Assessment of the greenness and whiteness degree of the *ex-situ/on-site* (µ-SPE)-SSETV-µCCP-OES methods by the AGREEprep and RGB-12 metrics (A2)

(Activity 2.4, CO-UBB)

The greenness of the (OFC)-SSETV-µCCP-OES and (µ-SPE)-SSETV-µCCP-OES methods was evaluated by means of the AGREEprep metric, based on the 10 principles of green sample preparation (GSP), using the freely available software, in which, in an input data template, the analyst includes the 10 principles (numerical values and qualitative options) and the corresponding weighting factors in the range 1–5, assigned for each section (Wojnowski et al., *TrAC, Trends Anal. Chem*., 2022, 116553, https://doi.org/10.1016/j.trac.2022.116553). The generated red-green pictogram allows both the rapid visual and numerical assessment of the greenness of each step and of the method as a whole. Unfortunately, the AGREEprep metric does not take into account several very important criteria, such as the analytical performance (LODs and LOQs, precision, reproducibility, accuracy, etc.), the cost- and time-efficiency, the utilities (energy and gas consumption) and the operational simplicity of the analytical method, which are taken into account in the metrics for the assessment of the whiteness degree of an analytical method and of the sample preparation as a whole. The whiteness of the method was evaluated using the RGB-12 metric, which considers 12 criteria, four for each of the red, green and blue components, representing the balance between the performance indicators grouped under these three colors and ultimately determining the overall whiteness of the method. Therefore, the RGB-12 procedure is comprehensive and highlights the most representative performance criteria, which define the red, green and blue components of the method. The software used for this evaluation is also freely available (Nowak et al., *TrAC, Trends Anal. Chem*., 2021, https://doi.org/10.1016/j.trac.2021.116223). The green scores of the OFC and *on-site* µ-SPE sample preparation procedure, using AGREEprep, and the red, green and blue scores obtained by RGB-12 and the *ex-situ* analysis by SSETV-µCCP-OES, were compared with other extraction procedures and analyses by SPE methods based on ICP-OES/MS. The toxicity of the reagents, the total reagent consumption, the waste generated and the energy consumption per sample, the analytical performance (scope of application, LODs, LOQs, precision and accuracy) and the practical applicability aspects (cost-efficiency and time-efficiency), the requirements and the operational simplicity (miniaturization, automation, portability, operator skills, etc.) were taken into account in the comparative study.

The greenness and whiteness degree of the (OFC)-SSETV-µCCP-OES method compared to ICP-OES in the analysis of food samples is presented in Fig. 1 and 2 (Mot et al. *Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d).

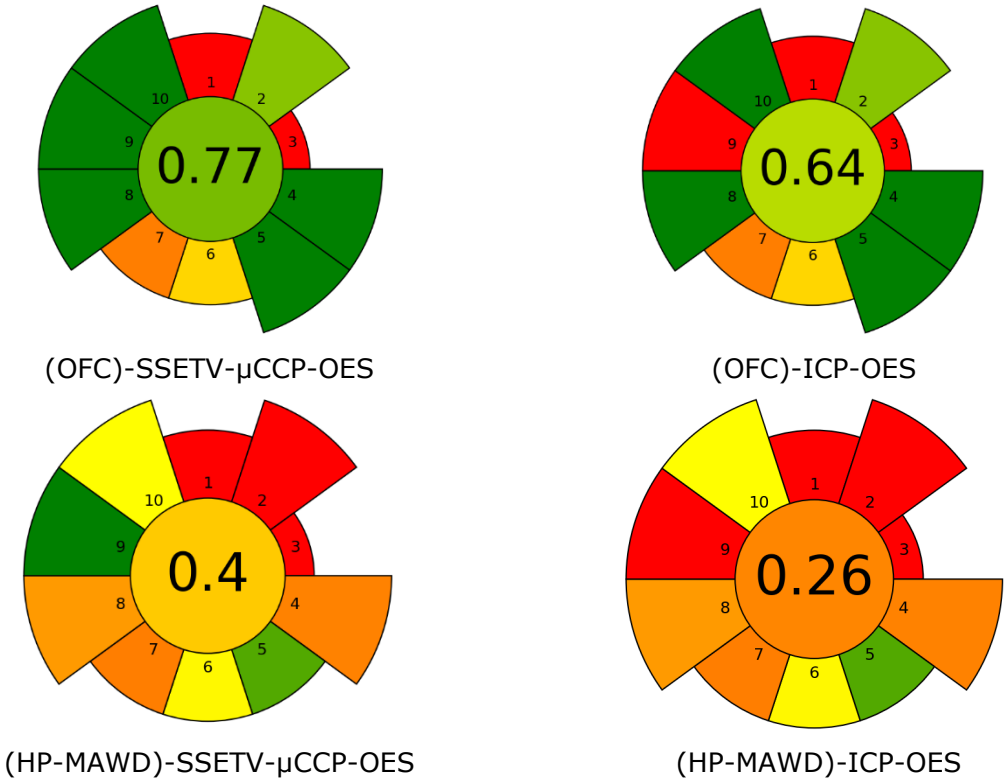
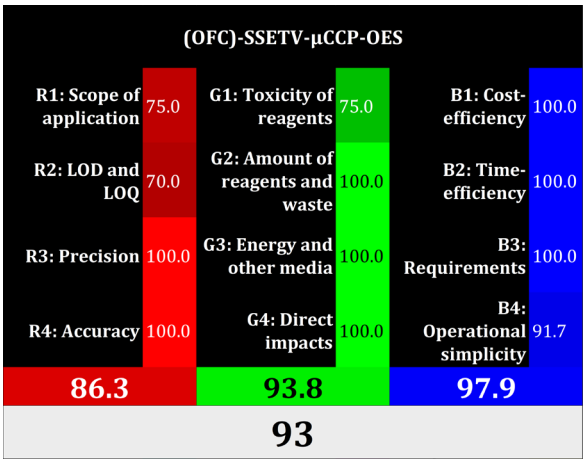
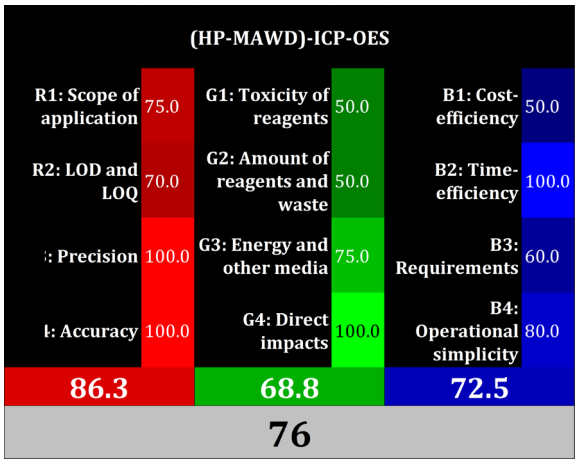


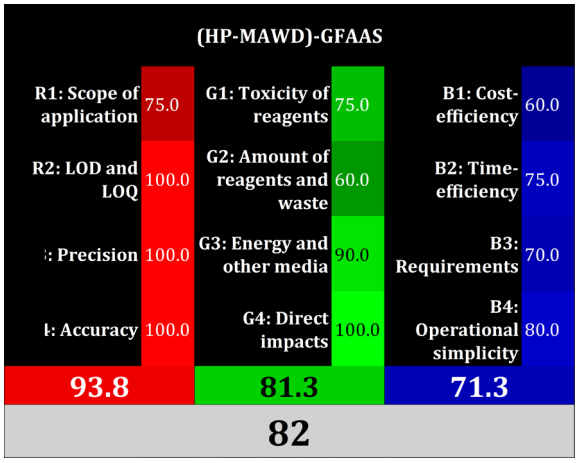
Fig. 1. Greenness degree of the (OFC)-SSETV-µCCP-OES method evaluated by the AGREEprep metric in comparison with the (OFC)-ICP-OES, (HP-MAWD)-SSETV-µCCP-OES and (HP-MAWD)-ICP-OES methods. (OFC)-SSETV-µCCP-OES – optical emission spectrometry in the capacitively coupled microplasma with electrothermal vaporization of the microsample and oxygen flask combustion assisted sample digestion; (HP-MAWD)-SSETV-µCCP-OES – optical emission spectrometry in the capacitively coupled microplasma with electrothermal vaporization of the microsample and high-pressure microwave-assisted sample digestion; (OFC)-ICP-OES – optical emission spectrometry in the inductively coupled plasma and oxygen flask combustion assisted sample digestion; (HP-MAWD)-ICP-OES – optical emission spectrometry in the inductively coupled plasma and high-pressure microwave-assisted sample digestion. (1. Sample preparation placement; 2. Hazardous materials; 3. Sustainability, renewability and reusability of materials; 4. Waste; 5. Size economy of the samples; 6. Sample throughput; 7. Integration and automation; 8. Energy consumption; 9. Post-sample preparation configuration for analysis; 10. Operator safety). (Mot et al. *Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d).



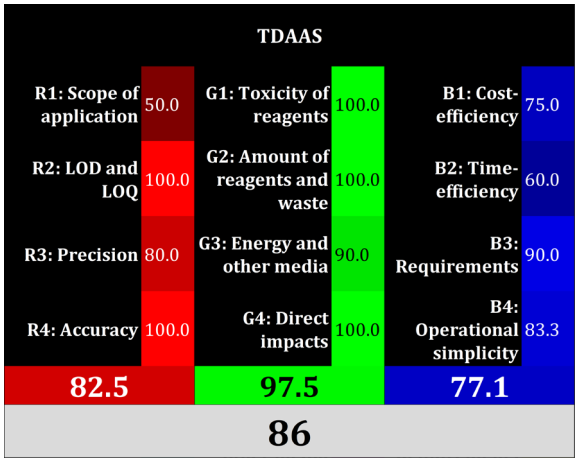
(OFC)-SSETV-µCCP-OES



(HP-MAWD)-ICP-OES



(HP-MAWD)-GFAAS

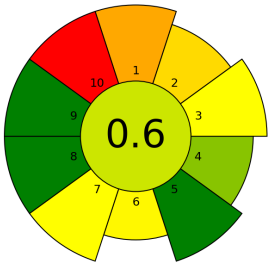


TDAAS

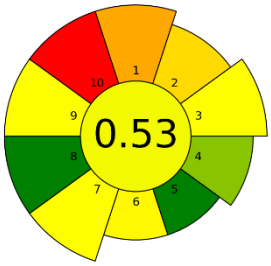
Fig. 2. Colors of the (OFC)-SSETV-µCCP-OES method evaluated by the RGB-12 algorithm in comparison with the (HP-MAWD)-ICP-OES, (HP-MAWD)-GFAAS and TDAAS methods. (OFC)-SSETV-µCCP-OES – optical emission spectrometry in the capacitively coupled microplasma with electrothermal vaporization of the microsample and oxygen flask combustion assisted sample digestion; (HP-MAWD)-ICP-OES – optical emission spectrometry in the inductively coupled plasma and high-pressure microwave-assisted sample digestion; (HP-MAWD)-GFAAS – graphite furnace atomic absorption spectrometry and high-pressure microwave-assisted sample digestion. (Mot et al. *Journal of Analytical Atomic Spectrometry*, 2026, https://doi.org/10.1039/d5ja00297d).

The red component of the whiteness degree was evaluated in relation to the intended application and the analytical performance, including LODs, precision and accuracy. The AGREEprep metric was used for the evaluation of the green component, taking into account the reagent toxicity, the amount of reagents and waste generated, the energy consumption during sample preparation and analysis and the operator safety. The blue component took into account the cost- and time-efficiency, the miniaturization of the instrument and the operational simplicity, the degree of automation, the *on-line* applicability, the portability and the potential for *on-site* measurements. The overall whiteness score was calculated as the average of the individual RGB scores. The green score of the (OFC)-SSETV-μCCP-OES method was evaluated at 77% and attributed both to the practically energy-free sample preparation by OFC (criterion 8) and to the use of simple miniaturized instrumentation based on a very low energy and Ar consumption for the generation of the microplasma and the multielement determination (criterion 9). In addition, the OFC preparation method demonstrates a high degree of greenness owing to the low sample consumption, the minimal waste generation and the increased operator safety (criteria 4, 5 and 10). The green score decreases to 64% when the analysis is performed using (OFC)-ICP-OES, owing to the complexity of the instrumentation and the high energy and Ar consumption of the plasma source. The green score decreases to 40% for (HP-MAWD)-SSETV-μCCP-OES, even though the analysis is performed on a simple microplasma-based instrument. This decrease is attributed to the significantly higher HNO_3 consumption, the larger sample volumes and waste generated, the high energy demand of HP-MAWD and the safety concerns arising from the potential risk of explosion of the digestion vessels (criteria 2, 4, 5, 8 and 10). The green score decreases to 26% when the sample preparation and analysis involve the (HP-MAWD)-ICP-OES method and chemical vapor generation for the determination of Hg, As and Se. The red score of the (OFC)-SSETV-μCCP-OES method is 86%, slightly lower than the 94% of the GFAAS method, owing to the poorer LODs associated with the microplasma source, despite its capacity for the simultaneous determination of several elements. The SSETV-μCCP-OES method provides a slightly higher red score owing to its multielement determination capacity, compared to TDAAS (red score of 82%), a method designed exclusively for the determination of Hg. The other performance parameters considered in the evaluation of the red score of the (OFC)-SSETV-μCCP-OES method, namely the precision and the recovery, were considered comparable to the traditional ICP-OES and GFAAS methods. The highest blue score (98%), obtained for the (OFC)-SSETV-μCCP-OES method, is attributed to the miniaturization and the low cost of the microplasma source and of the low-resolution microspectrometer, as well as to the relatively high speed of sample preparation by OFC and simultaneous analysis (minimum 6 samples/hour). The green score of 94% for the microplasma-based analytical method coupled with OFC sample preparation is higher than that of GFAAS (81%) and ICP-OES with chemical vapor generation (69%) and is consistent with the results obtained by the AGREEprep assessment presented above. Although TDAAS is used only for the determination of Hg, the method was evaluated at a green score of 98% in the RGB-12 algorithm, owing to its capacity to perform analyses directly on solids without reagent consumption. Overall, the whiteness score of the (OFC)-SSETV-μCCP-OES method, evaluated by the RGB-12 algorithm, was 93%, compared to 86%, 82% and 76% for TDAAS, GFAAS and ICP-OES, respectively.

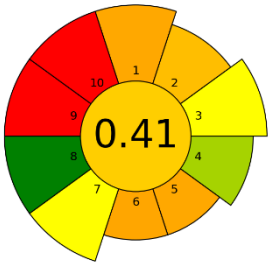
The greenness pictograms generated using the AGREEprep algorithm for the on-site μ-SPE sample processing and the (μ-SPE)-SSETV-μCCP-OES method, compared to the (μ-SPE)-GFAAS and (μ-SPE)-ICP-OES methods using the dithizone-functionalized C18 cartridge developed in our laboratory, are presented in Fig. 3 (Covaci et al., *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082).



(μ-SPE)-SSETV-μCCP-OES

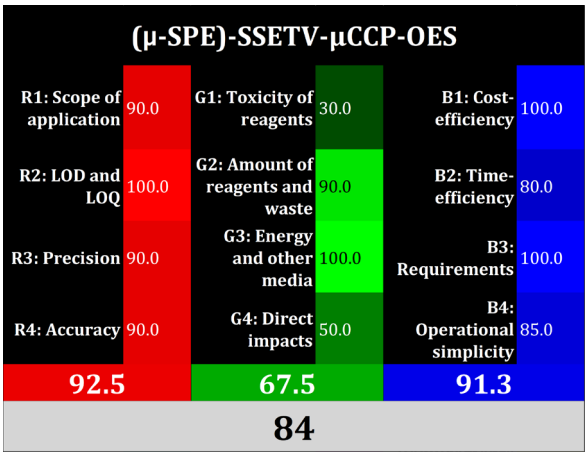


(μ-SPE)-GFAAS

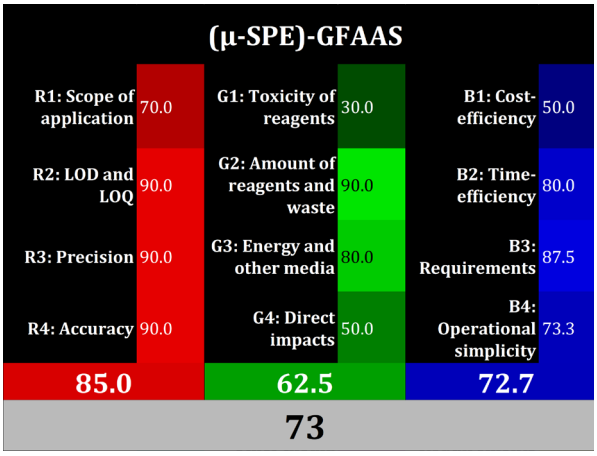


(μ-SPE)-ICP-OES

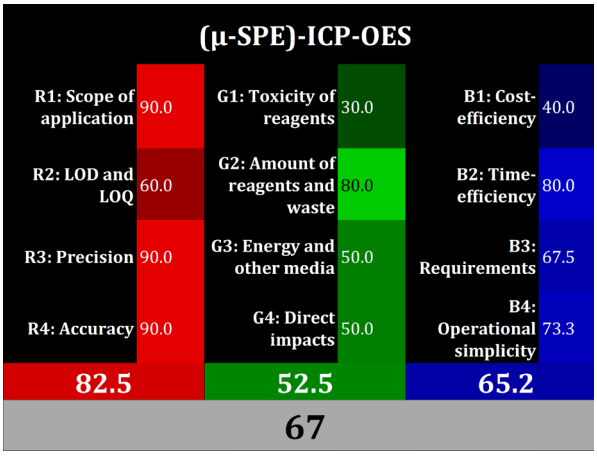
Fig. 3. AGREEprep pictograms obtained for the dithizone-functionalized C18 μ-SPE procedure for the *on-site* processing of river water samples and the analysis by SSETV-μCCP-OES, GFAAS and ICP-OES (1. Sample preparation placement; 2. Hazardous materials; 3. Sustainability, renewability and reusability of materials; 4. Waste; 5. Size economy of the samples; 6. Sample throughput; 7. Integration and automation; 8. Energy consumption; 9. Post-sample preparation configuration for analysis; 10. Operator safety). (Covaci et al., *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082)



(μ-SPE)-SSETV-μCCP-OES



(μ-SPE)-GFAAS



(μ-SPE)-ICP-OES

Fig. 4. Redness/greenness/blueness/whiteness degrees of the (μ-SPE)-SSETV-μCCP-OES, (μ-SPE)-GFAAS and (μ-SPE)-ICP-OES methods using *on-site* preparation of the river water samples and *ex-situ* analysis compared to GFAAS and ICP-OES. (Covaci et al., *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082)

The reagent consumption and the waste generated in the case of the (μ-SPE)-SSETV-μCCP-OES method were 0.521 g/sample, in comparison with the SPE methods based on ICP-OES/MS, which were in the range 0.467–2.981 g. Unfortunately, in the case of the (μ-SPE)-SSETV-μCCP-OES method the use of thiourea was necessary, although it is a sulfur-containing complexing reagent, relatively harmful for the operator safety compared to other complexing agents used in the ICP-OES/MS-based methods from the specialized literature. However, thiourea ensured an efficient elution for Cu(II) compared to elution in 1 mol L^{-1} HNO_3 without thiourea and provided a better protection against the oxidation of the dithizone immobilized on C18 during exposure to ambient light during use. Thus, the cartridge was more robust and could be used for a larger number of samples. All the methods considered as reference use HNO_3 or H_2SO_4 solutions at a concentration of 1 mol L^{-1}, but the amount used per sample is very small. On the basis of these considerations, as well as the qualitative and quantitative input data and their justification, the AGREEprep algorithm indicated a greenness score of 60% for the *on-site* μ-SPE river water sample processing procedure, followed by *ex-situ* SSETV-μCCP-OES analysis, in comparison with the scores of 53% for (μ-SPE)-GFAAS and 41% for (μ-SPE)-ICP-OES and 25–30% for the ICP-OES/MS-based methods. The higher greenness score of the (μ-SPE)-SSETV-μCCP-OES method was mainly attributed to the following criteria, considered in the AGREEprep algorithm: criterion 5 – size economy of the samples (the lowest sample volume, 0.01 mL); criterion 8 – energy consumption (energy-free operation owing to the on-site μ-SPE experimental configuration, powered by the photovoltaic-charged battery with sustainable energy autonomy); and criterion 9 – post-sample preparation configuration for analysis (use of the fully miniaturized SSETV-μCCP-OES, powered from the photovoltaic-charged battery, low Ar consumption for the generation of the microplasma (150 mL min^{-1}) and no special skills for the operator, compared to the more expensive and more complex laboratory instruments – ICP-OES or GFAAS); and criterion 4 – waste (low waste generation owing to the reduced reagent consumption). Criterion 3 – sustainability, renewability and reusability of materials – should also be taken into account in support of the overall greenness, owing to the reuse of the dithizone-functionalized C18 μ-SPE cartridges, although the reagents used are neither sustainable nor renewable. In addition, criterion 1 – sample preparation placement – contributes positively, since the procedure was implemented *on-site*. Unfortunately, criterion 10 – operator safety – represents a major limitation, which affects the greenness score of the method, owing to the use of thiourea, dithizone and HNO_3, associated with multiple hazard classifications (6 hazard statements). The greenness scores decreased to 53% and 41% when the analysis is performed in the laboratory using (μ-SPE)-GFAAS and (μ-SPE)-ICP-OES, even though the *on-site* μ-SPE river water sample preparation procedure was used. The lower greenness scores are mainly due to criterion 9 – post-sample preparation configuration for analysis (GFAAS and ICP-OES are bulky and expensive laboratory instruments, with a very high energy and Ar consumption). In the case of ICP-OES, criterion 5 related to the sample consumption is also significant, since the analysis requires at least 10 mL of sample for pneumatic nebulization, compared to only 10 μL for electrothermal vaporization in the microplasma system. The RGB-12 pictograms for the (μ-SPE)-SSETV-μCCP-OES method, in comparison with the reference methods GFAAS and μ-SPE based on ICP-OES/MS, are presented in Fig. 4. The (μ-SPE)-SSETV-μCCP-OES method exhibited redness/greenness/blueness degrees of 92/68/91%, resulting in an overall whiteness degree of 84%. The (μ-SPE)-GFAAS method exhibited redness/greenness/blueness/whiteness degrees of 85/62/73/73%, while the SPE methods based on ICP-OES/MS obtained scores of 79–91/34–61/61–66/59–70%.

The high redness degree (92%) (analytical performance) of the (μ-SPE)-SSETV-μCCP-OES method is mainly attributed to criterion R2 (substantial improvement of the LODs and LOQs through the high preconcentration factors of the analytes, the integration of the signal over the spectral line profile and a high flow rate of the microsample vaporized

into the plasma). The method meets the requirements of the intended purpose (R1) by allowing the simultaneous determination of the four elements in river water, as well as the criteria for precision (R3) and accuracy (R4). The greenness degree of the (μ-SPE)-SSETV-μCCP-OES method (68%) was mainly determined by criterion G3 (consumption of energy and other utilities), as shown previously. Even though SSETV-μCCP-OES was operated in the laboratory, the instrumentation proved to be efficiently energy autonomous, by being powered from a photovoltaic-charged battery. The weak point was attributed to criterion G1 – reagent toxicity (30%) and G4 – operator safety (50%). The blueness degree of the (μ-SPE)-SSETV-μCCP-OES method (91%) was determined in general by all four criteria, but in particular by B1 – cost-efficiency of the miniaturized instruments; B3 – requirements regarding a very low sample consumption and without requiring advanced technical expertise from the operator; and B4 – simple operation, through the fully miniaturized instrumentation, semi-automation and portability.

Degree of achievement of the objective. The objective *Assessment of the greenness and whiteness degree of the ex-situ/on-site developed methods (OFC)-SSETV-μCCP-OES and (μ-SPE)-SSETV-μCCP-OES by the AGREEprep and RGB-12 metrics* was achieved in accordance with the project implementation plan.

Results:

- Final report on the assessment of the greenness and whiteness degree of the *ex-situ/on-site* methods (OFC)-SSETV-μCCP-OES and (μ-SPE)-SSETV-μCCP-OES, single-element and multielement, with and without *ex-situ/on-site* μ-SPE
- 1 published ISI article (Covaci et al., *Talanta*, 2026, https://doi.org/10.1016/j.talanta.2026.130082) with impact factor 6.7
- 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/