Extension of the applicability range of the single-element and multielement SSETV-μCCP-OES methods with and without *ex-situ/on-site* μ-SPE at TRL4 level through application to real food and environmental samples (A2)

(Activity 2.3, CO-UBB, P-FI)

In the first stage of the project, an oxygen flask combustion (OFC) assisted sample digestion procedure was optimized, as well as an *on-site* procedure for the processing of river water by μ-SPE preconcentration on a dithizone-functionalized C18 cartridge, for the determination of the toxic elements from food and environmental samples (river water) by coupling with the SSETV-μCCP-OES method. The OFC-SSETV-μCCP-OES and (μ-SPE)-SSETV-μCCP-OES methods were then validated through LODs, accuracy (recovery and precision) by the analysis of food and water CRM samples and by comparison with the traditional ICP-OES and GFAAS methods used in chemical analysis and food and environmental quality control laboratories. In this stage, the results obtained following the extension of the applicability of the SSETV-μCCP-OES method to food analysis through sample combustion, to the *on-site* processing of river waters by μ-SPE preconcentration and elution, and through the reduction of the As-Cd spectral interference in samples with high As content, are presented.

The results obtained for the determination of the elements by OFC-SSETV-μCCP-OES in fish tissue, mushrooms and dietary supplements are presented in Tables 1 and 2 (Mot *et al. Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d). In the case of the dietary supplements, the concentration found for the essential elements (Cu, Zn and Se) was compared with the target values declared on the label. The concentration of the toxic elements (Cd, Pb, Hg and As) was below the method LOD. The results indicated a precision of 4.9–14.5% for the (OFC)-SSETV-μCCP-OES method on the basis of the combined uncertainty, which took into account the errors in the preparation of the calibration standards and of the samples, the calibration curve fitting and the analysis of the aliquots. The z scores calculated in accordance with the European Guide (Selection, Use and Interpretation of Proficiency Testing (PT) Schemes, ed. B. Brookman and I. Mann, 3rd edn., 2021, EPT_2021_P1_EN.pdf) are smaller than 2, indicating reliable results.

The results obtained for the determination of Cd, Pb, Cu and Zn from river water by ((μ-SPE)-SSETV-μCCP-OES) after *on-site* μ-SPE preconcentration from 200 mL of water (pH adjusted to 6.5 ± 0.2), at a flow rate of 10 mL min^{-1} and elution with 2 mL of 0.2 mol L^{-1} thiourea in 1 mol L^{-1} HNO_3 at 0.5 mL min^{-1} on a dithizone-functionalized C18 μ-SPE cartridge, compared to those obtained by GFAAS in the original samples without preconcentration, are presented in Table 3 (Covaci *et al.*, *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082). The precision estimated from the combined uncertainty, taking into account the individual errors associated with preconcentration and elution, the preparation of the standards and of the samples, the calibration curve fitting and the analysis of the aliquots for parallel sample processing and measurement (n = 3) was (%): 11–17 (Cd), 12–14 (Cu), 14–16 (Pb) and 12–15 (Zn). The largest contribution to the precision of the (μ-SPE)-SSETV-μCCP-OES method is associated with the *on-site* sample processing step. The paired t-test ($p > 0.05$) indicated the statistical validity of the results obtained by (μ-SPE)-SSETV-μCCP-OES versus GFAAS ($p = 0.348 – 0.611$), considering a concentration equal to half of the LODs of the GFAAS and (μ-SPE)-SSETV-μCCP-OES methods, for the samples in which the analysis could not be performed.

Table 1. Concentrations (mg kg^{-1}) in real fish muscle and mushroom samples obtained by (OFC)-SSETV-μCCP-OES with external calibration (Mot *et al. Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d)

Sample	Mean concentration ± U_{lab}[a] (mg kg^{-1})						
	Cu	Zn	Cd	Pb	Hg	Se	As
Fish tissue 1	3.51 ± 0.44	22.5 ± 3.2	< 0.01 (LOD)	< 0.07 (LOD)	< 0.01 (LOD)	< 1.20 (LOD)	< 1.00 (LOD)
Fish tissue 2	0.835 ± 0.125	8.00 ± 1.30	< 0.01 (LOD)	< 0.07 (LOD)	< 0.01 (LOD)	< 1.20 (LOD)	< 1.00 (LOD)
Mushroom 1	54.0 ± 11.5	118.4 ± 17.8	0.318 ± 0.044	0.423 ± 0.071	0.062 ± 0.018	< 3.96 (LOQ)	< 1.00 (LOD)
Mushroom 2	42.4 ± 7.5	129.8 ± 15.8	0.124 ± 0.019	0.794 ± 0.125	0.671 ± 0.157	< 3.96 (LOQ)	< 1.00 (LOD)
Mushroom 3	71.8 ± 7.0	116.0 ± 15.2	0.263 ± 0.057	< 0.231 (LOQ)	0.506 ± 0.073	< 3.96 (LOQ)	< 1.00 (LOD)
Mushroom 4	41.3 ± 9.2	85.8 ± 12.4	0.192 ± 0.024	0.345 ± 0.082	0.209 ± 0.042	< 3.96 (LOQ)	< 1.00 (LOD)
RSD (%)	4.9–11.1	6.1–8.1	6.3–10.8	7.9–11.9	7.2–14.5	-	-

[a] U_{lab} – is the expanded uncertainty in the laboratory (k = 2, 95% confidence level, n = 3 repeated measurements)

Table 2. Concentrations (mg/capsule) in dietary supplements obtained by (OFC)-SSETV-μCCP-OES with external calibration (Mot *et al. Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d)

Sample	Cu			Zn			Se		
	Declared value (mg/capsule)	Found value ± U_{lab}[a] (mg/capsule)	Recovery ± U_{lab}[a] (%)	Declared value (mg/capsule)	Found value ± U_{lab}[a] (mg/capsule)	Recovery ± U_{lab}[a] (%)	Declared value (μg/capsule)	Found value ± U_{lab}[a] (μg/capsule)	Recovery± U_{lab}[a] (%)
Supplement 1	0.500	0.490 ± 0.062	98 ± 13	5	4.64 ± 0.61	93 ± 13	45	49.1 ± 6.0	109 ± 12
Supplement 2	2	2.16 ± 0.30	108 ± 14	25	26.7 ± 3.3	107 ± 12	-	-	
Supplement 3	1.67	1.78 ± 0.28	107 ± 16	6.7	6.58 ± 0.69	98 ± 10	-	-	
Overall recovery ± U_{lab}[a] (%)			104 ± 14			99 ± 12			109 ± 12
Precision (%)			6.3–7.9			5.2–6.6			6.1
|z| score[b]		0.4–1.2		0.4–1.2				1.8	

[a] U_{lab} – is the expanded uncertainty in the laboratory (k = 2, 95% confidence level, n = 3 repeated measurements); [b] |*z*| score calculated in accordance with the European Guide (Selection, Use and Interpretation of Proficiency Testing (PT) Schemes, ed. B. Brookman and I. Mann, 3rd edn., 2021, EPT_2021_P1_EN.pdf)

Table 3. Results for Cd(II), Cu(II), Pb(II) and Zn(II) in river water samples obtained by (μ-SPE)-SSETV-μCCP-OES and GFAAS without preconcentration (Covaci *et al.*, *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082)

Sample	Cd Mean ± U_{lab}[a] (μg L^{-1})		Pb Mean ± U_{lab}[a] (μg L^{-1})		Cu Mean ± U_{lab}[a] (μg L^{-1})		Zn Mean ± U_{lab}[a] (μg L^{-1})	
	GFAAS [b]	(μ-SPE)-SSETV-μCCP-OES	GFAAS [b]	(μ-SPE)-SSETV-μCCP-OES	GFAAS [b]	(μ-SPE)-SSETV-μCCP-OES	GFAAS [b]	(μ-SPE)-SSETV-μCCP-OES
P1	0.17 ± 0.03	0.15 ± 0.04	< 0.6	0.22 ± 0.07	8.80 ± 1.23	9.36 ± 2.31	27.4 ± 3.5	25.2 ± 6.3
P2	< 0.12	0.020 ± 0.005	< 0.6	0.27 ± 0.08	2.21 ± 0.33	2.10 ± 0.55	10.1 ± 1.4	10.6 ± 3.0
P3	< 0.12	0.090 ± 0.020	< 0.6	0.14 ± 0.04	3.08 ± 0.40	3.00 ± 0.85	13.0 ± 2.1	14.3 ± 4.3
P4	0.48 ± 0.07	0.46 ± 0.12	2.59 ± 0.40	2.49 ± 0.70	2.74 ± 0.32	2.57 ± 0.68	7.25 ± 0.99	6.72 ± 1.67
P5	< 0.12	0.060 ± 0.020	< 0.6	< 0.0035	12.1 ± 1.6	11.0 ± 2.77	107 ± 11	116 ± 28
P6	0.49 ± 0.08	0.48 ± 0.14	< 0.6	0.54 ± 0.16	2.74 ± 0.29	2.91 ± 0.71	8.67 ± 1.47	9.39 ± 2.44

[a] U_{lab} – is the expanded uncertainty in the laboratory (k = 2, 95% confidence level, n = 3 parallel sample processing and measurements); [b] Measurements were carried out in the original water samples without preconcentration.

The dithizone-functionalized C18 cartridge can be reused for 7 days and exhibits a good stability against oxidation by oxygen under daylight conditions. The cartridge can be kept at 4 °C in the dark for 15 days. The portable assembly powered from a battery charged from a photovoltaic cell, used for the *on-site* processing of water samples by μ-SPE preconcentration on a dithizone-functionalized C18 cartridge, had an energy autonomy of 8 hours (the processing of 13 samples for 3 parallel field processings), while the miniaturized SSETV-μCCP-OES equipment with a microplasma source powered from the same battery had an autonomy of 5.5 h, a period in which 25 processed water samples can be analyzed (Covaci *et al.*, *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082).

An important issue observed within the WHIGREEN project regarding the miniaturized SSETV-μCCP-OES instrumentation, equipped with a low-power (15 W) and low-Ar consumption (150 mL min^{-1}) microplasma source, interfaced with a low-resolution microspectrometer (0.35 nm) such as the Maya2000 Pro used in the configuration, was the spectral interference of As 228.812 nm with the most sensitive line of Cd 228.802 nm, in fact the only one available for the determination of Cd. In order to overcome this limitation of the low-resolution instrumentation and to extend the analytical capability, a mathematical interference correction method was developed comprising the following steps: (i) determination of the As concentration on the basis of the calibration at the 189.042 nm line accessible in the vacuum ultraviolet range, since the spectrometer chamber is purged with 5.0 grade Ar; (ii) calculation of the As signal at 228.812 nm on the basis of the determined concentration and the calibration at this wavelength; (iii) correction of the sample signal at 228.802 nm by subtracting the As signal at 228.812 nm; (iv) calculation of the Cd concentration in the sample on the basis of the calibration at 228.802 nm. The calibrations are performed with single-element solutions of As and Cd without the sample matrix. Under these conditions, the simultaneous determination of As at 189.042 nm and Cd at 228.802 nm, the most sensitive line, is possible. The vaporization of the microsample takes place at a temperature of 1500 °C, which ensures the vaporization of the two elements. The method was validated through the analysis of CRM samples and applied to soil and water sediment samples with different As and Cd concentrations and different ratios. The results are presented in Tables 4 and 5 (Covaci *et al.*, *Analyst*, 2026, sent for publication).

The data in Table 4 indicate significant positive systematic errors for the CRM samples with high As/Cd concentration ratios (29–85), with recoveries between 124 and 182%, with a relative expanded uncertainty of ±(10–15)% (k = 2, 95% confidence level), which differ significantly ($p < 0.05$) from the certified concentrations. According to the Mann-Whitney test ($p < 0.05$), the As-Cd spectral interference led to Cd concentrations that differed significantly at this confidence level. The effectiveness of the mathematical method for the compensation of the As-Cd spectral interference in the SSETV-μCCP-OES analysis of the soil and sediment CRMs with high As/Cd concentration ratios was confirmed by the Dunnett statistical test, which indicated no significant differences ($p > 0.05$) between the values determined after mathematical correction and the certified ones. The recoveries after correction ranged from 94 to 105%, with a relative expanded uncertainty of ±(12–17)% (k = 2, 95% confidence level).

In contrast, for the CRM samples with As/Cd concentration ratios below 1 (0.4–1.12), the spectral interference of As at 228.812 nm did not significantly affect the accuracy of the Cd determination at 228.802 nm. The recoveries ranged from 98 to 106% before correction and from 98 to 105% after correction, with a relative expanded uncertainty of ±(9–12)%, indicating the absence of statistically significant differences between the determined and certified Cd concentrations according to the Mann-Whitney test ($p > 0.05$). The Dunnett test also confirmed the simultaneous and accurate determination of As at 189.042 nm and Cd at 228.802 nm using the mathematical compensation of the spectral interference. The recoveries ranged from 94 to 106% and did not differ significantly ($p > 0.05$) from the certified values for the analyzed CRM samples, with relative expanded uncertainties of ±(8–18)% (k = 2, 95% confidence level).

Table 4. Concentrations found and recoveries obtained for soil and water sediment CRMs, with and without mathematical correction of the As-Cd spectral interference at 228.802 nm in the simultaneous determination of As at 189.042 nm and Cd at 228.802 nm by SSETV-μCCP-OES using calibration based on matrix-free single-element solutions.

CRM	Certified concentration Mean ± U_{CRM}[a] (mg kg^{-1})		Found concentration Mean ± U_{lab}[b] (mg kg^{-1})			Recovery Mean ± U_{lab}[b] (%)		
	As	Cd	As	Cd Without correction	Cd With correction	As	Cd Without correction	Cd With correction
CRM048-050	104 ± 2.06	92.8 ± 1.55	106 ± 14.00	92.7 ± 10.70	91.8 ± 10.8	102 ± 13	100 ± 12	99 ± 12
CRM025-050	339 ± 20	369 ± 19	325 ± 45	390 ± 39	387 ± 41	96 ± 14	106 ± 10	105 ± 11
CC136a	21*	30*	22 ± 4	30 ± 3	30 ± 3.1	105 ± 18	100 ± 10	100 ± 10
SQC 001	43.1 ± 0.7	118 ± 2	42.5 ± 6.4	116 ± 10	116 ± 10	99 ± 15	98 ± 9	98 ± 12
Metranal 32	26.1 ± 1.1	0.28 ± 0.03	27.7 ± 3.3	0.51 ± 0.08	0.27 ± 0.05	106 ± 12	182 ± 16	96 ± 17
Metranal 34	42.4 ± 2.2	1.44 ± 0.07	41.5 ± 3.4	1.80 ± 0.20	1.41 ± 0.20	98 ± 8	125 ± 11	98 ± 14
BCR 280R	33.4 ± 2.9	0.85 ± 0.10	32.1 ± 5.1	1.17 ± 0.12	0.89 ± 0.13	96 ± 16	138 ± 10	105 ± 15
NCS DC 78301	56 ± 10	2.45 ± 0.30	59 ± 7	3.04 ± 0.49	2.53 ± 0.50	105 ± 12	124 ± 16	103 ± 17
GBW07424	8.9 ± 0.9	0.105 ± 0.013	8.4 ± 0.7	0.172 ± 0.017	0.099 ± 0.019	94 ± 8	164 ± 10	94 ± 12

[a] – U_{CRM} is the expanded uncertainty from the certificate (k = 2, 95% confidence level); [b] – U_{lab} is the expanded uncertainty in the laboratory for the found concentration in absolute units (k = 2; 95% confidence level for n = 5 parallel measurements); [c] – U_{lab} is the expanded uncertainty in the laboratory for the recovery in relative units (k = 2; 95% confidence level for n = 5 parallel measurements)

Table 5. Concentrations of As at 189.042 nm and Cd at 228.802 nm determined simultaneously in real soil and sediment samples by SSETV-μCCP-OES with mathematical correction of the As-Cd interference (Covaci et al., Analyst, 2026, sent for publication).

As (mg kg^{-1}) Mean ± U_{lab}[a] (mg kg^{-1})	Cd (mg kg^{-1}) Mean ± U_{lab}[a] (mg kg^{-1})		As/Cd report
	Without correction	With correction	
12.8 ± 1.4	0.900 ± 0.130	0.789 ± 0.130	16.2
18.9 ± 2.1	2.45 ± 0.33	2.28 ± 0.33	8.3
101 ± 12	14.1 ± 2.4	13.2 ± 2.4	7.7
547 ± 69	104 ± 19	99.2 ± 19	5.5
3.71 ± 0.35	1.43 ± 0.22	1.40 ± 0.22	2.7
17.9 ± 2.3	9.00 ± 1.60	8.83 ± 1.60	2.0
12.6 ± 1.4	7.86 ± 1.54	7.75 ± 1.54	1.6
8.23 ± 1.35	7.42 ± 0.92	7.35 ± 0.92	1.1
0.478 ± 0.085	5.07 ± 1.16	5.07 ± 1.16	0.1

[a] – U_{lab} is the expanded uncertainty in absolute concentration units (k = 2; 95% confidence level for n = 5 parallel measurements)

Table 5 presents the results obtained by SSETV-μCCP-OES for the simultaneous determination of As at 189.042 nm and Cd at 228.802 nm in real soil and water sediment samples, using the mathematical correction of the As-Cd spectral interference and the calibration based on matrix-free single-element solutions (Covaci *et al.*, *Analyst*, 2026, sent for publication). Arsenic concentrations in the range 0.478–547 mg kg^{-1} were determined with a precision of 4.7–8.9%, expressed as expanded uncertainty (k = 2, 95% confidence level). For the samples with an As concentration 5.5–8.3 times higher than that of Cd, a positive bias of the determined Cd concentration of 5–14% was observed, while for the samples with an As concentration up to 2.7 times higher than that of Cd, the positive bias for Cd was only 1.5–2.7%, which can be considered to fall within the error limits of the spectral method. These results indicate that the application of the mathematical correction is necessary for the samples in which the As concentration is more than 2 times higher than that of Cd, corresponding to the minimum concentration ratio previously established for an As concentration of 8.5 μg L^{-1}. The precision of the simultaneous determination of Cd in the presence of As, evaluated as expanded uncertainty (k = 2, 95% confidence level) after the application of the mathematical correction, was 5.5–8.9%. The Tukey test indicated statistically significant differences ($p < 0.05$) between the corrected and uncorrected Cd concentrations in the samples with As/Cd concentration ratios higher than 2.7.

Degree of achievement of the objective. The objective concerning the *Extension of the applicability range of the single-element and multielement SSETV-μCCP-OES methods with and without ex-situ/on-site μ-SPE at TRL4 level through application to real food and environmental samples* was achieved in accordance with the project implementation plan.

Results:

- Final report on the extension of the applicability range of the single-element and multielement SSETV-μCCP-OES methods with and without *ex-situ/on-site* μ-SPE at TRL4 level through application to real food and environmental samples
- 2 published ISI articles (Covaci *et al.*, *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082) with impact factor 6.7 and (Covaci *et al.*, *Analyst*, 2026, sent for publication) with impact factor 3.6
- 2 participations with 2 posters at the 52nd International Conference of the Slovak Society of Chemical Engineering – SSCHE 2026, Strbske Pleso, Slovakia, 26–29 May 2026 https://www.sschi.sk/en/event/ssche2026/