BABEȘ-BOLYAI UNIVERSITY OF CLUJ-NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING
CHEMISTRY DEPARTMENT
ANALYTICA RESEARCH CENTER

ABSTRACT OF PHD THESIS

Green and White Miniaturized Methods for the Determination of Metals by Capacitively Coupled Microplasma Optical Emission Spectrometry

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Keywords

Capacitively coupled microplasma

Miniaturized analytical intrumentation

Mercury and methylmercury determination

Diffusive gradients in thin-film

In-situ and *ex-situ* passive sampling

Surface water analysis

Soil analysis

Validation of the passive accumulation method

Green miniaturized methods

White miniaturized methods

List of Abbreviations

Abbreviation	Description in Romanian language	Description in English language
μCCP	Microplasma cuplată capacitiv	Capacitively coupled plasma microtorch
AFS	Spectrometrie de fluorescență atomică	Atomic fluorescence spectrometry
APGD	Microplasmă cu descărcare luminiscentă la presiune atmosferică	Atmospheric pressure glow discharge
CV	Derivatizare la vapori reci	Cold vapor generation
CV-μCCP-AES	Spectrometrie de emisie atomică în microplasma cuplată capacitiv și derivatizare la vapori reci	Cold vapor generation capacitively coupled plasma microtorch atomic emission spectrometry
CV-AFS	Spetrometrie de fluorescență atomică cu derivatizarea la vapori reci	Cold vapor generation atomic fluorescence spectrometry
CV-ETAAS	Spectrometrie de absorbție atomică şi vaporizare electrotermică cu derivatizarea la vapori reci	Cold vapor generation electrothermal vaporization atomic absorbtion spectrometry
CV-ICP-OES	Spectrometrie de emisie optică în plasmă cuplată inductiv cu derivatizarea la vapori reci	Cold vapor generation inductively coupled plasma optical emission spectrometry
CV-ICP-MS	Spectrometrie de masă în plasmă cuplată inductiv cu derivatizarea la vapori reci	Cold vapor generation inductively coupled plasma mass spectrometry
CV-QTAAS	Spectrometrie de absorbție atomică în tub de quarţ cu derivatizarea la vapori reci	Cold vapor generation quartz tube atomic absorbtion spectrometry
DBD	Microplasmă cu barieră de dielectric	Dielectric barrier discharge
dcGD	Microplasmă de curent continuu	Direct current glow discharge
DCGD	Microplasmă cu microelectrod picătură de lichid	Droplet cathode glow discharge
DGT	Difuzie cu gradient în straturi subțiri	Diffusive gradients in thin films
ELCAD	Microplasma cu descărcare luminiscentă şi catod electrolitic	Electrolyte cathode glow discharge
ETV	Vaporizarea electrotermică	Electrothermal vaporization
FLA	Anod lichid curgător	Flowing liquid anode
FLA-APGD	Microplasmă cu descărcare luminiscentă la presiune atmosferică cu anod lichid curgător	Flowing liquid anode atmospheric pressure glow discharge
FLC	Catod lichid curgător	Flowing liquid cathode
FLC-APGD	Microplasmă cu descărcare	Flowing liquid cathode

	luminiscentă cu catod lichid curgător	atmospheric pressure glow discharge
GAC	Chimie analitică verde	Green Analytical Chemistry
GD	Descărcare luminiscentă	Glow discharge
GFAAS	Spectrometrie de absorbție atomică în cuptor de grafit	Graphite furnace atomic absorption spectrometry
ICP	Plasma cuplată inductiv	Inductively coupled plasma
ICP-MS	Spectrometrie de masă în plasma cuplată inductiv	Inductively coupled plasma mass spectrometry
ICP-OES	Spectrometrie de emisie optică în plasma cuplată inductiv	Inductively coupled plasma optical emission spectrometry
LA	Ablație laser	Laser ablation
LCGD	Microplasmă cu descărcare luminiscentă cu catod lichid	Liquid cathode glow discharge
LOD	Limită de detecție	Limit of detection
LOQ	Limită de determinare	Limit of quantification
LS-APGD	Microplasma cu descărcare luminescentă la presiune atmosferică și eșantionare lichidă	Liquid sampling atmospheric pressure glow discharge
PD	Microplasmă punctiformă	Point discharge
PD-OES	Spectrometrie de emisie optică în microplasma punctiformă	Point discharge optical emission spectrometry
PTFE	Politetrafluoroetilenă	Polytetrafluoroethylene
SAGD	Microplasmă cu descărcare luminiscentă cu soluție anod	Solution anode glow discharge
SCGD	Microplasmă cu descărcare luminiscentă cu soluție catod	Solution cathode glow discharge
SSETV	Evaporator electrotermic miniaturizat	Small-sized electrothermal vaporization
SSETV-μCCP-OES	Spectrometrie de emisie optică în microplasmă cuplată capacitiv și vaporizare electrotermică	Small-sized electrothermal vaporization capacitively coupled plasma
WAC	Chimie analitică albă	White analytical chemistry

Introduction

Motivation of the Research Topic

The research topic was established considering the current level of knowledge in the microplasma technology and its applications in multielemental analyses of wide interest for the scientific and social community. The microplasma technology and the development of miniaturized methods with high green and white degree represent top research fields within the atomic spectrometry, as they present similar or even better performances for certain applications, compared to the traditional laboratory methods, such as graphite furnace atomic absorption spectrometry or thermal desorption. The literature data indicated that the direct analysis without derivatization of liquid samples is problematic, taking into account the low operating power of the microplasma, the limited excitation capacity and the presence of non-spectral interferences of the multi-mineral matrix. The literature data and the previous experience of the research group coordinated by the scientific advisor of the PhD thesis indicated that some previously reported analytical problems can be exceeded by the use of electrothermal vaporization of a liquid microsample from a metalic filament, as method for microsample introduction in the microplasma. Therefore, the topic of the thesis was chosen to be in the field of Green Analytical Chemistry and White Analytical Chemistry, for the development and widening of the applications of a capacitively coupled microplasma source and coupling with the passive sampling/accumulation methods by diffusive gradients in thin-film applied *in-situ* and *ex-situ* on environmental samples. Although the DGT technique has been known for 30 years, it was not coupled with a completely miniaturized instrumentation with microplasma source. Thus, it was desired to demonstrate that direct microsampling from liquid by selective vaporization of the analytes with or without DGT coupling represents a path for the development and implementation of highly sensitive miniaturized analytical methods without non-spectral and spectral interferences, in the use of microspectrometers, without chemical vapor derivatization of the analytes. The results demonstrated that, through these approaches, the widening of the area of applicability of the microplasma technology for samples with complex matrices, respectively the increase of the green and white level of the microanalytical methods with microplasma was possible. The technological and analytical progress via multiple directions could offer new opportunites for the development of faster and more efficient methods for analysis, with the fulfiment of the GAC and WAC requirements, using appropriate evaluation methods by cutting-edge algorithms (AGREEprep and RGB-12), and could represent a new research direction concerning the development of analytical methods with wide applicability in case of complex matrices which are hard to analyze with microplasmas. Specifically, this was the approach in the research program, which provides a high level of national and international novelty and originality to this PhD thesis.

Objectives and Research Methodology

1. The general objective of the thesis was the development and validation of some miniaturized analytical methods for the determination of some toxic elements (Hg, Cd, Pb, Cu, Zn, As, Bi, Sb, Sn, etc.), on a completely miniaturized instrumentation based on electrothermal vaporization capacitively coupled microtorch optical emission spectrometry, directly from the liquid microsample and detection by optical emission microspectrometry with a low resolution microspectrometer, coupled or not coupled with the diffusive gradients in thin-films passive sampling (DGT)-SSETV-μCCP-OES, without spectral and non-spectral interferences, with high green and white levels. The specific objectives of the thesis were the following:
2. Development of a method for the determination and speciation of mercury from aquatic organisms and sediments by SSETV-μCCP-OES, without cold vapor derivatization;
3. Development of a method for the simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn by SSETV-μCCP-OES by direct microsampling of the liquid sample, without chemical vapor derivatization;
4. Development of a method for the determination of the mobile fraction of Cd, Pb, Cu, Zn and Hg in surface waters by DGT-SSETV-μCCP-OES coupled with *in-situ* passive sampling;
5. Development of a method for the determination of total content and mobile fraction of Cd, Pb, Cu and Zn in soil by DGT-SSETV-μCCP-OES after *ex-situ* passive sampling.
6. The establishement of the specific objectives was based on some hypotheses, namely: (i) the elimination of the non-spectral interferences of the multi-mineral matrix by separation of the analytes by selective electrothermal vaporization and by separation by DGT passive sampling; (ii) improvement of the analytical performance of the methods (sensitivity and detection limits), by accumulation following passive sampling; (iii) coupling of the passive sampling by DGT with microplasma would be ideal, which, besides the improvement of the analytical performance, could ensure simultaneous determination with the use of low resolution spectrometer; (iv) the novel (DGT)-μCCP-OES methods could be alternatives to the traditional methods with similar or even better analytical performance, by widening the application of the microplasma for

liquid samples with complex multi-elemental matrices without chemical vapor derivatization. In order to achieve the objectives and validate the working hypotheses, the research methodology was the following:

1. Optimization of the working parameters of the SSETV-μCCP-OES instrumentation, with regard to the thermal evaporation scheme, which could ensure the separation of the analyte volatile elements of matrices, respectively the operating conditions of the microplasma (power, Ar flow and spectroscopic observation), for the simultaneous detection of the emission spectra;
2. Optimization of the parameters of *in-situ* and *ex-situ* passive sampling by DGT of Chelex-100 resin, which could ensure the selective accumulation of the analytes compared to the multi-elemental matrix of alkali and alkaline-earth metals, with applicability in case of river waters and soils;
3. Validation of the novel (DGT)-SSETV-μCCP-OES methods by LODs, accuracy and precision, by the analyses of certified reference materials and real samples, respectively by statistical comparison of the results with the graphite furnace atomic absorption spectrometry (GFAAS), by established statistical tests (Tukey, Dunnet, Bland and Altman);
4. Worldwide for the first time, the study of the coupling of the DGT *in-situ* and *ex-situ* with a microplasma source with applicability in surface water and soil monitoring, by the determination of the total content and mobile fraction of toxic elements, without spectral and non-spectral interferences;
5. Evaluation of the green and white degree of the novel (DGT)-SSETV-μCCP methods, by the novel approaches, namely AGREEprep and RGB-12;
6. Highlighting the originality and novelty elements towards the state of the art.

The PhD thesis is structured on a literature study (Chapter 1) and personal contributions, some of them with high novelty and originality level in the context of the current state of the art (Chapters 2-5). The last chapter is dedicated to the conclusions and the relevant contributions of the PhD thesis from the perspective of analytical practice.

Chapter 1 presents the current state of the art in the microplasma technology and the applications related to the analyses of gaseous, liquid and solid samples. The types microplasmas, analytical performance according to the nature of the samples and the introduction system are presented. The principals of the passive sampling, mainly by DGT, are discussed as well. The most novel approches of the evaluation of green and white degree are presented, mainly by AGREEprep and RGB-12, in the context of Green Analytical Chemistry (GAC) and White Analytical

Chemistry (WAC), underlying the objective evaluation of applicability and performance of the traditional methods but especially for the novel methods based on atomic spectrometry.

Chapter 2 presents the results obtained within the objective of the thesis, namely "*The Development of a novel method for the determination and speciation of mercury from aquatic microorganisms and sediments by electrothermal vaporization capacitively coupled microplasma optical emission spectrometry*". A novel method for the determination of total mercury and methylmercury from food samples and river sediment samples was developed and validated, by SSETV-µCCP-OES in a capacitively coupled microplasma for low power and reduced Ar consumption (10 W, 150 mL min^{-1}) without derivatization, using the procedure recommended by the European Committee for the preparation of the sample by liquid-liquid extraction in the HBr-toluene-L-cysteine system and the determination by thermal desorption atomic absorption spectrometry (TDAAS). The extraction method of CH_3Hg^+ of the European Committee was adapted for the SSETV-µCCP-OES method by the decrease of the reagents'consumption, and was applied later both for the determination of total Hg total and speciation of CH_3Hg^+, as alternative to the TDAAS method. The method was succefully validated and the determination was performed by external calibration of Hg wavelenght at 253.652 nm. It was emphasised that the 1300 °C vaporization temperature from the Rh filament of a microsample with 10 µL volume ensured the selective vaporization of Hg, and therefore, the absence of non-spectral effects, which allowed the use of external calibration solely with Hg^{2+} solutions both for the determination of total Hg and CH_3Hg^{3+}. The SSETV-µCCP-OES method was a validated with a limit of detection of 7 µg kg^{-1} for total Hg and 3.5 µg kg^{-1} for CH_3Hg^+, with the recovery degree in the 100 ± 7% interval, and the precision in the 1.6–12.8% interval. The use of the Maya 2000Pro microspectrometer with the chamber purged with Ar 5.0 resulted the improvement of the limit of detection, approximately 20 times better than in the case of the QE65 Pro microspectrometer with CCD detector cooled at – 20 °C with a Peltier element, previously reported by Butaciu Sînziana within her PhD thesis (https://teze.doctorat.ubbcluj.ro/doctorat/teza/fisier/6816).

The "*Development of a novel method for the simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn by electrothermal vaporization capacitively coupled microplasma optical emission spectrometry and the use of direct microsampling of the liquid sample without chemical vapor derivatization*" is presented in Chapter 3. The analytical excitation capacity of the elements with chemical vapor generation ability without derivatization by SSETV-µCCP-OES was demonstrated by the simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn, without non-spectral interferences in river and cave sediment samples, using the direct microsampling of the liquid sample with selective vaporization from the Rh, by controlled heating at 1300 °C and detection in the capacitively coupled microplasma operating at 15 W and 150 mL min^{-1} Ar with the low resolution Maya 2000Pro spectrometer. The emission spectrum of the corresponding elements was a simple one with lines with excitation energies below 7 eV, in the operating conditions of the microplasma. The use of the Maya 2000Pro microspectrometer with chamber purged with Ar allowed the investigation of the emission spectra of As, Bi, Se, Sb, and Sn, in the vacuum-UV range (180–210 nm), usually available in case of the ICP-OES instruments equipped with high resolution and high sensitivity spectrometers. The SSETV-µCCP-OES method was proved to be cost-effective and efficient for the avoidance of derivatization and overcoming the non-spectral interferences, which allowed the use of external calibration instead of standard addition, similarly with the method developed for the determination and speciation of Hg.

For the first time, Chapter 4 presents the "*Development of a novel method for the determination of the mobile fraction of Cd, Pb, Cu, Zn and Hg in surface waters using the electrothermal vaporization capacitively coupled microplasma optical emission spectrometry coupled with in-situ diffusive gradients in thin-film passive sampling with green and white degree*". A method for the simutaneous determination of Cd, Pb, Cu, Zn and Hg in surface waters is described, by the coupling of *in-situ* diffusive gradients in thin-film (DGT) passive sampling with *ex-situ* determination on the completely miniaturized SSETV-µCCP-OES instrumentation, worldwide for the first time. The *in-situ* passive sampling was conducted by using DGT devices with Chelex-100 resin, highly selective towards Cd, Cu, Pb and Zn, compared to alkali and alkaline-earth elements, respectively As. Thus, besides the improvement of LODs with one order of magnitude by the DGT-SSETV-µCCP-OES method, compared to the method without preconcentration, the non-spectral interferences were exceeded and the simultaneous determination of the corresponding elements was possible, by vaporization of the microsample at 1500 °C. By separating Cd from arsenate through passive sampling on Chelex-100 resin, the spectral interference of the Cd 228.802 nm line towards the As 228.812 nm line was also exceeded, which cannot be realized by low resolution microspectrometer. The proposed DGT-SSETV-µCCP-OES method was validated by analyses of CRM water samples and by comparison of the results in river waters with the GFAAS method for Cd, Cu, Pb and Zn and TDAAS for Hg. By applying the RGB-12 and AGREEprep algorithms, it was found that the DGT-SSETV-µCCP-OES method was characterized by red/green/blue/white scores of 100/80/98/93%, therefore, the method can be classified in the category of excellent green and white degree.

Chapter 5 presents the results of the "*Development of a novel method for the determination of total content and mobile fraction of Cd, Pb, Cu and Zn in soil by simultaneous electrothermal vaporization capacitively coupled microplasma optical emission spectrometry after passive accumulation by the diffusive gradients in thin-film technique*". The passive sampling procedure was similar with the one previously presented, used in case of river waters. The coupling of the DGT passive sampling with the SSETV-µCCP-OES instrumentation also provided improved LODs with at least one order of magnitude, compared to the procedure without preconcentration on the DGT gel. The validation of the DGT-SSETV-µCCP-OES method by statistical comparison towards the GFAAS by the Bland and Altman test indicated that the differences are not statistically significant.

Chapter 6 presents the general conclusions, originality and novelty elements of the thesis, especially in the case of the SSETV-µCCP-OES coupling with *in-situ* and *ex-situ* DGT passive sampling for the monitoring of waters and the toxic metal content in soil.

Current State of the Art

Literature Study

Chapter 1. Microplasma Sources, Characteristics and Applications

1.1. Characterization and Clasification of the Microplasma Sources

The term "Plasma" was first mentioned by Langmuir in his studies regarding the ionized gases in 1928.[1] Plasma is a gas which has undergone an ionization process, resulting electrons, ions and neutral particles, both in their fundamental and excited states. Plasma can be considered electrically neutral because the electron density is generally equal with the one of the cations, resulting from the ionization of the support gas. The plasmas used in analytical purposes are generated by the ionization of a support gas, typically inert, such as Ar and He, by supplying sufficiently high energy level from a power source to determine the reorganization of the electronic structure of the atoms of the support gas, which leads to the appearance of atoms and ions in excited states.[2] The microplasma technology and its analitycal applications represents a relatively new research field with high performance in the atomic spectrometry and even mass spectrometry.[3] Microplasmas are generated by the interaction of the support gas with an electric field at atmospheric pressure and have at least one of the dimensions (height, lenght or width) under 1 mm.[3] The research concerning the potential applicability of microplasmas as excitation sources in the analytical chemistry has increased significantly in the last 20 years.[4,5] Depending on coupling power to the microplasma and the operating parameters, the generation of the microplasmas at atmospheric pressure can happen in two ways: (i) by dielectric barrier discharge (DBD) and (ii) by glow discharge (GD).[6,7] Thus, compared with the ICP, the microplasma sources are more diverse, namely dielectric barrier discharge (DBD) microplasmas, direct current (DC) microplasmas, capacitively coupled (μCCP) microplasmas, point discharge (PD) microplasmas, atmospheric pressure glow discharge (APGD) microplasmas, electrolyte cathode glow discharge (ELCAD) microplasmas, flowing liquid cathode and flowing liquid anode (FLC and FLA) microplasmas or droplet cathode glow discharge (DCGD) microplasmas.[8-15] Figure 1.1 is a schematic presentation of the microplasma sources.

The DBD microplasma is a high frequency discharge (of kH order), generated at atmospheric pressure in a tube, capillary or a sandwich montage between two plane parallel, annular or annular – concentric electrodes, which do not have contact with the microplasma, as a consequence of the dielectric (insulator) layer between the electrodes and the microplasma (Figure 1.1A).[6] In contrast to the DBD microplasma, the direct current glow discharge (Figure 1.1B) or the capacitively coupled (Figure 1.1C) microplasma is developed in a tube/quartz capillary between two electrodes which have contact with the plasma, between which a potential difference in direct current is applied, respectively radiofrequency (MHz).[10,16] The point discharge (PD) microplasmas (Figure 1.1G) are generated between two electrodes, under a high voltage alternating current. This possesses a high electron density, concentrating the plasma energy at the peak of the microelectrodes.[11]

The atmospheric pressure glow discharge (APGD) microplasma was first proposed by Cserfalvi and coworkers in 1994, in which one of the electrodes is a liquid sample, therefore, in these terms, being a microplasma with unique characteristics.[13] The generation of the APGD microplasma can be realized in two ways: electrolyte cathode glow discharge (ELCAD) microplasma[17], which uses an electrolyte solution pumped in a capillary tube serving as cathode, and liquid sampling atmospheric pressure glow discharge microplasma (LS-APGD)[18] where the electrolyte is sprayed in the direction of the anode or cathode, at the end of a capillary tube (Figure 1.1F). The ELCAD type glow discharge microplasmas are whether flowing liquid cathode atmospheric pressure glow discharge (FLC-APGD) microplasmas[12], flowing liquid anode atmospheric pressure glow discharge (FLA-APGD) microplasmas[19], solution cathode glow discharge (SCGD) microplasmas[20], solution anode glow discharge (SAGD) microplasmas[21] (Figure 1.1E), respectively liquid cathode glow discharge (LCGD) microplasmas[22].

1.2. Introduction of Samples in the Microplasma

The introduction of the samples in the microplasma is not an easy task due to the low operating power and the low acceptance capacity of solvents which destabilize the discharge and restricts the excitation capacity of the microplasma. Therefore, due to the low operating power, a significant part of the dissipated power in the microplasma is used for the vaporization of the solvent and the matrix and not for the atomization and excitation of the atoms of the analyte. It is expected that the sample type and its quantity introduced in the microplasma will affect the analytical performance respectively the applicability of the miniaturized instrumentation with microplasma. However, the microplasma was used for the analysis of solid, liquid and gaseous samples with the performance depending on the system of introduction of the sample which is chosen depending on the nature of the sample. Figure 1.2 presents some of the methods of introduction of the samples in the microplasma.

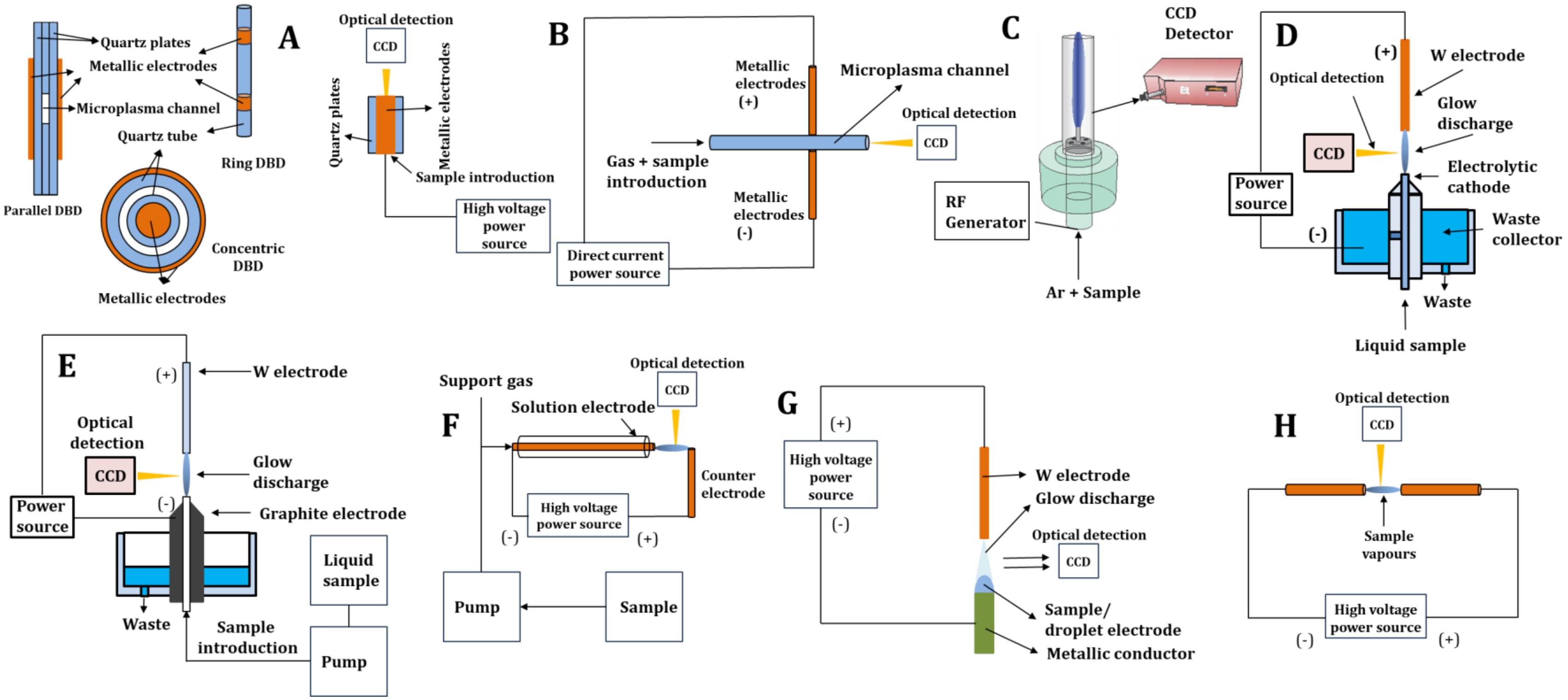


Figure 1.1. Schematic representation of the different microplasma sources. A – Dielectric barrier discharge (DBD) microplasma[23]; B – Direct current glow discharge (dcGD) microplasma[24]; C – Capacitively Coupled (μCCP) microplasma[16]; D – Electrolyte cathode glow discharge (ELCAD) microplasma[25]; E – Solution cathode glow discharge (SCGD) microplasma[26]; F – Liquid sampling atmospheric pressure glow discharge (LS-APGD) microplasma[27]; G – Droplet cathode glow discharge (DCGD) microplasma[15]; H – Point discharge (PD) microplasma[28]

Due to the low operating power, the microplasmas were usually used for the analysis of gaseous samples or the ones which could be easily converted into vapors. [29] The technique of derivatization to hydrides is used in the case of chemical vapor generating elements (As, Sb, etc.) and cold vapors in the case of mercury.[19] The liquid samples can be directly introduced in the microplasma with liquid electrode, which implies the *in-situ* generation of vapors from the sample, as a result of the expulsion phenomenon under the action of the Ar positive ions.[30,31] The liquid samples can be introduce by clasical methods as well, such as pneumatic nebulization and ultrasonic nebulization.[32,33] A much more adequate introduction of the liquid samples in the case of microplasma is the electrothermal vaporization (ETV) of a microsample from a metalic filament.[34,35] In this case, the energy of the microplasma is used much more efficiently, solely for the exication of the atoms of the elements, and not for the evaporation of the water.

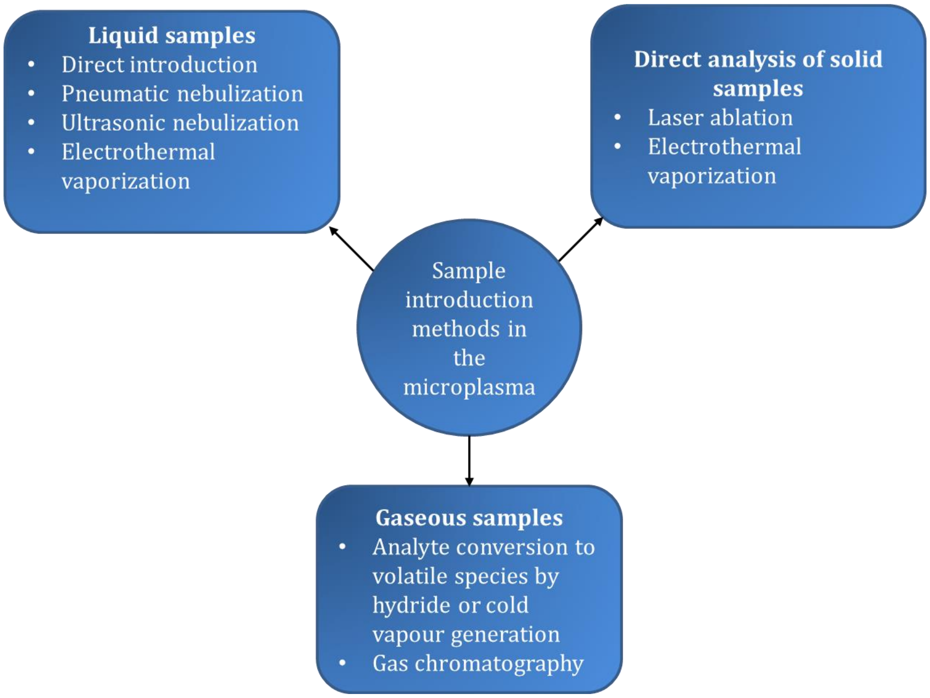


Figure 1.2. Methods for the introduction of samples in the microplasma

The solid samples can be dissolved and introduced after by one of the methods used for liquid samples or may be subjected to direct alanysis by the coupling of spectrometric methods with the microplasma sources with laser ablation (LA) or electrothermal vaporization (ETV), case where the aerosol is generated directly from the solid phase.[23,36]

1.3. Stage of Instrumental and Analytical Development of the Capacitively Coupled Microplasma

The first miniaturization of the capacitively coupled plasma (µCCP) was performed by the research group led by Professor Blades at the University of British Columbia, Vancouver, Canada, more than 20 years ago.[37] The in-depth development of the µCCP at international level was realized by an interdisciplinary research team from the Babeș-Bolyai University and the Research Institute for Analytical Instrumentation of Cluj-Napoca, launched by Professor Emil Cordoș and taken over by Professor Frențiu Tiberiu, the scientific advisor of the PhD thesis. The capacitively coupled microtorch consists of a microelectrode with Mo peak with the diameter of 1.25 mm, the Ar microplasma developing at the top of it in a quartz tube with the diameter of 5 mm and the length of 25 mm. The support argon of the plasma has also the role introducing the sample in the microplasma through 4 holes with the diameter of 0.75 mm, practiced around the Mo top microelectrode in the PTFE support. The capacitively coupled microplasma appears as a glow discharge of blue color and it develops in an electric field of 13.56 MHz radiofrequency, applied to the top microelectrode and requires a lower operating power (10 – 30 W) and reduce Ar consumption (100 – 200 mL min^{-1}). The microtorch and the diffuse aspect of the microplasma are presented in Figure 1.3.[16,38,39]

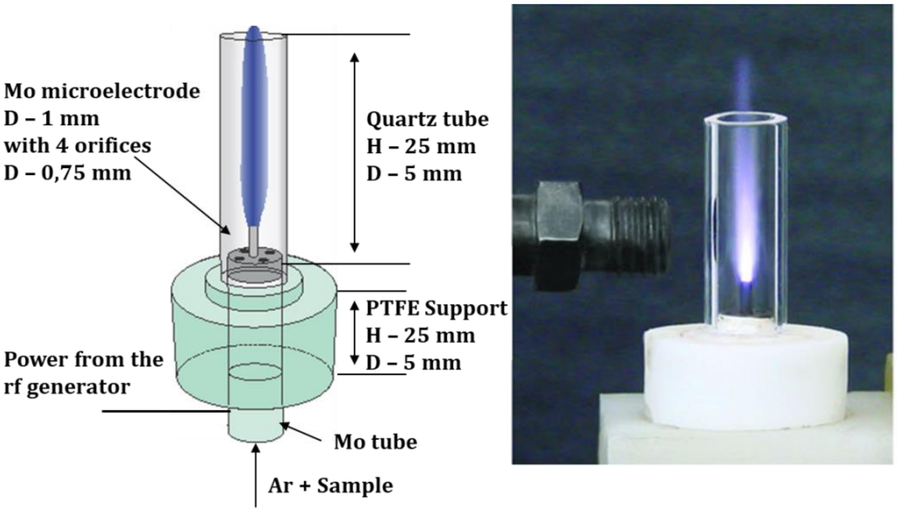


Figure 1.3. The microtorch and the aspect of the capacitively coupled microplasma[16,38,39]

The various analytical technologies developed on the capacitively coupled microplasma are based on the optical emission spectrometry (µCCP-OES). Two instrumental approaches were developed, OSIM patent granted, on the basis of the completely miniaturized µCCP-OES principle.[38,39] Some of the applications could be found in the following references.[16,40-48]

1.4. Passive Sampling based on Diffusive Gradients in Thin-Films

The passive sampling based of the diffusive gradients in thin-films (DGT) method was first proposed by Davison and Zhang[49] in 1994, for the detemination of mobile content of metlas in water. The DGT devices (Figure 1.4[50]) are composed of 3 thin layers: (i) a membrane filter with the role of retention of the particles in the sample suspension; (ii) a diffusion gel of polyacrylamide, agarose or polyacrylamide with agarose; (iii) an accumulation gel for the retention of the mobile species which were diffused through the diffusion gel.[49] According to the structure of the diffusion gel, the pore size and the nature of the complexing agent from the accumulation gel, it is ensured the selective diffusion of the mobile species and the selective retention on the accumulation gel.

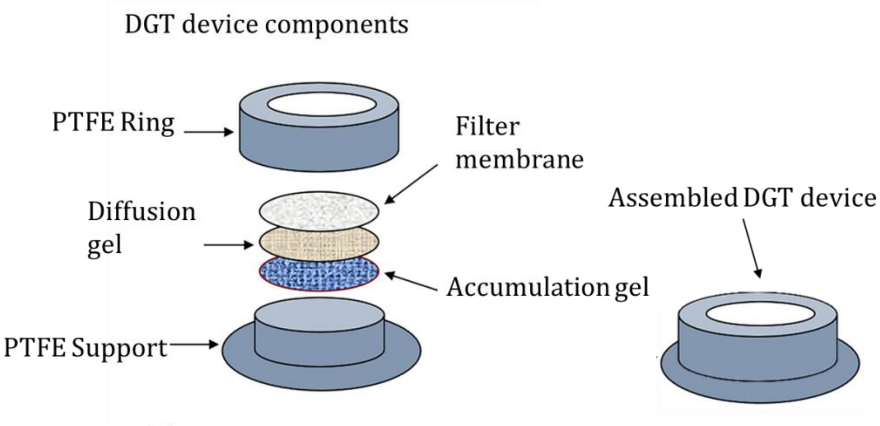
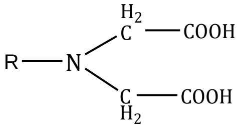
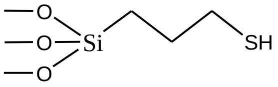


Figure 1.4. Schematic representation of a commercial DGT device, DGT Research Ltd., Lancaster, UK (https://www.dgtresearch.com/)[50]

The Chelex-100 gel with the polyacrylamide diffusion gel has a high selectivity in case of the ions of the divalent transition metals.[51-53] For the accumulation of the metalloids which form anions, the modified $Fe(OH)_3$, ZrO_2, TiO_2 silicagel is used, while for the simultaneous retention of of the ions of the divalent transition metals and the anions of the metalloids (As, Se, Sb, etc.) the modified silicagel is used with a mixture of Chelex-100 and $Fe(OH)_3$ at 1:2 ratio.[54-59] The accumulation and diffusion gels, respectively their selectivity, are presented in Table 1.1.

Table 1.1. The types of accumulation and diffusion gels, their selectivity and applicability

Accumulation gel	Formula	Diffusion gel	Retained analytes	Matrices	References
Chelex-100		Polyacrylamide linked to agarose	Cu, Pb, Cd, Co, Ni, Al, Zn	Water, Soil	[60-64]
3-MP		Agarose	As(III), CH_3Hg^+, Hg	Water, Seawater	[60,65-67]
Metsorb	TiO_2	Polyacrylamide	As, Se, Sb	Water, Soil	[59,68]
Zr oxide	ZrO_2	Polyacrylamide linked to agarose	As	Soil	[69]
Ferrihydride	$Fe(OH)_3$	Polyacrylamide linked to agarose	As, Se, V, Sb	Water	[70]
Mixture of Zr oxide and Chelex-100	-	Agarose	As, Cd, Pb, Cu, Zn	Soil	[71,72]
Mixture of Ferrihydride and Chelex-100	-	Polyacrylamide linked to agarose	As, Cd, Pb, Sb, Zn, Mn, P	Soil	[73,74]

The principle of the passive sampling by the DGT technique consists of the diffusion of the mobili metalic ions, A^{2+} in the present case, from the immersion solution, through the diffusion gel towards the accumulation gel, which contains a specific ligand which complexates A^{2+}, for an immersion period of the order of hours or days of the DGT device in the sample solution. The concentration of the analytical species at the interface between the solution and the accumulation gel is practically zero in the case of total retention on the linear accumulation period. In the case of the determination of the mobile species from the soil solution, the asambled DGT devices are immersed in a paste obtained by the mixing of the soil with water at a soil:water ratio at most 10:8, which represents the maximum water quantity which could be retained by the soil.[75] The accumulation of the metalic ions of the analyte A^{2+} by complexation in the accumulation gel by the anion of the ligand L^{2-}, is described by the following simplified reaction.[51]

$$A^{2+} + L^{2-} \leftrightarrow AL \tag{1.1}$$

Some of the details of the accumulation conditions for some of the applications are presented in Table 1.2.

After the accumulation time (t) of the mobile metalic species in the accumulation gel, the DGT device is extracted from the accumulation medium, is disassembled, and the accumulation gel is subjected to the elution of the analytes for 24 h.[54-59] The details of the elution conditions for some of the applications can be found in Table 1.2.

After obtaining the eluate, this is analyzed, and based on the concentration of the analytes in the eluate and on the accumulation parameters, their concentration in the sample solution (c_{DGT}) is calculated, in which the DGT device was immersed, according to the equation 1.1:

$$c_{DGT} = \frac{M \times \Delta g}{D \times A \times t} = \frac{c_e(V_{gel} + V_e)\Delta g}{D \times A \times t \times f_e} \tag{1.1}$$

Where: c_{DGT} represents the concentration of the mobile ions in the immersion solution; M_e is the mass of the analyte from the eluate (μg); Δg presents the thickness of the diffusion layer (cm); A represents the diffusion surface area; c_e represents the determined concentration in the eluate; V_{gel} represents the volume of the accumulation gel (mL); V_e represents the volume of the eluent solution (mL); f_e represents the elution factor; t represents the immersion time (s); D represents the diffusion coefficient ($cm^2\ s^{-1}$).

The elution factor can be experimentally determined by the ratio between the accumulated mass of metal and the mass extracted during elution, which is the difference between the metal quantity present in the solution before accumulation and after accumulation on the DGT gel, according to the equation 1.2:

$$f_e = \frac{M_e}{M_i - M_f} = \frac{c_e(V_{gel} + V_e)}{V(c_i - c_f)} \tag{1.2}$$

Where: M_e is the mass of the analyte from the eluate (μg); M_i is the initial mass of the analyte (mobile ion) in the immersion solution (μg); M_f is the final mass of the analyte (mobile ion) in the immersion solution (μg); c_i and c_f are the initial concentration of the analyte in the immersion solution and the concentration after the extraction of the DGT device ($\mu g\ cm^{-3}$); V is the volume of the solution in which the DGT device was immersed DGT (cm^3) all experimentally determined. The manufacturer of the DGT devices with Chelex-100 resin for the determination of divalent metals recommends an elution factor of 0.8 (DGT Research Ltd., Lancaster, UK, https://www.dgtresearch.com/).

Table 1.2. Working parameters for the passive sampling by DGT and the elution parameters of analytes from the used accumulation gel, for some applications.

Analyte[a]	Stage of accumulation from the immersion solution					Stage of elution of the analyte from gel		References
	Accumulation gel	Immersion solution pH	Temperature (°C)	Ionic strength $NaNO_3$ (mol L^{-1})	Diffusion coefficient x 10^{-6} (cm^2 s^{-1})	Solution for elution	Elution factor	
Cd Fe Mn Cu Co Ni Pb Zn	Silicagel modified with Chelex-100	5.0 – 8.5	25	0.01 – 1.0	-	HNO_3 1 - 2 mol L^{-1}	0.70 – 0.84	[49,76]
Total As As(III) As(V) DMA MMA Se(IV) Se(VI) V(V) Sb(V)	Silicagel modified with $Fe(OH)_3$; TiO_2; 3-MP; $ZnFe_2O_4$; ZrO_2	3.0 – 9.0;	25	0.01	3.34; 9.04; 7.32; 5.34; 4.23; 9.75; 6.11; 7.2; 4.2; 3.25; 5.95; 4.90; 6.83; 8.91; 5.25; 5.83; 6.26; 5.46;	HNO_3 15 mol L^{-1} NaOH 1 mol L^{-1} HNO_3 1 mol L^{-1} + KIO_3 0.01 mol L^{-1} H_3PO_4 0.8% la 90°C for 80 min; concentrated HCl;	0.63 – 0.97; 1.0;	[55,56,58,59,65,70]
Total As Cd Cu Pb Zn	Silicagel modified with a mixture of Chelex-100: $Fe(OH)_3$; 1:2	3.0 – 8.0	24	0.01	5.04	HNO_3 3 mol L^{-1}	0.68 – 0.79	[57]

[a] As(III) – arsenite; As(V) – arsenate; MMA – monomethylarsonic acid; DMA – dimethylarsenic acid; Se(IV) – selenite, Se(VI) – selenate; V(V) – vanadate; Sb(V) –antimonate

1.5. The Principles of Green and White Analytical Chemistry Applied to the Microplasma Technology

The concept of Green Analytical Chemistry (GAC) was proposed at the beginning of the 2000s, and it is based on 12 principles formulated for the first time by Gałuszka and coworkers in 2013.[77] These principles mainly focus on the preparation procedure of the sample and less on the method of analysis and pursue the decrease of the reagent consumption, the use of less toxic reagents in the analytical procedures, the decrease of the energy consumption, the decrease in the volum of waste resulted by the analytical procedures, the increased safety of the operator, the miniaturization and increase of the portability of the instrumentation.

There are several procedures for the evaluation of the green degree of an analytical method, however, the most objective and novel procedure is the AGREEprep proposed by Pena-Pereira an coworkers in 2020.[78] The AGREEprep evaluation algorithm of the green degree of an analytical method attributes to each GAC principle a score between 0 to 1 according to their influence, and the final result is obtained after the evaluation of each principle. Following the evaluation by the AGREEprep algorithm, a pictogram consisting of a circle results, in which the final score of green degree is displayed, and around, 10 circle segments with different colors from red to green, for the 12 GAC principles (Figure 1.5[79]). The criteria are presented in the reference, on the basis of which each GAC principle receives a score from 0 to 1.[80] A method is considered to have an excellent green degree if the general score is at least 0.75 (at least 75% on a scale of 0 to 100).

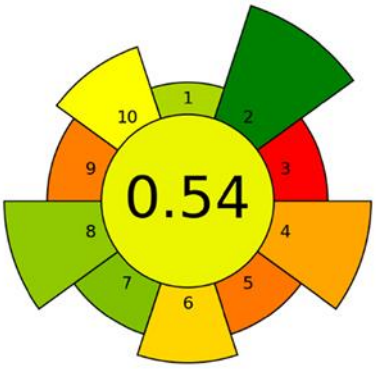


Figure 1.5. The representation of the green degree of an analytical method according to the AGREEprep algorithm[79]

The principle of the White Analytical Chemisrty (WAC) is an extension of the Green Analytical Chemistry (GAC). In the WAC principle, besides the green degree (G) of a method, the red degree (R) is also considered, which takes into account the analytical performance of the method, and the blue degree (B), which takes into account the applicability of the evaluated analytical method.[81,82] The 12 principles of WAC proposed by Nowak and coworkers[82] are based on the principles of GAC proposed by Gałuszka and coworkers[77] and to each degree (R, G and B) are assigned 4 principles from the 12 of the GAC concept, based on which the white degree shall be determined. The respective algorithm is called RGB-12 and it is synthesised in Figure 1.6.[81,82]

In the RGB-12 algorithm, a score (from 0 to 100) is assigned to each green, red and blue degree, and the white degree of the method comes from the average of the RGB scores.[81] An avegare of 100 of the RGB scores represents a maximum score of white (W), represented by the white color, similarly with the primary colors red, green and blue. The smaller the white degree, the color of the method will be more darker gray. The RGB-12 algorithm allows the simulateous evaluation of the white degrees of 10 methods by an Excel model.[82]

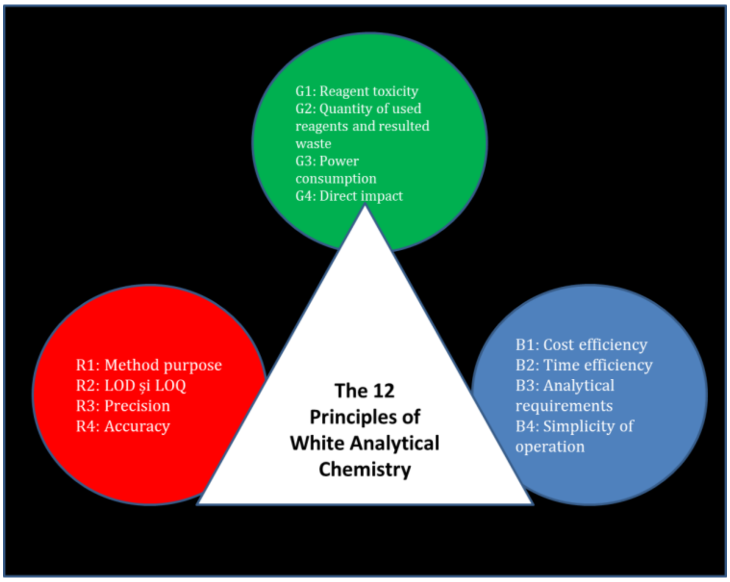


Figure 1.6. The schematic representation of the 12 principles of WAC[81,82]

Personal Contributions

Chapter 2. Determination and Speciation of Mercury from Aquatic Organisms and Sediments by Electrothermal Vaporization Capacitively Coupled Microplasma Optical Emission Spectrometry

2.1. Situation at International Level. Working Hypotheses and Objectives

The mercury present in the environment and foods represents a worldwide concern, and its presence generates an exposure risk with regards to the health of the population, as mercury exposure might have irreversible toxic effects. Besides, entire ecosystems can be affected by Hg pollution.[83] The negative impact of Hg was and currently still is recognized at international level, which lead to the addoption of the Minamata Convention in 2013.[84]

The prevailing methods in the literature for the determination and speciation of Hg are the spectrometric ones, coupled or not coupled with chromatographic or non-chromatographic techniques for the increase of sensitivity and the possibility of speciation. The literature annually presents a significant number of publications related to this topic[85,86]. Usually, the chromatographic methods are less accessible in the rutine laboratories because a complex, expensive and large instrumentation is necessary, respectively a complex preparation of the sample. An alternative to the chromatographic separation methods for the determination and speciation of Hg is represented by the spectrometric methods coupled with non-cromatographic techniques, especially based on cold vapor (CV) derivatization. In the case of the determination of methylmercury (CH_3Hg^+), the species with the highest toxicity, the separation by liquid-liquid extraction was applied, followed by derivatization and detection by electrothermal vaporization atomic absorption spectrometry (CV-ETAAS)[87], quartz tube atomic absorption spectrometry (CV-QTAAS)[88], inductively coupled plasma optical emission spectrometry (CV-ICP-OES)[89], inductively coupled plasma mass spectrometry (CV-ICP-MS)[90], or atomic fluorescence spectrometry (CV-AFS)[91].

2.2. SSETV-μCCP-OES Instrumentation

The analyses were performed by using a miniaturized optical emission spectrometer interfaced with a capacitively coupled microplasma and electrothermal vaporization (SSETV-μCCP-OES), presented in Figure 2.1, and is composed of a miniaturized device for the electrothermal vaporization of the microsample with a rhodium filament, a power source for the supply of the filament, a capacitively couple plasma microtorch of low power (10-30 W) and low Ar consumption (100-200 mL min^{-1}), a 13,56 MHz miniaturized RF generator and a

Maya 2000Pro microspectrometer (Ocean Optics, Dunedin, USA, 165-309 nm spectral domain).[47,92]

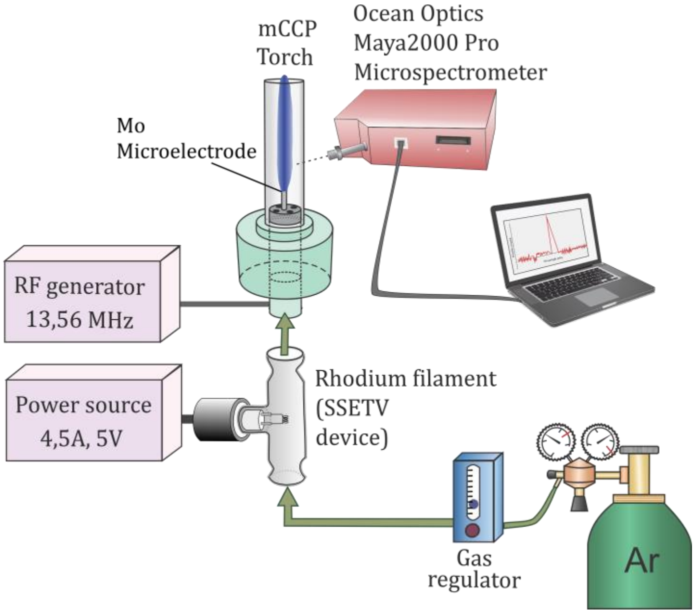


Figure 2.1. Scheme of the SSETV-µCCP-OES spectrometer[47]

2.3. Sampling and Sample Preparation

The preparation and work-up of the samples was carried out according to a standard procedure for the determination of CH_3Hg^+ in food from marine origin, recommended by the European Committee[93], but adapted by the decrease of the consumption of the HBr 47% solution from 10 mL to 5 mL, the toluene from 20 mL to 2 mL, and the L-cysteine 1% solution from 6 mL to 2 mL. The stages of the preparation procedure of food and environment samples for the determination of total Hg and CH_3Hg^+ by SSETV-µCCP-OES are presented in Figure 2.2.[94]

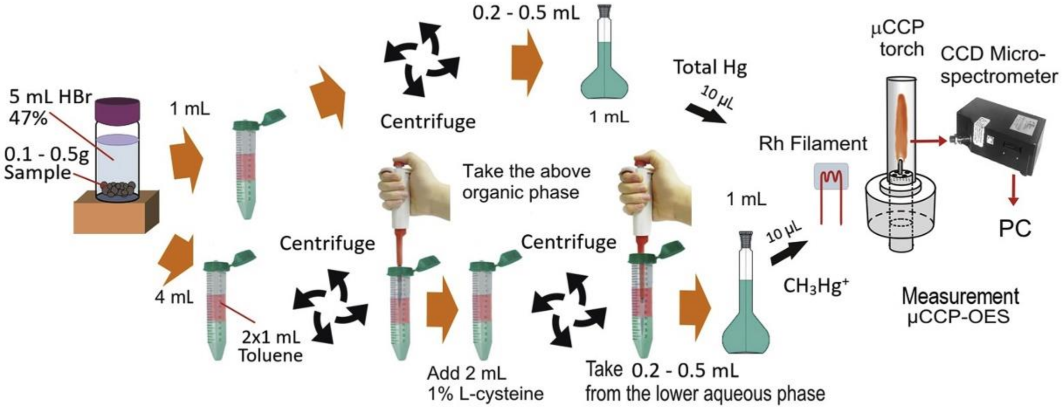


Figure 2.2. Scheme of the sample work-up for the determination of total Hg and CH_3Hg^+ by SSETV-µCCP-OES from food samples of fish muscle, mushroom and river sediment[94]

2.4. Optimization of the Working Parameters of the SSETV-μCCP-OES System

For the optimization of the working parameters of the miniaturized vaporizer the influence of the drying temperature in the 60-150 °C range, respectively the vaporization temperature in the 800-1400 °C range of a microsample with a volume of 10 μL from a Rh filament was evaluated, on the emission signal of the Hg deposited on the filament, for obtaining a larger emission signal. Therefore, the optimal drying, respectively vaporization temperature were: (i) drying of the sample at 70 °C for 180 s, (ii) vaporization of the sample at 1300 °C for 10 s, and (iii) cleaning of the filament by heating at 1400 °C for 10 s. The optimal operating parameters of the microplasma were 10 W power, Ar consumption of 150 min^{-1} and an observation height of 0.8 mm.

2.5. Validation of the SSETV-μCCP-OES Method for the Determination of total Hg and CH_3Hg^+

The SSETV-μCCP-OES method was characterized from the perspective of analytical performance, by limits of detection (LODs), limits of quantification (LOQs), linearity of the calibration curves, precision and accuracy. For the accuracy of the method, a series of certified reference materials (CRM) were subjected to analysis, for the determination of total Hg and CH_3Hg^+, using the external calibration and standar addition. The recovery degrees were calculated for 95% confidence level, and the statistical *t*-test was applied for the comparison of the results with the certified values.[95] The results of the analyses of the CRM samples obtained with the standard addition method and external calibration method are presented in Table 2.2.[94] Therefore, the recovery degree values were similar in case of external calibration and standard addition as well, being 101 ± 7% and 100 ± 7% for the external calibration, respectively 100 ± 7% and 100 ± 6% for the standard addition method. No significant differences were observed between the determined values and the certified ones, both for total Hg and CH_3Hg^+ ($t_{calc,95\%;\nu=4} = 0 - 2.085 < t_{tab} = 2.776$). Also, no significant differences were observed between the two calibration methods ($t_{calc,95\%;\nu=8} = 0 - 1.730 < t_{tab} = 2.306$). The similarity between the results obtained by the two calibration methods highlights the absence of the non-spectral interferences, and the SSETV-μCCP-OES method can be proposed to be considered as sustainable option for the determination of total Hg and CH_3Hg^+ from food samples and river sediment samples, using the external calibration with Hg^{2+} standards.

The limit of detection was calculated based on the $3s_{y/x}$ criterion, from the parameters of the calibration curve, using the equation 2.1[95], and the limit of quantification (LOQ) was calculated as being 3 times LOD.

$$LOD = \frac{3s_{y/x}}{m} \tag{2.1}$$

Where: $s_{y/x}$ is the standard deviation of the residual values of the signals (y), m is the slope of the calibration curve.

The parameters of the external calibration curve, LODs and LOQs for total Hg and CH_3Hg^+ are presented in Table 2.1.[94]

Table 2.1. Parameters of the external calibration curve, LOD and LOQ for total Hg and CH_3Hg^+ obtained by the SSETV-μCCP-OES method[94]

Calibration domain (ng ml^{-1} Hg)	Slope (a.u. mL ng^{-1})	Determination coefficient (R^2)	Standard deviation of residues $s_{y/x}$ (a.u.)	LOD Hg (ng mL^{-1})	LOQ Hg (ng mL^{-1})
0 – 50 (n = 7)	1233 ± 50	0.9994	285	0.70	2.10

An instrumental limit of detection of Hg total or CH_3Hg^+ of 0.70 ng mL^{-1} Hg can be observed in Table 2.1. In the solid samples were the LODs were 7.0 μg kg^{-1} for total and 3.5 μg kg^{-1} CH_3Hg^+, which allowed the determination of concentrations of over 21.0 μg kg^{-1} for total Hg and 10,5 μg kg^{-1} for CH_3Hg^+. These analytical performance meet the requirements of the European legislation with respect to the analytical methods used for food control, namely the LOD values should be 10 times smaller than the maximum permitted value for total Hg, namely 0.5 mg kg^{-1} in fish muscle[96-98] and 0.3 mg kg^{-1} in river sediments.[99] The results of the CRM samples are presented in Table 2.2.[94]

2.6. Real Sample Analysis of Fish Muscle, Mushrooms and River Sediments by SSETV-μCCP-OES

Table 2.3 presents the results for total Hg and CH_3Hg^+ in real samples of fish muscle, mushrooms and river sediments, analyzed by the SSETV-μCCP-OES method and the modifed extraction method.[94] The precision of the SSETV-μCCP-OES methos was in the 1.6 – 9.6% range, as regards to the determination of total Hg, respectively between 2.7 – 12.8% for CH_3Hg^+, and meets the guidelines of the European legislation with regards to the precision (<20%). Therefore, the preparation procedure of the samples in accordance with the recommendations of the European Committee but modified in the present PhD thesis by the decrease of reagent consumption, allowed the determination by the SSETV-μCCP-OES method in food and environmental samples with a precision and accuracy in accordance with the requirements imposed by the international legislation, even in samples in which one of the species present a higher proportion than 95%.

Table 2.2. Result of analyses of the CRM samples for the determination of total Hg and CH_3Hg^+ obtained by SSETV-μCCP-OES by external calibration and standard addition method (n = 5 parallel samples)[94]

CRM	Certified value ± U (mg kg^{-1})		External calibration				Standard addition			
			Determined value ± CI (mg kg^{-1})		Recovery ± CI (%)		Determined value ± CI (mg kg^{-1})		Recovery ± CI (%)	
	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+
BCR463	2.85 ± 0.16	3.04 ± 0.16[b]	2.85 ± 0.20	3.04 ± 0.21[b]	100 ± 7	100 ± 7	2.77 ± 0.27[b]	3.08 ± 0.09[b]	97 ± 10	101 ± 3
ERM-CE464	5.24 ± 0.10	5.50 ± 0.17[b]	5.18 ± 0.19	5.74 ± 0.32[b]	99 ± 4	104 ± 6	5.45 ± 0.39[b]	5.60 ± 0.40[b]	104 ± 7	102 ± 7
TORT-2	0.27 ± 0.02	0.152 ± 0.013	0.27 ± 0.02	0.156 ± 0.010	100 ± 7	103 ± 6	0.27 ± 0.01	0.151 ± 0.008	100 ± 4	99 ± 5
NIST SRM2976	0.0610 ± 0.0036	0.02809 ± 0.00031	0.0620 ± 0.0050	0.02770 ± 0.00191	102 ± 8	99 ± 7	0.0580 ± 0.0050	0.02736 ± 0.00206	95 ± 9	97 ± 8
CS-M-3	2.849 ± 0.104	-	2.911 ± 0.091	0.569 ± 0.170	102 ± 3	-	2.867 ± 0.067	-	101 ± 2	-
ERM-CC580	132 ± 3	0.075 ± 0.004	134 ± 13	0.072 ± 0.006	102 ± 10	96 ± 8	133 ± 6	0.077 ± 0.006	101 ± 5	103 ± 8
General recovery ± CI [a]					101 ± 7	100 ± 7			100 ± 7	100 ± 6

[a] CI confidence degree for n=5 parallel measurements and 95% conficence level

[b] Hg concentration expressed as CH_3Hg^+

Table 2.3. Results of the determination of total Hg and CH_3Hg^+ in fish, mushroom and river sediment samples obtained by SSETV-μCCP-OES with the external calibration method[94]

Sample	Average ± CI[a] (mg kg^{-1})		RSD (%)	
	Total Hg	CH_3Hg^+	Total Hg	CH_3Hg^+
Fish				
Trout	0.086 ± 0.009	0.020 ± 0.002	8.4	8.1
Hake	0.144 ± 0.010	0.118 ± 0.004	5.6	2.7
Tuna	0.210 ± 0.006	0.169 ± 0.015	2.3	7.1
Cod	0.360 ± 0.010	0.129 ± 0.015	2.2	9.4
Salmon	0.169 ± 0.011	0.126 ± 0.020	5.2	12.8
Tilapia	0.157 ± 0.014	0.148 ± 0.014	7.2	7.6
Hering	0.118 ± 0.014	0.086 ± 0.008	9.6	7.5
Nile perch	0.352 ± 0.033	0.240 ± 0.055	7.6	11.7
Mushroom				
Sample 1	0.156 ± 0.017	0.084 ± 0.004	8.8	3.8
Sample 2	0.508 ± 0.025	0.176 ± 0.011	4.0	5.0
River sediment				
Sample 1	4.614 ± 0.092	0.089 ± 0.010	1.6	9.1
Sample 2	1.000 ± 0.086	0.209 ± 0.025	6.9	9.6
Sample 3	4.525 ± 0.224	0.091 ± 0.012	4.0	10.6
Sample 4	1.157 ± 0.030	0.074 ± 0.010	2.1	10.9
Sample 5	1.809 ± 0.109	0.045 ± 0.006	4.9	10.7
Sample 6	2.107 ± 0.178	0.070 ± 0.011	6.8	12.7

[a] CI represents the confidence interval for n = 5 parallel measurements and 95% confidence level [b] Hg concentration expressed as CH_3Hg^+

2.7. Conclusions

According to the results obtained and presented in this chapter, the following conclusions can be listed:

1. A sustainable method was developed and characterized for the determination of total Hg and CH_3Hg^+, based on the the liquid-liquid successive extraction in HBr – toluene – L-cysteine from food samples from marine origin, mushrooms and river sediments, as alternative to the clasical TDAAS method and recommended by the European Committee, only for the determination of CH_3Hg^+.
2. In comparison with the original procedure recommended by the European Committee for the extraction of CH_3Hg^+ from food samples from marine origin, the toluene consumption was reduced 10 times, the cysteine 3 times, between others, being possible the determination of both total Hg and CH_3Hg^+, using the same SSETV-μCCP-OES instrumentation.

3. The complete mineralization of the sample for the determination of total Hg has been avoided, using only a simple extraction in HBr, which was used also for the pre-extraction of CH_3Hg^+.
4. The experimental determinations were performed using only external calibration, avoiding the non-spectral interferences of the multimineral matrix from the analyzed samples.
5. The control of the vaporization temperature at 1300 °C was proved to be essential because it allowed not only an efficient ans selective vaporization of Hg from the matrix but also contributing to the improvement of the sensitivity of the SSETV-μCCP-OES method at the same time, by ensuring a high Hg flow toward the plasma source in the absence of concomitants.
6. The increase of the sensitivity of the SSETV-μCCP-OES method was ensured also by the use of a microspectrometer with the purge of Ar 5.0, instead of a spectrometer with CCD detector cooled with a Peltier element (the improvement of LODs was over 20 times).
7. The validation of the SSETV-μCCP-OES method by the analysis of some certified reference materials of fish muscle, mushrooms and river sediment revealed that this satisfies the requirements concerning accuracy and precision, in accordance with the recommendations from the European legislation.
8. The applicability of the SSETV-μCCP-OES method was demonstrated on samples in which the major species was CH_3Hg^+ (over 95%) in the case of foods of marine origin, but also in the case of the samples in which the inorganic species Hg^{2+} was in majority (over 95%).
9. The advantages of the novel method for the determination of total Hg and CH_3Hg^+ were highlighted, by the efficiency of the extraction procedure by the decrease of solvent consumption, mainly organic solvents, but also by the efficiency of the costs of the miniaturized instrumentation, by the reduced energy and Ar consumption for the generation of the microplasma.

Chapter 3. Simultaneous Determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn by Electrothermal Vaporization Capacitively Coupled Microplasma Optical Emission Spectrometry and the Use of Direct Microsampling of the Liquid Sample without Chemical Vapor Derivatization

3.1. Situation at International Level. Working Hypotheses and Objectives

The interest for the determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn is related to their use in the field of emerging technologies for material synthesis but also in the medical field.[100-103] Furthermore, some of these elements might have significant roles in some enzymatic reactions.[104] On the other hand, Pb, Hg, As and Sn possess high toxicity towards living organisms, Pb and Hg being included in the category of the primary dangerous elements.[105-108] Therefore, the determination of these elements from food, biological, environmental and material samples by spectrometric methods represents a challenge due to the spectral lines, low sensitivity and spectral and non-spectral interferences, even for the high performance techniques for analysis, such as graphite furnace atomic absorption spectrometry (GFAAS), quartz tube atomic absorbtion spectrometry (QTAAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS).

As previously related in Chapter 2, by the controlled heating of the Rh filament at a lower temperature, namely at 1300 °C, the selective vaporization of Hg and the overcome of the non-spectral effects was possible, which allowed the development of a simple and sensitive SSETV-μCCP-OES method for the determination of total Hg and the speciation of CH_3Hg^+ in food samples from marine origin, mushrooms and environmental samples (river sediments), by the external calibration method, solely with Hg^{2+} solutions. Under these circumstances, it can be considered that the analytical performance of the miniaturized SSETV-μCCP-OES analytical system was not exploited sufficiently for the development of simultaneous and sensitive analytical methods for the determination of chemical vapor-generating elements, in the absence of derivatization, without non-spectral interferences, which could make possible the quantification by external calibration, more simpler and faster, compared to the standard addition method.

3.2. SSETV-µCCP-OES Instrumentation Interfaced with Two Microspectrometers

In this study, the analytical instrumentation used for the determination As, Bi, Sb, Se, Te, Hg, Pb, and Sn is similar with the one used in Chapter 2 (Figure 2.1), with the difference that the SSETV-µCCP-OES analytical system uses two low resolution microspectrometers simultaneously, namely QE65 Pro and Maya2000 Pro (Dunedin, FL, SUA), for the recording of the optical emission signals. The difference between the two spectrometers is that QE65 Pro uses a Peltier element for the cooling of the CCD detector at – 20 °C for the decrease of the background noise, while Maya2000 uses a chamber purged with Ar 5.0, which can also allow the investigation of the emission of the elements in the vacuum-UV range under 190 nm, where some of the elements, for which the development and validation of the method is desired, present spectral lines, being known that this spectral domain has a low background which could allow the improvement of the sensitivity of the SSETV-µCCP-OES instrumentation.[109]

3.3. Preparation of Sediment Samples

Quantities between 0.5 and 1 g of CRM sample or test sample of sediment were mineralized in 12 mL of *aqua regia* in a Berghof MW3 S+ microwave digestor (Berghof, Germania), according to the protocol previously used by Frențiu and coworkers.[16,46-48,94] After mineralization, ultrapure water was added until a final volume of solution of 25 mL was obtained and filtered then on a filter paper with 0.45 µm porosity. The filtered solution was therefore subjected to analysis by SSETV-µCCP-OES, using the external calibration and calibration by standard addition method.[109]

3.4. Optimization of the Working Parameters of SSETV-µCCP-OES

The optimal working parameters for the simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn are the following: drying temperature of 80 °C for 180 s; vaporization temperature of 1300 °C for 10 s; Ar flow of 150 mL min^{-1}; 15 W power; observation height of 0.8 mm over the peak of the Mo electrode.

3.5. Validation of the SSETV-µCCP-OES Method, LOD, Accuracy and Precision

The SSETV-µCCP-OES method was validated by the LODs, accuracy and pecision of the determinations. The parameters of the calibration curve and the LOD values were evaluated based of the area and height of the transient emission signals of the elements (Tables 3.1 and 3.2). The instrumental limits of detection were calculated based on the $3s_{y/x}$ criterion from the parameters of the calibration curve, according to the equation 2.1 (Section 2.5.).

According to Tables 3.1 and 3.2, it can be observed that the Maya2000 Pro microspectrometer provides better sensitivity and LODs for the SSETV-µCCP-OES method, compared with the QE 65Pro microspectrometer, regardless of the calculation procedure of the signals for calibration. The sensitivity and LODs are better in the integration mode of the signal area, compared to the ones obtained from maximum of the transient signal. Improvement of the LODs can also be observed with the Maya2000 microspectrometer, with at most 14 times, compared with the QE65 Pro microspectrometer with CCD detector. The curves have a good linearity, the determination coefficients of the curves were higher than 0.9991, for the calibration interval at most 5 µg mL^{-1} for each element.

The accuracy of the SSETV-µCCP-OES method was evaluated by the recovery degree by the alanysis of some soil and sediment CRM samples, the results being presented in Tables 3.3 and 3.4.[109] Also, from the analysis of the CRM samples, the presence of potential non-spectral interferences was evaluated, caused by the concomitant elements in the matrix of the sample, by comparing the recovery degrees of the analysis of the CRM samples by external calibration with the ones obtained with the standard addition method. In the case of missing certified values for some elements, the accuracy of the the method was evaluated by fortification of the sample with a well-know concentration of the element and calculation of the recovery degree of the fortification value.

Table 3.1. The parameters of the calibration curves and LODs obtained from the area of the transient signal of the elements by the SSETV-μCCP-OES method, using the Maya2000 Pro and QE65 microspectrometers.[109]

Element	λ (nm)	Maya2000 Pro					QE65 Pro				
		Slope (mL μg^{-1})	R^2	$s_{y/x}$ (a.u)	Instrum. LOD[1] (ng mL^{-1})	Method LOD[2] (mg kg^{-1})	Slope (mL μg^{-1})	R^2	$s_{y/x}$ (a.u)	Instrum. LOD[1] (ng mL^{-1})	Method LOD[2] (mg kg^{-1})
As	189.042	21398	0.9999	100	14	0.35	-	-	-	-	-
Bi	223.060	60608	0.9998	300	15	0.37	1560	0.9987	50	100	2.50
Sb	252.852	145462	0.9994	385	8	0.20	4623	0.9993	40	30	0.75
Se	196.026	22186	0.9999	100	13	0.33	1024	0.9991	52	152	3.75
Te	238.579	79690	0.9998	833	30	0.75	1660	0.9970	277	501	12.52
Hg	253.652	981663	0.9999	230	0.7	0.02	34235	0.9996	171	15[3]	0.37[3]
Pb	261.417	170830	0.9998	289	5	0.13	4506	0.9994	45	30	0.75
Sn	207.307	8116	0.9992	8	3	0.08	300	0.9990	1	10	0.25

[1] Instrumental limit of detection obtained on the basis of the residual standard deviation ($3s_{y/x}$) of the calibration curve

[2] Limit of detection of the method calculated in solid for 1.0000 g sample dissolved at a final volume of 25 mL

[3] Limit of detection available in ref.[47]

Table 3.2. The parameters of the calibration curves and LODs obtained from the height of the transient signal of the elements by the SSETV-μCCP-OES method, using the Maya2000 Pro and QE65 microspectrometers[109]

Element	λ (nm)	Maya2000 Pro					QE65 Pro				
		Slope (mL μg^{-1})	R^2	$s_{y/x}$ (a.u)	Instrum. LOD[1] (ng mL^{-1})	Method LOD[2] (mg kg^{-1})	Slope (mL μg^{-1})	R^2	$s_{y/x}$ (a.u)	Instrum. LOD[1] (ng mL^{-1})	Method LOD[2] (mg kg^{-1})
As	189.042	682	0.9993	16	70	1.75	-	-	-	-	-
Bi	223.060	7032	0.9994	70	30	0.75	153	0.9989	6	124	3.10
Sb	252.852	4322	0.9984	25	17	0.43	149	0.9954	10	200	5.00
Se	196.026	1970	0.9991	46	70	1.75	-	-	-	-	-
Te	238.579	3689	0.9972	69	56	1.40	100	0.9888	15	450	11.25
Hg	253.652	58955	0.9987	197	10	0.25	2165	0.9905	50	70	1.80
Pb	261.417	21508	0.9957	143	20	0.50	502	0.9913	14	80	2.00
Sn	207.307	450	0.9985	6	40	1.00	12	0.9932	1	250	6.25

[1] Instrumental limit of detection obtained on the basis of the residual standard deviation ($3s_{y/x}$) of the calibration curve

[2] Limit of detection of the method calculated in solid for 1.0000 s sample dissolved at a final volume of 25 mL

According to the results presented in Tables 3.3 and 3.4, it can be observed that the SSETV-μCCP-OES method presents the recovery degrees of the studied elements in the 92–108% interval, obtained by calibration with standard addition, respectively in the 86–116% interval in case of external calibration. Both calibration methods ensure general recovery degrees, whose confidence intervals contain 100% for each element. Therefore, the differences between the two approaches can be considered random. In the case of uncertified elements in the CRM sample, such as Te or Bi, their recovery degrees and the accuracy were evaluated by the fortification of the solution of the samples with the addition of at most 2 μg mL^{-1} Te or Bi. The recovery degrees obtained by external calibration for these two elements were found between 92% and 110%. The precision of the SSETV-μCCP-OES method was evaluated by the analysis of some real samples of cave and river sediments, the results being presented in Table 3.5.[109] The results of the analysis of the real samples indicate the fact that the repeatability of the method was found between 1.2% and 9.9% for n = 5 parallel measurements.

Table 3.3. Result of the analysis of soil and sediment CRM samples by the SSETV-μCCP-OES method with the Maya2000 Pro microspectrometer, using external calibration and the standard addition method for As, Hg, Pb, and Sb.[109]

CRM	Certified value ± U (mg kg^{-1}) [1]				Determined value ± CI (mg kg^{-1}) [2] Standard addition				Determined value ± CI (mg kg^{-1}) [2] External calibration			
	As	Hg	Pb	Sb	As	Hg	Pb	Sb	As	Hg	Pb	Sb
ERM–CC141	7.5 ± 1.4	0.08 ± 0.008	-	-	8.0 ± 1.5	0.08 ± 0.009	-	-	7.9 ± 1.5	0.07 ± 0.005	-	-
CRM04850G	123 ± 3.4	28 ± 1.13	86.9 ± 2.42	139 ± 13.9	122 ± 4.3	28 ± 1.60	86.9 ± 2.45	142 ± 14.1	124 ± 4.3	29 ± 1.62	84.9 ± 2.5	138.5 ± 14.0
LGC6141	13.2 ± 3.5	-	75.8 ± 16	-	12.1 ± 1.1		73.9 ± 2.30	-	13.8 ± 0.8	-	75.8 ± 3.65	-
Metranal–32	26.1 ± 1.1	0.120	35.5 ± 0.9	-	25.6 ± 1.1	0.13 ± 0.005	36.2 ± 2.5	-	25.7 ± 1.4	0.12 ± 0.01	33.4 ± 2.4	-
Metranal–34	42.4 ± 2.2	0.21	83.1 ± 2.3	-	43.0 ± 2.4	0.20 ± 0.012	82.6 ± 3.5	-	44.5 ± 2.3	0.22 ± 0.02	82.8 ± 2.7	-
BCR–142R	-	-	25.7 ± 1.6	-	-	-	25.8 ± 2.16	-	-	-	25.8 ± 1.7	-
BCR–287A	-	-	-	0.04 ± 0.015	-	-	-	<0.20	-	-	-	<0.20
NCSDC 78301	56 ± 10	0.22 ± 0.04	79 ± 12	-	55 ± 10	0.21 ± 0.05	79 ± 13	-	55 ± 12	0.19 ± 0.04	82 ± 13	-
ERM–CC580	-	132 ± 3	-	-	-	128 ± 8	-	-	-	125 ± 4	-	-
CRM025	339 ± 20	99.8 ± 18	1447 ± 88	<3.2	341 ± 20	104 ± 19	1366 ± 93	3.1 ± 0.2	357 ± 21	94.9 ± 18	1370 ± 92	3.5 ± 0.4
BCR–280R	33.4 ± 2.9	1.46 ± 0.2	-	-	35.0 ± 3.7	1.45 ± 0.3	-	-	29.6 ± 3.1	1.26 ± 0.2	-	-
RTCSQC001	43.1 ± 0.7	2.86 ± 0.1	144 ± 2	42.0 ± 4.1	45.2 ± 2.0	3.02 ± 0.2	134 ± 5	43.1 ± 4.7	44.2 ± 0.9	2.99 ± 0.1	139 ± 3	48.9 ± 4.2
Recovery degree interval (%)					92–107	95–108	93–102	97–103	89–105	86–105	94–104	100–116
General recovery degree (%)					101 ± 10	101 ± 13	98 ± 8	101 ± 9	101 ± 10	96 ± 12	98 ± 8	108 ± 10

[1] U represents the extended uncertainty for 95% confidence level (k = 2)

[2] CI represents the confidence interval for 95% confidence level for n = 5 parallel measurements

Table 3.4. Result of the analysis of soil and sediment CRM samples by the SSETV-μCCP-OES method with the Maya2000 Pro microspectrometer, using the external calibration and the standard addition method for Bi, Se, Te, and Sn[109]

CRM	Certified value ± U (mg kg^{-1}) [1]				Determined value ± CI (mg kg^{-1}) [2] Standard addition				Determined value ± CI (mg kg^{-1}) [2] External calibration			
	Bi	Se	Te	Sn	Bi	Se	Te	Sn	Bi	Se	Te	Sn
CRM048	-	178 ± 5.68	-	93.5 ± 3.26	-	176 ± 5.91	-	96.6 ± 3.26	-	176 ± 6.10	-	95.4 ± 6.07
BCR 142R	-	-	-	-	-	-	-	-	-		-	-
BCR 287	67.3 ± 1.1	-	-	-	67.3 ± 4.6	-	-	-	68.0 ± 5.3		-	-
NCSDC 78301	-	0.39 ± 0.1	-	-	-	0.35 ± 0.1	-	-	-	0.40 ± 0.1	-	-
CRM025	-	518 ± 31	-	-	-	536 ± 38	-	-	-	550 ± 32	-	-
SQC001	-	154 ± 3	-	215 ± 8	-	156 ± 7	-	212 ± 12	-	148 ± 7	-	203 ± 9
Recovery degree interval (%)					94–107	90–103	-	99–103	98–104	96–106	-	94–102
General recovery degree (%)					100 ± 7	98 ± 15	-	101 ± 5	101 ± 3	101 ± 13	-	98 ± 3

[1] U represents the extended uncertainty for 95% confidence level (k = 2)

[2] CI represents the confidence interval for 95% confidence level for n = 5 parallel measurements

Table 3.5. Results of the elemental analysis of real samples of river and cave sediments determined by the SSETV-µCCP-OES method with the Maya2000 Pro microspectrometer, using external calibration[109]

Origin of sample	Sample	Concentration ± CI (mg kg^{-1}) [1]							
		As	Bi	Sb	Se	Te	Hg	Pb	Sn
Leșu cave	1	<0.35	20.86 ± 0.53	7.02 ± 0.28	<0.33	8.38 ± 0.18	0.23 ± 0.01	588.19 ± 21.52	6.77 ± 0.33
	2	1.52 ± 0.05	36.90 ± 2.30	1.20 ± 0.06	<0.33	5.37 ± 0.13	0.27 ± 0.01	10.29 ± 0.44	9.63 ± 0.19
Movile cave	3	<0.35	107.34 ± 2.65	6.53 ± 0.26	6.07 ± 0.27	8.49 ± 0.15	0.17 ± 0.01	16.13 ± 0.41	5.94 ± 0.30
Muierii cave	4	1.72 ± 0.04	57.79 ± 1.91	2.61 ± 0.04	3.39 ± 0.13	5.93 ± 0.30	1.49 ± 0.04	83.25 ± 4.26	4.83 ± 0.17
	5	2.20 ± 0.06	130.87 ± 6.85	37.19 ± 3.06	11.91 ± 0.74	11.54 ± 0.59	0.11 ± 0.01	76.87 ± 3.60	3.97 ± 0.09
	6	1.88 ± 0.15	54.78 ± 3.97	16.95 ± 0.19	1.11 ± 0.05	36.29 ± 2.90	0.22 ± 0.01	1.74 ± 0.06	4.67 ± 1.34
	7	6.10 ± 0.17	63.13 ± 2.55	22.40 ± 1.38	25.76 ± 0.91	9.68 ± 0.37	0.21 ± 0.02	67.83 ± 2.68	4.52 ± 0.15
	8	1.08 ± 0.03	7.66 ± 0.21	<0.20	<0.33	4.42 ± 0.16	0.28 ± 0.01	7.70 ± 0.45	3.51 ± 0.14
	9	1.54 ± 0.03	12.97 ± 0.26	3.34 ± 0.10	3.43 ± 0.14	4.94 ± 0.23	0.26 ± 0.02	10.43 ± 0.32	2.58 ± 0.12
Topolnița cave	10	1.23 ± 0.02	45.20 ± 0.72	19.44 ± 0.75	5.24 ± 0.24	6.18 ± 0.25	0.26 ± 0.01	12.46 ± 0.59	4.62 ± 0.18
	11	0.81 ± 0.02	9.79 ± 0.26	<0.20	<0.33	2.75 ± 0.17	0.14 ± 0.01	<0.13	2.10 ± 0.11
Arieș river	12	5.39 ± 0.13	129.51 ± 7.55	<0.20	0.87 ± 0.03	65.92 ± 3.07	30.70 ± 0.79	<0.13	1.64 ± 0.12
	13	5.02 ± 0.22	135.98 ± 8.72	2.38 ± 0.06	2.09 ± 0.07	<0.75	30.33 ± 1.55	<0.13	2.00 ± 0.12
	14	1.74 ± 0.08	94.19 ± 4.36	<0.20	<0.33	<0.75	15.45 ± 0.82	<0.13	0.97 ± 0.03
	RSD (%) [2]	1.2–9.3	1.6–8.3	1.3–9.4	1.3–7.1	2.0–9.1	2.8–9.9	2.6–6.4	1.3–8.1

[1] CI represents the confidence interval for 95% confidence level (n = 5)

[2] RSD represents the relative standard deviation for n = 5 parallel measurements

3.6. Conclusions

Following the research carried out in this chapter, the following conclusions resulted:

1. The analytical applicability of a completely miniaturized intrumentation was demonstrated, in which the central element was a capacitively coupled plasma microtorch operated at 15 W and 150 mL min^{-1} Ar, interfaced with electrothermal vaporizer with Rh microfilament and two low resolution microspectrometers (Maya 2000Pro and QE 65Pro), for the simultaneous determination of some chemical vapor generating elements (As, Bi, Sb, Se, Te, Hg, Pb and Sn) without derivatization;
2. The capacitively couple plasma microtorch was interfaced with two low resolution microspectrometers. If was found that the Maya 200Pro microspectrometer, with the chamber purged with Ar, ensured better improvement of the sensitivity and limits of detection, in comparison with the QE65 Pro microspectrometer with CCD detector cooled at -20 °C with a Peltier element;
3. The Maya 2000Pro microspectrometer allowed also the investigation in the vacuum-UV spectral range (180-210 nm interval), where resonance and non-resonance lines sensitive for As, Bi, Sb, Se and Te were identified;
4. The study highlighted the selective vaporization of the volatile analyte elements from the sample matrix at a controlled temperature of 1300 °C, which allowed the overcome of the non-spectral effects and the use of external calibration instead of standard addition;
5. The analysis of some certified reference materials and real samples of river sediments and cave sediments revealed the possibility of the determination of chemical vapor generating elements by vaporization of a microsample with a volume of 10 μL from a Rh filament, without the necessity of derivatization or preconcentration, currently applied at the determination of As, Sb, Bi, Se, Te, Hg, Pb and Sn by other methods;
6. The analytical performance obtained demonstrated that the novel SSETV-μCCP-OES method, based on the selective vaporization of the microsamples, without derivatization, represents a sustainable alternative to the clasical analytical laboratory systems based on GFAAS, which are sequential and slow by excellence, whereas the determinations in the microplasma were simultaneous and have speeded up the time of the analysis;
7. The research in the field of sensitivity improvement and the overcominh of non-spectral effects from the side of matrix with miniaturized instrumentation with microplasma source, can be continued by the application of preconcentration/accumulation methods by active and passive sampling and separation from the matrix, which offers new opportunities of relevant research at internation level, for the determination of elements of interest with toxic effects on the environment and health, which will be presented in the next chapters.

Chapter 4. Determination of the Mobile Fraction of Cd, Pb, Cu, Zn and Hg in Surface Waters Using Electrothermal Vaporization Capacitively Coupled Microplasma Optical Emission Spectrometry Coupled with *In-situ* Diffusive Gradients in Thin-Film Passive Sampling with Green and White Degree

4.1. Situation at International Level. Working Hypothesis and Objectives

The determination of primary dangerous elements, Cd, Pb and Hg, alongside other potentially toxic elements, for example Cu and Zn, in surface waters by spectral methods continues to be difficult due to the fact that the direct determination is not always possible, even by highly sensitive methods such as GFAAS and ICP-MS. In case of the *off-line* analysis methods various laboratory operations are carried out after the collection of the samples, namely the filtration and conservation of the water sample, transportation to the laboratory.[110] Although the sample collection in vessels can be easily achieved, the analysis cannot provide representative information, especially if these are part of a dynamic system, such as surface running waters, coastal and underground waters. The results of such analyses can highlight only the concentration of the elements in the moment of the sampling, and these can be often underestimated or overestimated, in comparison with the concentrations obtained in real timel.[111-115] To compensate the disadvantages of the *off-line* methods coupled with active preconcentration procedures, both *ex-situ* and *in-situ* passive sampling methods were proposed. Among these, the most innovative method was and currently is the one proposed by Davison and Zhang in 1994, namely the diffusive gradients in thin-film (DGT) technique.[49,116]

Based on the current state of the art at international level, as from analytical point of view, the coupling of DGT passive sampling with a microplasma source for simultaneous multielemental applications was not explored, the aim of this study was the exploration of the *in-situ* DGT coupling with the capacitively coupled plasma microtorch optical emission spectrometry with electrothermal vaporization from a Rh filament as *ex-situ* method (DGT-SSETV-µCCP-OES), in order to develop, worldwide for the first time, some methods with high green and white degree, for the monitoring of surface water quality. Therefore, the implementation of the novel DGT-SSETV-µCCP-OES method was envisaged, for the determination an monitoring of Cd, Cu, Pb, Zn and Hg with highest toxicity, in the mobile fraction in river waters.

4.2. DGT-SSETV-μCCP-OES Instrumentation

The DGT passive sampling was conducted *in-situ* by the immersion of the DGT devices in the river water, and the work-up of the samples and the analyses were done *ex-situ* using the SSETV-μCCP-OES equipment.[117] The used instrumentation is similar with the one described in Chapter 2, section 2.2. The DGT devices with Chelex-100 resin with polyacrylamide diffusion gel for the accumulation of Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+} and Hg^{2+} were purchased from DGT Research Ltd., Lancaster, UK (https://www.dgtresearch.com/). The characteristics of the DGT devices were the effective thickness of the diffusion layer ($\Delta g = 0.094$) and the exposure area ($A = 3.14$ cm^2). The gels were asambled in the laboratory in the teflon support, according to the manufacturer's recommendations, and transported separately in plastic bags at the place of the immersion for sampling.

4.3. Sampling and Work-up of Samples for the Mobile Fraction Determination of Cd, Cu, Pb, Zn and Hg in Water

The *in-situ* passive sampling of the metals was performed by the immersion of some DGT devices in several sampling points along the Arieș river. The DGT devices (3 for each sampling point), were installed in polycarbonate supports and protected with a nylon net (1 cm mesh size),[112] and were immersed at a depth of 30 cm in 10 sampling points along Arieș river. These remained immersed for 7 days (168 hours). At the end of the passive sampling period, the devices were extracted, washed with ultrapure water, deposited at 4 °C and transported to the laboratory for analysis. The devices were disassembled, and the accumulation resin was immersed in 1 mL HNO_3 1 mol L^{-1} solution for the elution of the analytes by SSETV-μCCP-OES for the determination of Cd, Cu, Pb, Zn and Hg, by GFAAS using a PerkinElmer PinAAcle 900T spectrometer (Norwalk, SUA)[118] for the determination of Cd, Cu, Pb and Zn, and by TDAAS (Direct Hydra-C, Teledyne Instruments, Leeman Labs, Hudson, New Hampshire, SUA) for the determination of.[119] The results obtained by GFAAS and by TDAAS were used for the validation of the DGT-SSETV-μCCP-OES method. The average concentration in time of the mobile fraction (c_{DGT}), was calculated according to the equation 1.1 (Chapter 1). The water samples collected at each samplig point were also analyzed after filtration (0,45 μm) and acidification with 2% HNO_3 (v/v) by SSETV-μCCP-OES, GFAAS and TDAAS, for the determination of the total dissolved content of Cd, Cu, Pb, Zn and Hg.

4.4. Validation of the DGT-SSETV-µCCP-OES Method

The DGT-SSETV-µCCP-OES method was validated by the evaluation of LOD, LOQ, precision and accuracy, and the results obtained at the analysis of the water CRM sample and the river water samples were compared with the ones obtained at the analysis by DGT-GFAAS and DGT-TDAAS, by the application of the Tukey test and Dunnett test ($p < 0.05$).[120,121]

The instrumental limits of detection were evaluated on the basis of the slope (m), calibration curves and standard deviation of the background signals (s_b) (LOD = 3 x s_b/m). The accuracy of the DGT-SSETV-µCCP-OES, DGT-GFAAS and DGT-TDAAS methods was verified by the analysis of water CRM sample, for the determination of Cd, Cu, Pb, Zn and Hg. The existence of significant differences ($p < 0.05$), betweem the results obtained by the DGT-SSETV-µCCP-OES and DGT-GFAAS (for Cd, Cu, Pb and Zn) or DGT-TDAAS (for Hg) methods, was verified by the application of the statistical Tukey test. The Dunnett test was applied for the statistical comparison of the concentration values determined in the CRM sample with the certified values of the elements of interest within this study. The recovery degrees were also calculated, with their confidence interval, for 95% confidence level (R ± U_{lab}%, k = 2). Besided, the results obtained by DGT-SSETV-µCCP-OES were compared with the ones obtained by DGT-GFAAS as regards to the average concentrations and U_{lab} (k = 2). The precision of the DGT-SSETV-µCCP-OES method was evaluated by the analysis of real samples of river water and the calculation of the relative standard deviation (RSD%) based on the u_c value for the results obtained after DGT passive sampling in triplicate for each sampling place. The precision of the method was considered acceptable, if the RSD value was <30%.[112,122,123]

4.5. Study of the Elimination of the Spectral Interference of Cd – As in SSETV-µCCP-OES by the DGT Selective Accumulation of Cd^{2+} on Chelex-100 Resin

The disadvantage of the use of the low resolution Maya2000 Pro microspectrometer is the appearance of the spectral interference by the overlap of the emission line of Cd at 228.802 nm (5.42 eV) with the one of As at 228.812 nm. This interference can cause systematical errors and the most intense line of Cd 228.802 nm cannot be used. Therefore, the possibility of the elimination of the spectral interference was studied, by separating Cd from As. This study was performed by using the DGT passive sampling technique with Chelex-100 accumulation resin, and applying the DGT-SSETV-µCCP-OES method for the analysis of some synthetic solutions, monoelement and mixture of Cd and As. At the analysis of the obtained eluate after the immersion of the DGT device in the 100 µg L^{-1} As solution, the determined concentration of Cd was below LOD (0.12 µg L^{-1}). Under these circumstances, În aceste condiții, consistent with the separation of the Cd^{2+} ions from the arsenate, ensured by the Chelex-100 resin, results the possibility of Cd determination by SSETV-µCCP-OES at the most sensitive emission line from 228.802 nm.

4.6. Accumulation Kinetics of Cd, Cu, Pb, Zn and Hg in the Chelex-100 Gel, Determination of the Elution Factors and Diffusion Coefficients and the Influence of pH on the Accumulation

The experimentally obtained values of the diffusion coefficients (D_e) and elution factors (f_e) are presented in Table 4.1.[117] The influence of the pH on the accumulation of Cd, Cu, Pb, Zn and Hg in the Chelex-100 gel was studied by the analysis of some immersion solutions with the pH in the pH = 4 – 7 range. The influence of the pH was expressed as the ratio between the concentration determined by DGT-SSETV-µCCP-OES and the concentration determined directly in the immersion solution (c_{DGT}/c_{sol}). Therefore, the values of the c_{DGT}/c_{sol} ratio were between 0.83 and 1.13 and falls within the limit of ± 20% for accuracy[117], and it was observed that the DGT technique can be applied in the case of water samples with a pH in the 4 – 7 interval.

Table 4.1. Elution factor values for Cd, Cu, Pb, Zn and Hg in 1 mL HNO_3 1 mol L^{-1} solution for 24 hours and diffusion coefficient values in the presence of 0.01 mol L^{-1} $NaNO_3$ at pH = 5[117]

Parameter	Cd	Cu	Pb	Zn	Hg
f_e (average ± CI)[a]	0.85 ± 0.05	0.85 ± 0.05	0.85 ± 0.05	0.90 ± 0.05	0.88 ± 0.05
D_e (cm^2 s^{-1}) (average ± CI)x10^{-6} [b]	4.45 ± 0.24	4.49 ± 0.19	5.57 ± 0.25	4.32 ± 0.24	5.64 ± 0.26

[a] The effective value of the the elution factor and the confidence interval (CI) for 95% confidence level (n = 3).

[b] The effective values of the diffusion coefficient and the confidence interval (CI) for 95% confidence level calculated with the standard deviation of the accumulation speed curve.

4.7. Analytical Performance and the Accuracy of the SSETV-µCCP-OES Method with and without Coupling with the DGT Accumulation/Sampling Technique

The instrumental limits of detection for SSETV-µCCP-OES, GFAAS and TDAAS, with and without DGT accumulation, were evaluated based on the 3σ (LOD = 3 s_b/m) criterion, where (s_b) represents the standard deviation of the background signals and (m) represents the slope of the calibration curve. The limits of detection for the DGT-SSETV-µCCP-OES, DGT-GFAAS and DGT-TDAAS couplings were calculated for an accumulation period of

24 hours, with the f_e and D_e values (Table 4.1). The limits of quantification (LOQ) were determined as 3xLOD. The LOD values obtained by the SSETV-μCCP-OES method without coupling with the DGT technique (in μg L^{-1}) were 0.05 for Zn, 0.12 for Cd, 0.14 for Hg, 0.20 for Cu and 0.80 for Pb.[117] By the DGT-SSETV-μCCP-OES coupling, the LODs were improved with over 10 times at most, obtaining the LOD values (in μg L^{-1}): 0.01 for Hg, Cd and Pb, 0.02 for Cu and 0.07 for Pb, for an accumulation time of 24 hours.[117] The LOD values obtained by DGT-SSETV-μCCP-OES were better in case of Hg in comparison with TDAAS, better in case of Cd, Cu and Zn, in comparison with the ones obtained by GFAAS, respectively similar for Pb. The result of the analysis of the water CRM sample are presented in Table 4.2.[117]

Table 4.2. Results obtained at the *ex-situ* analysis of the ERM-CA713 water CRM sample by different methods[117]

Element	Certified value Average ± U (μg L^{-1})[a]	Determined value Average ± U$_{lab}$ (μg L^{-1})[b]			Recovery degree Average ± U$_{lab}$ (%)[b]		
		DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-TDAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-TDAAS
Cd	5.09 ± 0.20	5.18 ± 1.46	5.43 ± 1.45	-	102 ± 28	107 ± 28	-
Cu	101 ± 7	97 ± 27	99 ± 25	-	96 ± 28	98 ± 25	-
Pb	49.7 ± 1.7	51.2 ± 13.8	50.8 ± 14.4	-	103 ± 27	102 ± 28	-
Zn	78[c]	80 ± 27	89 ± 24	-	103 ± 33	114 ± 30	-
Hg	1.84 ± 0.11	1.75 ± 0.49	-	1.77 ± 0.47	95 ± 28	-	96 ± 27

[a] U represent the extended uncertainty for 95% confidence level (k = 2).
[b] U$_{lab}$ represents the laboratory extended uncertainty (k = 2, n = 3 parallel measurements) at 95% confidence level
[c] Indicative value

The accuracy of the method with a microplasma source was evaluated by the analysis of a water CRM sample by DGT-SSETV-μCCP-OES, using the passive sampling by the immersion of the Chelex-100 DGT device, in a CRM sample volume of 50 mL for 168 h, at a pH equal with 5, followed by the analysis of the eluate of 1 mol L^{-1} nitric acid and the calculation of the concentration found in the immersion solution of the CRM sample. The proposed method is characterized with good accuracy, with recovery degrees of 95-103%, and fidelity of 27-33%. These values are similar with the ones of the DGT-GFAAS method (89-114% accuracy, 25-30% fidelity) and the DGT-TDAAS method for the determination of Hg (96 ± 23%). By the application of the Tukey test for the comparison of the values determined by DGT-SSETV-μCCP-OES, DGT-GFAAS and DGT-TDAAS, it was highlighted that the values determined by these methods do not differ significantly ($p < 0.05$). Also, by comparing the determined values with the certified ones by the Dunnett test, it was concluded that there is no significant difference.

4.8. Applicability of the DGT-SSETV-µCCP-OES Method for the Determination of Cd, Cu, Pb, Zn and Hg in River Waters

The data of Table 4.3 show that the precision calculated on the basis of u_c for the determination of Cu and Zn by DGT-SSETV-µCCP-OES, was of 15.3-20.1% and 16.0-21.1%, respectively. The determination of Cu and Zn from river water by SSETV-µCCP-OES, without coupling with the DGT technique provided a precision of 4.8 – 9.8% for the determination of Cu and 4.5 – 9.3% for the determination of Zn. In the case of Cd and Pb (Table 4.4), the precision of their determination was 16.4 – 22.4% for Cd and 15.4 – 20.7%, for Pb.[117] For the SSETV-µCCP-OES method without coupling with the DGT technique, the precision of the dtermination of Cd and Pb was 5.4 – 10.8%, respectively 9.3%. The determination of Hg could not be realised due to its presence in concentrations lower than LOD, even for an accumulation period of 7 days on the DGT devices.

4.9. Evaluation of the Green and White Degree of the DGT-SSETV-µCCP-OES Method

The white degree of the DGT-SSETV-µCCP-OES method for the analysis of river water samples was evaluated based on the RGB-12 algorithm presented in Chapter 1, section 1.5.[82] The DGT-SSETV-µCCP-OES method used for the determination of the labile fraction of Cd, Cu, Pb and Zn in river water is characterized by high scores of Red (100%), Green (80%), Blue (98%) and White (93%). The green degree of the method was evaluated by the AGREEprep software recommended by Wojnowski and coworkers[79], and a 80% green degree was obtained for the DGT-SSETV-µCCP-OES method. The green degree of the DGT-SSETV-µCCP-OES method can be improved by automation of the method, such as the implementation of an automated system for the deposition of the microsample on the filament. The results are presented in Figure 4.1.[117]

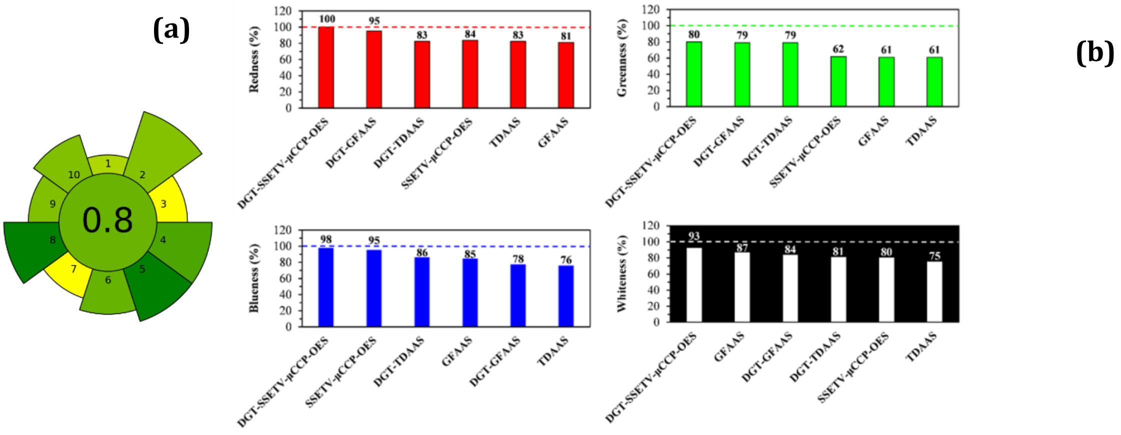


Figure 4.1. Green (a) and white (b) degrees of the DGT-SSETV-µCCP-OES method in comparison with the GFAAS and TDAAS methods with or without DGT passive sampling[117] .

Table 4.3. Results of the analysis of river water samples (average ± U_{lab})[a] for the determination of Cu and Zn[117]

Sampling point	Concentration determined in river water (μg L^{-1})[b]		Calculated labile fraction concentration in river water (μg L^{-1})[c]		Concentration determined in the elaute (μg L^{-1})[d]	
	GFAAS	SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES
Cu						
P1	4.40 ± 1.32	4.70 ± 1.40	3.60 ± 1.29	3.91 ± 1.53	241 ± 87	262 ± 103
P2	7.00 ± 3.06	8.00 ± 4.00	5.05 ± 1.85	5.20 ± 2.09	339 ± 124	349 ± 140
P3	5.90 ± 1.34	5.90 ± 1.46	4.31 ± 1.71	4.49 ± 1.75	289 ± 116	301 ± 117
P4	20.4 ±9.2	19.8 ± 8.9	14.9 ± 4.0	14.5 ± 4.9	999 ± 271	972 ± 332
P5	38.0 ± 13.6	37.2 ± 11.2	31.5 ± 8.8	30.3 ± 9.2	2110 ± 590	2030 ± 620
P6	28.1 ± 11.8	25.0 ± 14.1	27.4 ± 7.3	26.3 ± 8.8	1840 ± 490	1760 ± 590
P7	27.0 ± 11.4	29.4 ± 12.1	28.9 ± 8.1	30.2 ± 9.4	1940 ± 550	2030 ± 630
P8	21.9 ± 9.2	19.3 ± 8.1	14.3 ± 4.6	13.3 ± 4.9	959 ± 310	892 ± 326
P9	24.5 ± 10.9	24.7 ± 9.1	25.9 ± 8.2	25.0 ± 8.4	1740 ± 550	1680 ± 560
P10	22.0 ± 8.7	22.6 ± 9.0	20.7 ± 7.3	19.4 ± 6.6	1390 ± 490	1300 ± 440
RSD(%)[e]	3.3 – 9.6	4.8 – 9.8	10.0 – 17.8	15.3 – 20.1	10.0 – 17.8	15.3 – 20.1
Zn						
P1	6.49 ± 3.23	6.19 ± 3.04	4.34 ± 1.47	4.81 ± 1.96	296 ± 109	329 ± 134
P2	7.52 ± 3.80	7.07 ± 3.09	5.00 ± 1.72	4.73 ± 1.96	342 ± 126	323 ± 134
P3	8.28 ± 3.55	6.81 ± 3.73	6.48 ± 1.84	6.68 ± 2.31	443 ± 149	456 ± 157
P4	6.59 ± 3.17	6.39 ± 2.44	6.53 ± 1.75	6.22 ± 2.26	446 ± 157	425 ± 155
P5	8.19 ± 3.87	8.30 ± 3.08	7.68 ± 2.10	7.58 ± 2.77	525 ± 159	518 ± 189
P6	8.21 ± 5.24	8.12 ± 3.87	6.09 ± 2.29	6.02 ± 2.30	416 ± 151	411 ± 157
P7	6.79 ± 2.38	6.68 ± 3.60	4.24 ± 1.22	4.16 ± 1.62	290 ± 105	284 ± 111
P8	5.92 ± 2.63	6.27 ± 2.23	4.24 ± 1.28	4.16 ± 1.69	290 ± 115	284 ± 120
P9	6.22 ± 3.04	6.33 ± 3.21	5.60 ± 1.58	5.47 ± 2.23	382 ± 146	374 ± 156
P10	6.47 ± 2.46	6.28 ± 3.78	6.56 ± 1.59	6.55 ± 2.09	448 ± 141	447 ± 143
RSD(%)[e]	2.2 – 7.5	4.5 – 9.3	6.9 – 15.9	16.0 – 21.1	6.9 – 15.9	16.0 – 21.1

[a] U_{lab} represents the laboratory extended uncertainty (k = 2, n = 3 parallel measurements and 95 % confidence level).
[b] Analysis of the samples collected on daily basis (n = 7 days).
[c] Average concentration in time in river water calculated from the determined concentration in the eluate.
[d] Eluate volume: 1.15 mL; 1 mL volume of eluent + 0.15 mL volume of accumulation gel.
[e] Relative standard deviation obtained on the basis of the composed uncertainty (n = 3 parallel samples, k = 2).

Table 4.4. Results of the analysis of river water samples (average ± U_{lab}) for the determination of Cd and Pb[117]

Sampling point	Concentration determined in river water (μg L^{-1})[b]		Calculated labile fraction concentration in river water (μg L^{-1})[c]		Concentration determined in the elaute (μg L^{-1})[d]	
	GFAAS	SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES
Cd						
P1	< LOD[e]	< LOD[e]	0.02 ± 0.01	0.02 ± 0.01	1.13 ± 0.48	1.20 ± 0.50
P2	< LOD	< LOD	0.07 ± 0.03	0.08 ± 0.03	4.85 ± 1.88	4.98 ± 2.10
P3	< LOD	< LOD	0.04 ± 0.02	0.04 ± 0.02	2.59 ± 1.08	2.46 ± 1.10
P4	0.18 ± 0.06 (< LOQ[e])	0.17 ± 0.07(< LOQ[e])	0.13 ± 0.05	0.13 ± 0.04	8.64 ± 3.11	8.64 ± 2.90
P5	0.60 ± 0.28	0.66 ± .025	0.36 ± 0.11	0.34 ± 0.11	23.9 ± 7.3	22.6 ± 7.4
P6	0.48 ± 0.26	0.50 ± 0.24	0.39 ± 0.12	0.34 ± 0.12	25.9 ± 8.0	22.6 ± 7.8
P7	0.31 ± 0.13(< LOQ[e])	0.31 ± 0.09(< LOQ[e])	0.25 ± 0.09	0.23 ± 0.09	16.6 ± 5.7	15.3 ± 5.9
P8	< LOD	< LOD	0.08 ± 0.03	0.08 ± 0.04	5.32 ± 2.07	5.30 ± 2.30
P9	0.18 ± 0.09(< LOQ[e])	0.22 ± 0.11(< LOQ[e])	0.16 ± 0.06	0.18 ± 0.07	10.6 ± 3.8	12.0 ± 4.7
P10	< LOD	< LOD	0.12 ± 0.04	0.13 ± 0.05	7.97 ± 2.59	8.64 ± 3.30
RSD(%)[e]	4.3 – 11.0	5.4 – 10.8	12.3 – 20.1	16.4 – 22.4	12.3 – 20.1	16.4 – 22.4
Pb						
P1	< LOD[e]	< LOD[e]	0.31 ± 0.11	0.28 ± 0.10	25.8 ± 9.4	23.3 ± 8.4
P2	< LOD	< LOD	0.24 ± 0.09	0.30 ± 0.12	20.0 ± 7.6	25.0 ± 9.7
P3	< LOD	< LOD	0.43 ± 0.12	0.50 ± 0.17	35.8 ± 9.9	41.6 ± 14.0
P4	< LOD	< LOD	0.19 ± 0.07	0.22 ± 0.08	15.8 ± 6.1	18.3 ± 6.7
P5	< LOD	< LOD	0.47 ± 0.16	0.42 ± 0.13	39.1 ± 12.9	34.9 ± 11.1
P6	< LOD	< LOD	0.30 ± 0.11	0.35 ± 0.12	25.0 ± 9.3	29.1 ± 10.2
P7	< LOD	< LOD	0.25 ± 0.10	0.30 ± 0.11	20.8 ± 8.3	25.0 ± 9.1
P8	< LOD	< LOD	0.68 ± 0.19	0.63 ± 0.19	56.6 ± 16.0	52.4 ± 16.1
P9	< LOD	< LOD	0.24 ± 0.08	0.24 ± 0.08	20.0 ± 7.1	20.0 ± 6.9
P10	1.09 ± 0.50 (< LOQ[e])	1.20 ± 0.64 (< LOQ[e])	0.61 ± 0.18	0.58 ± 0.23	50.7 ± 15.3	48.2 ± 20.0
RSD(%)[e]	8.5	9.3	10.3 – 17.7	15.4 – 20.7	10.3 – 17.7	15.4 – 20.7

[a] U_{lab} represents the laboratory extended uncertainty (k = 2, n = 3 parallel measurements and 95 % confidence level).

[b] Analysis of the samples collected on daily basis (n = 7 days).

[c] Average concentration in time in river water calculated from the determined concentration in the eluate.

[d] Eluate volume: 1.15 mL; 1 mL volume of eluent + 0.15 mL volume of accumulation gel.

[e] LOD/LOQ – 0.12/0.36 μg L^{-1} for Cd by GFAAS and SSETV-μCCP-OES; 0.80/2.40 μg L^{-1} for Pb by SSETV-μCCP-OES and 0.60/1.80 μg L^{-1} for Pb by GFAAS

[f] Relative standard deviation obtained on the basis of the composed uncertainty (n = 3 parallel samples, k = 2).

4.10. Conclusions

According to the results obtained, the following conclusions can be listed:

1. Worldwide for the first time, a method with a high green and white degree was developed and characterized for the simultaneous determination of Cd, Cu, Pb, Zn and Hg in surface waters, by optical emission microspectrometry on a completely miniaturized *ex-situ* instrumentation with a microplasma source, operated at 15 W and 150 mL min^{-1} Ar consumption, a low resolution microspectrometer, an electrothermal vaporizer for the introduction of the microsample, coupled with the *in-situ* passive samplin/accumulation by DGT with Chelex-100 resin;
2. By the coupling of the *in-situ* DGT passive sampling with the SSETV-μCCP-OES intrumentation, the advantages of both techniques were combined, namely the relatively highly factors of selective multielemental preconcentration, and the excitation capacity and simultaneous measurement, by optical emission in microplasma;
3. Following the preconcentration and *in-situ* passive sampling by the DGT technique, the analytical performance of the SSETV-μCCP-OES miniaturized spectral instrumentation were substantially improved, namely, LODs were improved with at least one order of magnitute, which allowed also the determination of Cd and Pb, found in the river water at lower concentrations than instrumental LODs;
4. By the use of the DGT sampling the spectral interference of the most intense Cd line in the microplasma at 228.802 nm with the spectral line of As 228.812 nm was solved, which could not be achieved with a low resolution microspectrometer, such as Maya 2000Pro;
5. Although the DGT-SSETV-μCCP-OES method was also validated for the determination of Hg, unfortunately, it could not be determined in river water because the Chelex-100 resin has a reduced affinity for the Hg^{2+} ions;
6. The study of the validationof the DGT-SSETV-μCCP-OES method by the analysis of water CRM samples indicated that the uncertainty of the determination of the certified concentrations and in river water, by passive sampling of Cd, Cu, Pb, Cu and Zn, falls within the interval ± 20 or at most ± 30%, considered acceptable for a microanalytical spectral method coupled with *in-situ* DGT passive sampling;
7. The application of the AGREEprep and RGB-12 algorithms highlighted that the DGT-SSETV-μCCP-OES method is characterized by a high green degree, due to the *in-situ* DGT passive sampling, the decreased reagent consumption and the small quantity of

generated chemical waste, red degree of 100% by substantially improved LODs by the DGT sampling and the simultaneous determination of the elements, respectively a high blue degree, by the low cost of the miniaturized intrumentation with microplasma, the high speed of the analysis by the simultaneous detection and the miniaturization of all devices of the SSETV-µCCP-OES instrumentation;

8. Due to the high analytical performance and the high green and white degrees, which cover all the aspects of and analytical procedure, the DGT-SSETV-µCCP-OES can be considered as another attractive instrumental concept and an alternative option for the clasical laboratory instrumentation, used among others for the monitoring of surface waters, such as GFAAS and TDAAS;
9. Additional studies are necessary in order to improve automation by the coupling with an autosampler for the deposition of the microsample on the filament in the scope of vaporization, respectively the control of the Ar flow towards the plasma in the drying and vaporization stages of the microsample, which will contribute to the improvement of the precision and LODs of the method.

Chapter 5. Determination of Total Content and Mobile Fraction of Cd, Pb, Cu and Zn in Soil by Electrothermal Vaporization Capacitively Coupled Microplasma Optical Emission Spectrometry after Passive Accumulation by Diffusive Gradients in Thin-Film

5.1. Situation at International Level. Working Hypotheses and Objectives

Due to the concerns related to the exposure of the population and the environment to toxic or potentially toxic elements, such as Cd, Pb, Hg, As, Cu, Zn and Cr, the development of some sampling and sample work-up methods represent a challenge for the routine research laboratories, but also the development of some analytical methods for determination based on atomic spectrometry or mass spectrometry, in various types of samples, such as water, soil, food and biological samples, etc.[124,125]

Taking into consideration the interest at international level concerning the widening of the application area of the miniaturized instrumentation with microplasma sources and the personal experience in the use of the DGT passive accumulation from surface waters, the research presented in this chapter is a continuation of the research presented in Chapter 5, namely, the development and validation of a novel DGT-SSETV-μCCP-OES simultaneous analytical method, without non-spectral interferences for the determination of the total content and mobile fraction of Cd, Cu, Pb and Zn, with the highest toxicity, in soil, also using the DGT passive sampling on Chelex-100 resin. Besides the total content, the mobile fraction from soil was chosen to be determined, because the diffusion and accumulation on the gel simulates the passive accumulation of the metalic ions from the soil solution towards plants, by absorption through roots, which implies transfer mechanisms of metalic ions from soil to the soil solution, and, ultimately, in the plant through the roots. Furthermore, in studies concerning the bioavailability of the toxic metals for plants, it was observed that the the mobile fraction (c_{DGT}) determined by DGT technique from the soil solution was the best indicator of the bioavailability of metals from soil toward plant, demonstrated by the statistically significant correlation coefficients between the metal content in plants and concentrations (c_{DGT}), determined by DGT accumulation in the soil solution or soil.[126-129]

5.2. DGT-SSETV-μCCP-OES Instrumentation

The SSETV-μCCP-OES instrumentation, interfaced with the DGT passive sampling used for the determination of the total content and mobile fraction of Cd, Cu, Pb and Zn in soil is similar with the one used for the simultaneous determination of Cd, Cu, Pb, Zn and Hg in surface waters, previously presented in Chapter 4, with the mention that the DGT accumulation experiments were performed solely *ex-situ*.[132]

5.3. Sample Preparation for the Determination of the Total Content of Cd, Pb, Cu and Zn in Soil

Quantities of approximately 0.1000 g soil sample were mineralized in 12 mL *aqua regia*, and the digestion was performed in a microwave digestor, following the thermal protocol previously used by Frențiu and coworkers.[16] The solutions used for the determination of the total content of Cd, Pb, Cu and Zn by DGT-SSETV-μCCP-OES were prepared by the dilution of the sample mineralized in *aqua regia* with ultrapure water, 5 or 10 times, in a volumetric flask of 100 mL and the adjustment of the pH of the solution at 4.0 ± 0.1 value with a solution of 0.1 mol L^{-1} NH_3. The DGT devices were immersed in the solution with pH 4.0, for a period of accumulation of 24 hours, in triplicate, at a temperature of 21 ± 1 °C. After accumulation, the devices were were subjected to the elution and analysis procedure described in Chapter 4, section 4.3. Based on the concentration in the eluate, the concentration in the immersion solution was calculated with the equation 1.1, Chapter 1, and based on the preparation protocol, the total content of metals in soil was calculated.

5.4. Sample Preparation for the Determination of Mobile Fraction Content of Metals

For the determination of mobile fraction content of metals in soil, the soil samples were mixed with a quantity of *aqua regia* equal to its maximum retention capacity (MWHC), at a soil:water ratio of 10:8. The samples with paste aspect were left to stabilize for 48 hours at a temperature of 21 ± 1 °C in vessels covered with polyethylene foil. The DGT devices with Chelex-100 resin were immersed in the resulted paste with the exposure window down, to maintain the contact with the soil solution. The devices were left in the soil sample for 24 hours, at a temperature of 21 ± 1 °C. The accuracy of the DGT-SSETV-μCCP-OES method for the determination of the total content was evaluated by the analysis of some soil CRM samples, according to the procedure described previously, by the immersion of the DGT devices in the solution with pH 4.0 ± 0.1 for 24 hours.

In parallel with the determination of the mobile fraction, an identical soil-water paste was prepared, and the soil solution was separated by centrifugation at 3000 rotations per minute. The separated solution was analyzed for the total dissolved content of Cd, Pb, Cu and Zn in the soil solution (c_{sol}), by SSETV-μCCP-OES and by GFAAS. The pH was determined in this solution, and the c_{sol} value was used as reference for the evaluation of the mobility of metals from soil in the soil solution.[75]

5.5. Validation of the DGT-SSETV-μCCP-OES Method for the Determination of Total and Mobile Content of Metals in Soil

The precision of the DGT-SSETV-μCCP-OES method was evaluated based on the absolute extended uncertainty (U_{lab}) and the average values of the results obtained at the analysis of real samples, by the external calibration curve, and the comparison of the result with the ones obtained at the analysis by GFAAS. The comparison of the results was conducted by the application of the statistical Bland and Altman test.[130]

The instrumental LOD values of the SSETV-μCCP-OES method were calculated according to the 3σ ($LOD = 3s_b/m$) criterion, where (s_b) represents the standard deviation of the blank, and (m) represents the slope of the calibration curve. The LOD values of the method in soil were calculated taking into consideration the preparation protocol of the sample, the instrumental limits of detection of the DGT-SSETV-μCCP-OES method and were calculated for a passive accumulation of 24 hours. The accuracy of the SSETV-μCCP-OES and DGT-SSETV-μCCP-OES methods was evaluated by the analysis of some CRM samples. The recovery degrees (R%) were calculated and the relative extended uncertainty (U_{lab}%) was determined, respectively the confidence interval of recovery (R ± U_{lab}%, k = 2), for the procedures with and without DGT accumulation.

The value of the concentration c_{DGT} in the immersion solution/soil was calculated by the use of the experimentally determined values of the diffusion coefficient and elution factor (D_{exp}, f_{exp}), but also the values recommended by the manufacturer. The accuracy of the method was considered acceptable for a recovery degree (R ± U_{lab}%) in the 100 ± 30% interval.[122,123] The precision of the method for the determination of total and mobile content of metals in soil was evaluated based on the RSD (%) values, calculated from the composed uncertainty (u_{lab}) value, based on the analysis of 3 parallel samples subjected to DGT passive sampling. The precision of the method was considered acceptable for a limit value of 30%, previously proposed for spectral methods coupled with DGT passive sampling.[122,123]

5.6. Working Parameters for the Application of the DGT Passive Accumulation Technique of Cd, Cu, Pb and Zn for Determination from Soil. Determination of Experimental Diffusion Coefficients (D_{exp}) and Elution Factors (f_{exp})

The passive accumulation of Cd, Cu, Pb and Zn by the DGT technique on Chelex-100 resin with the scope of the determination of the total and mobile content from soil by DGT-SSETV-µCCP-OES was validated by the experimental determination of D_{exp} and f_{exp} similarly with the procedure described in section 4.6, under the laboratory working parameters for soil CRM samples prepared according to the presentation in section 5.3. The immersion solutions were prepared by dilution (1:10) of the solution from the digestion and the adjustment of the pH in the 4.0 ± 0.1 interval. The DGT devices were immersed in the obtained solutions for periods of time in the 8 – 48 h interval at a temperature of 21 ± 1 °C. The experimental elution factors were obtained as the ratio between the mass of the found element by analysis in the eluent and the mass retained in the Chelex-100 gel, calculated according to the procedure used for the study of the accumulation kinetics, previously presented. The D_{exp} and f_{exp} values were compared with the ones recommended by the manufacturer and presented in Table 5.1.[131]

Table 5.1. Values of the diffusion coefficients and elution factors experimentally determined by the analysis of soil CRM samples and comparison with the values recommended by the manufacturer[131]

Parameter	Experimental values (Average ± U_{lab})[a,b]				Values recommended by the manufacturer (Average ± U_{lab})[a,b]			
	Cd	Pb	Cu	Zn	Cd	Pb	Cu	Zn
D ($cm^2\ s^{-1}$)10^{-6}	5.00 ± 0.38	7.60 ± 0.50	5.50 ± 0.40	5.82 ± 0.38	5.46 ± 0.32	7.19 ± 0.40	5.58 ± 0.32	5.44 ± 0.30
f_e	0.89 ± 0.06	0.91 ± 0.08	0.76 ± 0.10	0.81 ± 0.08	0.80 ± 0.06	0.80 ± 0.06	0.80 ± 0.06	0.80 ± 0.06

[a] Represents the diffusion coefficient determined at pH = 4.0 ± 0.1 and temperature of 21 ± 1 °C; U_{lab} represents the absolute extended uncertainty (k = 2) evaluated on the basis of the uncertainty of the kinetic accumulation slope and the influence of temperature for a variation of ± 1 °C;

[b] Represents the elution factor of metals from Chelex-100 in 1 mL solution of HNO_3 1 mol L^{-1} for 24 hours; U_{lab} represents the extended uncertainty (k = 2) for n = 3 parallel measurements for each CRM sample.

5.7. Limits of Detection for the DGT-SSETV-μCCP-OES Method

The DGT-SSETV-μCCP-OES method presents LODs with values between 0.03 mg kg^{-1} for Zn and 0.40 mg kg^{-1} for Pb, for an accumulation time of 24hours. For the DGT-GFAAS method, values of LODs between 0.10 mg kg^{-1} for Cd and 0.40 mg kg^{-1} for Cu were obtained. Besides, LODs obtained by the coupling with the DGT technique are with an order of magnitude better than the ones determined without the coupling with the DGT accumulation technique.In the case of the determination of Cd, Pb, Cu and Zn without coupling with DGT technique, the SSETV-μCCP-OES method allows the determination in soil only for total concentrations higher than 3.6 mg kg^{-1} Cd, 24 mg kg^{-1} Pb, 6 mg kg^{-1} Cu and 1.5 mg kg^{-1} Zn. For the determination pf the mobile fraction metal content in soil, LOD values of 0.008 μg L^{-1} Cd, Cu and Zn and 0,024 μg L^{-1} Pb were obtained in the soil solution, or 0.01 μg kg^{-1} Cd, Cu and Zn and 0,03 μg kg^{-1} Pb, in the dried mass of the sample at an accumulation time of 24 h. The LOD values obtained by GFAAS with and without DGT accumulation, were similar for Cd and Pb, and better for the determination of Cu and Zn, compared with the SSETV-μCCP-OES coupled or not with the DGT technique.[131]

5.8. Accuracy of the DGT-SSETV-μCCP-OES Method for the Determination of Total Content of Metals in Soil

In case of the analysis of CRM samples by SSETV-μCCP-OES without coupling with the DGT technique (Table 5.2[131]), the recovery degrees in the 58-83% interval resulted, with an extended uncertainty of 12 – 37% (k = 2). The Dunnett test highlighted the statistically significant differences between the found and certified values ($p > 0,05$). The recovery degrees obtained at the analysis of the soil CRM samples by DGT-SSETV-μCCP-OES and DGT-GFAAS (Table 5.3[131]) were of 85–123%, with a relative extended uncertainty of 19–35%, k = 2, respectively 84–122%, with a relative extended uncertainty of 18–28%, k = 2. The similarity of the recovery degrees obtained by the two methods indicates the absence of the non-spectral interferences of the multielemental matrix at the determination of Cd, Pb, Cu and Zn by DGT-SSETV-μCCP-OES, respectively the absence of the spectral interference of As on the Cd line in the same method.

Table 5.2. Results of the determination of the cotal content of Cd, Pb, Cu and Zn in soil CRM samples by SSETV-μCCP-OES and GFAAS with external calibration without coupling with the DGT technique[131]

CRM/Analyte	Certified concentration Average ± U_{CRM} (mg kg^{-1})[a]	Concentration found without coupling with the DGT technique Average ± U_{lab} (mg kg^{-1})[b]		Accuracy Recovery ± U_{lab} (%)[b]	
		SSETV-μCCP-OES	GFAAS	SSETV-μCCP-OES	GFAAS
SQC001-30G					
Cd	118 ± 2	73 ± 14	137 ± 23	62 ± 19	116 ± 17
Pb	144 ± 2	94 ± 28	124 ± 17	65 ± 30	86 ± 14
Cu	330 ± 4	246 ± 47	287 ± 41	75 ± 19	87 ± 14
Zn	874 ± 11	546 ± 199	902 ± 148	62 ± 37	103 ± 16
CRM048-50G					
Cd	92.8 ± 1.55	69.1 ± 12.03	80.6 ± 17.82	74 ± 17	87 ± 22
Pb	320 ± 6.27	233 ± 76.22	268 ± 44.56	73 ± 33	84 ± 17
Cu	84.3 ± 1.45	70.3 ± 16.83	71.5 ± 12.33	83 ± 24	85 ± 17
Zn	425 ± 9.14	304 ± 36.80	403 ± 52.39	72 ± 12	95 ± 13
Metranal 34					
Cd	1.44 ± 0.07	0.99 ± 0.26	1.53 ± 0.26	69 ± 26	106 ± 17
Pb	83.1 ± 2.3	54.2 ± 12.1	75.1 ± 13.8	65 ± 22	90 ± 18
Cu	167 ± 1	115 ± 30	153 ± 23	69 ± 26	92 ± 15
Zn	198 ± 6	148 ± 39	215 ± 38	75 ± 26	108 ± 18
CRM025–050					
Cd	369 ± 19	276 ± 45	325 ± 52	75 ± 16	88 ± 16
Pb	1447 ± 88	841 ± 225	1596 ± 198	58 ± 27	110 ± 12
Cu	7.76 ± 0.73	5.79 ± 1.34	8.63 ± 1.27	75 ± 23	111 ± 15
Zn	51.8 ± 3.35	33.5 ± 4.32	42.3 ± 6.42	65 ± 13	82 ± 15

[a] – U_{CRM} represents the extended uncertainty of the certified concentration ($k = 2$; at 95% confidence level)

[b] – U_{lab} represents the absolute and relative uncertainty in the laboratory ($k = 2$, $n = 3$ parallel measurements and 95% confidence level)

Table 5.3. Results of the determination of the total content of Cd, Pb, Cu and Zn in soil CRM samples by the SSETV-μCCP-OES and GFAAS methods with coupling with the DGT technique, at an accumulation time of 24 h at pH = 4.0 ± 0.1 and a temperature of 21 ± 1 °C using the experimental accumulation D_{exp} and f_{exp} values, and external calibration[131]

CRM/ Analyte	Certified concentration Average ± U_{CRM} (mg kg^{-1})[a]	Experimental sdffusion coefficient and elution factor				Recommended diffusion coefficient and elution factor			
		Determined concentration Average ± U_{lab} (mg kg^{-1})[b]		Accuracy Recovery ± U_{lab} (%)[b]		Determined concentration Average ± U_{lab} (mg kg^{-1})[b]		Accuracy Recovery ± U_{lab} (%)[b]	
		DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS
SQC001-30G									
Cd	118 ± 2	123 ± 28	113 ± 28	105 ± 23	95 ± 25	126 ± 25	115 ± 25	107 ± 19	98 ± 22
Pb	144 ± 2	147 ± 49	145 ± 38	102 ± 33	101 ± 26	176 ± 56	175 ± 40	123 ± 32	121 ± 23
Cu	330 ± 4	363 ± 94	359 ± 102	110 ± 26	109 ± 28	341 ± 69	337 ± 79	103 ± 20	102 ± 23
Zn	874 ± 11	827 ± 278	921 ± 260	95 ± 34	105 ± 28	902 ± 280	1004 ± 252	103 ± 31	115 ± 25
CRM048-50G									
Cd	92.8 ± 1.55	93.7 ± 32.00	102.8 ± 27.18	101 ± 34	111 ± 26	96.0 ± 30.81	105.2 ± 25.17	103 ± 32	113 ± 24
Pb	320 ± 6.27	327± 103.38	324 ± 87.04	102 ± 32	101 ± 27	394 ± 113.62	390 ± 89.40	123 ± 29	122 ± 23
Cu	84.3 ± 1.45	78.4 ± 19.33	80.5 ± 19.61	93 ± 25	95 ± 24	73.7 ± 13.92	75.6 ± 14.00	87 ± 19	90 ± 19
Zn	425 ± 9.14	428 ± 148.41	414 ± 103.64	101 ± 35	97 ± 25	466 ± 147.94	451 ± 93.54	110 ± 32	106 ± 21
Metranal 34									
Cd	1.44 ± 0.07	1.33 ± 0.38	1.38 ± 0.36	93 ± 28	96 ± 26	1.37 ± 0.34	1.41 ± 0.32	95 ± 25	98 ± 22
Pb	83.1 ± 2.3	75.9 ± 24.4	71.9 ± 17.6	91 ± 32	87 ± 24	91.3 ± 27.4	86.5 ± 17.9	110 ± 30	104 ± 21
Cu	167 ± 1	151 ± 49	153 ± 41	91 ± 33	92 ± 27	142 ± 40	144 ± 31	85 ± 28	86 ± 22
Zn	198 ± 6	168 ± 56	170 ± 38	85 ± 33	86 ± 22	180 ± 55	182 ± 32	91 ± 31	92 ± 18
CRM025–050									
Cd	369 ± 19.0	372 ± 107.1	375 ± 94.0	101 ± 29	101 ± 25	381 ± 100.4	383 ± 85.5	103 ± 26	104 ± 22
Pb	1447 ± 88	1234 ± 372	1216 ± 315	85 ± 30	84 ± 26	1487 ± 415	1465 ± 319	103 ± 28	101 ± 22
Cu	7.76 ± 0.73	7.99 ± 2.32	8.38 ± 2.01	103 ± 29	108 ± 24	7.53 ± 1.96	7.92 ± 1.82	97 ± 26	102 ± 23
Zn	51.8 ± 3.35	50.1 ± 13.41	46.1 ± 13.00	97 ± 27	89 ± 28	54.6 ± 12.63	50.3 ± 12.45	105 ± 23	97 ± 25

[a] – U_{CRM} represents the extended uncertainty of the certified concentration (k = 2; 95% confidence level)

[b] – U_{lab} represents the laboratory absolute and relative uncertainty (k = 2, n = 3 parallel measurements and 95% confidence level)

5.9. Determination of Total and Mobile Content of Cd, Pb, Cu and Zn in Soil by DGT-SSETV-μCCP-OES. Evaluation of Precision

According to Table 5.4[131], the total content of Cd, Pb, Cu and Zn in soil, was determined by DGT-SSETV-μCCP-OES with 10 – 19% accuracy, calculated based on the composed uncertainty. It can be observed that the accuracy of the DGT-SSETV-μCCP-OES and DGT-GFAAS methods are similar.

The results of the determination of the mobile fraction of Cd, Cu, Pb and Zn in soil are presented in Table 5.5[131], alongside the dissolved total concentrations of these metals in the soil solution, separated by centrifugation. The mobile content of Cd, Cu, Pb and Zn in soil was determined by DGT-SSETV-μCCP-OES with a precision of 10 – 15%, similar with the one of the DGT-GFAAS method.

The results obtained at the determination of the total and mobile content of metals in soil by DGT-SSETV-μCCP-OES and DGT-GFAAS were compared by the application of the statistical Bland and Altman test, which does not show any significant difference at the determination of total and mobile fraction of Cd, Pb, Cu, Zn in soil for ($p > 0.05$). Furthermore, following the analysis of the total dissolved concentration of Cd, Pb, Cu, Zn in the soil solution, it was found that the analyzed soils were characterized by an average mobility of Cd, with R values of 0.13 – 0.50. In the case of Pb and Cu, the mobility was small to average, with values of 0.03 – 0.22, respectively 0.03 – 0.26. Zinc had an increased mobility in 5 of the 10 analyzed samples, and a small mobilty in the remaining samples, with values of 0.03 – 0.96. These results were confirmed by the low percentage of the total dissolved metals in the soil solution compared to the total content of the soil sample, namely 0.2% for Cd, Cu and Zn, and 0.08% for Pb.[131]

Table 5.4. Results of the determination of the total content of Cd, Pb, Cu and Zn from soil samples by DGT-SSETV-μCCP-OES and DGT-GFAAS[131]

Sample	Total content of Cd Average ± U_{lab} (mg kg^{-1})[a]		Total content of Pb Average ± U_{lab} (mg kg^{-1}) [a]		Total content of Cu Average ± U_{lab} (mg kg^{-1}) [a]		Total content of Zn Average ± U_{lab} (mg kg^{-1}) [a]	
	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS	DGT-SSETV-μCCP-OES	DGT-GFAAS
S1	4.1 ± 1.2	4.8 ± 1.2	95.2 ± 29.4	104 ± 33	142 ± 33	131 ± 33	321 ± 95	306 ± 93
S2	4.8 ± 1.5	6.0 ± 1.5	71.0 ± 21.6	69.5 ± 14.8	59.0 ± 15.7	63.4 ± 18.9	135 ± 34	111 ± 31
S3	14.5 ± 3.3	14.1 ± 3.9	171 ± 37	160 ± 37	432 ± 137	420 ± 128	359 ± 90	372 ± 89
S4	4.4 ± 0.9	5.6 ± 1.5	35.6 ± 9.3	37.0 ± 8.0	75.2 ± 28.0	83.6 ± 23.4	86.4 ± 32.2	64.5 ± 20.2
S5	7.3 ± 1.8	7.2 ± 2.2	74.2 ± 20.3	71.3 ± 15.0	78.2 ± 22.3	79.4 ± 19.8	246 ± 68	255 ± 52
S6	8.9 ± 2.3	8.6 ± 2.3	71.0 ± 17.0	77.1 ± 18.2	85.8 ± 29.1	78.0 ± 22.9	202 ± 62	179 ± 44
S7	11.1 ± 2.8	11.9 ± 3.7	91.4 ± 28.5	92.4 ± 23.4	49.7 ± 13.1	50.6 ± 18.4	114 ± 32	125 ± 35
S8	4.9 ± 1.5	5.6 ± 1.4	228 ± 57	215 ± 69	74.8 ± 18.7	70.6 ± 20.0	152 ± 46	162 ± 50
S9	13.5 ± 3.7	13.2 ± 4.7	146 ± 31	146 ± 31	79.3 ± 25.1	79.8 ± 22.2	227 ± 57	207 ± 64
S10	8.1 ± 1.9	9.5 ± 2.3	128 ± 30	135 ± 38	135 ± 40	145 ± 36	223 ± 54	204 ± 55
RSD (%)[b]	12–19	12–18	10–16	12–18	11–16	11–16	12–19	10–16

[a] – U_{lab} represent the laboratory absolute extended uncertainty for the determined concentrations (k = 2, n = 3 parallel measurements and 95% confidence level)

[b] - RSD represents the relative standard deviation calculated from the composed uncertainty (n = 3 parallel measurements and 95% confidence level)

Table 5.5. Results of the determination of the mobile content of Cd, Pb, Cu and Zn in soil samples, obtained by DGT-SSETV-µCCP-OES and DGT-GFAAS after 24 hours of accumulation in the soil paste:water (10:8) and comparison with the total content of dissolved metals determined in the soil solution without coupling with the DGT technique[131]

Sample	Mobile content of Cd Average ± U_{lab} (µg kg^{-1})[a]		Dissolved content of Cd Average ± U_{lab} (µg kg^{-1})[a]		Mobile content of Pb Average ± U_{lab} (µg kg^{-1})[a]		Dissolved content of Pb Average ± U_{lab} (µg kg^{-1})[a]	
	DGT-SSETV-µCCP-OES	DGT-GFAAS	SSETV-µCCP-OES[b]	GFAAS	DGT-SSETV-µCCP-OES	DGT-GFAAS	SSETV-µCCP-OES[b]	GFAAS
S1	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	2.7 ± 0.6	2.3 ± 0.6	75.6 ± 9.8	88.0 ± 12.2
S2	0.7 ± 0.2	0.8 ± 0.1	1.4 ± 0.2	1.0 ± 0.2	2.8 ± 0.8	3.2 ± 0.7	17.9 ± 3.9	14.2 ± 3.8
S3	1.5 ± 0.4	1.9 ± 0.4	3.4 ± 0.5	2.9 ± 0.6	9.5 ± 2.4	7.9 ± 1.7	43.3 ± 8.1	45.1 ± 8.5
S4	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	1.8 ± 0.5	1.7 ± 0.4	8.5 ± 1.6	6.3 ± 0.9
S5	0.8 ± 0.2	1.0 ± 0.2	4.9 ± 0.7	1.5 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	20.2 ± 2.6	21.0 ± 6.1
S6	0.8 ± 0.2	0.8 ± 0.2	4.6 ± 0.8	4.1 ± 0.5	2.5 ± 0.6	2.7 ± 0.9	39.2 ± 6.6	31.0 ± 4.1
S7	0.7 ± 0.1	0.7 ± 0.1	4.4 ± 0.8	4.0 ± 0.7	0.8 ± 0.2	0.9 ± 0.2	28.3 ± 3.8	21.2 ± 3.0
S8	1.3 ± 0.4	1.6 ± 0.3	10.4 ± 2.1	7.2 ± 1.0	18.4 ± 4.4	18.1 ± 4.0	157 ± 26	150 ± 36
S9	2.0 ± 0.4	1.7 ± 0.3	6.8 ± 1.0	5.4 ± 0.8	15.0 ± 3.5	15.4 ± 4.6	114 ± 16	128 ± 25
S10	1.6 ± 0.4	1.6 ± 0.3	5.2 ± 0.9	6.4 ± 1.5	3.5 ± 0.7	4.1 ± 1.0	42.7 ± 6.5	46.9 ± 6.9
RSD (%)[c]	10–14	8–12	7–10	7–12	10–15	11–16	6–11	7–14
Sample	Mobile content of Cu Average ± U_{lab} (µg kg^{-1})[a]		Dissolved content of Cu Average ± U_{lab} (µg kg^{-1})[a]		Mobile content of Zn Average ± U_{lab} (µg kg^{-1})[a]		Dissolved content of Zn Average ± U_{lab} (µg kg^{-1})[a]	
S1	2.4 ± 0.6	1.9 ± 0.4	70.0 ± 14	51.4 ± 10.8	12.0 ± 2.6	14.9 ± 3.1	81.7 ± 10.5	51.4 ± 15.2
S2	5.4 ± 1.5	5.0 ± 1.2	75.3 ± 16.2	78.2 ± 16.9	24.3 ± 5.7	21.3 ± 4.9	25.2 ± 5.2	28.2 ± 9.2
S3	56.3 ± 13.5	81.7 ± 18.3	295 ± 49	345 ± 63	15.9 ± 4.5	19.5 ± 4.5	116 ± 18	120 ± 25
S4	7.4 ± 1.6	8.6 ± 2.1	76.4 ± 10.1	78.2 ± 15.8	21.9 ± 5.5	21.8 ± 4.1	27.3 ± 5.4	28.2 ± 5.3
S5	8.7 ± 2.4	7.9 ± 2.0	100 ± 22	67.2 ± 13.0	60.6 ± 12.2	70.2 ± 11.9	70.8 ± 10.1	67.2 ± 8.6
S6	15.5 ± 4.3	12.2 ± 2.7	85.8 ± 12.6	120 ± 23	31.1 ± 7.9	24.3 ± 5.4	38.2 ± 4.6	40.3 ± 4.1
S7	7.5 ± 2.1	6.4 ± 1.4	60.7 ± 10.7	67.6 ± 13.3	26.7 ± 6.9	30.4 ± 5.1	33.6 ± 4.1	37.6 ± 4.5
S8	20.1 ± 4.9	23.6 ± 6.2	166 ± 22	175 ± 25	9.4 ± 2.5	11.9 ± 2.8	282 ± 64	282 ± 57
S9	13.8 ± 3.1	12.1 ± 3.7	105 ± 23	107 ± 25	10.0 ± 2.6	13.2 ± 2.5	198 ± 24	197 ± 54
S10	22.3 ± 5.2	26.1 ± 6.8	84.6 ± 17.7	90.6 ± 21	19.7 ± 4.8	15.9 ± 4.0	92.6 ± 12.1	90.6 ± 11.5
RSD(%)[c]	11–14	11–15	7–11	7–12	10–15	11–16	6–11	5–16

[a] – U_{lab} represents the laboratory absolute extended uncertanty for the determined concentrations (k = 2, n = 3 parallel measurementss and 95% confidence level)

[b] – Concentration determined by calibration with standard addition

[c] - RSD represents the relative standard deviation calculated from the composed uncertainty (n = 3 parallel measurements and 95% confidence level)

5.10. Conclusions

According to the results obtained, the following conclusions can be listed:

1. For the first time, a simultaneous method for the determination of total and mobile content of Cd, Cu, Pb and Zn in soil was developed and analytically characterized, by the coupling of of DGT passive sampling with the SSETV-µCCP-OES miniaturized instrumentation;
2. Two aspects of the analytical methods based on microplasma optical emission spectrometry were solved, namely the improvement of the limits of detection with at least one order of magnitude, and the elimination of the non-spectral interferences of the multielemental matrix composed by alkali and alkaline-earth metals, respectively the elimination of the spectral interference of As on the most intense Cd line, which cannot be solved by low resolution microspectrometers, and the use of external calibration was possible, instead of standard addition;
3. Due to the separation of Cd, Cu, Pb and Zn from the matrix of the sample, the overcome of the non-spectral interferences was possible, and also the use of a higher vaporization temperature was possible, namely 1500 °C, necessary for the less volatile elements, such as Cu and Zn;
4. The improvement of LODs by the DGT accumulation allowed the determination of the mobile fraction of Cd, Cu, Pb and Zn with the highest toxicity towards plants;
5. The novel DGT-SSETV-µCCP-OES method was validated for the determination of the total and mobile fraction by analyses of CRM samples, and by comparison with DGT-GFAAS, by application of the statistical Tukey, Dunnet, Bland and Altman tests, which indicated the statistical similarity of the results;
6. Significant differences were observed between the results obtained by SSETV-µCCP-PES and GFAAS at the determination of the total dissolved content of metals. Further studies would be necessary in order to solve this issue, by the mineralization of the soil solution, for the oxidation of organic matter;
7. The passive accumulation by DGT on the Chelex-100 gel was not influenced by the matrix of the sample and therefore, the obtained diffusion coefficients were similar for different CRM samples;
8. The SSETV-µCCP-OES method coupled with the DGT technique for sampling was proved to be versatile and represents a research opportunity for the determination of other elements, such as As, Se, Sb or Hg, by the use of specific accumulation resins, such as the ones modified with ZrO_2, $(Fe)_2O_3$, TiO_2 or silicagel modified with 3-mercaptopropyl.

Chapter 6. Elements of Originality and Personal Contributions. General Conclusions

As a result of the PhD program, personal contributions with high scientific impact were made in the field of the development of microanalytical methods, based on a completely miniaturized instrumentation which used a capacitively couple plasma microtorch of low power and low Ar consumption and simultaneous detection by optical emission spectrometry with a low resolution microspectrometer. The obtained results show novelty elements and absolute originality in the internation community with concerns within the development of methods with high green and white degree, which became a critical field of research in the atomic spectrometry in the last years. The result and novelty elements and personal contributions are the following:

1. Four novel methods were developed and validated on the SSETV-μCCP-OES, as follows: (i) the method for the determination of total Hg and speciation of CH_3Hg^+ in food samples of marine origin, mushrooms and river sediments; (ii) the method for the simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn without derivatization in environmental samples, namely river sediments and cave sediments; (iii) the method for the determination of mobile fraction of Cd, Cu, Pb, Zn and Hg in surface waters (river) by the coupling of *in-situ* passive accumulation by DGT (Chelex-100 resin) with the *ex-situ* measurement by SSETV-μCCP-OES; (iv) the method for the determination of the total content and mobile fraction of Cd, Cu, Pb and Zn from soil, using the coupling of passive accumulation by DGT on Chelex-100 resin and determination by SSETV-μCCP-OES;
2. Improvements were made with regards to LODs, and the overcome of the non-spectral effects was realized due to the use of the following combined approaches: (i) separation by selective vaporization of the analyte volatile elements/species from a Rh filament; (ii) selective accumulation/preconcentration of the analyte elements by DGT technique on Chelex-100 resin; (iii) the use of the Maya 2000Pro microspectrometer;
3. It was demonstrated that a temperature of 1300 °C allowed an efficient but selective vaporization of total Hg, CH_3Hg^+, As, Bi, Sb, Se, Te, Hg, Pb and Sn, from the matrix, which also allowed a good excitation in the microplasma, by the efficient use of dissipated power for the atomization and simultaneous determination of the analytes;
4. The coupling with the DGT technique of the SSETV-μCCP-OES miniaturized analytical instrumentation improved the LODs for Cd, Cu, Pb, Zn and Hg with at least one order of magnitude for and accumulation time of 24 hours, in comparison with the LODs of the

instrumentation without DGT coupling, due to the relatively high preconcentration capacity on the Chelex-100 gel;

5. It was observed that the sensitivity and LODs of the SSETV-μCCP-OES instrumentation are influenced by the sensitivity and type of the microspectrometer;
6. The use of the Maya2000 Pro microspectrometer allowed the approach of the vacuum-UV spectral range, in the interval of 180-210 nm, where resonance and non-resonance lines sensivity for As, Bi, Sb, Se, and Te were identified. This discovery can be considered a novelty in the literature, taking into account that the vacuum-UV range is rather accessible to the performant ICP-OES instrumentation, and less to the miniaturized instrumentation with a low resolution microspectrometer.
7. It was observed that the low power (15 W) and reduced Ar consumption (150 mL min^{-1}) plasma was able to excite only the elements whose excitation energy of the spectral lines usually do not exceed 7 eV, which could represent a disadvantage at first sight, but in reality this presents an important advantage, because the simple emission spectrum of the elements allowed the use of a low resolution microspectrometer, ideal for the coupling with a microplasma in a miniaturized instrumentation;
8. Although the Maya2000 Pro microspectrometer, due to its low resolution, was not able to solve the spectral interferente of Cd 228.802 nm, overlapped with the emission line of As 228.812 nm, this spectral interference was still solved by the selective accumulation of Cd^{2+} ions on the Chelex-100 resin, which has an increased affinity toward the cations of divalent metals in comparison with arsenate. This discovery will be patented in the near future (patent application no: A00226/29.04.2024).
9. The developed methods on the SSETV-μCCP-OES instrumentation were characterized by high red, green, blue and white degree, as results of the reduced ragent consumption and reduced generated waste, DGT passive accumulation, *ex-situ* or *in-situ*, substantially improved LODs, especially in the case of DGT passive sampling, the completely miniaturized intrumentation, the simultaneous recording of the spectrum compared to the sequential mode in GFAAS, the low energy and Ar consumption for the support of the microplasma.
10. The green degree of the SSETV-μCCP-OES method for the determination of total Hg and CH_3Hg^+ results from the miniaturized instrumentation and the low consumption of utilities but also from the adjustment of the extraction and separation procedure, recommended by the European Committee, by the decrease of the necessary reagent

volumes, namely the use of only 5 mL HBr 47%, 2 mL toluene, and 2 mL L-cysteine 1% solution, in comparison with the consumption of 10 ml HBr, 20 mL toluene and 6 mL L-cysteine solution in the case of the procedure proposed by the European Committe for the determination of CH_3Hg^+ by TDAAS.

11. In the case of the determination of total Hg and speciation of CH_3Hg^+, the SSETV-µCCP-OES procedure, based on the extraction recommended by the European Committee in the HBr-toluene-L-cysteine system, was extended at other samples than the food samples and it was demonstarted the determination of total Hg and CH_3Hg^+ can be realised by using a single external calibration with solutions of Hg^{2+}.
12. Although the DGT passive sampling technique requires a longer time for preconcentration, in the case of the analytical methods developed within the PhD thesis, was at least 24 h, the coupling with the SSETV-µCCP-OES instrumentation was proved to be essential in the development of microanalytical methods with high sensitivity, without non-spectral and spectral interferences.
13. The research field initiated within the PhD thesis can be continued by the approach of some topics of speciation of inorganic As and Hg in the form of Hg^{2+} and CH_3Hg^+ in food samples using preconcentration on the silicagel gel modified with 3-mercaptopropyl, which has a high selectivity for the retention of inorganic As(III) species and Hg species, in comparison with the Chelex-100 gel used in this PhD thesis, which was proved to be effiecient solely for the divalent cations of Cd, Cu, Pb and Zn.
14. The result obtained in the PhD thesis were published in 4 ISI articles, as first author, with the sum of the impact factors of 20.20 and relative influence factors of 6.366. Between which, 3 were published in Q1 journals, after the impact or influence factor, and one article in Q2 journal, after the impact or influence factor. The results were presented at 8 conferences (3 national and 5 international, 4 oral presentations and 4 posters). An application for OSIM invention patent was submitted (patent application no: A00226/29.04.2024).

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List of Published Articles in ISI Journals during the PhD Program

No.	Article	Impact factor	Relative influence factor
1	**S. B., Angyus**, E., Darvasi, M., Ponta, D., Petreus, R., Etz, M., Senila, M., Frentiu și T., Frentiu Interference-free, green microanalytical method for total mercury and methylmercury determination in biological and environmental samples using small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometry *Talanta*, 2020, 217, 121067.	6.100 (Q1) (Place 11)	1.707 (Q1)
2	**S. B., Angyus**, E., Levei, D., Petreus, R., Etz, E., Covaci, O. T., Moldovan, M., Ponta, E., Darvasi și T., Frentiu Simultaneous determination of As, Bi, Sb, Se, Te, Hg, Pb and Sn by small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometry using direct liquid microsampling *Molecules*, 2021, 26, 2642.	4.600 (Q2)	1.452 (Q2)
3	**S. B., Angyus**, M., Senila, T., Frentiu, M., Ponta, M., Frentiu și E., Covaci In-situ Diffusive Gradients in thin-films passive sampling coupled with ex-situ small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometry as green and white method for the simultaneous determination of labile species of toxic elements in surface water *Talanta*, 2023, 259, 124551.	6.100 (Q1) (Place 10)	1.707 (Q1)
4	**S. B., Angyus**, M., Senila, E., Covaci, M., Ponta, M., Frentiu și T., Frentiu Simultaneous determination of Cd, Pb, Cu and Zn as total and labile fractions in soil using a small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometer after diffusive gradients in thin-film passive accumulation *Journal of Analytical Atomic Spectrometry*, 2024, 39, 141–152.	3.400 (Q1) (Place 8)	1,.00 (Q1) (Place 10)
	Sum of impact factorst and relative influence factors	20.20	6.366

OSIM patent application

No.	Patent application
1	M., Șenilă, T., Frențiu, D., Petreuș, E., Covaci, **S. B., Angyus**, M., Roman, M., Frențiu, O.A., Cadar, M., Ponta Procedeu de eliminare a interferentei spectrale a arsenului la determinarea cadmiului prin spectrometria de emisie optică în microplasme echipate cu microspectrometre de joasă rezoluție Patent application no: A00226/29.04.2024

List of Scientific Communications where Results of the PhD Thesis Were Disseminated

No.	Conferences	Dissemination
1	**S. B.**, **Angyus,** T., Frențiu Determinarea mercurului din probe alimentare și de mediu utilizând spectrometria de emisie optică în microplasmă cuplată capacitiv și vaporizare electrotermică (ETV-μCCP-OES) *Conferința Națională a Școlilor Doctorale din Consorțiul UNIVERSITARIA* – Ediția a II-A, 11 – 14.11.2019, Timișoara.	Oral presentation
2	**S. B.**, **Angyus,** E., Covaci, T., Frentiu Use of small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometry for direct liquid microsampling and determination of vapor generating elements in environmental samples *Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE III)*, 04 – 05.06.2021 – Cluj Napoca.	Oral presentation
3	E., Covaci, Z., Sandor, **S. B.**, **Angyus,** M., Senila, T., Frentiu Optical emission spectrometry by electrothermal vaporization in a capacitively coupled plasma microtorch as a specific detector in simultaneous multielemental passive sampling by diffusive gradients in thin films (DGT-SSETV-μCCP-OES): Preliminary studies on the optimization of working parameters *Conferinta Nationala de Chimie, editia XXXVI, secțiunea Chimie anorganică, chimie-fizică și analitică*, 04–07.10.2022 Călimănești-Căciulata.	Oral presentation
4	Z., Sandor, E., Covaci, **S. B.**, **Angyus,** M., Senila, T., Frentiu Microanalytical method for monitoring of toxic elements in surface waters using passive sampling in diffusive gradients in thin films and detection by capacitively coupled plasma microtorch optical emission spectrometry with electrothermal vaporization (DGT-SSETV-μCCP-OES) *Conferinta Nationala de Chimie, editia XXXVI, secțiunea Chimie anorganică, chimie-fizică și analitică*, 04–07.10.2022 Călimănești-Căciulata.	Poster
5	**S. B.**, **Angyus,** M., Senila, E., Covaci, T., Frentiu, M., Frentiu Monitoring of toxic trace metals in river water using in-situ diffusive gradients in thin film passive sampling and small-sized electrothermal vaporization capacitively coupled plasma microtorch optical emission spectrometry *49th International Conference of SSCHE* 15 – 18.05 2023, Tatranské Matliare, Slovakia.	Poster
6	E., Covaci, **S. B.**, **Angyus,** M., Senila, M., Frentiu, T., Frentiu Evaluation of green and white degree of a method based on in-situ diffusive gradients in thin film passive sampling coupled with ex-situ microplasma optical emission spectrometry set-up for determination of toxic elements in river water *49th International Conference of SSCHE* 15 – 18.05 2023, Tatranské Matliare, Slovakia.	Poster
7	E., Covaci, **S. B.**, **Angyus,** M., Senila, M., Frentiu, T., Frentiu Elimination of spectral interference between Cd and As in their monitoring in water by using in-situ diffusive gradients in thin film passive sampling and detection by ex-situ microplasma optical emission spectrometry set-up equiped with a low-resolution microspectrometer *49th International Conference of SSCHE*, 15 – 18.05 2023, Tatranské Matliare, Slovakia.	Poster
8	**S. B.**, **Angyus,** M., Șenilă, E., Covaci, T., Frențiu Diffusive gradients in thin film and electrothermal vaporization capacitively coupled plasma optical emission spectroscopy method for the evaluation of bioavailable Cu, Zn, Cd and Pb fraction in agricultural soils *4th Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE IV)* 01-03.06.2023, Debrecen, Hungary.	Oral presentation

List of Publications during the PhD Studies which Did Not Present Contribution in the Elaboration of the Thesis

No.	Article	Impact factor	Relative influence factor
1	E., Covaci, **S. B., Angyus**, M., Senila, M., Ponta, E., Darvasi, M., Frentiu și T., Frentiu Eco-scale non-chromatographic method for mercury speciation in fish using formic acid extraction and UV–Vis photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry *Microchemical Journal,* 2018, 141, 155–162.	4.800 (Q2)	1.270
2	E., Covaci, M., Senila, C., Tanaselia, **S. B., Angyus**, M., Ponta, E., Darvasi, M., Frentiu și T., Frentiu A highly sensitive eco-scale method for mercury determination in water and food using photochemical vapor generation and miniaturized instrumentation for capacitively coupled plasma microtorch optical emission spectrometry *Journal of Analytical Atomic Spectrometry*, 2018, 33, 799–808.	3.400 (Q1)	1.500
3	M., Senila, O., Cadar, L., Senila, A., Bezce, M., Roman, **S. B., Angyus** și G., Bruj A straightforward method for determination of Ba and Sr total content in natural zeolites based on microwave-assisted digestion and inductively coupled plasma optical emission spectrometry *Studia UBB Chemia*, 2021, 66, 105–116.	0.300 (Q4)	0.108
4	A. I., Torok, C., Tanaselia, A., Molovan, **S. B., Angyus**, E. A., Levei și C., Roman Lead isotopic ratio determination in cave sediments using triple-quadrupole inductively coupled plasma mass spectrometry *Studia UBB Chemia*, 2021, 66, 23–31.	0.300 (Q4)	0.108
5	A. I., Torok, E. A., Levei, S., Constantin, O. T., Moldovan, M., Senila, O., Cadar, D., Casoni, **S. B., Angyus**, C., Tanaselia, E., Covaci și T., Frentiu Application of Inductively Coupled Plasma Spectrometric Techniques and Multivariate Statistical Analysis in the Hydrogeochemical Profiling of Caves—Case Study Cloșani, Romania *Molecules*, 2021, 26, 6788.	4.600 (Q3)	1.270
6	M., Senila, O., Cadar, L., Senila și **S. B., Angyus** Simulated Bioavailability of Heavy Metals (Cd, Cr, Cu, Pb, Zn) in Contaminated Soil Amended with Natural Zeolite Using Diffusive Gradients in Thin-Films (DGT) Technique *Agriculture*, 2022, 12, 321.	3.600 (Q2)	1.491
7	I., Nesterovschi, I., Marica, E. A., Levei, **S. B., Angyus**, M., Kenesz, O., T. Moldovan și S. C., Pînzaru Subterranean transport of microplastics as evidenced in karst springs and their characterization using Raman spectroscopy *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2023, 298, 122811.	4.400 (Q2)	1.226
8	M., Senila, E. A., Levei, T., Frentiu, C., Mihali și **S. B., Angyus** Assessment of mercury bioavailability in garden soils around a former nonferrous metal mining area using DGT, accumulation in vegetables, and implications for health risk *Environmental and Monitoring Assessment*, 2023, 195, 1554.	3.000 (Q3)	0.712