UNIVERSITY OF BELGRADE
Technical Faculty in Bor

PROCEEDINGS

32nd International Conference

Ecological Truth & Environmental Research

24-26 June 2026

Editor
Prof. Dr Ana Radojević

EcoTER'26

Hotel Sunce, Sokobanja, Serbia

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PREFACE

The 32nd International Conference *Ecological Truth & Environmental Research – EcoTER'26* has been dedicated for more than three decades to presenting the latest scientific achievements, research findings, and innovative approaches in the fields of ecology, environmental protection, and sustainable development. In 2026, the conference was held in Sokobanja, Serbia, at Hotel Sunce, from 24 to 26 June.

The primary aim of the conference is to bring together leading experts, researchers, professionals, and young scientists from diverse fields in order to foster the exchange of knowledge, strengthen scientific collaboration, and promote innovative solutions to contemporary environmental challenges. By connecting science, education, industry, and society, EcoTER'26 seeks to inspire the transformation of attitudes and practices in everyday life, as well as within economic and industrial systems, contributing to a more sustainable future.

The 32nd International Conference *Ecological Truth & Environmental Research – EcoTER'26* is organized by the University of Belgrade, the Technical Faculty in Bor, with the co-organization of the University of Banja Luka, the Faculty of Technology; the University of Montenegro, the Faculty of Technology – Podgorica; the University of Zagreb, the Faculty of Metallurgy – Sisak; the University of Pristina, the Faculty of Technical Sciences – Kosovska Mitrovica; and the Association of Young Researchers – Bor (Serbia).

The Proceedings of EcoTER'26 include 98 scientific papers authored by researchers, academics, and industry representatives from universities, research institutions, and organizations across the following countries: Bosnia and Herzegovina, Bulgaria, the Czech Republic, Finland, Italy, Montenegro, North Macedonia, Pakistan, Romania, Slovenia, the Netherlands, the United Kingdom, the United States of America, Uzbekistan, and Serbia. It is our great honour and pleasure to welcome all participants and contributors and to express our sincere appreciation for their valuable scientific contributions.

Within this year's conference programme, the 7th Student Section – EcoTERS'26 was also organized. We highly value the contribution of students and their mentors, whose enthusiasm, creativity, and dedication represent an important foundation for the advancement of scientific knowledge. We encourage young researchers to continue exploring, questioning, and expanding the boundaries of knowledge, recognizing that education and scientific discovery are lifelong processes.

The support of our donors, together with their commitment, trust, and willingness to cooperate, has significantly contributed to the successful organization of the conference. The Organizing Committee expresses its deepest gratitude to the Platinum donors of the conference – Serbia Zijin Mining DOO Bor and Serbia Zijin Copper DOO Bor; the Gold donors – HBIS SERBIA, the Serbian Chamber of Engineers, and Elixir Prahovo; and the Silver donor – Rudex Explosives.

We extend our sincere appreciation to all authors who contributed to these Proceedings. Special recognition is given to the members of the Scientific, Organizing, and Honorary Committees, reviewers, invited speakers, session chairs, and all participants whose dedication and support have contributed to the success and continuity of EcoTER.

Our sincere gratitude goes to everyone who has contributed to the organization and delivery of this conference. Your efforts and collaboration continue to strengthen the mission of EcoTER and its role in promoting scientific excellence, environmental responsibility, and sustainable development.

Prof. Dr Ana Radojević

President of the Organizing Committee

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QUANTIFYING SAMPLING CONTRIBUTION TO MEASUREMENT UNCERTAINTY IN MICROBIOLOGICAL GROUNDWATER ANALYSIS

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Abstract

Sampling is often an underestimated source of measurement uncertainty in microbiological water quality assessment. This study quantifies the contribution of groundwater sampling to overall measurement uncertainty using a balanced split-level design and statistical approaches recommended by ISO 29201 (Annex H) and EURACHEM (Annex D). Groundwater samples were collected during 2023–2024 across different seasons following the EN ISO 19458 sampling protocol. Ten duplicate samples were taken under reproducible field conditions by two operators at the same location within a short time interval. Each sample and its replica were analyzed in duplicate for aerobic mesophilic bacteria (CFU·mL^{-1}) using Standard Method 9215B. Data were processed using range statistics (split-level design), classical one-way ANOVA, and robust ANOVA (RANOVA) after logarithmic transformation. Results showed that the relative standard uncertainty of sampling (23.35–24.45%) was almost twice the analytical uncertainty (12.53%) and approached the total measurement uncertainty (26.50%). The expanded sampling uncertainty (k=2.95% confidence) reached 46.7%, demonstrating that sampling is a dominant contributor to uncertainty in microbiological groundwater assessment. Although EN ISO 19458 showed acceptable performance (RSD < 30%), the results reveal that sampling uncertainty is frequently underestimated and should be routinely included in ISO/IEC 17025 uncertainty evaluations for microbiological groundwater analysis.

Keywords: groundwater sampling, measurement uncertainty, microbiological analysis, sampling uncertainty.

INTRODUCTION

A reliable evaluation of microbiological groundwater quality depends not only on laboratory analysis but also on the representativeness of the collected sample. Microbiological assessment of groundwater quality relies on the assumption that collected samples accurately represent *in situ* conditions. While laboratories routinely evaluate analytical uncertainty in accordance with ISO/IEC 17025, [1] the uncertainty introduced during sampling is rarely quantified in practice, despite clear requirements in ISO 29201 to address it [2].

Variability associated with groundwater sampling may arise from field conditions, operator influence, seasonal changes, and the applied sampling technique. Although EN ISO 19458 [3] provides detailed procedural guidance for microbiological water sampling, its performance is

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typically assumed rather than experimentally verified through estimation of sampling uncertainty under real field conditions.

The present study addresses this gap by experimentally evaluating the contribution of groundwater sampling to overall measurement uncertainty using a balanced split-level design and statistical approaches recommended by ISO 29201 and EURACHEM guidelines [2,4].

MATERIALS AND METHODS

Study design

A balanced split-level (duplicate) design recommended by ISO 29201 (Annex H) and EURACHEM guidelines was applied in this study [2,4]. Ten independent duplicate samples were collected by two operators at the same sampling location, within a short time interval and under identical field conditions. Each sample and its replica were transported to the laboratory and analyzed independently. The structure of this experimental design is presented in Figure 1.

In this approach, two independent field samples (A and B) were collected at the same location. Each sample was subsequently analyzed in duplicate in the laboratory (A1, A2 and B1, B2), enabling separation of analytical and sampling variability through statistical evaluation.

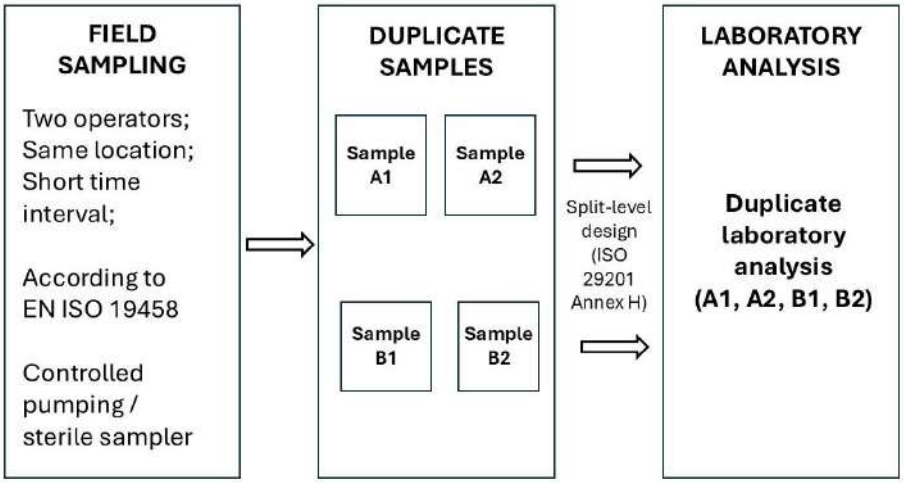

Figure 1 *Balanced split-level design for estimation of sampling and analytical uncertainty according to ISO 29201 Annex H*

Sampling procedure

Groundwater sampling was conducted in accordance with EN ISO 19458 [3]. Sterile 500 mL glass sampling bottles were used and protected from contamination until sampling. Depending on well conditions, sampling was performed either after controlled pumping through an installed outlet or using a sterile metal sampler for wells without permanent pumping systems. Extended pumping was applied when the objective was to characterize groundwater quality, ensuring stabilization of temperature and conductivity prior to sampling. Samples were transported to the laboratory in portable coolers for immediate analysis.

Microbiological analysis

Microbiological testing was performed using Standard Method 9215B for the determination of aerobic mesophilic bacteria (CFU·mL^{-1}) [5]. Each sample and its replica were analyzed in duplicate. Serial dilutions were prepared in phosphate dilution water and inoculated onto nutrient agar plates. Quality control of the method was ensured using reference strains (A. viridans ATCC 11563, B. spizizenii ATCC 6633, E. faecalis ATCC 29212, E. coli ATCC 25922, S. aureus ATCC 25923). Incubation was carried out under controlled conditions at 35 ± 0.5°C.

Verification approach for sampling uncertainty

Sampling uncertainty was verified using statistical approaches recommended by ISO 29201 Annex H and EURACHEM guidelines [2,4]. Data from duplicate sampling and duplicate laboratory analyses were processed after logarithmic transformation assuming lognormal distribution of microbiological counts.

Evaluation was performed using:

- range statistics within the split-level design,
- one-way ANOVA,
- robust ANOVA (RANOVA) using the Royal Society of Chemistry statistical tool [6].

Sampling standard deviation was derived using the split-level range statistics approach according to ISO 29201 Annex H [2] (Eq. 1):

$$S_{sampl}^2 = S_{meas}^2 - \left(\frac{S_{anal}}{\sqrt{2}}\right)^2 \quad (1)$$

Relative standard uncertainties (RSD, %) were calculated for analytical, sampling, and total measurement components. Expanded uncertainty was determined using a coverage factor k=2, corresponding to a 95% confidence level.

Control range charts (R-charts) were constructed during validation and internal quality control to monitor variability between duplicate results and to confirm the stability of the sampling process throughout the study, in accordance with ISO/IEC 17025 requirements [1]. Quality control included duplicate analyses performed under identical incubation conditions, using the same equipment and consumables to ensure reproducibility. R-charts were used to monitor variability between duplicate results in accordance with laboratory quality procedures and ISO/IEC 17025 requirements [1].

RESULTS AND DISCUSSION

The statistical evaluation of duplicate groundwater samples and their laboratory replicates enabled the separation of analytical, sampling, and total measurement uncertainty in microbiological analysis. Data were processed after logarithmic transformation using range statistics (split-level design), one-way ANOVA, and robust ANOVA (RANOVA), in accordance with ISO 29201 (Annex H) and EURACHEM recommendations [2,4].

The obtained relative standard uncertainties (RSD) showed clear differences between the analytical and sampling components, as summarized in Table 1. The relative analytical uncertainty was 12.53%, while the relative sampling uncertainty ranged between 23.35% and

24.45%, depending on the statistical approach applied. The relative standard uncertainty of the overall measurement was 26.50%.

These results demonstrate that the variability introduced during sampling is almost twice as large as the variability originating from laboratory analysis. Moreover, the sampling uncertainty approaches the total measurement uncertainty, indicating that sampling represents a dominant contributor to the variability of microbiological groundwater results.

The expanded sampling uncertainty (k=2.95% confidence level) reached 46.7%, as presented in Table 1, confirming that nearly half of the uncertainty associated with microbiological groundwater results originates from the sampling stage. This finding is particularly important in the context of ISO/IEC 17025 uncertainty evaluation, where laboratories often focus primarily on analytical sources of variability [1].

Comparison of classical ANOVA, robust ANOVA, and range statistics showed consistent results, confirming the reliability of the applied statistical model and the suitability of the balanced split-level design for evaluating sampling uncertainty under real field conditions [2,4,6]. The agreement between statistical approaches also validates the applicability of ISO 29201 Annex H for microbiological groundwater sampling [2].

The results further confirm that the application of the EN ISO 19458 sampling protocol under controlled conditions provides acceptable performance (RSD < 30%), in accordance with EURACHEM and NORDTEST recommendations for sampling quality [3,4,7]. However, despite proper adherence to the sampling standard, significant variability remains inherent to the sampling process due to field conditions, operator influence, and groundwater heterogeneity. These findings highlight that sampling cannot be considered a negligible step in microbiological groundwater assessment. Instead, it must be recognized as a major source of variability that should be experimentally quantified and routinely included in uncertainty evaluation [1,2].

Table 1 *Relative standard uncertainties of analytical, sampling and total measurement components in microbiological groundwater analysis*

Component	RSD (%)
Analytical uncertainty (Uanal)	12.53
Sampling uncertainty (Usampl)	23.35–24.45
Measurement uncertainty (Umeas)	26.50
Expanded sampling uncertainty (k=2)	46.7

CONCLUSION

This study demonstrates that sampling is a major contributor to overall uncertainty in microbiological groundwater analysis and cannot be treated as a minor or secondary step in the assessment process. By applying a balanced split-level design recommended by ISO 29201 Annex H, it was possible to experimentally separate analytical and sampling components of variability under real field conditions.

The results showed that sampling uncertainty was nearly twice as large as analytical uncertainty and approached the total measurement uncertainty, confirming that a significant

part of the variability in microbiological groundwater results originates from the sampling stage rather than from laboratory procedures.

Although the EN ISO 19458 protocol provided acceptable performance (RSD < 30%), the findings indicate that inherent field variability remains substantial even when the sampling procedure is properly implemented. These results emphasize the need to recognize sampling as a dominant source of uncertainty and to include experimentally determined sampling uncertainty in routine ISO/IEC 17025 uncertainty evaluations for microbiological groundwater analysis.

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