrs,x) = 2^{n-3}r(sn+2s-2)x^{2^{n-2}rs}$
3	$PI_v'(1) = 2^{2n-5}r^2s(sn+2s-2)$
4	$PI_v''(1) = 4^{n-3}r^2s\,(2^{n-1}rs-2)(sn+2s-2)$
Cluj polynomial and its derivatives	
5	$CJS(TUrs,x) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^{n-1}r\left[x^{2^{n-2}ri}+x^{2^{n-2}r(s-i)}\right]+\left[2^{n-2}r\left(sn+1+(-1)^s\right)\right]x^{2^{n-3}rs}$
6	$CJS'(1) = 2^{2n-5}r^2s(sn+2s-2)$
7	$CJS''(1) = \frac{4^{n-4}}{3}r^2s\left[2^n r(3s^2n+8s^2-12s+4)-24(ns+2s-2)\right]$
Szeged polynomial and index	
8	$Sz(TUrs,x) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^{n-1}r\,x^{4^{n-2}r^2i(s-i)}+2^{n-3}r\left(sn+1+(-1)^s\right)x^{4^{n-3}r^2s^2}$
9	$Sz(TUrs) = \sum_{i=1}^{\left\lfloor\frac{s-1}{2}\right\rfloor} 2^{3n-5}r^3i(s-i)+2^{3n-9}r^3s^2\left(sn+1+(-1)^s\right)$

Table 4: Examples: *PIv; PIv`* and *PIv``*; number of vertices and edges in TU45

n	*PIv* Polynomial	*PIv`*	*PIv``*	v	e
3	$36x^{20}$	720	13680	20	36
4	$92x^{40}$	3680	143520	40	92
5	$224x^{80}$	17920	1415680	80	224
6	$528x^{160}$	84480	13432320	160	528
7	$1216x^{320}$	389120	124129280	320	1216

Table 5: Examples: Cluj polynomial and its derivatives in TU45

n	Cluj polynomial	*CJ*`(1)	CJ``(1)
3	$8x^{16}+8x^{12}+40x^{10}+8x^{8}+8x^{4}$	720	7120
4	$16x^{32}+16x^{24}+120x^{20}+16x^{16}+16x^{8}$	3680	75040
5	$32x^{64}+32x^{48}+320x^{40}+32x32+32x^{16}$	17920	739840
6	$64x^{128}+64x^{96}+800x^{80}+64x^{64}+64x^{32}$	84480	7001600
7	$128x^{256}+128x^{384}+1920x^{160}+128x^{128}+128x^{64}$	389120	64491520

Table 6: Examples: Szeged polynomial and index in TU45

n	Szeged polynomial	Szeged index
3	$20x^{100}+16x^{384}+8x^{64}$	3280
4	$60x^{400}+16x^{384}+16x^{256}$	34240
5	$160x^{1600}+32x^{1536}+32x^{1024}$	337920
6	$400x^{6400}+64x^{6144}+64x^{4096}$	3215360
7	$960x^{25600}+128x^{24576}+128x^{16384}$	29818880

Table 7: Examples: PI_v; PI_v` and PI_v``; number of vertices and edges in TU68

n	PI_v Polynomial	PI_v`	PI_v``	v	e
2	$90x^{48}$	4320	203040	48	90
3	$228x^{96}$	21888	2079360	96	228
4	$552x^{192}$	105984	20242944	192	552
5	$1296x^{384}$	497664	190605312	384	1296
6	$2976x^{768}$	2285568	1753030656	768	2976

Table 8: Cluj polynomial and its derivatives in **TU68**

n	Cluj polynomial	*CJ*`(1)	CJ``(1)
2	$12\left(x^{42}+x^{36}+x^{30}+x^{18}+x^{12}+x^{6}\right)+108x^{24}$	4320	111456
3	$24\left(x^{84}+x^{72}+x^{60}+x^{36}+x^{24}+x^{12}\right)+312x^{48}$	21888	1125504
4	$48\left(x^{168}+x^{144}+x^{120}+x^{72}+x^{48}+x^{24}\right)+816x^{96}$	105984	10842624
5	$96\left(x^{336}+x^{228}+x^{240}+x^{144}+x^{96}+x^{48}\right)+2016x^{192}$	497664	101246976
6	$192\left(x^{672}+x^{576}+x^{480}+x^{288}+x^{192}+x^{96}\right)+4800x^{384}$	2285568	924917760

Table 9: Examples; Szeged polynomial and index in TU68

n	Szeged polynomial	Szeged index
2	$54x^{576}+12x^{540}+12x^{432}+12x^{252}$	45792
3	$156x^{2304}+24x^{2160}+24x^{1728}+24x^{1008}$	476928
4	$408x^{9216}+48x^{8640}+48x^{6912}+48x^{4032}$	4700160
5	$1008x^{36864}+96x^{34560}+96x^{27648}+96x^{16128}$	44679168
6	$2400x^{147456}+192x^{138240}+192x^{110592}+192x^{64512}$	414056448

CONCLUSION

In this paper we presented the calculation of Cluj polynomial in hypercube hypertubes TU*rs*. Definitions and relations with other polynomials and their corresponding topological indices, were given. Analytical formulas as well as examples were tabulated.

REFERENCES

1. H. Hosoya, *Discrete Appl. Math*., **1988**, 19, 239.
2. M. V. Diudea, *J. Chem. Inf. Comput. Sci.,* **1997**, 37, 300.
3. M. V. Diudea, *Croat. Chem. Acta.,* **1999**, 72, 835.
4. M. V. Diudea, A. E. Vizitiu and D. Janežič, *J. Chem. Inf. Model.*, **2007**, 47, 864.

5. M. V. Diudea, *J. Math. Chem.*, **2009**, *45*, 295.
6. M. V. Diudea, N. Dorosti, A. Iranmanesh, Cluj Cj Polynomial and Indices in a Dendritic Molecular Graph, *Studia Univ. "Babes-Bolyai"*, *Chemia*, 55 (4), **2010**, 247-253.
7. M. V. Diudea, *Novel Molecular Structure Descriptors-Theory and Applications I*, **2010**, 191.
8. M. V. Diudea, *Novel Molecular Structure Descriptors-Theory and Applications II*, **2010,** 57.
9. I. Gutman and S. Klavžar, *J. Chem. Inf. Comput.,* **1995**, 35, 1011.
10. M. V. Diudea and S. Klavžar, *Acta. Chem. Sloven.*, **2010**, 57, 565.
11. P. V. Khadikar, *Nat. Acad. Sci. Lett.,* **2000**, 23, 113.
12. M. H. Khalifeh, H. Yousefi-Azari and A. R. Ashrafi, *Linear Algebra Appl.*, **2008** 429, 2702.
13. A. R. Ashrafi, M. Ghorbani and M. Jalali, *J. Theor. Comput. Chem.*, **2008**, 7, 221.
14. T. Mansour and M. Schork, *Discr. Appl. Math.*, **2009**, 157, 1600.
15. I. Gutman , *Graph Theory Notes,***1994**, 27, 9.
16. A. Parvan-Moldovan, M. V. Diudea, *Iran. J. Math. Chem.*, **2015**, 6, 163.
17. K. Fathalikhani, A. Pîrvan-Moldovan, M. V. Diudea, *Studia Univ. Babes-Bolyai, Chemia*, **2016**, 61, 291.
18. O. Ursu, M. V. Diudea, **TOPOCLUJ** software program, *Babes-Bolyai University*, **2005**.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 495-504)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoş
on the occasion of his 80th anniversary

EVALUATION OF MASS TRANSFER PARAMETERS FOR UREA DISSOLUTION IN FIXED-BED WITH DOWNWARD FLOW OF WATER

SZABOLCS FOGARASI[a], FLORICA IMRE-LUCACI[b], SIMION DRĂGAN[a], ARPAD IMRE-LUCACI[a,*]

ABSTRACT. The current study aimed to evaluate the mass transfer parameters for the dissolution of spherical urea particles in fixed bed with downward flow of water. The impact of several operating parameters (particle size, height of the bed, liquid flow rate) on the key mass transfer parameters was investigated in isothermal conditions and atmospheric pressure. It was found that the mass transfer coefficient and urea dissolution degree increases by flow rate increase and decreases with the increase of particle size and bed height. The experimental values of the mass transfer coefficients were in good agreement with the ones found in the literature and the ones predicted by the Cussler equation.

Keywords: *urea dissolution, mass transfer coefficient, dissolution degree, packed bed, downward flow*

INTRODUCTION

The study and understanding of fundamental aspects of two phase solid-liquid mass transfer is a key issue in the design of catalytic and non-catalytic chemical and biochemical reactors with application in wastewater treatment, liquid–solid circulating fluidized beds, treatment of solid–liquid mixtures [1-3]. Among different types of industries, the dissolution and extraction of valuable minerals also requires the identification of key mass transfer parameters [4]. Fixed and fluidized bed reactors are one of the

[a] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Department of Chemical Engineering, 11 Arany Janos str., RO-400028, Cluj-Napoca, Romania*
[b] *Babeş-Bolyai University, Interdisciplinary Research Institute on Bio-Nano-Sciences, Physico-Chemical Analyses Center, 42 Treboniu Laurian Street, Cluj-Napoca, RO-400271*
[*] *Corresponding author: aimre@chem.ubbcluj.ro*

most important equipments of the chemical industry having a wide range of industrial and environmental applications [5, 6]. Depending on the structure of the bed of particles, these reactors offer a potential solution for process intensification by increasing the surface contact area between the two phases [7]. In addition to the increase of the heterogeneous reaction rate the increase of surface contact area per unit volume also improves the heat transfer parameters and lowers the capital costs involved in the acquisition of different process equipment [5, 8]. According to the literature, the most common approaches for the study of mass transfer in solid - liquid systems using fixed and/or fluidized beds are: dissolution, adsorption, ion exchange, electrochemical methods, using columns with short or long bed of active particles [9, 10]. In many situations the active particles consist of spherical or cylindrical cores coated with a thin layer of the melted active substance [11, 12]. The most important techniques used in the literature for the evaluation of key mass transfer parameters involve fix and fluidized bed dissolution, rotating disc method and plug flow systems [13]. The dissolution techniques are applied in isothermal and isobaric conditions, mainly at atmospheric pressure and ambient temperature with upward or downward flow of the fluid. According to the literature distilled water is the most common solvent but if the technology requires aqueous solution of the active substance or other impurities may be also used as dissolution medium [14]. Heterogeneous mass transfer is also of great importance in the case of nutrient uptake and assimilation in plants. It is necessary to understand and control the dissolution of different fertilizers in order to achieve efficient plant growth and high production yields [15].

Considering the industrial and agriculture importance of urea dissolution, this paper studies the dissolution of solid urea in fixed bed with downward flow of the dissolution medium. The study revealed the dependence of key mass transfer parameters on bed height, particles diameter and solvent flow rate.

RESULTS AND DISCUSSION

The identification of the mean mass transfer coefficients at different experimental conditions was based on the following assumptions:

- During the dissolution urea particles were considered perfectly spherical of the same diameter and density.
- The initial number of particles remains constant and the change in the particle size is uniform in the bed.
- The dissolution rate is the same for all the particles and changes similarly in time for all of them.

Based on the above assumptions, the *number of particles* (N_p) in the bed was determined from the amount of material introduced into the column:

$$N_p = \frac{6 \cdot m_o}{\pi \cdot \rho_p \cdot d_o^3} \tag{1}$$

where: m_o - amount of urea introduced into the column, (kg); ρ_p - urea density, (kg·m^{-3}); d_o - initial diameter of the urea particles, (m).

The amount of non-dissolved urea ($m_{col,i}$) can be determined by subtracting the quantity of dissolved urea over a period of time from the initial amount. As a result the diameter of the particle (d_i) may be determined from the following equation:

$$d_i = \left(\frac{6 \cdot m_{col,i}}{\pi \cdot \rho_p \cdot N_p} \right)^{1/3} \tag{2}$$

The mass transfer area is variable during the dissolution and can be calculated from the number and diameter of the particles found in the column:

$$A_i = \pi \cdot N_p \cdot \overline{d}_i^{\,2} \tag{3}$$

where: A_i - the particles surface, (m^2) and $\overline{d}_i$ - the average particle diameter for the period of time for which the contact surface area is calculated, (m).

The mass transfer coefficient can be determined from the experimental results using the equation:

$$k = \frac{\Delta m}{A_i \cdot \Delta t \cdot \Delta C_{med}} \tag{4}$$

where: k - mean mass transfer coefficient for period Δt, (m·s^{-1}); Δm - amount of dissolved urea over a period of time Δt, (kg); Δt - dissolution time period, (s) and ΔC_{med} - log mean driving force, (kg·m^{-3}).

According to the literature the driving force can be calculated as the difference between the saturation concentration and the concentration of urea at the exit of the column or by the log mean driving force defined by eq. (5). In particular when the solute concentration at the entrance is equal to zero both methods give comparable values for the driving force. However, the driving force for urea dissolution was determined by eq. (5), considering that even in this particular situation several studies recommend the use of the log mean driving force.

$$\Delta C_{med} = \frac{\left(C^* - C_i\right) - \left(C^* - C_f\right)}{\ln \frac{C^* - C_i}{C^* - C_f}} \tag{5}$$

where: C^* - saturation concentration of urea, (kg·m^{-3}); C_i - initial concentration of urea, (kg·m^{-3}) and C_f - final concentration of urea, (kg·m^{-3}).

The results presented in Fig. 1 show that the mean mass transfer coefficients, calculated for the dissolution of urea particles with diameter of 1.25 mm, are almost constant in time but are significantly influenced by bed height and flow rate variations. It can be seen that flow rate increase has a positive impact on the mean mass transfer coefficients, which is related to the intensification of the process due to the greater liquid velocity in the intergranular spaces. In contrast, the increase of the particle bed height reduces the mean mass transfer coefficients due to the fact that the mean driving force increases as well as the values of urea concentration in the effluent.

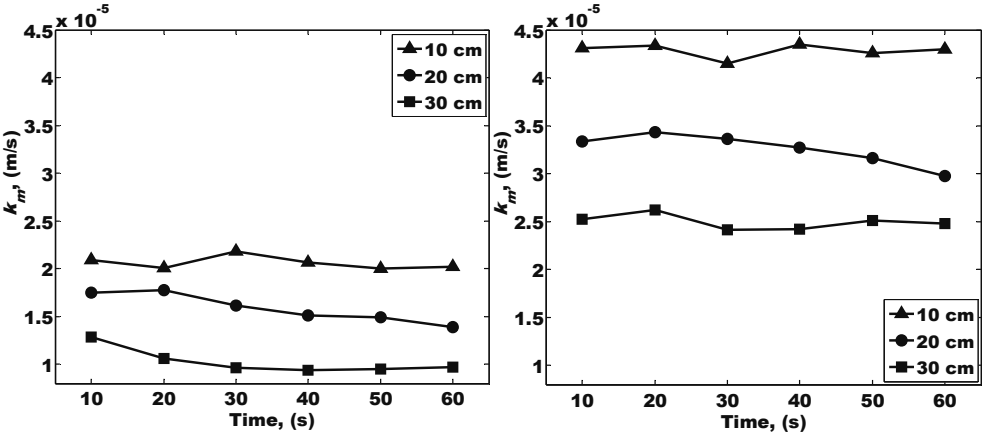


Figure 1. Mass transfer coefficient vs. time at different bed heights using particles with diameter of 1.25 mm and flow rates of (a) 3.5 L/h and (b) 7 L/h.

Increasing the diameter of the urea particles from 1.25 mm to 2 mm leads to the decrease of mass transfer coefficients regardless the applied flow rate or particle bed height. Similarly, at constant bed height, flow rate increase from 3.5 L/h to 7 L/h almost doubles the mean mass transfer coefficient, while bed height increase from 10 cm to 30 cm halves it regardless the flow rate (Fig. 2). It is also important to note that the results found in the current study regarding the dependence of the mean mass

transfer coefficients on flow rate, particle size and bed height are in good agreement with the ones found in the literature [7].

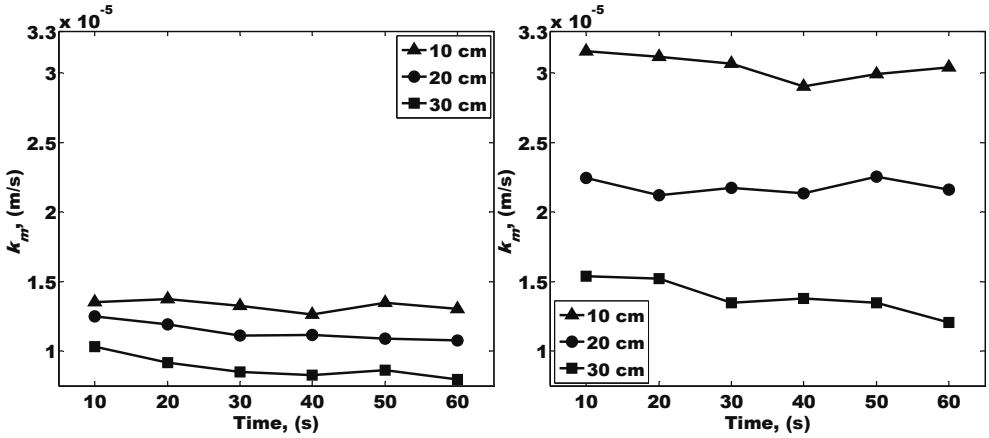


Figure 2. Mass transfer coefficient vs. time at different bed heights using particles with diameter of 2 mm and flow rates of (a) 3.5 L/h and (b) 7 L/h.

The experimental mean mass transfer coefficients were compared with the theoretical ones given by Cussler's equation:

$$k = 1.17 \cdot v_0 \cdot \left(\frac{d \cdot v_0}{\nu} \right)^{-0.42} \cdot \left(\frac{D}{\nu} \right)^{0.66} \tag{6}$$

where: v_0 - fluid velocity in the column, ($m \cdot s^{-1}$); d - urea particle diameter, (m); ν - kinematic viscosity of the solution, ($m^2 \cdot s^{-1}$) and D - diffusion coefficient of urea in water, ($m^2 \cdot s^{-1}$).

For instance, as it's shown in Fig. 3, the evolution in time of the experimental and theoretical mass transfer coefficients at the same flow rate of 7 L/h. It can be noticed that in the first part of the experiments the measured and predicted mass transfer coefficients values are close to each other, while in the final stage of the experiment the difference between the theoretical and experimental values increases significantly. This can be accounted to the fact that particle characteristics become more diverse as the dissolution process advances in time, especially at low particle diameters. For this reason, the discrepancies between the measured and predicted mass transfer coefficient values are even greater for the experiments with 1.25 mm particle diameter.

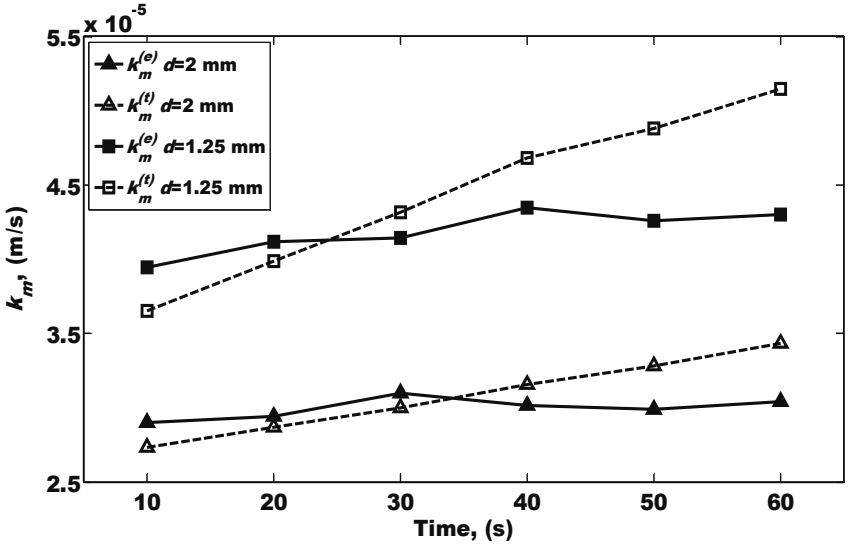


Figure 3. Experimental ($k_m^{(e)}$) and theoretical ($k_m^{(t)}$) mass transfer coefficients vs. time at the flow rate of 7 L/h using particles with diameter of 1.25 mm and 2 mm.

In order to evaluate the efficiency of urea dissolution, the mean dissolution degree of urea was determined for all the experimental conditions. According to eq. (7), the mean dissolution degree of urea (η_i) over a period of time (Δt_i) was defined as the ratio between the amount of dissolved urea (Δm_i) and the amount of urea introduced into the column at the beginning of the experiment (m_o):

$$\eta_i = 100 \cdot \left(\frac{\Delta m_i}{m_o} \right) \tag{7}$$

Fig. 4 shows that the mean dissolution degree of urea for a constant particle diameter of 1.25 mm increases in time regardless the applied flow rate or initial bed height. It can be also observed that the decrease of the bed height of particles for both flow rates leads to a more rapid increase of the mean dissolution degree. However, it seems like at the lowest flow rate the mean dissolution degree is more sensitive to bed height variations than at highest flow rate.

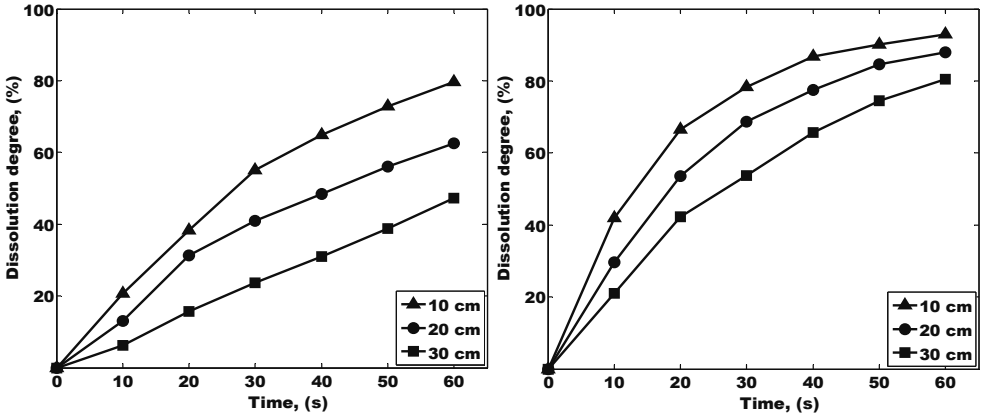


Figure 4. Dissolution degree vs. time at different bed heights; particles diameter of 1.25 mm and flow rates of (a) 3.5 L/h and (b) 7 L/h.

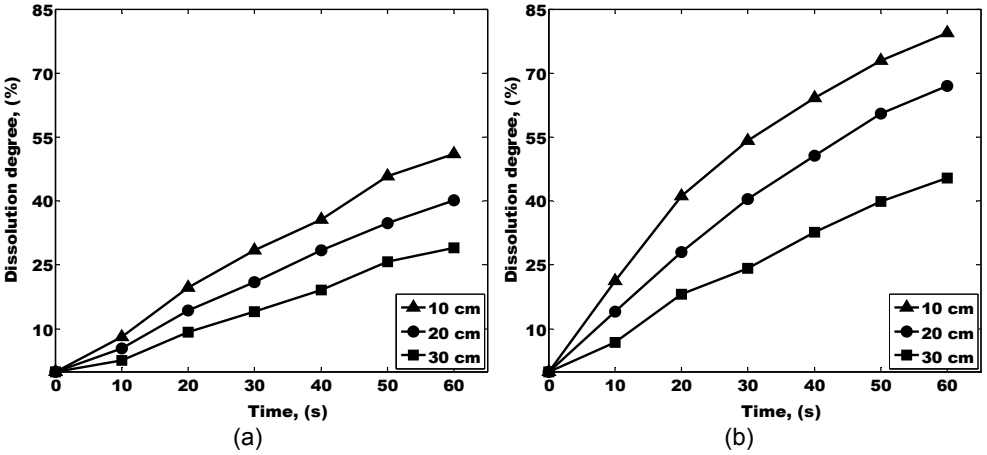


Figure 5. Dissolution degree vs. time at different bed heights using particles with diameter of 2 mm and flow rates of (a) 3.5 L/h and (b) 7 L/h.

As can be seen from Fig. 5, the mean dissolution degree follows similar tendency at the dissolution of particles with an initial diameter of 2 mm. Still, in the same experimental conditions the mean dissolution degree values are smaller for the particles with 2 mm than for the ones with 1.25 mm. This can be accounted to the fact that the urea particles with 1.25 mm diameter offer a larger solid-liquid contact surface area at the

same bed height than the ones with a diameter of 2 mm. For the same reason, the mean dissolution degree values differ more between the highest and lowest bed heights for the experiments with particle diameter of 2 mm than for the ones with 1.25 mm diameter. This difference is the most obvious at the flow rate of 7 L/h where for the experiments with particles of 2 mm diameter the mean dissolution degree increases with 75 % between the bed heights of 10 cm and 30 cm, while for the experiments with particles of 1.25 mm diameter it increases with only 15 % for the same variation of bed height.

CONCLUSIONS

The key mass transfer parameters were identified for the dissolution of spherical urea particles in fixed bed with downward flow of water. The experimental results indicate that the increase of flow rate increases the mean mass transfer coefficients as well as the dissolution degree of urea regardless the applied operating conditions. It was also found that the increase of particle diameter and height of the fixed bed have a negative impact on the key mass transfer parameters. The study also revealed that mean mass transfer coefficients can be predicted by the Cussler's equation considering the good agreement between the experimental and theoretical values given by Cussler's equation.

As an overall conclusion it can be stated that the dissolution of spherical urea particles occurs the most efficiently by using the highest flow rate, lowest bed height and particle diameter.

EXPERIMENTAL SECTION

The experiments were performed at room temperature and atmospheric pressure using a cylindrical glass column with an internal diameter of 2.1 cm and a height of 50 cm. The granular material was sustained by the perforated plate found at the bottom of the column.

The dissolution study was conducted in a fixed bed at different bed heights (10, 20, 30 cm) using two urea fractions with particles of 1.25 and 2.00 mm diameter.

Distilled water was used as the dissolution medium and a centrifugal pump ensured the necessary flow rates (3.5 and 7.0 L/h) through the column (Fig. 6).

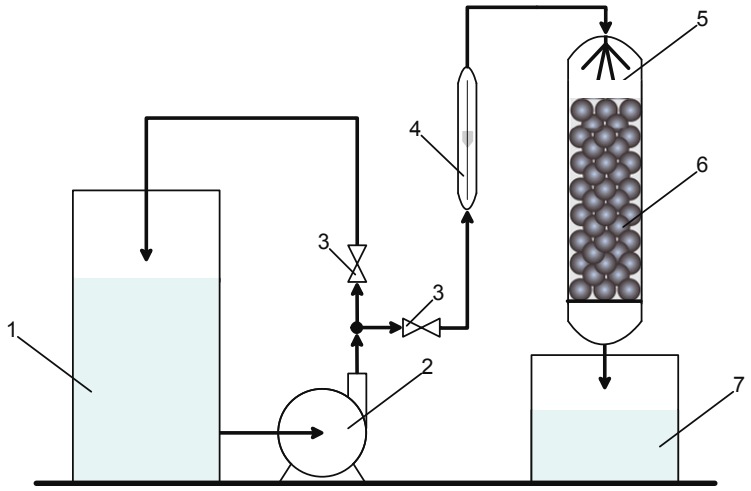


Figure 6. The experimental setup: 1 - water tank; 2 - pump; 3 - valve; 4 - rotameter; 5 - dissolution column; 6 - granular urea; 7 - solution tank.

At different time intervals, the solution exiting the column was sampled in order to determine the concentration of urea and the amount of dissolved urea.

Sample concentration (C) was determined based on a calibration curve (Fig. 7) using refractive index (n) measurements:

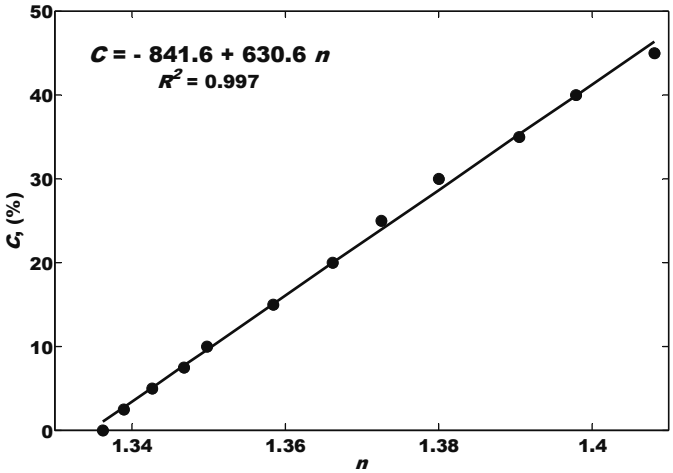


Figure 7. The calibration curve used for measurements

REFERENCES

1. A. Dib, L. Makhloufi, *Chem. Eng. Proc.: Proc. Inten.*, **2004**, *43*, 10, 1265.
2. L. Zeng, Y. Zhang, Q. Liu, L. Yang, J. Xiao, X. Liu, Y. Yang, *Chem. Eng. J.*, **2016**, *289*, 452.
3. R.E. Tătaru-Farmuş, M. Drăgan, S. Drăgan, I. Siminiceanu, *Studia UBB Chemia*, **2013**, *58*, 4, 113.
4. R.V. Garić-Grulović, Ž.B. Grbavčić, N. Bošković-Vragolović, Z.L. Arsenijević, *Powder Technol.*, **2009**, *189*, 1, 130.
5. M.H. Abdel-Aziz, M. Bassyouni, I.A.S. Mansour, A. Nagi, *J. Ind. Eng. Chem.*, **2014**, *20*, 5, 2650.
6. S. Drăgan, *Studia UBB Chemia*, **2015**, *60*, 3, 183.
7. C.A. Horoba, I. Mămăligă, *Rev. Chim.*, **2014**, *65*, 8, 907.
8. I. Mohammed, T. Bauer, M. Schubert, R. Lange, *Chem. Eng. Sci.*, **2014**, *108*, 223.
9. S. Petrescu, I. Fechete, M. Ciubotariu, *Chem. Eng. J.*, **1998**, *69*, 39.
10. J.J. Derksen, G. Reynolds, A. Crampton, Z. Huang, J. Booth, *Chem. Eng. Res. Des.*, **2015**, *93*, 66.
11. T. Elperin, A. Fominykh, *Heat Mass Transfer.*, **2004**, *41*, 5, 442.
12. M. Doichinova, O. Lavrenteva, C. Boyadjiev, *Int. J. Heat Mass Transfer*, **2011**, *54*, 13-14, 2998.
13. L.A. Calcada, L.A.A. Martins, C.M. Scheid, S.C. Magalhães, A.L. Martins, *J. Petrol. Sci. Eng.*, **2015**, *126*, 97.
14. A.A. Harraz, I.A. El Gheriany, M.H. Abdel-Aziz, T.M. Zewail, A.H. Konsowa, G.H. Sedahmed, *Biochem. Eng. J.*, **2015**, *103*, 1.
15. W. Huang, H. Shao, W. Li, H. Jiang, Y. Chen, *Aquat. Bot.*, **2016**, http://dx.doi.org/10.1016/j.aquabot.2016.04.003

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 505-514)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

EVALUATION OF THE ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOLS IN DIFFERENT FRUIT TEAS

IOAN SIMON[a], DORINA SIMEDRU[b], LUCIAN DORDAI[b, c], EMIL LUCA[c], VANDA FUSS[b], ANCA BECZE[b]

ABSTRACT. A diet rich in antioxidants is a heath choice most people make when it comes to options in fighting free radicals, that are formed natural in the body, but that cause damage to DNA and are connected to aging. The study was done in order the evaluate fruit teas that are a sources of antioxidants that can be easily introduce in the diet and are found all year along. 20 tea samples were analyzed to evaluate the antioxidant capacity and total polyphenols content. For the antioxidant capacity a photochemiluminescence methods was used and the Photochem instrument from AnalytikJena and for the total polyphenols content the Folin-Ciocalteu method was used. The average results where compared to the results obtained from black tea. The antioxidant capacity values obtained where from 0,53 to 2,27 mg/l Ascorbic Acid equivalents. The total polyphenols content was between 54 to 96 mg/l Gallic Acid equivalents. A positive correlation of 0.9664 was obtained between the antioxidant capacity and the total polyphenols content. A negative correlation of -0.962031201 was obtained between the antioxidant capacity and the number of the ingredients found in the tea samples.

Key words*: antioxidants, tea, polyphenols, Gallic acid, Ascorbic acid, antioxidant capacity*

INTRODUCTION

One important aspect in our modern culture is to live longer and to age slower. Scientists have been searching for answers regarding the ageing

[a] *University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Department of Surgery, 18 Republicii Street, 400015 Cluj-Napoca, Romania, isimon@umfcluj.ro;*
[b] *INCDO-INOE2000, Research Institute for Analytical Instrumentation, ICIA Cluj-Napoca Subsidiary, 400293 Cluj-Napoca, Romania*
[c] *University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania*

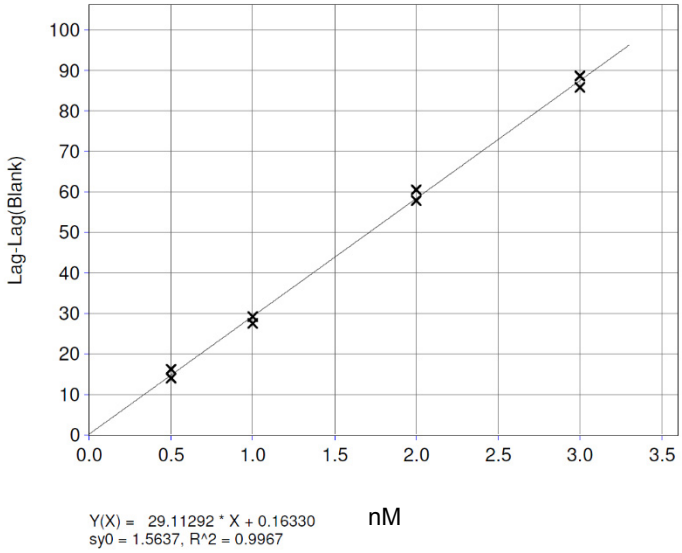
process. The result of these researches is the free radical theory of aging (FRTA) [1, 2] which states that organisms age because cells accumulate free radical damage over time [1, 3]. Free radicals are atoms with unpaired electrons that are the byproduct of normal cell function. The free radical theory of aging asserts that free radicals are linked to DNA damage, to protein cross-linking and other changes that accumulate over time and causes us to experience aging.[2 - 4] A chemical chain reaction of radical production occurs when a free radical pulls an electron off a neighboring molecule, causing the affected molecule to become a free radical itself, the new free radical can then pull an electron off the next molecule and so one. [2, 4] The free radicals produced in such reactions often terminate by removing an electron from a molecule which becomes changed or cannot function without it. Such an event causes damage to the molecule, and thus to the cell that contains it (since the molecule often becomes dysfunctional).[5] Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule. [2] They are electron donors that can break the free radical chain reaction by donating one electron, but without turning into free radicals themselves. [2] Studies have shown that increasing the amount of antioxidants in the diets of mice and other animals can slow the effects of aging. Since the creation of free radicals cannot be stop it is become more and more important to enrich our diets with higher quantities of antioxidants.[6, 7] A lot of food products have high concentrations of antioxidants like red wine, blueberries, red berries, dark green veggies, sweet potatoes, orange vegetables, tea, coffee, etc. [8 - 11] The problem with some food sources of antioxidants is that they are not available all year long like blueberries and raspberries and some are dietary restriction for people with different medical conditions like red wine, black tea and coffee.[12] The free radical theory of aging does not fully explain all the changes that occur during aging. It is likely that free radicals are only one part in the aging equation.

The purpose of the study was to evaluate the antioxidant capacity of fruit tea found on the local market and to compeer those result with similar products that have high antioxidant content like black tea. Fruit teas are available all year long, are not part of dietary restrictions, can be easily incorporated in a day to day diet, come in many different flavors and can be enjoy by all age groups.[13] All these characteristics make fruit tea an easy and enjoyable source of antioxidants. The antioxidant capacity of the fruit tea was also correlated with the total polyphenols content and number of ingredients of the tea samples.

RESULTS AND DISCUSSIONS

The calibration curve in 4 points obtained using Photochem for vitamin C is presented in figure 1. The slope of the calibration curve was 0,9967.

The calibration curve in 5 points obtained using Lambda Spectrophotometer for Gallic acid is presented in image 2. The slope of the calibration curve obtained was 0,9993.



***** The interception point of the tangent with the x coordinate defines the Lag phase- Lag

Figure 1. Calibration curve of vitamin C

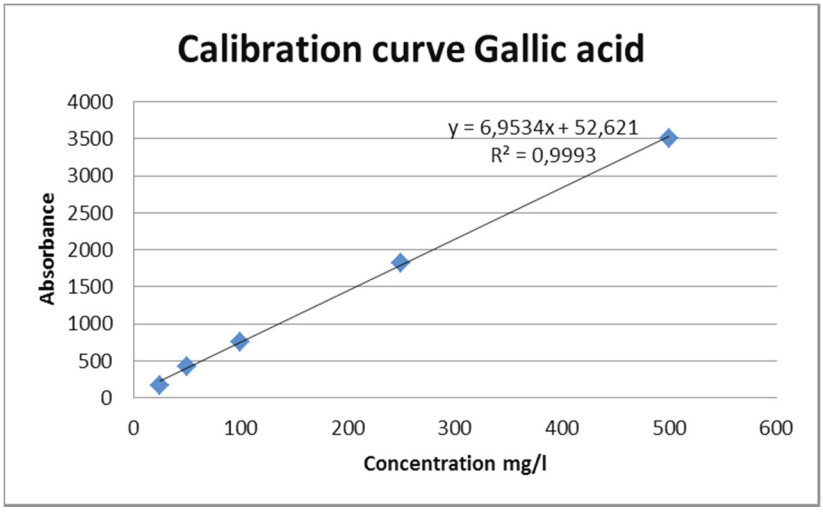


Figure 2. Calibration curve of Gallic acid

The average results obtained for the analysis of the 20 tea samples done in triplicates are presented in table 1.

Table 1. Antioxiodant capacity and polyphenol content of the 20 tea samples

Sample name	Antioxidant capacity mg/l Ascorbic Acid equivalents	Total polyphenols mg/l Gallic Acid equivalents	Total ingredients
Sample 1	1.57±0,007	93±0,57	7
Sample 2	2.27±0,009	96±0,55	6
Sample 3	2.23±0,09	96±0,53	6
Sample 4	2.19±0,009	96±0,53	6
Sample 5	1.25±0,006	76±0,44	7
Sample 6	0.53±0,003	66±0,41	9
Sample 7	0.56±0,003	67±0,39	9
Sample 8	0.58±0,003	68±0,39	9
Sample 9	0.67±0,004	68±0,40	9
Sample 10	1.07±0,005	90±0,53	7
Sample 11	1.26±0,006	81±0,51	7
Sample 12	1.32±0,005	71±0,48	8
Sample 13	0.75±0,004	70±0,48	9
Sample 14	0.74±0,004	72±0,48	9
Sample 15	1.56±0,007	95±0,56	7
Sample 16	0.68±0,004	54±0,35	11
Sample 17	0.98±0,005	63±0,39	9
Sample 18	1.17±0,005	75±0,48	8
Black tea 1	4.67±0,011	175±0,73	1
Black tea 2	3.96±0,009	149±0,69	1

The correlation between the results and between the number of ingredients and the results is presented in table 2, fig 3, fig 4 and fig. 5.

Table 2. Value of correlation factors

Crt. nr.	Antioxidant capacity	Polyphenols	Total ingredients
1	1		
2	0.9664	1	
3	-0.9620	-0.9702	1

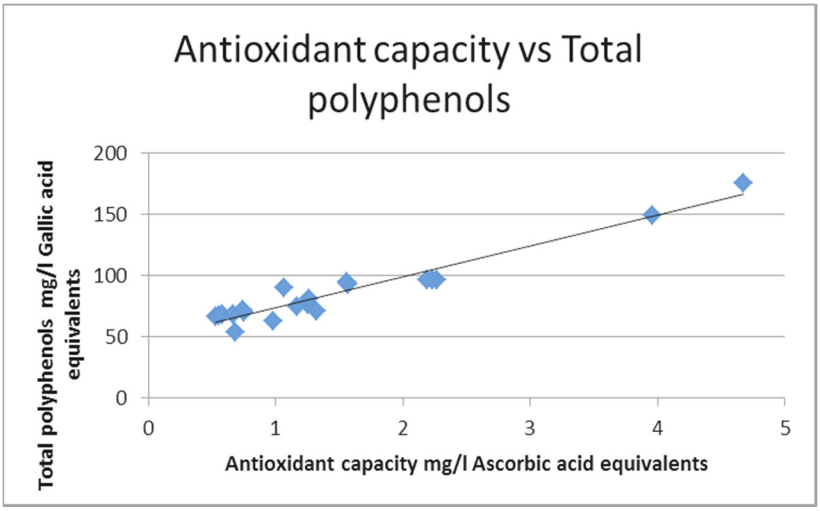


Figure 3. Trend line correlation between antioxidant capacity and total polyphenols

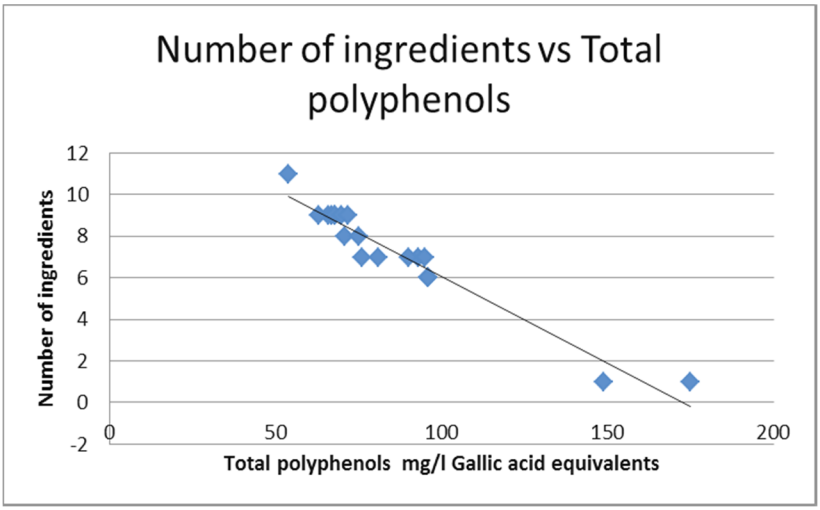


Figure 4. Trend line correlation between number of ingredients and total polyphenols

The values obtained correlating the results are significantly different from 0 which prove that the antioxidant capacity of the fruit tea is proportional with the total polyphenols content. The data also shows that there is an indirect correlation between ingredients and antioxidant capacity/ total polyphenols content. The more ingredients a tea has the less antioxidant capacity and total polyphenols content values where obtained.

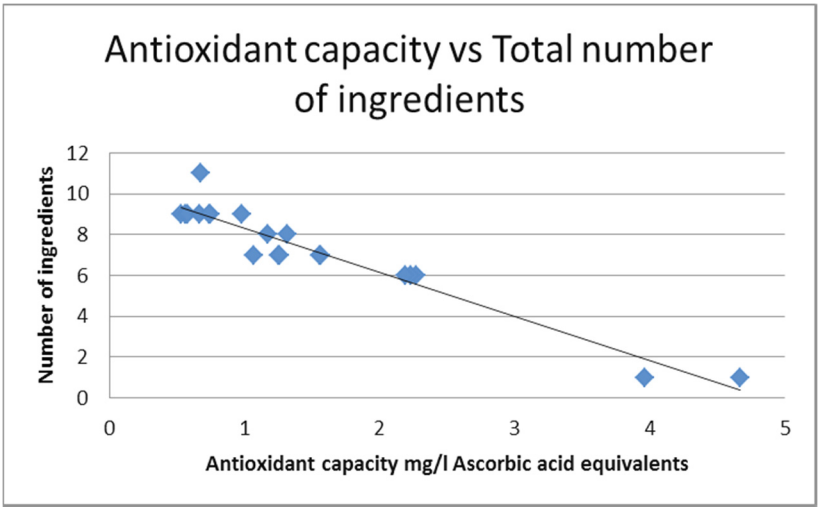


Figure 5. Trend line correlation between number of ingredients and antioxidant capacity

The antioxidant capacity of the black tea was significantly higher than that of fruit tea also the total polyphenols content. (figure 6, 7)

The antioxidant capacity was more them 3,5 higher than that of the average values obtained for fruit tea, but the total polyphenols was only 2 times higher.

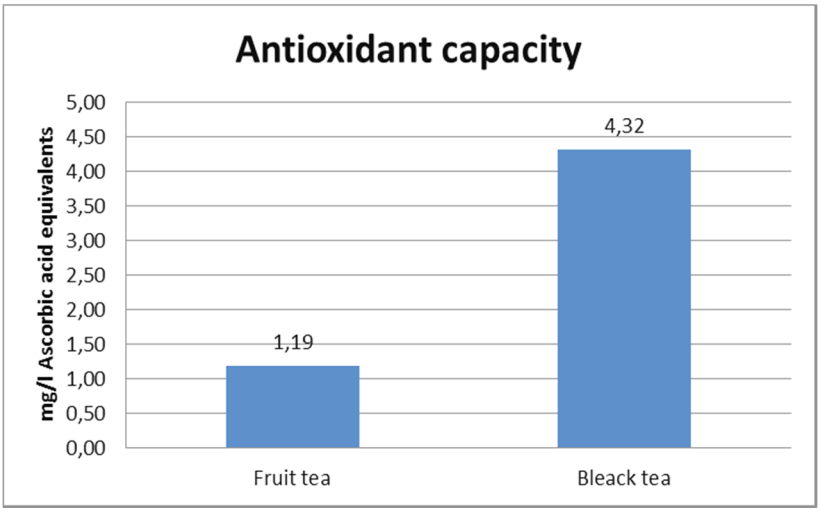


Figure 6. Average antioxidant capacity values for fruit tea and black tea

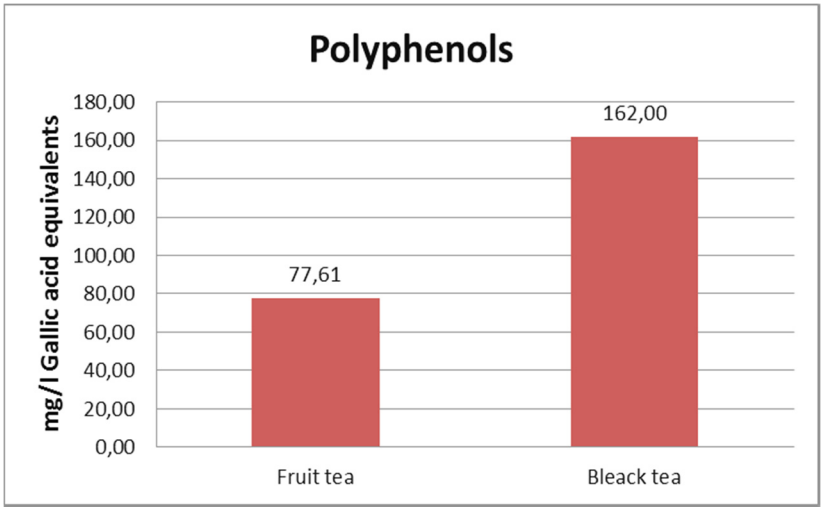


Figure 7. Average total polyphenols content for fruit tea and black tea

CONCLUSIONS

The antioxidant capacity values obtained where from 0,53 to 2,27 mg/l Ascorbic Acid equivalents and the total polyphenols content was between 54 to 96 mg/l Gallic Acid equivalents.

The highest antioxidant capacity values obtained was 2,27 mg/l Ascorbic Acid equivalents the tea had only 6 ingredients and it had the heights total polyphenols content of 96 mg/l Gallic Acid equivalents.

The lowest antioxidant capacity values was obtained for a tea with 9 ingredients, 0,58 mg/l Ascorbic Acid equivalents, that had total polyphenols content of 68 mg/l Gallic Acid equivalents.

The lowest total polyphenols content was obtained for a tea with 11 ingredients, 54 mg/l Gallic Acid equivalents that had antioxidant capacity value of 0.68 mg/l Ascorbic Acid equivalents.

The extra ingredients added to the fruit tea did not give a higher antioxidant capacity or a higher total polyphenols content which mean that the 3 base ingredients hibiscus, blackberry leaves (*Rubus suavissimus*) and apple are the ones that give the antioxidant capacity, all the other ingredients add lower the content of the basic tea and thus lower the antioxidant capacity. The extra ingredients have no antioxidant capacity because either they don't have it as raw material, the compounds that are antioxidants are no extracted in water or the hot water temperature denatures the compounds.

The types of polyphones present in the teas have different antioxidant capacity of that is way the antioxidant capacity was more them 3,5 higher than that of the fruit tea and the total polyphenols content was only 2 times higher.

EXPERIMENTAL SECTION

Tea samples

18 fruit tea samples where purchase from different local shops they had different flavors but the same base notes (hibiscus and apple) and 2 black tea samples. Samples consisted of two batches of each commercial tea.

Reagents

The fallowing reagents where used in the analysis: Folin-Ciocalteu solution (Supelco), Gallicacid (Supelco), sodium carbonate (Merck), ultra-pure water (EVOQVA), Ethanol (Supelco), ACW kit from AnalytikJena, HPLC grade Methanol (Merck).

Preparation of tea infusion

The infusion was prepared using a bag of tea or 2 g of tea in 200 ml of ultra-pure water at 97°C. After 5 minutes of infusion the tea was filter and left to cool for 1h in an Erlenmeyer flask with lid to prevent any water evaporation. Every sample was done in triplicate.

Antioxidant capacity

For the determination of the antioxidant capacity expressed in equivalents of vitamin C the Photochem instrument from AnalytikJena was used and the specially design ACW kits. ACW kit contains a standard solution of vitamin C, stock solution (Photo sensitizer and detection reagent), a buffer solution and a diluting solution. The instrument uses the fast photochemical excitation of radical formation combined with sensitive luminometric detection. The measurment is done in 2 steps:

1. Optical excitation of a photosensitizer substance S and subsequent generation of the superoxide anion radicals

$$S + hv + O2 \rightarrow [S^*O2] \rightarrow S^{\bullet+} + O2^{\bullet -}$$

2. Detection of the radicals (left after the reaction with antioxidants) by means of a chemiluminogenic substance.

A calibration curve of vitamin C was done in 4 points 0,5nM, 1 nM, 2 nM and 3 nM. The samples were diluted 1:20-1:50 to be in the range of the calibration curve.

The formula used for calculation is:

$$Concentration\ \left[\frac{\mu g}{ml}\right] = \frac{Quantity \cdot Dilution \cdot M}{Pipetted\ Volume}$$

Where:
Quantity - nmol Ascorbic acid
M - Molar mass Ascorbic acid = 176,13 ng/nmol
Pipetted volume: used volume in the vial in µl
Dilution - at 1:10 dilution factor = 10.

Total polyphenols analysis

For the determination of polyphenols a modified version of the Folin-Ciocalteu method was used. Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [14].

0,5 ml of sample, was pipette in a 10 ml volumetric flask, which contained 0.5 ml Folin-Ciocalteu solution, 5 ml ultra-pure water and 1,5 ml sodium carbonate solution (20%), the flask was filled up to the mark with ultrapure water. The volumetric flasks samples were left 90 de minutes and where then measure at 765 nm using the Spectrophotometer UV/VIS Lambda 25 from Perkin Elmer. Measurements were compared to a calibration curve of Gallic acid (25, 50, 100, 250, 500 ppm), and the results were expressed as Gallic acid equivalents. All samples where done in triplicate.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme “Increase of Economic Competitiveness”, Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164

REFERENCES

1. S. Hekimi, J. Lapointe, Y. *Trends In Cell Biology*. **2011**, *21(10)*, 569.
2. D.N. Aubrey J. de Grey, “Molecular Biology Intelligence Unit 9”, R.G. Landes Company, **1999**.
3. E.J. Masoro, Handbook of physiology, Section 11: Aging. Oxford University Press, **1995**, 3-21.

4. B.Halliwell, *Lancet*, **1994**, 344 (8924), 721.
5. D. Dreher, A. F. Junod, *European Journal of Cancer*,**1996**, 32A (1), 30.
6. C. J. Dufresne, E. R Farnworth, *Journal of Nutritional Biochemistry*, **2001**, *12*, 404.
7. A. Scalbert, C. Manach, C. Morand, C. Rémésy, *Critical Reviews in Food Science and Nutrition,* **2005**, *45*, 287.
8. U. Imeh and S. Khokhar, *Journal of Agricultural and Food Chemistry*, **2002,** *50(22)*, 6301.
9. T. P. Whitehead, D. Robinson, S. Allaway, J. Syms, *Journal of Clinical Chemistry,* **1995**, *41*, 32.
10. S. Şahin, *Antioxidants*, **2013**, *2*, 206.
11. K. Joo-Shin, *Food and Nutrition Sciences*, **2013**, *4*, 177.
12. A. Pekal, P. Dróźdź, M. Biesaga, K.Pyrzynska, *European Journal of Nutrition*, **2011**, *50*, 681.
13. C. D. WU, G. X. WEI, *Nutrition and Oral Health*, **2002**, 18, 443.
14. E. Sarıburun, S. Şahin, C.Demir, C.Türkben, V. Uylaşer, *Journal of Food Science,* **2010**, *75*, 328.

STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 515-522)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoş
on the occasion of his 80th anniversary

IDENTIFICATION AND CLASSIFICATION OF WHISKEY ALCOHOLIC DRINKS USING MASS SPECTROMETRY AND CHEMOMETRIC TOOLS

RAZVAN PODEA[a], MONICA CULEA[b], RAMONA BLEIZIFFER[b], SONIA SUVAR[b], PAULA PODEA[a,*]

ABSTRACT. Adulteration of alcoholic drinks is often common, due to the products high prices and large commercialization. Using Mass Spectrometry and a Multi-Variate Statistical Package soft with PCA analysis application, whiskey from different sources was identified and compared in order to create a successfully method for identification possible adulterants. For PCA analysis the m/z abundance values (obtained from fingerprints mass spectra) were used. The results showed that the complex spectrum is a good chemical fingerprint of whiskey sample, indicating its origin and type and facilitate a good differentiation of different samples. By classification the models obtained for all whiskey samples, a method for adulterants detection was created.

Keywords: whiskey*, GC-MS, PCA analysis, spectrum fingerprint.*

INTRODUCTION

Whiskey is a high quality alcoholic beverage obtained by the distillation of spirits from various cereals. Scotch wihskey is the one produced from barley malt in Scotland and matured for three years in oak casks[1] and American whiskey is made from a corn steep, rye and some malt barley and aged in new oak barrels burned inside for at least two years. There are also blended whiskey which is obtained by a mixture of several varieties of whiskey, often more than 30, to get the same uniform taste always which has moderate quality[2]. The flavours and the signiatures of different types whiskey are the product of manufacturing process, period of fermentation processes, distillation

[a] *Faculty of Chemistry and Chemical Engineering, 1 Kogălniceanu str., 400084 Cluj-Napoca, Romania Babeş-Bolyai University.*
[b] *Faculty of Physics, 1 Kogălniceanu str., 400084 Cluj-Napoca, Romania, Babeş-Bolyai University.*
[*] *Corresponding author: mpaula@chem.ubbcluj.ro*

and aging[3]. The natural process of aging for at least two years in oak barrels, will change the odor, flavor and color of whiskey. The chemical profile of whiskey is a complex range of flavor compounds of which the most important are carbonyl compounds, alcohols, carboxylic acids and their esters, sulfur and nitrogen compounds (pyridine, pyrazine), polyphenolic compounds, terpenes, and fatty esters.[4,5] Phenolic acids are extracted in oak whiskey, and their concentration can be influenced by the time that was in contact with wood[6].

Determination of authenticity of food products and also identification of the origin of food products is an important aspect in food quality control. Adulteration of alcoholic drinks is often common, due to the products high prices and large commercialization. Being an expensive alcoholic liquor, whiskey is often exposed to adulteration. The certification of authencity of whiskies is the main interest of producers, dealears and consumers. To prove the authencity and origin of whiskey different methods have been reported. Some of them are using gas chromatography (GC) for determination of the mixture of flavouring compunds or for analysis of isotopic *ratio*[1] of ^{13}C and ^{12}C and furfural and 5-hydroxymethyl-2- furaldehyde *ratio* as whisky markers[7]. Analysis of the tracing metals is also considered an indicator of whiskey's authencity.[8] Several GC-MS methods were used to analyze the volatiles profile in whiskey.[9,10,11,12] ESI-MS methods with direct injection of samples coupled with chemometric analysis have been shown as an efficient technique for determination of authencity of whiskies.[13,14] Whiskey has a complex variety of compounds and is a good matrix to use multivariate techniques, such as Principal Component Analysis (PCA). Different programs used in chemometric analysis were used together with analytical methods to group or to identify adulteration of whiskey sample[15].

In this study whiskey from different sources was classified and compared using a new Gas-Cromatography-Mass Spectrometry method coupled with a Multi-Variate Statistical Package soft with PCA analysis application, in order to create a rapid and efficient method for identification of possible adulterants by mixing alcoholic beverages.

RESULTS AND DISCUSSION

Four types of authentic samples of whiskey commercialized in Romania: (Jack Daniel's, (whi5), Ballantine's (whi6), Jim Beam (whi7) and Johnnie Walker (whi8)) were liquid-liquid extracted and analyzed. Another two samples of blended whiskey obtained by mixing Jack Daniel's and Ballantine's (1:1, v/v), (whi 5.6) and Jack Daniel's, Ballantine's, Jim Beam (2:1:1, v/v/v), (whi5,6,7) were extracted and analyzed**.** The whiskey extracts were analyzed by GC-MS to obtain the reconstituted chromatograms. Using the soft provided by the MS instrument, a fingerprint mass spectrum for the all analyzed samples was obtained by summing up the mass spectra taken

every minute from 5 minutes up to 30 minutes. The obtained complex mass spectrum will be named fingerprint and the corresponding abundances m/z will be variables that will differentiate the samples. In Figure 1 the reconstructed chromatogram are shown for the Jack Daniel's whiskey extract, beside the fingerprint mass spectrum. This is a very complex chromatogram, more than 100 components being detected. Similarities between the samples are observed also in fingerprints. All whiskies fingerprints display 67 common ions. The most important are 53, 55, 57, 61, 83, 84, 85, 88, 91, 97, 101, 127, 129 and 143 m/z, with variable intensities, which can be considered diagnostic ions for whiskey. Fingerprints of different whiskey sources display also some individual variation. For example, Jack Daniel's ion of m/z 88 is the most intense and some others are unique ions (m/z 149, 165, 191, 208, 298); Ballantine's most intense ion is m/z 55, while the ion of m/z 88 appearing in fingerprint has a weak intensity. Ballentine's fingerprint has also some characteristic ions of m/z like 138, 162, 274; the intense ion of Jim Beam whiskey is m/z 55, but also ion of m/z 88 has a high intensity. Johnnie Walker whiskey fingerprint has ion of m/z 55 of highest intensity and some unique ions of m/z like 242 and 267. All authentic samples fingerprints possessed many common ions which are valuable for stabilize a common chemical label of whiskey. The used method demonstrates that is possible to identify common ions of samples and also different variables in samples.

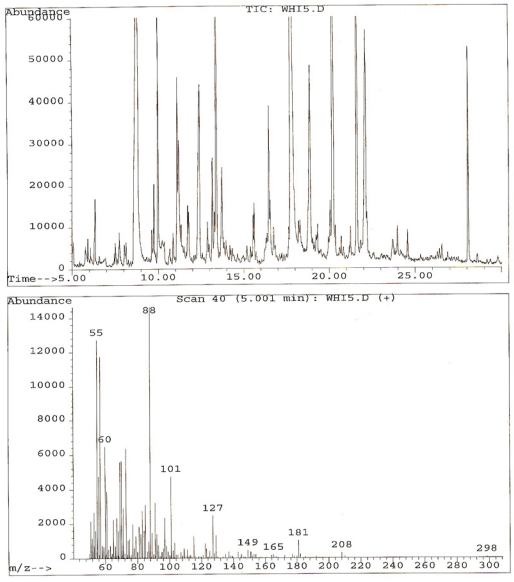


Figure 1. Reconstructed chromatograms for extract of Jack Daniel's whiskey beside the fingerprint mass spectrum

Validation of analytical method

For validation of the analytical method three samples from the same Jack Daniel's whiskey extract were analysed: whi5, whi5.1 and whi5.2 by GC-MS. For each mass spectrum fingerprint obtained, abundance fragments m/z were collected. To calculate the reproducibility, the fingerprint spectra abundances were compared for all three injections. Calculating standard deviation (SD) and relative standard deviation (RSD) in each of the three injections of the same extract (whi5), an average standard deviation (0.82) and an average relative standard deviation (9.09) were obtained. These values indicate a medium reproducibility, but given the large number of variables can be considered as acceptable. Further, the collected data were statistically interpreted.

Statistical analysis of data

The correlation of multiple mass spectra is simplified by using multivariate statistical techniques and is essential due to the large number of variables. The principal component analysis subtracted the extension of data set of highly correlated variables, so the number of variables will be reduced by constructing a new set of coordinates[15].

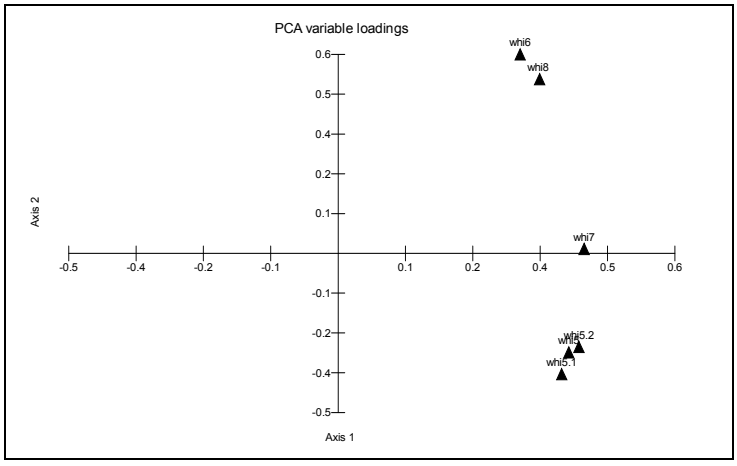


Figure 2. The graphical representation of the two main components obtained by the 67 variables (values m/z from fingerprint mass spectrum) for three samples of Jack Daniel's (whi5; whi5.1; whi5.2), Ballantine's (whi6), Jim Beam (whi7), and Johnnie Walker (whi8).

The PCA analysis variables are abundances of fragments (m/z) from mass spectrum fingerprint obtained by summing the abundances of ions from mass spectra obtained every minute. PCA method search for correlations between abundances values of m/z and extract linear combinations of the

nearest abundance (major components) which define the difference between samples. To construct the complex matrix, 67 variables (the percentage values of reports m/z spectra fingerprint) were considered. Following the interpretation of the results obtained by mass spectrometry using the MVSP soft with PCA analysis application, the resulting graphs, obtained from analysis of three injections of whiskey Jack Daniel's (whi5; whi5.1; whi5.2) layout very close to each other, which prove that the method is reproducible. Also the representation graph for all four types of whiskey shows that samples can be differentiated (Figure 3).

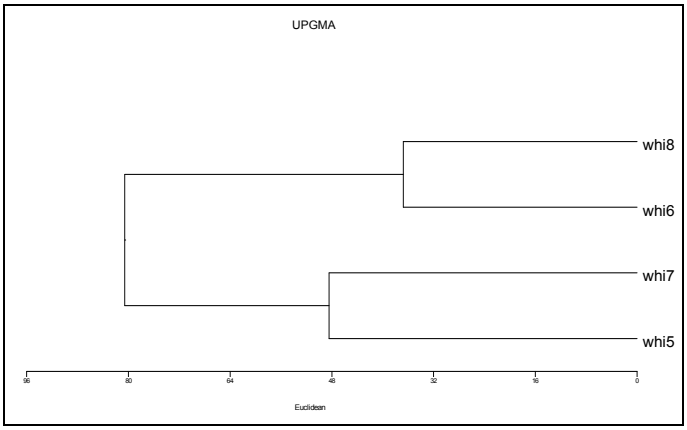


Figure 3. The dendogram obtained by measuring the Euclidean distances for the four extracts of whiskey Jack Daniel's (whi5), Ballantine's (whi6), Jim Beam (whi7), Johnnie Walker (whi8) based on the values of m/z from fingerprint mass spectra

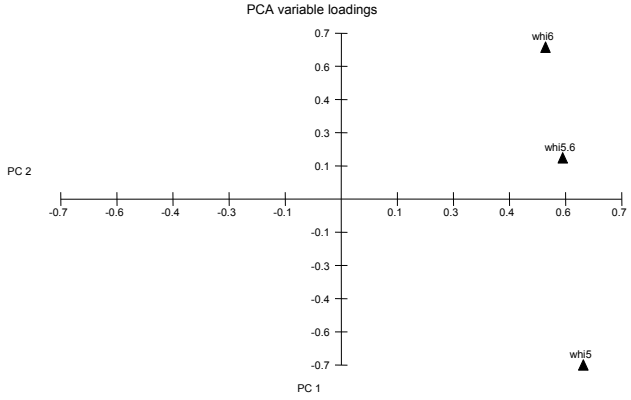


Figure 4. The graphical representation of the two main components obtained by the 67 variables (the percentage values of reports m/z from fingerprint mass spectrum) for extracts of Jack Daniel's (whi5), Ballantine's (whi6) and mixtures of the two in equal proportions (whi5.6).

The cluster analysis shows us the degree of relatedness by the m/z values from mass spectra fingerprint. This similarity of samples can be attributed to their different origins: Jack Daniel's (whi5) and Jim Beam (whi7) comes from the US (Bourbon) and Ballantine's (whi6) and Johnnie Walker (whi8) come from Scotland (Scotch).

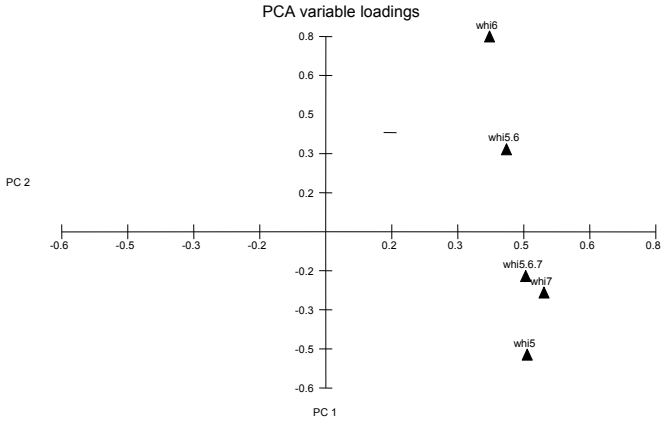


Figure 5. The graphical representation of the two main components obtained by the 67 variables (the percentage values of reports m/z from fingerprint mass spectrum) for extracts of Jack Daniel's (whi5), Ballantine's (whi6), Jim Beam (whi7) mixture Jack Daniel's 50% Ballantine's 50% + (whi5.6) and 50% mixture Jack Daniel's (whi5), 25% Ballantine's (whi6) and 25% Jim Beam (whi7): (whi5.6.7)

The analysis of the main components from mixtures obtained by combining samples of whiskey, shows that samples can be differentiated from the originals from which they are derived using this fingerprint obtained by mass spectrometry. The described developed method can be successfully used to detect a mixture or a fake made by mixing two or three original drinks. (Figure 4-5)

CONCLUSIONS

The obtained results showed that the recorded complex spectrum is a good chemical fingerprint of whiskey sample and facilitate a good diferentiation of whiskey samples in terms of origin and type. By classification models obtained for all whiskey samples, a method for adulterants detection was created. The proposed method can be applied successfully to identify the origin of alcoholic beverages and by creating a database it is possible to detect forgeries drinks.

EXPERIMENTAL SECTION

Materials and methods

Samples of whiskey

Original Jack Daniel's, Ballantine's, Jim Beam, and Johnnie Walker were purchased from a liquor store from Romanian. The blended whiskey was obtained by mixing of 50% original Jack Daniel's and 50% original Ballantine's whiskey and other blended combining 50% original Jack Daniel's, 25% original Ballantine's, 25% original Jim Beam.

Method of extraction

Whiskey samples were liquid-liquid extracted. As extraction solvent, a mixture of ethyl acetate: *n*-hexane: dichloromethane (5:1:1, v/v/v) was used. 20 mL sample of whiskey were extracted with 1 mL of solvent mixture and analyzed by GC-MC.

General experimental procedure

The GC-MS analysis were done using a GC-MS Hewlett Packard 5890 (EI mode) using Rtx-5MS capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, using a temperature program from 50 ^{o}C, 2 min, 3^{o}C /min at 180^{o}C, 30^{o}C /min at 220^{o}C, then 220 ^{o}C, for 5 min. The flow rate of helium, the carrier gas was 1 mL/min. The injector, interface, ions source and quadrupole are operated at 250°C, 280°C, 200°C and 100°C. The mass spectrometer was used in electron impact mode; electron energy of 70 eV and electron emission 300μA. 2μL of each extract of whiskey (whi5,6,7 or 8) were injected into the GC-MS to yield for each of them the reconstituted one chromatogram. Using the mass spetrometer soft fingerprints mass spectra for the comparison sample were obtained by summing up the mass spectra taken every minute from 5 minutes up to 30 minutes.

Statistical analysis of data

To classify the whisky samples, the obtained mass spectra were converted in samples chemical fingerprints, which were interpreted and compared using a Multi-Variate Statistical Package (MVSP) soft with PCA analysis application. MVSP is an easy to use program that performs a number of multivariate numerical analyses. It can also perform cluster analysis, with 23 different distance and similarity measures and seven clustering strategies.[16]

REFERENCES

1. C. N Rhodes, K. Heaton, I. Goodall, P. A Brereton, *Food Chemistry*, **2009**, *114* 697.
2. N Christoph, C. Bauer-Christoph, *Flavours and Fragrances Chemistry, Bioprocessing and Sustainability,* Springer Berlin Heidelberg, **2007**, 219.
3. K. Y. M. Lee, A. Paterson, J. R. Piggott and G. D. Richardson, *Journal of Institute of Brew*ing, **2001**, *107*, 287.
4. H.Maarse, *Volatile Compounds in Food and Beverages,* CRC press, **1991**, 548.
5. H.D.Belitz, S. Peter, G.Werner, *Food Chemistry***,** Springer-Verlag Berlin Heidelberg, **2004**, 936.
6. M.S.Bronze, L.F.Vilas Boas, A.P.Belchior, *Journal of Cromatography A,* **1997**, *768*, 143.
7. J. Jaganathan, S. M. Dugar, *Journal of AOAC International*., **1999**, *82(4)*, 997.
8. T. Adam, E. Duthie, J. Feldmann*, Journal of Institute of Brew*ing, **2002**, *108*, 459.
9. G. Fitzgerald, K.J. James, K.MacNamara, M.A. Stack, *Journal of Chromatography A*, **2000**, *896*, 351.
10. J H. Kahn, P. A. Shipley, E. G. Laroe, H A. Conner, *Journal of Food Science*, **1969**, *34(6)*, 587.
11. *P*. Wisniewska, T. Dymerski, W. Wardencki, J. Namiesnik, *Journal of the Science of Food and Agriculture*, **2015**, *95(11)*, 2159.
12. J. S. Camara, J. C. Marques, R. M. Perestrelo, F. Rodrigues, L. Oliveira, P. Andrade, M. Caldeira, *Journal of Chromatography A*, **2007**, *1150*, 198.
13. J. K. S. Møller, R. R. Catharino , M. N. Eberlin, *Analyst*, **2005**, *130*, 890.
14. J. S. Garcia, B. G. Vaz, Y. E. Corilo, C. F. Ramires, S. A. Saraiva, G. B. Sanvido, E. M. Schmidt, D. R. J. Maia, R. G. Cosso, J.J. Zacca, M. Nogueira Eberlin, *Food Research International*, **2013,** *51*, 98.
15. L. M. Headley J. K. Hardy, *Journal of Food Science*, **1992**, *57*, 4.
16. https://www.kovcomp.co.uk/mvsp/