

From Science to Citizen Science: Assessing Microplastic Particle Counting in Water Samples

The outcome of three Erasmus Maris interlaboratory comparisons

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Abstract

The Joint Research Centre produced two well characterised microplastic (MP) Test Materials (TM) and coordinated interlaboratory comparisons (ILC) to support the evaluation of a harmonised analytical method for the determination of MP in water. This report presents the ring trial validation of the Erasmus Maris Standard Operating Procedure (SOP) for counting microplastic particles in water samples. This exercise involving trained teachers assessed recovery and precision using sea water (SW) and river water (RW) samples spiked with TM-062. For 'SW + TM-062' and 'RW + TM-062 + algae', recoveries of ca. 83 % were obtained, with precisions of ca. 18 %, demonstrating satisfactory performance.

An ILC with expert laboratories applying more sophisticated analytical methods showed good agreement with assigned values, confirming that results from the simplified SOP fall within the range of established approaches. The subsequent proficiency testing (PT) round with laboratories of the Technical Group on Marine Litter (TG-ML) demonstrated overall satisfactory performance despite methodological diversity.

The findings indicate that the Erasmus Maris SOP is fit for purpose for educational and screening applications. Variability is mainly linked to particle recovery during transfer and filtration and to visual counting. Continued quality assurance through refresher exercises for teachers and participation in PT rounds is recommended.

Acknowledgements

The thirty organisations listed below are kindly acknowledged for their participation in one of the interlaboratory comparisons (EM-RT-2025; EM-ILC-2025 or EM-PT-2025) described in this report. Thanks are extended to Georg Hanke for facilitating contacts with Member State expert laboratories associated with the Technical on Marine Litter (TG-ML), and to Ursula Vincent and Robert Koeber for their thorough review of the report and helpful suggestions.

Organisation	Country
Campus MAX STEM, Tessenderlo	Belgium
European School Brussels I	Belgium
European School Brussels II	Belgium
European School Mol	Belgium
Flanders Marine Institute (VLIZ), Ostende	Belgium
Institute of Natural Sciences, Sint Jozef Geel	Belgium
University of Gent	Belgium
Department of Ecoscience, Aarhus University	Denmark
Tallinn University of Technology	Estonia
The Finnish Environment Institute (SYKE), Helsinki	Finland
European School Frankfurt	Germany
European School Karlsruhe	Germany
European School Munich	Germany
University of Hamburg	Germany
Hellenic Centre for Marine Research (HCMR), Anavyssos	Greece
University of Patras	Greece
European School Varese	Italy
Institute for Environmental Protection and Research (ISPRA), Roma	Italy
Latvian Institute of Aquatic Ecology, Riga	Latvia
The Norwegian Institute for Water Research (NIVA), Oslo	Norway
Institute of Meteorology and Water Management (IMGW), Warsaw	Poland
National Institute for Marine Research and Development (RMRI), Constanta	Romania
National Institute of Chemistry (KI)	Slovenia
Centre for Studies of Ports and Coasts (CEPYC)	Spain
IES Joan Corominas, Benicarló	Spain
IES Ramón Cid, Castelló	Spain
IES Torre Del Rei, Orpesa	Spain
Instituto Español de Oceanografía (IEO-CSIC), Vigo	Spain
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Executive summary

The Joint Research Centre (JRC) organised and coordinated a series of interlaboratory comparison exercises to evaluate the performance of the Erasmus Maris Standard Operating Procedure (SOP) for the detection and counting of microplastic (MP) particles in water samples. For this purpose, the JRC produced and characterised two test materials based on polyethylene and polypropylene particles (JRC Test Materials TM-062 and TM-062bis). The materials - designed to mimic samples collected using manta nets - were prepared and verified for homogeneity and stability prior to distribution.

The overall objective was to assess the analytical performance of the simplified and low-cost SOP designed for educational and citizen science applications, to compare its performance with that of expert laboratories applying routine analytical procedures, and to benchmark it against a wider network of laboratories involved in monitoring of floating microlitter. This enabled the evaluation of its suitability for generating robust scientific data while remaining simple and accessible.

Ring trial validation study for trained teachers

A ring trial was conducted with trained teachers applying the Erasmus Maris SOP. Sea water (SW) and river water (RW) samples were spiked with TM-062, corresponding to an assigned value of 83 MP particles per sample. Performance was evaluated using ANOVA in accordance with ISO 5725-1:2023 to estimate recovery, repeatability (RSD_r) and reproducibility (RSD_R). For 'SW + TM-062' and 'RW + TM-062 + algae', recoveries of approximately 83 % were obtained, with RSD_r of ca. 14 % and RSD_R of ca. 18 %, demonstrating satisfactory precision. Unexpectedly, poorer results were observed for 'RW + TM-062', with lower recovery of ca. 75 % and higher variability (precision of ca. 30 %). Across datasets, a general tendency towards underestimation was observed. This behaviour is commonly reported in visual MP particle counting and is likely attributable to particle losses during transfer or filtration, and to partial retention on equipment surfaces.

Interlaboratory comparison with expert laboratories

An interlaboratory comparison (ILC) involving laboratories from the Erasmus Maris Scientific Alliance (EMSA) - with established expertise in MP analysis - was organised to benchmark the simplified SOP against well-established methodologies. Participating laboratories applied different analytical procedures, including different filtration materials, digestion steps, and spectroscopic identification techniques (ATR-FTIR, μ FTIR, Raman, LDIR). Despite these differences, the reported results showed good agreement with the assigned target values and fell within the range of values observed in the ring trial. This shows that the performance achieved using the simplified Erasmus Maris SOP is consistent with that obtained using more advanced laboratory approaches.

Proficiency testing round for Member State laboratories

A proficiency testing (PT) round was subsequently organised for Member State laboratories associated with the Technical Group on Marine Litter (TG-ML). Two samples were distributed: 'SW + TM-062' (containing ca. 83 MP particles), and 'SW + TM-062bis' (containing ca. 62 MP particles). Performance was expressed as percent deviation ($D\%$) in accordance with ISO 17043:2023 and ISO 13528:2022. All participating laboratories achieved satisfactory performance. Although dispersion was larger for the lower-level sample, the majority of results were in agreement with the

assigned or informative values, demonstrating adequate analytical capability despite methodological diversity.

Conclusions and recommendations

The results demonstrate that the Erasmus Maris SOP is fit for purpose for screening applications and educational use, providing satisfactory recovery and precision while remaining simple and accessible. The principal sources of variability are related to particle recovery during transfer or filtration and to visual counting of the particles. To ensure sustained quality, attention should be given to optimising transfer and filtration steps, harmonising counting criteria, and maintaining competence among trained teachers through periodic refresher exercises or participation in PT schemes.

Beyond method validation, the results also indicate the feasibility of integrating trained non-expert actors into a quality-assured monitoring framework. The approach shows potential for broader deployment across European educational institutions, supporting the generation of harmonised and comparable data. In this context, it may also contribute to improved coordination of monitoring activities and support regular measurements over time.

The experience gained through this work provides a structured basis for future harmonisation of MP monitoring approaches within European networks underpinning key Directives, such as the Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

1. Introduction

The Joint Research Centre (JRC), in collaboration with Ayam Sailing Europe (ASE), organised a series of interlaboratory comparisons (ILCs) under the Collaboration Agreement No. 500006 [1]. The ILCs were conducted as part of the Erasmus Maris citizen science initiative, which served as a catalyst for this work [2]. The exercise was coordinated by the Food and Feed Compliance Unit (JRC.F.5) and involved both expert laboratories and secondary school science teachers. Two microplastic (MP) test materials were prepared and characterised by the JRC Reference Materials Unit (JRC.F.6).

The objectives were twofold: (i) to ensure that citizen science monitoring activities conducted by secondary schools' science teachers across the EU generate reliable and fit-for-purpose data for the analysis of surface water samples collected using manta nets. This was achieved using a simplified, single-laboratory validated Standard Operating Procedure (SOP); and (ii) to strengthen quality assurance and quality control (QA/QC) approaches in support of the Marine Strategy Framework Directive (MSFD) [3] and the Water Framework Directive (WFD) [4]. This initiative also contributes to broader international efforts, including the Global Treaty on Plastic Pollution, by promoting harmonised and scientifically sound monitoring practices of floating MP particles.

The Erasmus Maris initiative

This initiative contributes to international efforts, including the Global Treaty on Plastic Pollution, by promoting harmonised and scientifically sound monitoring of floating microplastic (MP) particles [5]. It can also support EU-wide monitoring by complementing existing professional networks, particularly where approaches are not yet fully harmonised across Member States, and by increasing data coverage over time across the EU through a distributed and standardised framework.

Beyond method validation, the Erasmus Maris initiative represents a model for distributed environmental monitoring, integrating citizen science, education systems and expert laboratories within a quality-assured framework. By bridging education, research and policy, the approach enables the generation of comparable data at scale through harmonised protocols, while using schools as distributed monitoring nodes for long-term observation. It is also relevant in the context of the river-sea continuum, where monitoring under the Water Framework Directive (WFD) [4] and the Marine Strategy Framework Directive (MSFD) [3] currently operates in parallel, highlighting the need for more coordinated and harmonised approaches to microplastic monitoring across environmental compartments [5-7].

Three groups of participants were identified and invited to participate:

- Trained teachers from the European Schools (Schola Europaea) network and other national secondary schools applied the validated SOP to analyse MP particles in water samples. The results of this ring-trial (RT) validation study (EM-RT-2025) were used to establish the performance characteristics of the simplified analytical method.
- Expert laboratories from the Erasmus Maris Scientific Alliance (EMSA) analysed the same samples using their established protocols. Their results obtained in the dedicated interlaboratory comparison (EM-ILC-2025) were used to verify the accuracy and robustness of the simplified SOP.
- Expert laboratories from EU Member States linked to the Technical Group on Marine Litter (TG-ML), coordinated by the Ocean and Water Unit (JRC.D.2), analysed the samples using their routine methods. Their results were evaluated in a dedicated proficiency testing (PT) round (EM-PT-2025) to assess laboratory performance.

The three interlaboratory comparisons described in this report serve not only to assess method performance, but also to demonstrate the feasibility of this integrated approach, in line with broader EU efforts to harness citizen science for environmental monitoring and policy [2,6,7].

2. Set up of the exercise

While this exercise was not conducted under formal accreditation, it was organised in accordance with recognised quality assurance principles, and the reported results were evaluated following appropriate statistical procedures. The framework applied for the organisation of the ILCs ensured that the identity of the participants and the information provided by them are treated as confidential. Each participant was assigned a unique laboratory code (Rxx for the RT, Lxx for the ILC, or Pxx for the PT), which is used throughout this report to maintain anonymity.

2.1. Time frame

All three interlaboratory exercises were announced by invitation emails sent on 6 March 2025, with a registration deadline of 31 March 2025 (Annexes 1.a–1.c). The EM-ILC-2025 exercise involved members of the EMSA, while the EM-PT-2025 round targeted Member State laboratories associated with the Technical Group on Marine Litter (TG-ML). For the EM-ILC-2025 and EM-PT-2025 rounds, the test items were dispatched to participants by courier in April 2025, together with accompanying letters placed in the parcels (Annexes 3.a-b). Detailed instruction letters describing the analytical requirements and reporting procedures were subsequently sent by email (Annexes 2.a-b). Participants were requested to submit their results through an online reporting interface (Annexes 4.b-c) available from 23 April 2025, with a reporting deadline set for 31 May 2025 at 16:00. In contrast, the EM-RT-2025 ring-trial validation study involved trained teachers who performed the experiments during dedicated sessions organised in Burriana (Spain) on 16–17 September 2025 and in Geel (Belgium) on 8 October 2025. For this exercise, samples and relevant instructions were provided on site, and results were reported using the dedicated reporting interface (Annex 4.a).

2.2. Distribution

For the EM-ILC-2025 and EM-PT-2025 exercises, the test items were distributed by courier to the participating laboratories. Each participant received:

- Four bottles containing approximately 0.1 L of water sample spiked with MP particles, comprising two bottles for each of the two test items; and
- An accompanying letter placed in the parcel describing storage conditions and handling requirements (Annexes 3.a and 3.b).

Participants were instructed to store the bottles at 4 °C in the dark (e.g. in a refrigerator) upon receipt and to keep them under these conditions until analysis.

For the EM-RT-2025 ring-trial validation study, similar water samples containing MP particles were distributed directly to the participating teachers at the experimental venues, where the experiments were carried out. The samples and the relevant instructions were provided on site.

2.3. Instructions to participants

Detailed instructions were provided to participants in the “Instruction letter” distributed to the laboratories (Annexes 2.a and 2.b). Participants were requested to apply their routine analytical procedures used for the monitoring of floating micro-litter in water samples. For each test item, they were asked to identify the type(s) of polymers present in the MP particles and to report the total number of MP particles per bottle within the specified size ranges.

Participants received an individual laboratory password, which was used to access the online reporting interface and to submit their measurement results together with a brief description of the analytical procedure applied. The reporting form also allowed participants to provide additional technical information related to the measurement conditions (Annex 4). Random laboratory codes were assigned and communicated to participants by email to ensure confidentiality of the reported results.

For the EM-RT-2025 ring-trial validation study, similar analytical instructions were provided on site during the experimental sessions, where the trained teachers performed the analyses using the standard operating procedure (SOP) introduced during the training activities (Annex 5).

3. The Erasmus Maris SOP for microplastic particle counting

The Erasmus Maris Standard Operating Procedure (SOP) for monitoring MP in marine and fresh waters (Annex 5) was developed within the RAISE-CS project [2] in collaboration with Ayam Sailing Europe (www.ayamsailing.eu), building on existing methodologies, and subsequently single-laboratory validated by the JRC under the Collaboration Agreement mentioned above [1]. The original protocol focuses on samples collected using manta nets and supports harmonised, reliable data collection by schools and research partners using standard laboratory equipment.

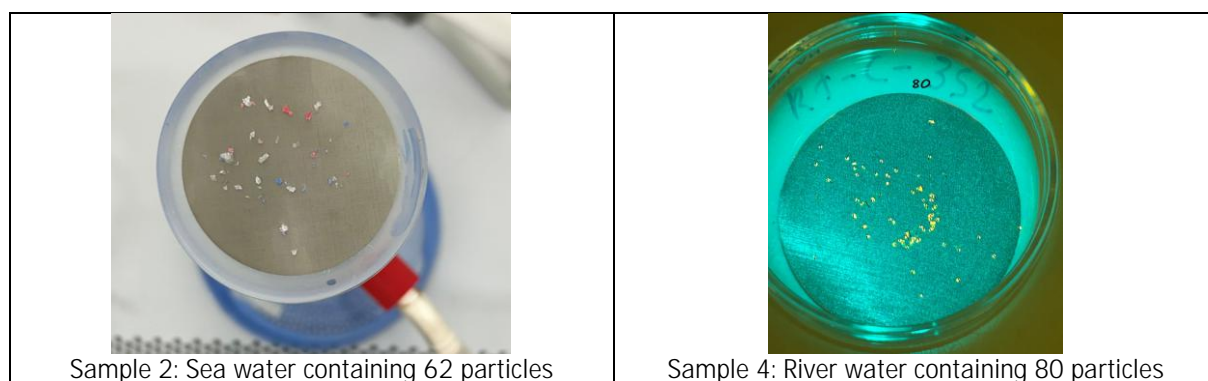
The SOP described below provides the workflow for sample preparation, density separation and particle counting, ensuring accurate and comparable results. While maintaining scientific rigour, the method has been adapted for use in upper secondary school settings and relies on commonly available equipment. The analytical procedure applied for the homogeneity and stability studies, as well as in the ring-trial validation performed by trained teachers, followed the original “Microplastics Counting SOP” (Steps 3–5). Steps 1 and 2 - covering, respectively, the preparation of manta net samples and the digestion of organic contaminants using commercial bleach at room temperature - were performed in advance by the JRC to allow sufficient time for digestion; hence, these steps were not within the scope of this study. Participants received ready-to-use test items consisting of microplastic particles suspended in seawater (SW) and/or fresh river water (RW), with or without added organic load, as detailed in Section 5.1.

3.1. SOP applied by trained teachers in the ring-trial

The ready-to-use suspension of MP particles in water (Samples 1–4) was transferred into a separation funnel and diluted/rinsed with NaCl solution (≈ 1.10 g/ml). After settling (5–15 min), the lower two-thirds of the liquid were discarded. A second density separation was then carried out using a 25 % m/m NaCl solution (density ≈ 1.2 g/ml), allowing mineral particles to sediment while MP remained in the supernatant. After settling, the lower fraction was again discarded and the remaining suspension was collected.

The retained fraction was filtered onto a 10 µm stainless steel filter (diameter 47 mm) using vacuum filtration. All particles were quantitatively transferred and the filter was thoroughly rinsed. Fluorescent staining with Nile Red was then performed, followed by imaging under Royal Blue light (450 nm) with a 500 nm long-pass filter. The entire filter was photographed with a smartphone, ensuring sharp focus and full coverage. Fluorescent particles were counted and results were reported in the provided data sheet. The photographs (Figure 1) were taken with a mobile phone camera (iPhone 13), and particles were counted on a computer screen using a photo editor.

Figure 1. Examples of filters containing MP particles illuminated with white (left) or Royal Blue (right) lights



Source: JRC

3.2. In-house validation

The method for counting MP particles ($> 300 \mu\text{m}$, $< 1 \text{ mm}$) in sea and fresh river water samples was validated using a nested experimental design deployed over two days, using two matrices (filtered SW and RW) and two independent operators, with 24 spiked samples prepared using JRC Test Material-062 (see Section 5.1). The single-laboratory validation performed by the JRC assessed repeatability, intermediate precision and trueness in line with predefined performance criteria [8]. Statistical evaluation (ANOVA) demonstrated no significant day or matrix effects, while operator variability contributed to the intermediate precision. The following satisfactory performance characteristics were obtained: an overall recovery of 90 %, corresponding to 74 particles recovered from 83 spiked, and a repeatability and intermediate precision relative standard deviations (RSD_r and RSD_{ip}) of 6.1 % and 11 %, respectively. These results meet the predefined acceptance criteria and confirm that the method is fit for purpose.

4. Test items

4.1. Preparation of the main ‘ingredients’

The JRC Test Material 062 (TM 062), used for spiking MP into water samples, was prepared from polyethylene (PE) and polypropylene (PP) by suspending the particles in a 10 % NaCl solution, followed by freeze drying, according to the procedure described by Seghers *et al.* [9].

The white and green PE starting materials were purchased from Alfa Aesar (Haverhill, US) and Novolab (Geraardsbergen, BE), while the blue and red PP starting materials were purchased from VWR (Radnor, US). Each portion of the starting material (10 g) was cryo-milled separately using a SPEX 6875 Freezer/Mill (Metuchen, NJ, USA).

Four different stock suspensions of each milled polymer material were prepared with 1 % Triton X 100 (m/m) and stirred for 15 minutes before sequential wet sieving through 500 μm and 300 μm stainless steel sieves. The particles retained on the 300 μm mesh were washed with tap water and ultrapure water (Milli-Q, ASTM Type 1). The fraction larger than 500 μm was discarded and the resulting fraction larger than 300 μm was resuspended. Wet sieving of the resuspended material was repeated twice to remove fine and coarse particles. Each stock suspension was then filtered over a 0.8 μm track etched polycarbonate 47 mm filter, rinsed with Milli-Q water and dried at 60 °C. To confirm the chemical identity of each material the remaining particles were characterised using micro-Fourier transform infrared (FTIR) spectroscopy.

The four particle types were combined in a suspension containing 10 % NaCl and 0.05 % Triton X 100 (m/m). The number of particles per mL was verified by pipetting a 5 mL aliquot, filtering over a black polycarbonate track etched membrane (Whatman Cyclopore), and counting the retained particles. Counting was performed by three operators, each in two replicates. No adjustment of the intended particle number concentration was required.

A total of 1200 amber glass vials (10 mL, Figure 2) were filled by pipetting 5 mL aliquots of the stock suspension under continuous stirring with a strong vortex. The pipet tip opening was selected to have a diameter at least three times the size of the largest particle in the suspension. Each aliquot was taken with the tip oriented towards the vortex flow. These measures were necessary to avoid size discrimination of larger particles and resulted in a sufficiently homogeneous material, with particles exhibiting a minimum Feret diameter ($\text{Feret}_{\text{min}}$) between 300 and 500 μm . After filling with the NaCl suspension, the contents of the vials were freeze-dried to remove water and immobilise the MP particles, resulting in MP particles embedded in a solid NaCl carrier.

The vials were sealed with aluminium caps and grey rubber inserts coated with a black Teflon layer, which is sufficiently hard to prevent contamination by additional MPs. If contamination were nevertheless to occur, black particles could be distinguished from the red, green, blue and white PE and PP particles.

Half of the vials (every second vial) were allocated to this project to constitute the JRC Test Material 062 (TM 062), while the remaining vials were retained for another JRC project.

Sixty litres of sand filtered seawater (SW) were collected via a pipeline by the Flanders Marine Institute (VLIZ) located on the North Sea coast in Ostend, Belgium. The water was further filtered over a 20 μm sieve and stored at +4 °C until use.

Forty litres of fresh river water (RW) were collected from a nearby river in Mol, Belgium. The water was further filtered over a 20 μm sieve and stored at +4 °C until use.

The organic load to be spiked consisted of algae collected from an infiltration basin at the JRC Geel site. The algae material was washed, then cut using a ceramic knife on a stainless-steel plate. No MP particles were detected in the material. The cleaned algae were then suspended in 3 L of filtered fresh river water and allowed to grow (Figure 2).

4.2. Background microplastic assessment

All water used for the preparation of the test materials was subjected to appropriate filtration prior to use. Ultrapure water was filtered through 0.1 μm and 0.22 μm cartridges, while the different environmental water matrices were passed through a 20 μm stainless steel mesh, which remains substantially smaller than the added MP particles (300 μm to 5 mm). Six blank samples per water matrix were analysed following digestion (where applicable), Nile Red staining and inspection under Royal Blue light. Across all matrices, no systematic presence of MP particles was observed. Only isolated single particles were observed in a few samples. These results demonstrate that background contamination by MP was negligible and confirm the quality of the ingredients used, ensuring that the integrity and quality of the final test items were not compromised.

4.3. Preparation of the test samples

Table 1 presents the four types of samples produced, namely:

- Sample 1: SW spiked with TM-062,
- Sample 2: SW spiked with TM-062bis (containing MP particles up to 5 mm),
- Sample 3: RW spiked with TM-062, and
- Sample 4: RW spiked with TM-062 and algae.

Samples (1, 3 or 4) were prepared by filling a 0.5 L borosilicate glass bottles with 100 mL of filtered water (SW or RW, as appropriate) and spiking with TM-062, which was quantitatively introduced by dissolving the NaCl carrier in filtered water (< 0.2 μm) and pouring the resulting suspension into the bottle. The vial, insert and neck were rinsed sequentially, and the rinsates were added to ensure complete recovery of the MP particles.

For Sample 4, the algae (organic load described above) were harvested using a 32 μm stainless-steel sieve, cut into pieces of approximately 10 mm, and re-suspended in water. An aliquot of 10 mL, containing 1.2 g (wet mass, corresponding to 60 mg dry matter) of the prepared algae suspension, was then added to the previously prepared Sample 3: RW + TM-062 (see Figure 2).

Unlike the other samples, which were prepared from freeze-dried material, Sample 2 was produced using a liquid suspension approach. Polypropylene particles (300 – 500 μm) and larger cryo-milled polyethylene particles (300 to 5000 μm) were combined in seawater pre-filtered through a 20 μm mesh and containing 0.05 % Triton-X 100. A 7 mL aliquot of the resulting suspension was added to 100 mL of SW to prepare the test items.

A total of 50 to 60 bottles equipped with blue polypropylene screw caps were produced per sample type. This was sufficient to cover the needs of the three interlaboratory comparisons, as well as the homogeneity and stability studies, including a limited number of spare samples for potential replacement requests.

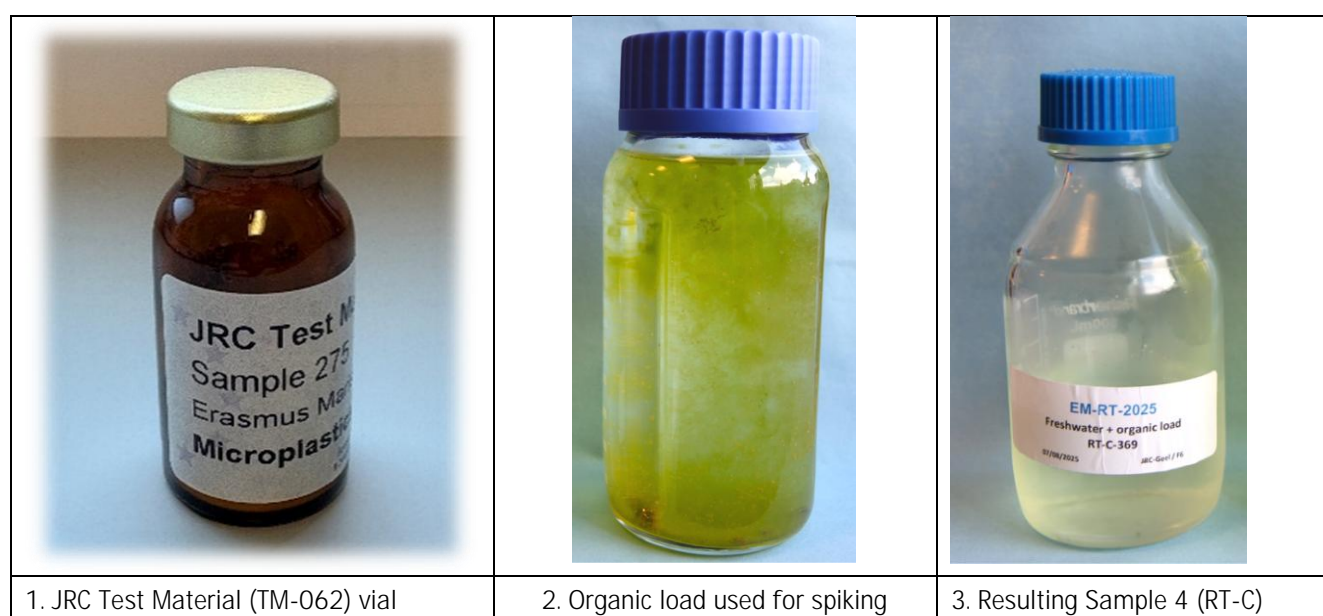
Table 1. Sample composition and allocation by participant type.

Sample	Water	TM	Algae	RT	ILC	PT	
S1	SW	062	No	RT-A	ILC-A	PT-A	
S2	SW	062bis	No			PT-B	
S3	RW	062	No	RT-B			
S4	RW	062	Yes	RT-C	ILC-B		
				<i>Participants</i>	<i>Trained teachers</i>	<i>EMSA</i>	<i>TG-ML labs</i>
				<i>Timing</i>	<i>Sep - Oct.</i>	<i>Apr. - Jul.</i>	<i>Apr. - Jul.</i>

SW: seawater; RW: river water; TM: test material; RT: ring trial; ILC: interlaboratory comparison; PT: proficiency test round; Apr., Jul., Sep., and Oct. = April, July, September and October.

Source: JRC

Figure 2. Ingredients used to produce Sample 4



Source: JRC

4.4. Transfer Efficiency of Microplastic Particles

The efficiency of transferring MP particles from the NaCl carrier (JRC TM-062) to the final test items was experimentally verified. Eight vials were processed according to the standard rinsing protocol. To identify any particles potentially lost during transfer, 1 mL of Nile Red solution was applied directly onto the salt cake to stain the MP particles immobilised within it. Following transfer and filtration, particles were examined under both Royal Blue light (440–460 nm, 500 nm long-pass filter) and white light, yielding consistent counts. In total, 681 particles were detected on the recipient filters. Only three particles were located elsewhere: two remaining in the original vials and one on the transfer funnel. This corresponds to a loss of 0.44 %, equivalent to a transfer efficiency of 99.56 %. The transfer can therefore be considered quantitative, and no correction for particle loss was required.

4.5. Homogeneity assessment of the test samples

Three out of the four sample batches (S1: SW + TM-062; S2: SW + TM-062bis; and S4: RW + TM-062 + Algae) were experimentally assessed at the JRC for between-bottle heterogeneity with respect to the number of MP particles. No homogeneity testing was performed for S3 (RW + TM-062), as it was considered comparable to S1 and, owing to its matrix composition, less complex than S4.

For each material, six to seven bottles were selected across the filling sequence and analysed by the JRC using an experimental protocol similar to the SOP described earlier. The pooled homogeneity study results are presented in Figure 3 as box plots with individual data points. The optical (manual) counting procedure used throughout this study had previously been validated to derive a repeatability relative standard deviation of 5.6 % [10].

Since the whole NaCl carrier content of each TM-062 vial had to be analysed at once, no replicate subsampling within a vial was possible, and classical ANOVA for the determination of the within- and between-bottle variance components could not be applied. Homogeneity was therefore assessed by subtracting the relative variance of method repeatability from the observed relative variance of the between-bottle results, in accordance with ISO 33405:2025.

The homogeneity assessment demonstrated consistent performance across the tested materials. For S1, an observed relative standard deviation of 10.9 % corresponded to a corrected relative between-bottle heterogeneity of 9.3 %. For S4 (stained with Nile Red), the corrected relative between-bottle heterogeneity was 6.3 %, indicating that a substantial proportion of the observed variability originated from counting repeatability rather than intrinsic material inhomogeneity. For S2, formal repeatability data for larger particles was not established; therefore, as a conservative estimate, the relative standard deviation of the seven analysed bottles (approximately 6 %) was used as an indicator of between-bottle heterogeneity. In addition, the mean values obtained for each sample type confirmed the assigned target values, as illustrated in Figure 3 by the horizontal reference lines (solid line: 83 MP particles for samples spiked with TM-062; dashed line: 62 MP particles for samples spiked with TM-062bis).

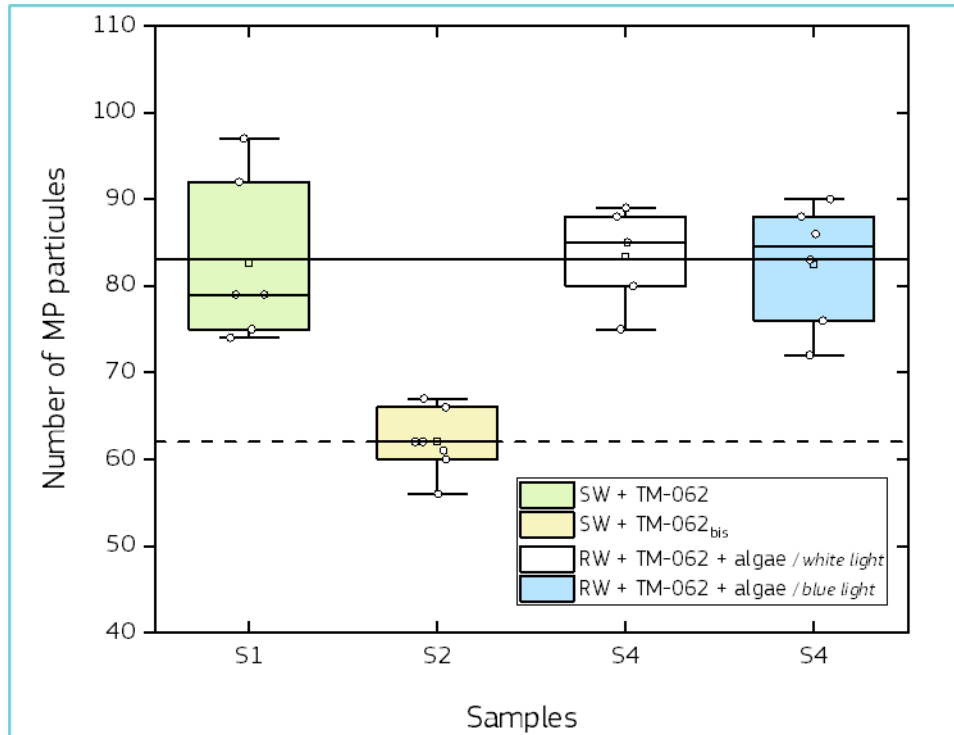
Overall, the pooled results presented in Figure 3 demonstrate sufficient homogeneity for tested materials S1, S2 and S4, with relative between-bottle heterogeneity values in the range of approximately 6 to 9 %. S3 is expected to exhibit comparable homogeneity to S1.

4.6. Stability assessment of the test samples

The ring-trial validation study with the trained teachers was conducted in September and October 2025, while the two other exercises (ILC and PT) were performed between May and July 2025, using samples prepared in April 2025.

To verify that the integrity of the test materials was maintained throughout these periods, stability was assessed by analysing selected units/bottles at the time of preparation and again at the end of the respective measurement period. The pooled stability results for S1, S2 and S4 - obtained by the JRC using the SOP described earlier - are presented in Figure 4.

Figure 3. Homogeneity results for samples S1, S2 and S4

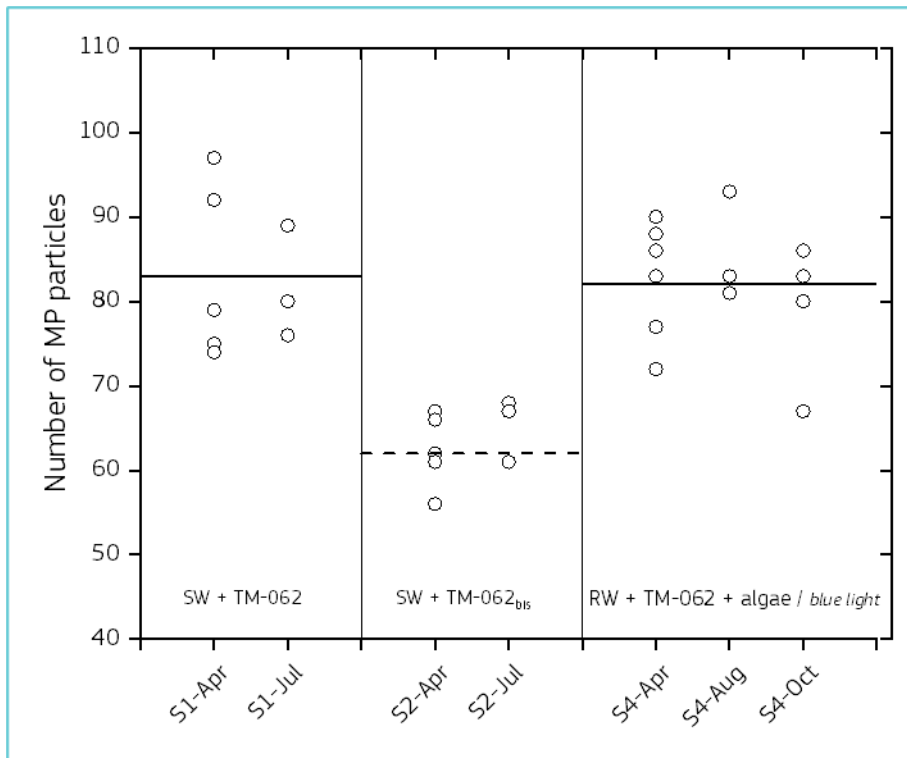


Source: JRC

No systematic trends or significant shifts in particle counts were observed over the investigated time periods for any of the materials (S1, S2, and S4). The dispersion of the data points is consistent with the previously established between-bottle heterogeneity of TM-062 and, in the case of S2, with the heterogeneity observed for TM-062_{bis}. The observed variability reflects the material heterogeneity and counting repeatability rather than instability. In addition, the mean values obtained for each sample type confirmed the assigned target values, as illustrated in Figure 4 by the horizontal reference lines (solid line: 83 MP particles for samples spiked with TM-062; dashed line: 62 MP particles for samples spiked with TM-062_{bis}).

These results confirm that the investigated samples are stable and that any uncertainty contribution from storage during this exercise is negligible. Owing to its lower matrix complexity, S3 is expected to be at least as stable as S4. Overall, all four sample types remained fit for purpose throughout the interlaboratory comparison, and instability did not contribute significantly to the measurement uncertainty.

Figure 4. Stability results for samples S1, S2 and S4 obtained after production and at the end of the measurement campaigns



Source: JRC

4.7. Assigned values

As described above, Samples 1, 3 and 4 were prepared by dissolving and reconstituting the NaCl carrier of TM-062 in 100 mL water. The number of MP particles in TM-062 was previously determined within another JRC project (*in progress*) as 83 MP particles per vial/bottle, with an associated expanded uncertainty below 15 %.

For Sample 2, prepared using TM-062bis, the assigned value was derived by pooling the results of the homogeneity and stability studies, yielding an indicative value of 62 MP particles per bottle, with an estimated standard uncertainty of approximately 25 %, considered to be conservative.

5. Results and discussion

5.1. Ring trial validation study

In the ring trial, all trained teachers analysed the test items using the Erasmus Maris SOP, presented in Annex 6. Participants analysed two bottles per sample and reported one result per bottle. The samples were spiked with JRC Test Material-062 (TM-062), for which an assigned value of 83 MP particles per bottle was established. This value is indicated by a horizontal line in Figure 5 and by a red dashed vertical line in the kernel density plots (Annex 6).

For each water sample, the reported results were evaluated using analysis of variance (ANOVA), according to ISO 5725-1:2023 [11], to estimate the recovery as well as the repeatability and reproducibility relative standard deviations (RSD_r and RSD_R). Where necessary, incomplete datasets or statistical outliers were excluded before calculation.

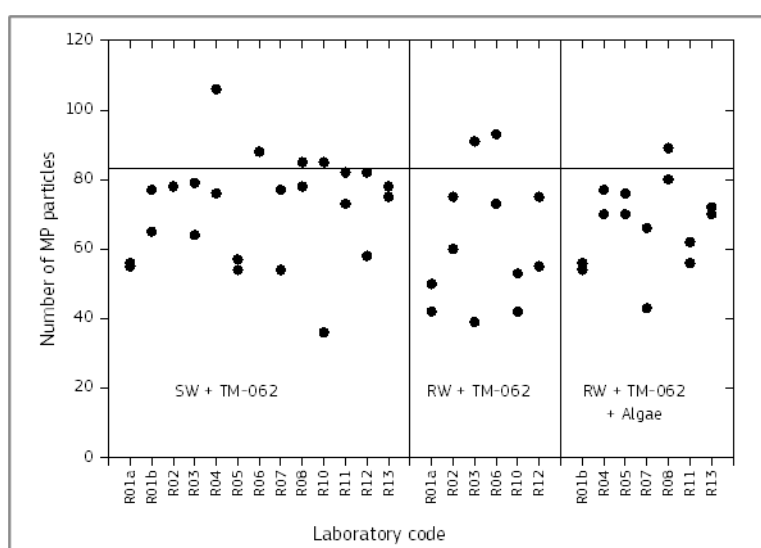
5.1.1. Sample S1: SW + TM-062

Thirteen trained teachers (including two participants from the same school, identified as R01a and R01b) submitted results for Sample S1. The results are presented in Annex 6.1.

The reported results show a moderate dispersion among participants. The kernel density plot suggests a bimodal distribution, indicating the presence of a subset of underestimated results compared with the assigned value. Two participants (R02 and R06) reported only one result because particles from one of the bottles were lost during the transfer and/or filtration step. In addition, the duplicate results submitted by participants R04 and R10 were identified as a Grubbs outlier, exhibiting a significantly larger standard deviation. Consequently, the corresponding datasets (R02, R04, R06 and R10) were excluded from the ANOVA calculation.

Based on the remaining (9x2) results, the estimated recovery was 84 %, with a repeatability relative standard deviation RSD_r of 14 % and a reproducibility relative standard deviation RSD_R of 17 %, indicating satisfactory performance when applying the common SOP.

Figure 5. Overview of the ring-trial validation study results



Source: JRC

5.1.2. Sample S3: RW + TM-062

Six trained teachers reported results for Sample S3, providing a total of twelve results (Annex 6.2). The reported results show a relatively large dispersion among participants and replicates. The kernel density distribution indicates that the largest mode lies below the assigned value, suggesting that a substantial proportion of the results underestimated the number of particles present in the samples.

All the reported results were retained for the statistical evaluation. The estimated recovery was 75 %, with RSD_r of 29 % and RSD_R of 30 %. The higher variability observed for this sample may reflect increased variability in particle recovery during filtration and transfer steps.

5.1.3. Sample S4: RW + TM-062 + Algae

Seven trained teachers reported results for Sample S4, resulting in fourteen results (Annex 6.3). The reported results show a moderate dispersion among participants. The kernel density distribution is unimodal, with the mode slightly below the assigned value, indicating a general tendency towards low (underestimated) results, likely due to losses of particles during sample preparation. Despite the increased matrix complexity due to the presence of algae, all datasets were retained for the statistical evaluation.

The estimated recovery was 81 %, with RSD_r of 11 % and RSD_R of 19 %. These values are comparable to those obtained for Sample S1, indicating that the presence of algae did not affect the robustness of the analytical procedure.

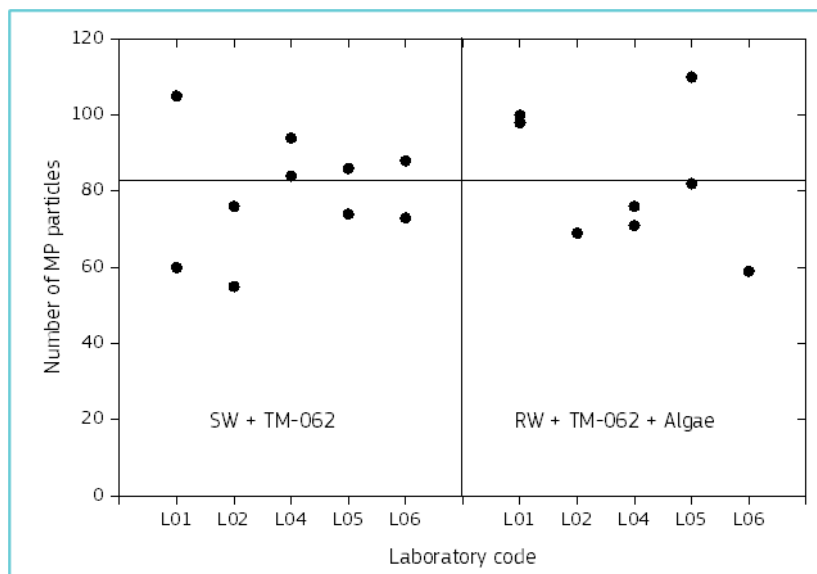
5.2. Interlaboratory comparison

As part of the overall evaluation of the Erasmus Maris method, an interlaboratory comparison (ILC) was organised within the Erasmus Maris Scientific Alliance (EMSA), involving laboratories with established expertise in MP analysis. The objective of this exercise was to compare the results obtained using the simple and low-cost Erasmus Maris method with those obtained by expert laboratories using their more advanced analytical methods. This comparison provides an indication of whether the results produced by the simplified method fall within the range obtained by expert laboratories.

The participating laboratories did not apply the Erasmus Maris method. Instead, they analysed the test items using their analytical methods. All six participating laboratories analysed the entire test item and the corresponding transfer rinse. None of the applied methods were accredited. Filtration was performed using different filter materials and pore sizes, including PTFE (10 μm), GF/A glass fibre (1.6 μm), platinum-coated polycarbonate membranes (30 μm) and steel filters (10 μm). Polymer identification was primarily based on FTIR spectroscopic techniques (μFTIR microscopy and ATR-FTIR), with spectral matching against reference libraries. Quantification approaches varied and included fluorescence microscopy following chemical digestion and Nile Red staining, multispectral imaging, and FTIR-assisted particle recognition combined with manual verification. Although the overall analytical strategy was comparable across laboratories, differences were observed in sample preparation, degree of automation and particle counting strategies. See Annex 9.a for details.

As in the ring trial, the samples analysed by the ESMA were spiked with JRC Test Material-062 (TM-062), corresponding to an assigned value of 83 MP particles per bottle. This value is indicated by the horizontal line in the scatter plots (Annex 7.1 and 7.2). The reported results presented in Figure 6 indicate overall consistency with the target level of 83 MP particles.

Figure 6. Overview of the results reported by the EMSA laboratories



Source: JRC

5.2.1. Sample 1: SW + TM-062

Five laboratories from the EMSA reported results for Sample 1, corresponding to ten individual measurements (Annex 7.1). Results ranged from 55 to 105 particles per bottle, with most values between 70 and 90 particles, with a few values falling below this range and one higher result above 100 particles.

The kernel density distribution exhibits a main mode close to the assigned value, with a minor mode around 60 particles. Nevertheless, the overall mean value corresponds to an observed recovery of approximately 96 %.

5.2.2. Sample 4: RW + TM-062 + Algae

Five laboratories reported results for Sample 4 (Annex 7.2). One laboratory (L02) reported only a single result due to particles lost during the filtration step, resulting in nine reported measurements. Results ranged from 59 to 110 particles per bottle, with most values between 70 and 100 particles, indicating a slightly larger dispersion compared with Sample 1.

The kernel density distribution shows a bimodal distribution, with the main mode slightly below the assigned value and another mode around 100 MP particles. Nevertheless, the mean reported value, corresponding to an average recovery of approximately 98 %.

Overall, the results obtained by the expert laboratories for the two samples fall within the same range as those observed during the ring trial, demonstrating consistency between the more advanced analytical methods and the simplified Erasmus Maris method.

5.2.3. Type of polymers identified

The polymer identification results reported by the EMSA laboratories show a high consistency across all samples. Polyethylene (PE) and polypropylene (PP) were identified in all cases, with no other polymers reported, confirming accurate characterisation of the test materials.

5.3. Proficiency testing round

A proficiency testing (PT) round was organised to assess the performance of Member State laboratories associated with the Technical Group on Marine Litter (TG-ML) in the determination of MP particles in seawater samples. The TG-ML group, coordinated by JRC D.2, contributes to the implementation of the Marine Strategy Framework Directive (MSFD, [3]). This exercise aims to support the harmonisation of measurement results and to demonstrate the application of quality assurance and quality control (QA/QC) procedures for the monitoring of floating microlitter, which represents only one component of the MSFD monitoring framework.

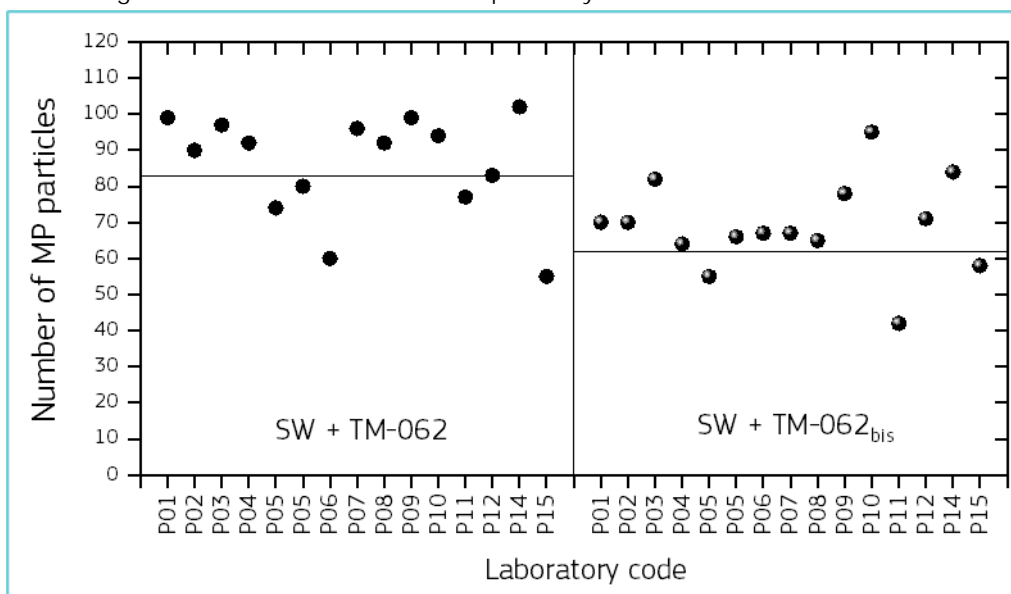
In contrast to the ring trial, participants were free to apply their routine analytical methods, thereby reflecting the diversity of approaches typically used in MP analysis laboratories. All laboratories analysed the entire sample, and almost all included the bottle rinse; none of the methods were accredited. Filtration was performed using a wide range of filter materials (metal meshes, stainless steel sieves, glass fibre, cellulose, polycarbonate and gold-coated membranes) with pore sizes spanning from sub-micrometre to several hundred micrometres. In several cases, chemical digestion and/or Nile Red staining were applied prior to analysis. Polymer identification was based on ATR-FTIR, μ FTIR, LDIR or Raman spectroscopy, often following visual or fluorescence-based pre-selection. Quantification was performed by manual stereomicroscopy, fluorescence microscopy or automated image analysis. Although the overall workflow (filtration, visual screening and spectroscopic confirmation) was comparable across laboratories, substantial variability was observed in sample preparation, size fraction considered and degree of automation. See Annex 9.b for details.

The PT evaluation focused on laboratory performance, expressed as the relative deviation ($D_{\%}$) from the assigned or informative values, calculated in accordance with ISO 17043:2023 [12] and ISO 13528:2022 [13].

Two samples were distributed. The first sample consisted of sea water spiked with JRC Test Material TM-062 (SW + TM-062), corresponding to an assigned value of 83 MP particles per bottle. The second sample consisted of SW spiked with TM-062bis, for which an informative value of 62 MP particles per bottle was derived from the homogeneity and stability studies. The assigned or informative values are indicated by the horizontal lines in the scatter plots.

The reported results summarised in Figure 7, while more details can be found in Annex 8.

Figure 7. Overview of the results reported by the Member State laboratories



Source: JRC

5.3.1. Sample 1: SW + TM-062

Fourteen laboratories reported results for Sample 1 (Annex 8.1). Laboratory P05 analysed both bottles provided and reported two independent results. Reported values range from 55 to 102 MP particles per bottle, indicating moderate inter-laboratory variability.

Most results were between 80 and 100 particles, close to the assigned value of 83 particles. The kernel density distribution (Annex 8.1) exhibits a main mode slightly above the assigned value, suggesting a tendency towards overestimation by several laboratories.

The calculated $D_{\%}$ values range from -34% to $+23\%$. Overall, the majority of results fall within $\pm 25\%$ of the assigned value, indicating a generally satisfactory level of performance among the participating laboratories.

5.3.2. Sample 2: SW + TM-062bis

The same laboratories reported results for Sample 2 (Annex 8.2). Again, Laboratory P05 analysed both bottles provided and reported two independent results. Reported values range from 42 to 95 MP particles per bottle, showing a broader dispersion compared with Sample 1, likely due to the lower number of particles present in the sample.

The kernel density distribution (Annex 8.2) exhibits a main mode close to the informative value of 62 MP particles, with results lying above and below this value. The calculated $D_{\%}$ values range from -32% to $+53\%$, indicating a wider spread of results compared with Sample 1. Despite this broader spread, most results remain reasonably close to the informative value, indicating an overall satisfactory level of performance in the detection and counting of MP particles in the samples.

5.3.3. Type of polymers identified

The polymer identification results reported by the Member State laboratories show greater variability compared to those of the EMSA laboratories. While most laboratories correctly identified polyethylene (PE) and polypropylene (PP) in both samples, several participants reported additional polymers, including polyethylene terephthalate (PET), polyamide (PA) and polystyrene (PS), whereas others did not report any polymer types.

6. Conclusions and Recommendations

Conclusions

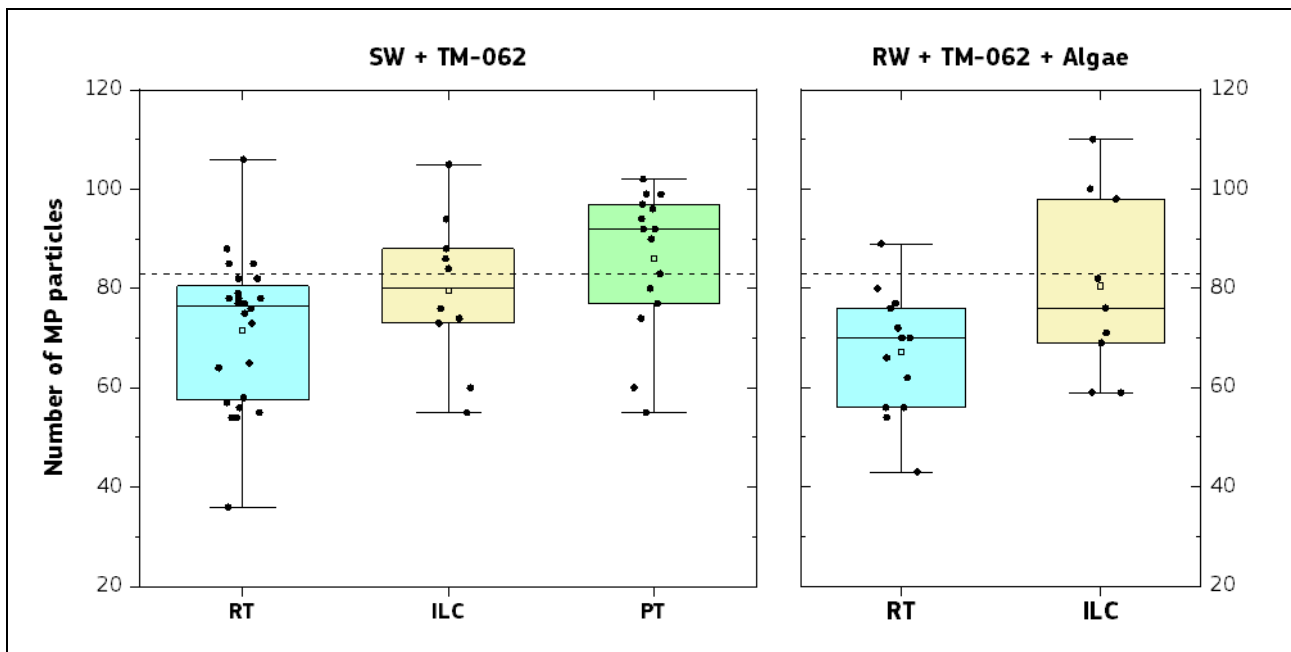
The comparability of the results across the different exercises was ensured through the use of well-characterised reference materials. Figure 8 summarises the results obtained for the common samples S1 (SW + TM-062) and S4 (RW + TM-062 + algae) across the three exercises (RT, ILC and PT), based on the analysis of identical test materials. A consistent pattern can be observed: trained teachers (RT) generally underestimated particle numbers, in line with the recovery values discussed earlier; expert laboratories of the EMSA (ILC) produced results centred around the assigned values; and Member State laboratories associated with the Technical Group on Marine Litter (PT) showed a slight tendency towards overestimation.

Overall, the results are coherent across exercises, with differences mainly related to methodological complexity and operator experience. They indicate that the simplified and operationally accessible SOP can produce reproducible and comparable microplastic results across operators when supported by appropriate quality assurance measures and well-characterised test materials.

The ring trial validation study demonstrates that the simple and operationally accessible Erasmus Maris SOP can be applied consistently and provides satisfactory performance characteristics for the determination of MP particles in water samples. For S1 and S4, recoveries of approximately 83 % were obtained, with repeatability relative standard deviations (RSD_r) of about 14 % and reproducibility relative standard deviations (RSD_R) of ca. 18 %, indicating acceptable precision for the SOP. The tendency to underestimate particle numbers is consistent with known limitations of visual MP counting, including particle losses during transfer or filtration. This reflects systematic and predictable bias associated with the simplified analytical approach applied in this study. Within screening applications, this bias may be considered acceptable, provided that it is well characterised and addressed through appropriate quality assurance and calibration strategies. Despite this tendency, the recovery and precision estimates indicate that the overall analytical performance remains satisfactory.

Unexpectedly, poorer performance was observed for Sample S3 (RW + TM-062), with lower recovery (≈ 75 %) and higher variability ($RSD_r \approx 29$ %, $RSD_R \approx 30$ %), suggesting that handling-related effects may have influenced the results. In contrast, the comparable repeatability and reproducibility observed for the algae-containing sample (S4) indicate that the presence of organic material did not significantly affect the method performance.

Figure 8: Results reported for S1 (SW + TM-062) and S4 (RW + TM-062 + Algae), in the frame of the three exercises



Source : JRC

The interlaboratory comparison conducted with expert EMSA laboratories further confirms the quality of the simplified SOP. Despite the use of more advanced and heterogeneous analytical approaches, the results showed good agreement with the assigned target levels and fell within the same range as those obtained using the Erasmus Maris protocol.

Similarly, the proficiency testing (PT) round organised for the Member State laboratories associated with the TG-ML demonstrated satisfactory performance. For both distributed samples, the majority of results were in agreement with the assigned or informative values. The results obtained by the expert laboratories confirm the quality of the simplified SOP, indicating that it provides a consistent basis for microplastic screening, with outcomes within the range of those achieved using more sophisticated analytical methods.

This evaluation is based on controlled test materials and specific experimental conditions; further work is required to assess performance across a wider range of environmental matrices and particle types.

Beyond method validation, the results indicate the feasibility of integrating citizen science actors into a quality-assured European monitoring framework. The approach shows potential for broader deployment across European educational institutions, supporting the generation of harmonised and comparable data over time across the EU, and enabling more coordinated monitoring across the river–sea continuum, thereby contributing to improved coherence between monitoring efforts under the WFD and MSFD.

Recommendations

The following three main sources of results variability were identified:

1. Particle recovery. To minimise particle losses during transfer and filtration, it is recommended to:
 - ensure thorough and quantitative rinsing of bottles and filtration equipment,
 - optimise filtration procedures to reduce retention on apparatus surfaces,
 - document and, where feasible, verify recovery performance through internal checks.
2. Quality of particle counting. Given the potential subjectivity of visual counting, the following measures are recommended:
 - application of clear and harmonised counting criteria,
 - use of reference images and standardised training materials,
 - periodic duplicate counting or peer verification,
 - participation in interlaboratory exercises to monitor performance over time.
3. Maintaining competence of trained trainers. As trained trainers may not apply the SOP regularly, continued competence should be supported through:
 - periodic refresher exercises or simplified ring trials,
 - participation in PT schemes when available,
 - short online refresher modules focusing on critical procedural steps,
 - exchange of experience within the network, and
 - access to high-quality test materials comparable to the JRC test materials used in this project.

Overall, the Erasmus Maris SOP can be considered fit for purpose for screening applications and educational use, providing satisfactory precision and recovery while remaining simple, robust and accessible.

In addition to the methodological recommendations outlined above, this study identifies further opportunities to support the implementation and sustainability of the Erasmus Maris approach:

- Further explore the role of the SOP in preliminary screening, as a complement to advanced laboratory analyses.
- Consider establishing a coordinated Erasmus Maris monitoring network at European level, building on existing structures (e.g. EMSA) and fostering synergies with initiatives of the Technical Group on Marine Litter (TG-ML), to support harmonised data collection.
- Develop or adapt data management tools to facilitate the collection, validation and sharing of results, ensuring interoperability with existing European reporting systems (e.g. MSFD).
- Promote integration of the SOP into educational systems, including school curricula and teacher training programmes, to support long-term capacity building.
- Consider a voluntary recognition scheme (e.g. “Erasmus Maris recognised school/laboratory”) to strengthen quality assurance, visibility and engagement.

- Explore digital tools (e.g. image recognition, AI-assisted particle counting, automated quality control) to improve data consistency, reduce operator-dependent variability and support scalability.
- Further assess the role of the Erasmus Maris framework in enhancing coordination between freshwater and marine monitoring (WFD-MSFD, including through pilot activities and cross-framework exchanges).

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- [12] ISO/IEC 17043 "*Conformity assessment – General requirements for proficiency testing*". Issued by the International Organisation for Standardization, Geneva, Switzerland.
- [13] ISO 13528:2022 "*Statistical methods for use in proficiency testing by interlaboratory competences*". Issued by the International Organisation for Standardization, Geneva, Switzerland.

List of abbreviations and definitions

Abbreviations	Definitions
ASE	Ayam Sailing Europe
ATR_FTIR	Attenuated Total Reflectance Fourier Transform Infrared spectroscopy
$D_{\%}$	Percent deviation from the assigned value
EMSA	Erasmus Maris Scientific Alliance
ILC	Interlaboratory comparison
ISO	International Organization for Standardization
JRC	Joint Research Centre
JRC.D.2	Water and Marine Resources Unit of the JRC
JRC.F.5	Food and Feed Compliance Unit of the JRC
JRC.F.6	Reference Material Unit of the JRC
LDIR	Laser Direct Infrared (Imaging Spectroscopy)
μ FTIR	Micro-Fourier transform infrared spectrometry
MP	Microplastic
MSFD	Marine Strategy Framework Directive
PT	Proficiency Test
QA/QC	Quality Assurance / Quality Control
RT	Ring trial
RW	(fresh) River Water
SOP	Standard Operating Procedure
SW	Sea Water
TG-ML	Technical Group on Marine Litter
TM-062	JRC Test Material 062
TM-062bis	JRC Test Material 062bis
WFD	Water Framework Directive
x_i	reported value by participant "i"
x_{pt}	assigned value

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Annexes

Annex 1.a Invitation email for the Ring-Trial validation study

From: ROBOUCH Piotr (JRC-GEEL)

Sent: Thursday, March 6, 2025

To: <xxxx>

Cc: <xxxx>

Subject: Invitation to participate to the Ring Trial validation study (EM-RT-2025)

Dear Trained Teachers, Dear Colleagues,

On behalf of the Joint Research Centre (JRC) we are pleased to invite you to participate in the upcoming ring trial validation study (EM-RT-2025) for the determination of performance characteristics of the Standard Operating Procedure (SOP) for counting of micro plastic particles sampled with a Manta Net.

You previously attended a training session jointly organised by the JRC and ASE (Ayam Saling Europe), where you became familiar with the experimental protocol. You also demonstrated good performance by successfully completing the practical work and providing satisfactory results.

Study details

- Samples: You will receive two bottles of each of three different water samples containing microplastic particles.
- Dispatch date: Estimated for September 2025.
- Analysis period: September - October 2025.
- Location: At your school or during a dedicated meeting (to be confirmed).
- Procedure: Please follow the provided SOP strictly, without modifications or deviations.
- Reporting requirement: Report the total number of microplastic particles in each bottle.
- Deadline for reporting results: To be confirmed (October - November 2025).

Important note

Please ensure that you have access to all equipment, chemicals, and solvents required to perform the analysis according to the SOP.

Registration

To confirm your participation, please reply to this email by 31 March 2025, providing your postal address for sample dispatch and future correspondence. Send your confirmation to: <xxxx> and specify "EM-RT-2025" in the subject of the mail.

Further information, including (i) confirmation of registration, (ii) detailed instructions for the exercise, and (iii) reporting templates, will be provided shortly after your registration.

Confidentiality

The procedures for organising this ring trial comply with the requirements of ISO/IEC 17043:2023, ensuring that both participant identities and all data provided will be kept strictly confidential.

Do not hesitate to contact us for additional information.

Kind regards, Piotr Robouch
(signature)

Annex 1.b Invitation email for the Scientific Alliance

From: ROBOUCH Piotr (JRC-GEEL)

Sent: Thursday, March 6, 2025

To: <xxxx>

Cc: <xxxx>

Subject: Invitation to participate the Inter Laboratory Comparison (EM-ILC-2025)

Dear Members of the Erasmus Maris Scientific Alliance, Dear Colleagues,

On behalf of the Joint Research Centre (JRC), we are pleased to invite you to participate in the upcoming interlaboratory comparison (EM-ILC-2025) for the characterisation of a test material (MP-RM) containing microplastic particles. Your scientific and technical expertise in this field will be highly valuable to the success of the project.

Study details

- Samples: You will receive two bottles of each of two different water samples containing microplastic particles “up to 1 mm”.
- Dispatch date: Estimated early April 2025.
- Analysis period: April-May 2025.
- Procedure: Please apply the validated methods you would use for environmental monitoring.
- Reporting requirement: (i) identity of the plastic material; (ii) total number of microplastic particles in each bottle.
- Deadline for reporting results: Mid-June 2025.

Registration

To confirm your participation, please reply to this email by 31 March 2025, providing your postal address for sample dispatch and future correspondence. Send your confirmation to: <xxx> and specify “EM-ILC-2025” in the subject of the mail.

Further information, including (i) confirmation of registration, (ii) detailed instructions for the exercise, and (iii) reporting templates, will be provided shortly after your confirmation.

Confidentiality

The procedures for organising this ring trial comply with the requirements of ISO/IEC 17043:2023, ensuring that both participant identities and all data provided will be kept strictly confidential.

Do not hesitate to contact us for additional information.

Kind regards, Piotr

Piotr ROBOUCH, PhD
Senior Scientific Officer
European Commission
Directorate-General Joint Research Centre
Directorate F – Health and Food
Food & Feed Compliance Unit (F.5)

Annex 1.c Invitation email for the Proficiency Testing round

From: ROBOUCH Piotr (JRC-GEEL)

Sent: Thursday, March 6, 2025

To: <xxxx>

Cc: <xxxx>

Subject: Invitation to participate to the Proficiency Test (EM-PT-2025)

Dear Members of the Technical Group on Marine Litter, Dear Colleagues

On behalf of the Joint Research Centre (JRC), we are pleased to invite you to participate in the upcoming proficiency testing round (EM-PT2025) for the identification and counting of microplastic particles in sea water samples. This PT is organised to support the Marine Strategy Framework Directive (MSFD). It aims to assess the performance of the laboratories for this type of analysis

Study details:

- Samples: You will receive two bottles of each of two different water samples containing microplastic particles (up to 1 mm and 5 mm, respectively).
- Dispatch date: Estimated early April 2025.
- Analysis period: April-May 2025.
- Procedure: Please apply either the validated methods you would use for environmental monitoring, or the Standard Operating Procedure developed by the Erasmus Maris project.
- Reporting requirement: (i) identity of the plastic material; (ii) total number of microplastic particles in each bottle.
- Deadline for reporting results: Mid-June 2025.
- Participation fee: Participation is free of charge.

Registration

To confirm your participation, please reply to this email by 31 March 2025, providing your postal address for sample dispatch and future correspondence. Send your confirmation to: <xxx> and specify "EM-PT-2025" in the subject of the mail.

Further information, including (i) confirmation of registration, (ii) detailed instructions for the exercise, and (iii) reporting templates, will be provided shortly after your confirmation.

Confidentiality

The procedures for organising this ring trial comply with the requirements of ISO/IEC 17043:2023, ensuring that both participant identities and all data provided will be kept strictly confidential.

Do not hesitate to contact us for additional information.

Kind regards, Piotr

Piotr ROBOUCH, PhD
Senior Scientific Officer
European Commission
Directorate-General Joint Research Centre
Directorate F – Health and Food
Food & Feed Compliance Unit (F.5)

Annex 2.a Instruction letter for the Scientific Alliance



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health and Food
Food and Feed Compliance

Geel 14/04/2025
JRC.F.5/PR

XXXX@XXXX.XX

Sent by email

Subject: EM-ILC-2025 Instruction letter

Dear xxxxx

Thank you for enrolling in the inter-laboratory comparison (ILC) round EM-ILC-2025, focused on the identification and quantification of microplastic particles in seawater samples. This ILC is organised in support of the implementation of the Marine Strategy Framework Directive (MSFD) and the Erasmus initiative. Your scientific and technical expertise in this field will be highly valuable to the success of the project.

You have been assigned the following Laboratory Code: LXX

You will shortly receive a parcel containing four (4) bottles, comprising of two (2) bottles for each of the two (2) test items. Upon arrival, please verify that all bottles are intact and were not damaged during transport. Should you find any broken or leaking bottles, notify us immediately. We will promptly replace any damaged test items.

Please store the bottles at 4 °C in the dark (e.g. in the refrigerator) as soon as possible after arrival, and keep them under these conditions until analysis.

Test item description and Instructions for analysis

Two bottles, labelled “ILC-A”, each containing approximately 0.1 L of filtered seawater, spiked with a known amount of microplastic particles in the size` ranging from about 0.3 to 1 mm. Similarly, the remaining two bottles, labelled “ILC-B”, each containing ca. 0.1 L of filtered water, spiked with organic matter and a known amount of microplastic particles in the size` ranging from about 0.3 to 1 mm. (*) *The size fractions are operationally defined using a sequence of sieves with different mesh sizes, which limits the ability to define precise cut-offs exactly at 300, or 1000 µm.*

For each bottle, you are requested to perform the following steps:

1. Transfer the entire contents (solution and all contained microplastic particles) from each bottle for analysis in a single step. The samples are intended for single-use – no sub-sampling is permitted.
2. Rinse each bottle thoroughly to recover all the particles, including those that may adhere to the inner walls and screw cap.

Note: Take care to avoid loss of particles during rinsing, filtration and transfer steps. These particles are highly hydrophobic and may adhere to glassware or agglomerate to form clusters.

3. Apply your routine analytical procedure as used for the monitoring of floating micro-litter particles in water.

4. For each test item (A and B), identify and report the type(s) of microplastic particles present.
5. For each test item (A and B), **quantify and report the “total number of microplastic particles per bottle”** with sizes ranging from 0.05 to 1 mm contained in each bottles.
6. Report the following via the online submission form available from 23/04/2025 at <https://bit.ly/4iddT9j>. You will be asked to submit:
 - (i) The identified “type of microplastic(s)”;
 - (ii) The quantified “total number of microplastic particles” per bottle.
Note: If your quantification method is able to distinguish particle sizes, report the number of particles for the size ranges, as requested.
 - (iii) The estimated corresponding measurement uncertainty (recommended);
 - (iv) A brief description of the sample preparation and analytical procedure used, including relevant technical information (e.g. reagents, instrumentation, settings).

Please ensure that you finalise your submission by clicking on the “Submit” button.

You are encouraged to save or print a copy of your submission as a PDF for your records.

Result submission and evaluation

The deadline for submission of results is 31 May 2025 @ 16:00.

The deadline could be extended upon request.

Your results will be used for the characterisation of the two test items, applying the statistical methods described in the ISO 13528:2022 standard.

Participants are reminded that collusion — i.e. the sharing of results or coordination of responses between laboratories — is contrary to scientific integrity and undermines the quality of this inter-laboratory comparison. Independent analysis is essential to ensure the reliability and comparability of results.

Should you require further information, do not hesitate to contact the organisers. Your participation to this exercise is greatly appreciated.

We wish you a successful experimental campaign,

Yours sincerely,

Piotr ROBOUCH

on behalf of the EM-ILC-2025 Coordination team

c.c.: U. Vincent; H. Emteborg, A. Arcot Rajashekar; S. Garcia Ruiz.

Annex 2.b Instruction letter for the Proficiency Testing round



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health and Food
Food and Feed Compliance

Geel 14/04/2025
JRC.F.5/PR

XXXX@XXXX.XX

Sent by email

Subject: EM-PT-2025 Instruction letter

Dear xxxxx

Thank you for enrolling in the proficiency testing (PT) round EM-PT-2025, focused on the identification and quantification of microplastic particles in seawater samples. This PT is organised in support of the implementation of the Marine Strategy Framework Directive (MSFD) and aims to evaluate the performance of participating laboratories for measurement of floating micro-litter.

You have been assigned the following Laboratory Code: PXX

You will shortly receive a parcel containing four (4) bottles, comprising of two (2) bottles for each of the two (2) test items. Upon arrival, please verify that all bottles are intact and were not damaged during transport. Should you find any broken or leaking bottles, notify us immediately. We will promptly replace any damaged test items.

Please store the bottles at 4 °C in the dark (e.g. in the refrigerator) as soon as possible after arrival and keep them under these conditions until analysis.

Test item description and Instructions for analysis

Two bottles, labelled “PT-A”, each containing approximately 0.1 L of filtered seawater, spiked with a known amount of microplastic particles in the size* ranging from about 0.3 to 1 mm. Similarly, the remaining two bottles, labelled “PT-B”, each containing ca. 0.1 L of filtered water, spiked with a known amount of microplastic particles in the size* ranging from about 0.3 to 5 mm. (*) *The size fractions are operationally defined using a sequence of sieves with different mesh sizes, which limits the ability to define precise cut-offs exactly at 300, 1000, or 5000 µm.*

For each bottle, you are requested to perform the following steps:

1. Transfer the entire contents (solution and all contained microplastic particles) from each bottle for analysis in a single step. The samples are intended for single-use – no sub-sampling is permitted.
2. Rinse each bottle thoroughly to recover all the particles, including those that may adhere to the inner walls and screw cap.
 1. *Note: Take care to avoid loss of particles during rinsing, filtration and transfer steps. These particles are highly hydrophobic and may adhere to glassware or agglomerate to form clusters.*
 2. Apply your routine analytical procedure as used for the monitoring of floating micro-litter particles in water.

4. For each test item (A and B), identify and report the type(s) of microplastic particles present.
5. For each test item (A and B), **quantify and report the “total number of microplastic particles per bottle”** with sizes ranging from 0.05 to 1 mm (A) or from 0.05 to 5 mm (B) contained in one of the two bottles.

Note: The second bottle could be used for training/testing purposes.

6. Report the following via the online submission form available from 23/04/2025 at <https://europa.eu/INCDJpC>. You will be asked to submit:

- (i) The identified “type of microplastic(s)”;
- (ii) The quantified “total number of microplastic particles” per bottle.

Note: If your quantification method is able to distinguish particle sizes, report the number of particles for the size ranges, as requested.

- (iii) The estimated corresponding measurement uncertainty (optional);
- (iv) A brief description of the sample preparation and analytical procedure used, including relevant technical information (e.g reagents, instrumentation, settings).

Please ensure that you finalise your submission by clicking on the “Submit” button.

You are encouraged to save or print a copy of your submission as a PDF for your records.

Result submission and evaluation

The deadline for submission of results is 31 May 2025 @ 16:00. The deadline could be extended upon request.

Your results will be used for the characterisation of the two test items, applying the statistical methods described in the ISO 13528:2022 standard.

Participants are reminded that collusion — i.e. the sharing of results or coordination of responses between laboratories — is contrary to scientific integrity and undermines the quality of this inter-laboratory comparison. Independent analysis is essential to ensure the reliability and comparability of results.

Should you require further information, do not hesitate to contact the organisers. Your participation to this exercise is greatly appreciated.

We wish you a successful experimental campaign,

Yours sincerely,

Piotr ROBOUCH
on behalf of the EM-PT-2025 Coordination team

c.c.: U. Vincent; H. Emteborg, A. Arcot Rajashekar; S. Garcia Ruiz.

Annex 3.a Accompanying letter for the Scientific Alliance



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health and Food
Food and Feed Compliance

Geel 10/04/2025
JRC.F.5/PR

(placed in the parcel together with the samples)

Subject: EM-ILC-2025 Accompanying letter

Dear Member of the Scientific Alliance, Dear Colleague,

Thank you for enrolling in the inter-laboratory comparison (ILC) EM-ILC-2025, focused on the identification and quantification of microplastic particles in seawater samples. This ILC is organised in support of the implementation of the Marine Strategy Framework Directive (MSFD) and the Erasmus Maris initiative. Your scientific and technical expertise in this field will be highly valuable to the success of the project. The parcel you received contains four bottles, comprising two bottles for each of the two test items. These bottles should be stored as soon as possible at 4 °C in the dark (e.g. in a refrigerator) until analysis.

Bottles labelled "ILC-A" contains about 0.1 L of filtered fresh seawater, spiked with a known amount of microplastic particles in the size ranging from about 0.3 to 1.0 mm.

Similarly, Bottles "ILC-B" contains about 0.1 L of filtered fresh water, spiked with organic matter and a known amount of microplastic particles in the size ranging from about 0.3 to 1.0 mm.

On Monday, you will receive a personalised e-mail containing important information, including: - the laboratory code assigned to you – to be used when reporting results; - the link to the online reporting interface for submitting your results and describing your experimental procedure (make sure to transfer the whole amount from the bottle); the definition of the measurand to which your results should conform; and - the tasks and analytical requirements to be completed.

Please note that these samples are intended for single-use only: you must process the entire volume in each bottle in a single operation. Sub-sampling is not permitted.

The deadline for submission of results is 31/05/2025. Final performance assessment will be disclosed towards the end of the year, as your results will be compared with those from other rounds conducted through October 2025.

Should you require any further information, do not hesitate to contact <xxxx>

We wish you a successful experimental campaign.

Best Regards,

Piotr ROBOUCH
on behalf of the EM-PT-2025 Coordination team

c.c.: U. Vincent; H. Emteborg, A. Arcot Rajashekar; S. Garcia Ruiz.

Annex 3.b Accompanying letter for the Proficiency Testing round



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health and Food
Food and Feed Compliance

Geel 10/04/2025
JRC.F.5/PR

(placed in the parcel together with the samples)

Subject: EM-PT-2025 Accompanying letter

Dear Member State Laboratory, Dear Colleague,

Thank you for enrolling in the proficiency testing (PT) round EM-PT-2025, focused on the identification and counting of microplastic particles in seawater samples. This PT is organised in support of the implementation of the Marine Strategy Framework Directive (MSFD) and aims to evaluate the performance of participating laboratories for measurements of floating micro-litter. The parcel you received contains four bottles, comprising two bottles for each of two distinct PT items. These bottles should be stored as soon as possible at 4 °C in the dark (e.g. in a refrigerator) until analysis.

Bottles labelled "PT-A" contains about 0.1 L of filtered fresh seawater, spiked with a known amount of microplastic particles in the size ranging from about 0.3 to 1.0 mm.

Similarly, Bottles "PT-B" contains about 0.1 L of filtered fresh seawater, spiked with a known amount of microplastic particles in the size ranging from about 0.3 to 5.0 mm.

On Monday, you will receive a personalised e-mail containing important information, including: - the laboratory code assigned to you – to be used when reporting results; - the link to the online reporting interface for submitting your results and describing your experimental procedure (make sure to transfer the whole amount from the bottle); the definition of the measurand to which your results should conform; and - the tasks and analytical requirements to be completed.

Please note that these samples are intended for single-use only: you must process the entire volume in each bottle in a single operation. Sub-sampling is not permitted.

The deadline for submission of results is 31/05/2025. Final performance assessment will be disclosed towards the end of the year, as your results will be compared with those from other rounds conducted through October 2025.

Should you require any further information, do not hesitate to contact <xxx> .

We wish you a successful experimental campaign.

Best Regards,

Piotr ROBOUCH
on behalf of the EM-PT-2025 Coordination Team

c.c.: U. Vincent; H. Emteborg; A. Arcot Rajashekar; S. Garcia Ruiz

Annex 4.a EU Survey reporting portal for the Ring-Trial validation study



Dear Colleague,

Thank you for contributing to the ring trial round organised by the Joint Research Centre in the framework of the Erasmus Maris Project.

Please indicate the date and session (morning or afternoon) when you carried out your measurements. Do not forget to specify the name of the sample and to report the result (expressed as the number of microplastic particles) for each of the four samples.

Your results will be treated confidentially. We trust that you submitted them independently, without discussing with colleagues from other schools, in order to avoid collusion that could compromise the overall quality of the trial.

We greatly appreciate your contribution and look forward to the positive outcome of this ring trial, which will provide satisfactory performance characteristics for the SOP.

Kind regards,
Piotr
on behalf of the Erasmus Maris team

* School Name

* Email (for follow-up if needed)

* Specify which session you attended

- 16/09/2025 pm
 17/09/2025 am
 08/10/2025 pm

Table of results

	Sample Name (e.g. RT-A-007)	Result: Nb of particles (numerical value)
*1		
*2		
*3		
*4		

Please upload the pictures of the filters used for counting? (optional)

Select file(s) to upload

Do you have further comments/suggestions?

Source: JRC

Annex 4.b EU Survey reporting portal for the Scientific Alliance

1. Introduction

Dear Participant,

Thank you for participating in this inter laboratory comparison exercise for the identification and quantification of microplastic particles in seawater samples.

Please complete all required fields in this questionnaire and ensure that your results are submitted **no later than 31 May 2025**.

If you have any questions, feel free to contact:

Piotr Robouch [Piotr.ROBOUCH@ec.europa.eu]

Arcot Rajashekar Ananda [Anandasagari.ARCOT-RAJASHEKAR@ec.europa.eu]

2. Participant Information

* Lab code assigned to you

* Contact email address

* Country

Is your method accredited

- Yes
 No

If yes, specify the standard (e.g. ISO/IEC 17025)

Experimental Details

3.1 Sample preparation

* Did you use entire volume (0.1 L) of the sample for analysis?

- Yes
 No

After transferring the sample from the glass bottle, did you rinse the bottle and collect the rinse water for analysis?

- Yes
 No

3.2 Filtration Procedure

If a filtration step was applied, specify the filter characteristics:

Filter Specification	Value
Filter type	
Diameter (mm)	
Mesh size (μm)	

3.3. Analytical Techniques

3.3.1. Identification

Specify analytical technique used

Describe the experimental procedure for identification

3.3.2. Quantification

Specify analytical technique used

Describe the experimental procedure for quantification

Results

	1	2	3	4
Sample code (e.g. ILC-A-XXX or ILC-B-XXX)	*	*	*	*
Type(s) of polymer identified (Reported as A; B;..or None)	*	*	*	*
Total number of microplastic particles (MPP)	*	*	*	*
Number of MPP 1000-4999* µm *Feret _{max}				
Number of MPP 300-999 µm				
Number of MPP 100-299 µm				
Number of MPP 50*-99 µm *Feret _{min}				
Measurement uncertainty (MU)				
Method of MU estimation				

Comments

Provide any additional comments or observations:

Source: JRC

Annex 4.c EU Survey reporting portal for the Proficiency Testing round

1. Introduction

Dear Participant,

Thank you for participating in this proficiency testing exercise for the identification and quantification of microplastic particles in seawater samples.

Please complete all required fields in this questionnaire and ensure that your results are submitted **no later than 31 May 2025**.

If you have any questions, feel free to contact:

Piotr Robouch [Piotr.ROBOUCH@ec.europa.eu]

Arcot Rajashekar Ananda [Anandasagari.ARCOT-RAJASHEKAR@ec.europa.eu]

2. Participant Information

* Lab code assigned to you

* Contact email address

* Country

Is your method accredited

- Yes
 No

If yes, specify the standard (e.g. ISO/IEC 17025)

3. Experimental Details

3.1. Sample Preparation

* Did you use entire volume (0.1 L) of the sample for analysis?

- Yes
 No

After transferring the sample from the glass bottle, did you rinse the bottle and collect the rinse water for analysis?

- Yes
 No

3.2. Filtration Procedure

If a filtration step was applied, specify the filter characteristics:

Filter Specification	Value
Filter type	
Diameter (mm)	
Mesh size (μm)	

3.3. Analytical Techniques

3.3.1. Identification

Specify analytical technique used

Describe the experimental procedure for identification

3.3.2. Quantification

Specify analytical technique used

Describe the experimental procedure for quantification

4. Results

	1	2
Sample code (e.g. PT-A-XXX or PT-B-XXX)	* <input type="text"/>	* <input type="text"/>
Type(s) of polymer identified (Report as A; B;... or None)	* <input type="text"/>	* <input type="text"/>
Total number of microplastic particles (MPP)	* <input type="text"/>	* <input type="text"/>
Number of MPP 1000-4999* μm *Feret _{max}	<input type="text"/>	<input type="text"/>
Number of MPP 300-999 μm	<input type="text"/>	<input type="text"/>
Number of MPP 100-299 μm	<input type="text"/>	<input type="text"/>
Number of MPP 50*-99 μm *Feret _{min}	<input type="text"/>	<input type="text"/>
Measurement uncertainty (MU)	<input type="text"/>	<input type="text"/>
Method of MU estimation	<input type="text"/>	<input type="text"/>

5. Comments

Provide any additional comments or observations:

Source: JRC



MICROPLASTICS COUNTING SOP

SOP (Standard Operating Procedure) for counting of microplastic particles after sampling with a Manta Net from sea- or inland waters.

Note: Depending on the availability of running water, the entire Step 1 of the SOP can be performed on the sampling site or in the lab.

Step 1: Sample preparation

1. Stack two stainless steel sieves on top of each other. The sieve with the largest mesh (1 mm) should be placed on top of the sieve with the smallest mesh size (300 μm). The collection pan normally placed under the smallest sieve should be omitted.
2. Pour the collected sample from the COD-end (Manta Net) over the stacked sieves. Rinse out the COD-end or sample container with filtered water (< 25 μm) or tap water until all solid material is transferred.
3. Rinse the top sieve thoroughly. You can use your fingers or tweezers to move/disperse oversized particles around. Make sure to rinse your fingers above the sieve, after use. All solids larger than 1 mm will remain on the top sieve. Optionally, large plastic particles/pieces sitting on top sieve can be counted in this step.
4. Remove the top sieve. All solids ranging from 300 to 1000 μm (microplastics included) are collected on the lower sieve. Rinse the 300 μm sieve thoroughly with filtered water to remove all particles smaller than 300 μm .
5. Collect the particles ranging from 300 μm to 1000 μm on one side of the sieve by holding the sieve at an angle while rinsing it with filtered water.
6. Transfer the contents of the sieve quantitatively into a glass jar of at least 500 ml volume. Do this by tilting the sieve and flushing out the particles with a wash bottle. Use around 100 ml of filtered water.

This document was produced within the framework of the Raise-CS project.



Step 2: Digestion of organic contaminants

IMPORTANT: Wear protective gloves, lab coat, and goggles while handling highly concentrated oxidizing solutions!



Corrosive



Hazardous to the environment

1. After transferring the sample to the jar, add 200 mL of 15° (equivalent to 4.5 % m/m) NaClO solution (commercially available bleach from any supermarket) so that about 300 ml of liquid is present in the jar. This corresponds to about 3 % m/m NaClO in the final solution. If the available commercial bleach has a different concentration, recalculate the equivalent volume of bleach using the bleach conversion table at the end of this document (Annex 1).
2. Close the jar with the lid and allow at least 48 hours at room temperature for digestion before continuing. If the organic matter load in the sample is still high after 48 hours, add 100 ml extra bleach and allow digestion for extra time. The digestion is also facilitated by increasing temperature until a maximum of 40 °C [Pfeiffer F. and E. K. Fischer (2020)].

Step 3: Separate microplastics from bleach solution by density segregation

After digestion, the solution holding the microplastics has a density ≥ 1.06 g/ml, while the microplastics sampled with the Manta Net have a density < 1 g/ml. Hence the particles will float on the surface while the inorganic and residual organic materials with a density higher than the one of the solution will settle at the bottom.

1. Shake and transfer the content of the jar into a separation funnel. If the solution volume is bigger than the volume of the separation funnel, split the solution into two portions and treat them separately. Use filtered water to rinse the jar and the lid, making sure that all particles are transferred to the separation funnel.

Allow the solution to settle (minimum 5 to 15 minutes) and make sure not to shake the separation funnel afterwards.

2. Drain and discard the liquid from the bottom of the separation funnel, until ca. 70 ml solution remains in the funnel.

Pfeiffer F. and E. K. Fischer (2020), *Various digestion protocols within microplastic sample processing – Evaluating the resistance of different synthetic polymers and the efficiency of biogenic organic matter destruction*, Front. Environ. Sci., Vol. 8, Art. 572424.

Step 4: Density separation to remove mineral fraction

1. Add 150 ml of saturated NaCl solution (approx. 25 % m/m, density 1.19 g/ml) to the separation funnel and gently agitate the suspension inside the funnel.

Allow the solution to settle (minimum 5 to 15 minutes) and make sure not to shake the separation funnel afterwards.

The resulting solution holding the microplastics will have a density of around 1.17 g/ml, thus allowing the separation by density segregation (described earlier).

2. Drain and discard the liquid from the bottom of the separation funnel, until ca. 70 ml solution remains in the funnel.
3. Collect the remaining of the solution into a beaker.
Rinse the wall of the separation funnel with filtered water to make sure that all particles are transferred into the beaker. Add rinsing solution to the beaker. Repeat if necessary until there are no microplastic particles on the wall of the separation funnel.
Collect all fractions of the solution together before filtration

Step 5: Filtration

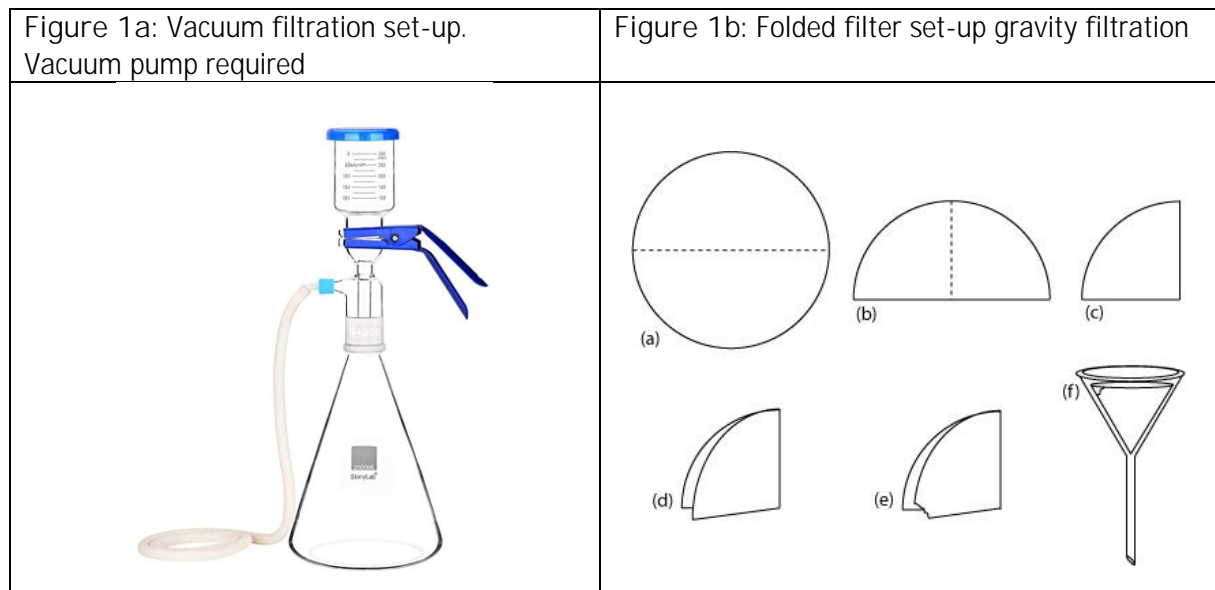
1. Take a 47mm metallic filter fitting a vacuum filtration setup with a clamp (see Figure 1a) or 47 mm diameter filter paper (or another size) that fits any vacuum filtration setup available. If no vacuum filtration setup is available, fold a 110 mm diameter filter paper as shown in Figure 1b. Metallic filters have some advantages over paper filters: easier filtration, better contrast with particles, and better staining. Check filtration properties with water before starting the actual filtration.

IMPORTANT: write the sample details/ID either on a petri dish or on the paper filter with a pencil, to indicate for example name, date, sample number, and location. In addition, one could draw a grid on the paper filter before use (e.g. 1 x 1 cm squares) to facilitate the counting.

Wet the filter paper with water, before placing it in the funnel.

2. Filter the contents of the beaker. Rinse the beaker well using a wash bottle to transfer all the particles onto the filter. Make sure no microplastic particles are sticking on the funnel wall. Rinsing with Ethanol, if available, facilitates the collection of the microplastics on the filter.
3. After filtration, remove the funnel and check for microplastics.
Transfer any remaining plastic particles on the funnel onto the filter with a set of tweezers.

Figure 1: Filtration set-up



Step 6: Counting of particles without staining

1. Use a smartphone camera and focus on the filter paper making sure that the entire filtered area is inside the frame before taking a picture (see example in Figure 2).
2. Transfer the photo to a PC, laptop, or tablet. Use any photo editor (such as MS Paint). Count and mark the microplastic particles, using the zoom function and the pencil tool.
3. Fill data in the reporting sheet for further evaluation.

Figure 2: Example of an optimal photo of filter taken with a smartphone.



Note: A photo of poor quality will have a severe impact on the result when counting the microplastic particles!

Mobile phone cameras of different brands produce images of different quality. If you are not satisfied with your photo, take a new one and/or ask to use another phone camera.

Step 7: Fluorescent labelling using a Nile Red staining solution

1. Ask your teacher for the Nile Red solution.
The Nile Red (NR) stock solution will be prepared by the teacher:
 - Dissolve 1 mg Nile red in 10 ml of acetone.
 - Add 90 ml hexane to the 10 ml NR/Acetone solution, to get a 10 mg/l working solution.

Note: Work with hexane and acetone in a fume hood or use an extraction fan.

Hexane:



Serious health hazard



Hazardous to the environment

Acetone and hexane:



Flammable



Health hazard / Hazardous to the ozone layer

2. Using a pipette or a dropper bottle filled with Nile red, wet the microplastic particles on the filter with max. 1 to 2 ml Nile Red.
3. Wait c.a. 10 to 20 min or until the solvents evaporate. Meanwhile, the Nile Red would have adhered to the surface of the microplastic particles.
4. Place the filter under a Royal Blue light (450 nm). Use a 500 nm overpass colour filter (yellow filter) to observe the fluorescence light emitted by the stained microplastic particles.

Step 8: Counting of the particles after staining

1. Use a smartphone camera and focus on the filter paper making sure that the entire filtered area is inside the frame (see Figure 2 above). Take a picture through the 500 nm yellow long-pass filter.
2. Transfer the photo to a PC, laptop or tablet. Use any photo editor (e.g. MS Paint). Count and mark the (fluorescent) microplastic particles, using the zoom function and the pencil tool.
3. Fill data in reporting sheet for further evaluation and discussion.
4. Check the results you reported before submission.

STEP 9

Clean metallic filters, do not discard them! Tap it over a bin to remove all bigger particles. Rub it with your fingers and hold it under a stream of water until it is clean.

Clean your laboratory bench after the experiment.

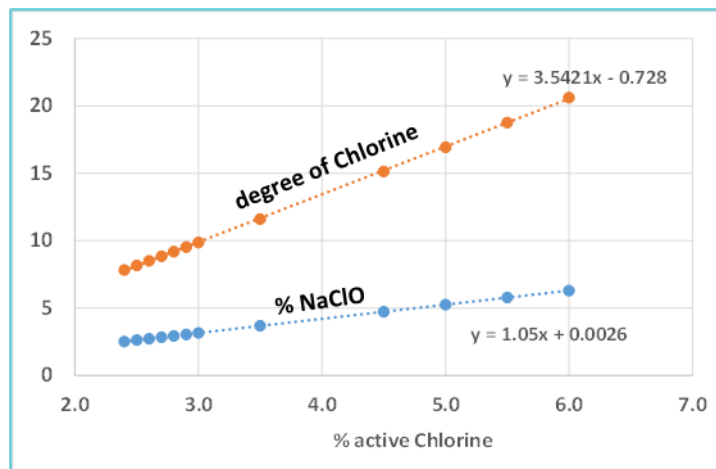
This document was produced within the framework of the Raise-CS project.



Co-funded by
the European Union

Annex 1: Bleach Conversion table

% active chlorine	% NaClO	degree of Chlorine
2.40	2.52	7.83
2.50	2.63	8.17
2.60	2.73	8.51
2.70	2.84	8.84
2.80	2.94	9.19
2.90	3.05	9.53
3.00	3.15	9.87
3.50	3.68	11.60
4.50	4.73	15.13
5.00	5.25	16.93
5.50	5.78	18.77
6.00	6.30	20.62



MICROPLASTICS COUNTING SOP

Source: JRC and Ayam Sailing Europe

Annex 6. Results reported during the Ring Trial validation study

Determination of precision parameters

A total of g laboratories participated in the study. Each laboratory ' i ' reported two independent replicate results, denoted $x_{i,1}$ and $x_{i,2}$.

The reporting unit was the number of microplastic (MP) particles.

While the grand mean of all reported results is denoted \bar{x}_{gd} , the assigned value of the test item was set to: $x_{pt} = 83$ MP particles.

The recovery rate ($R_{rec,\%}$), expressed as a percentage, was calculated as: $R_{rec,\%} = 100 * \frac{\bar{x}_{gd}}{x_{pt}}$

Estimation of precision components

Precision parameters were estimated using a one-way ANOVA model, performed in Microsoft Excel (balanced design with two replicates per laboratory). The ANOVA output provides: (i) degrees of freedom (df), (ii) sum of squares (SS), and (iii) mean squares (MS), for (a) between-laboratory effects (MS_b), (b) within-laboratory effects (MS_w), and (c) total variation.

The standard deviation of repeatability (s_r), representing the within-Laboratory variability, was calculated as: $s_r = \sqrt{MS_w}$

The between-laboratory standard deviation (s_b) was calculated as: $s_b = \sqrt{(MS_b - MS_w)/2}$ since duplicates analyses were performed

The standard deviation of reproducibility (s_R), representing the combined effect of within- and between-laboratory variability, was calculated as: $s_R = \sqrt{s_r^2 + s_b^2}$

The corresponding relative standard deviations, expressed as percentages, were calculated as:

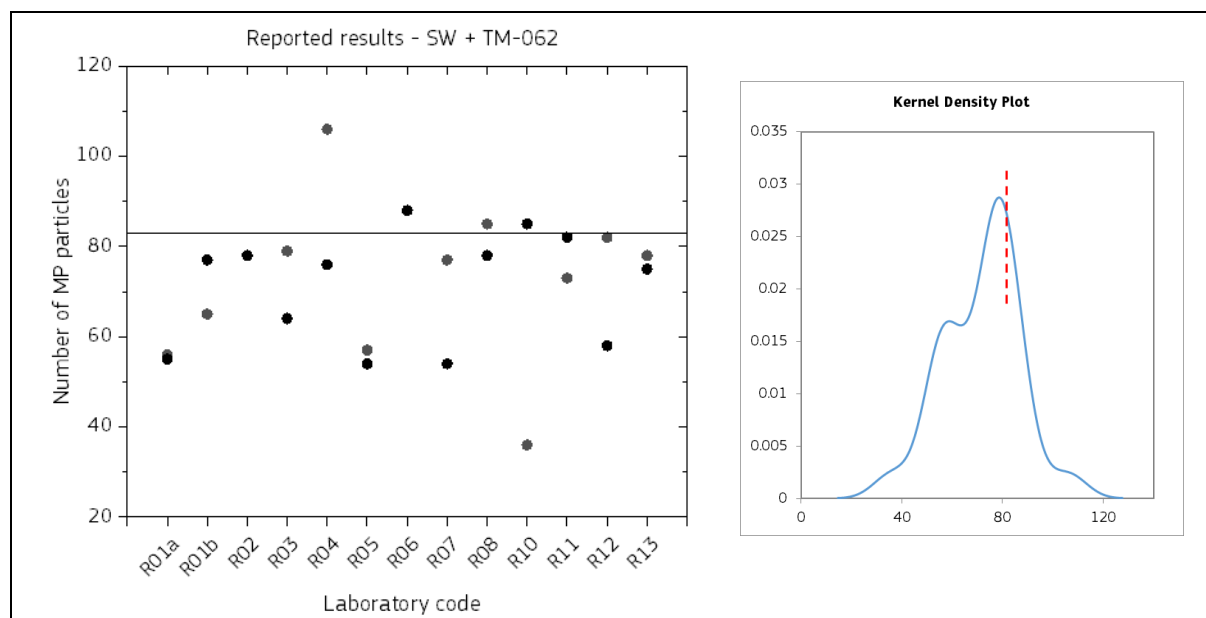
$$RSD_{r,\%} = 100 * s_r / \bar{x}_{gd} \quad \text{and} \quad RSD_{R,\%} = 100 * s_R / \bar{x}_{gd}.$$

Numerical examples illustrating these calculations are provided hereafter.

Annex 6.1. Results reported for S1: SW + TM-062

Lab.Code	1 st sample	Result 1	2 nd sample	Result 2	Note
R01a	T-335	56	T-361	55	
R01b	T-365	65	T-362	77	
R02	T-322	'lost'	T-330	78	Excluded (only 1 result)
R03	RT-A-129	79	T-332	64	
R04	T-329	106	T-327	76	Excluded (Grubbs outlier)
R05	T-364	57	RT-A-262	54	
R06	T-326	'lost'	T-328	88	Excluded (only 1 result)
R07	RT-A-157	77	RT-A-092	54	
R08	RT-A-208	85	RT-A-174	78	
R10	RT-A-151	36	RT-A-221	85	Excluded (Grubbs outlier)
R11	RT-A-243	73	RT-A-206	82	
R12	RT-A-161	82	RT-A-103	58	
R13	T-325	78	T-334	75	

'lost': a significant number of particles were lost during the filtration step.



ANOVA calculation for S1: SW + TM-062

(laboratories) g	9
(replicates) m	2

	df	SS	MS	Variance	s	F
between	8	1342.7	167.84	38.840	6.232	1.86
Within	9	811.5	90.166	90.166	9.495	3.22
total	17	2154.2	126.72	83.923	9.160	

Fcrit

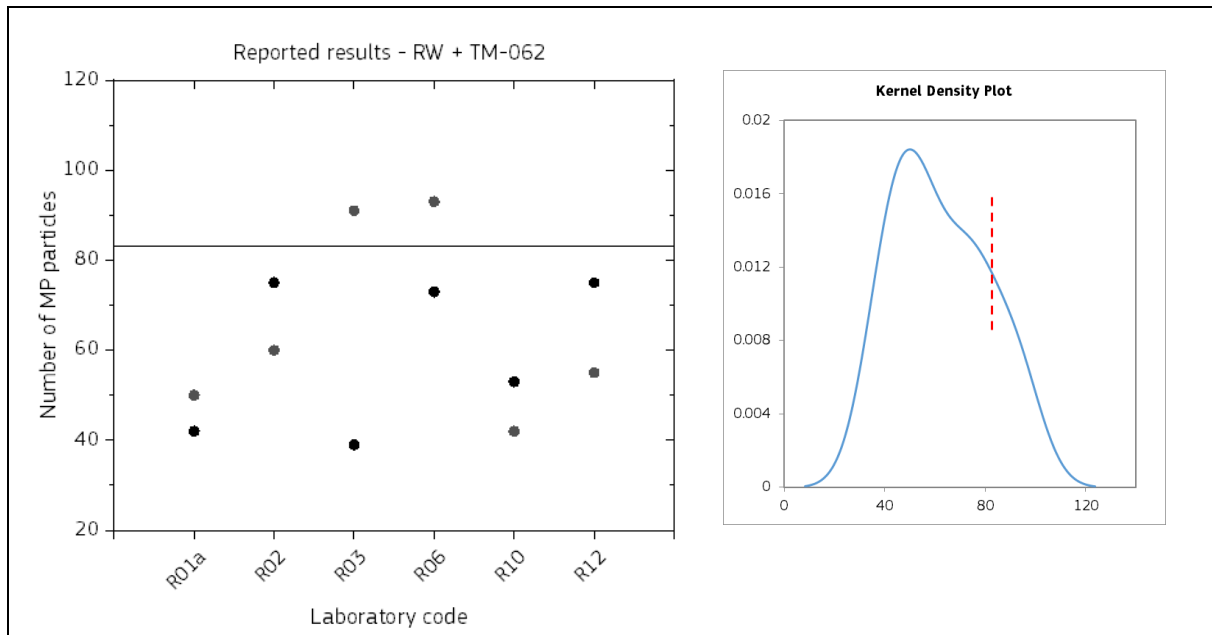
	Recovery	s_r	Sb	s_R	(particles)
Grand average	69.4	0.836	9.5	6.2	11.4
Assigned value	83	84%	13.7%	9.0%	16.4%

Where s_r : repeatability standard deviation; s_R : reproducibility standard deviation

Source: JRC

Annex 6.2. Results reported for S3: RW + TM-062

Lab.Code	1 st sample	Result 1	2 nd sample	Result 2
R01a	RT-B-346	50	RT-B-349	42
R02	RT-B-359	60	RT-B-360	75
R03	RT-B-341	91	RT-B-344	39
R06	RT-B-338	93	RT-B-340	73
R10	RT-B-336	42	RT-B-337	53
R12	RT-B-342	55	RT-B-343	75



ANOVA calculation for S3: RW + TM-062

(laboratories) g	6
(replicates) m	2

	df	SS	MS	Variance	s	F
between	5	1909.6	381.93	27.883	5.280	1.17
Within	6	1957	326.16	326.166	18.060	4.38
total	11	3866.6	351.51	190.966	13.819	

F_{crit}

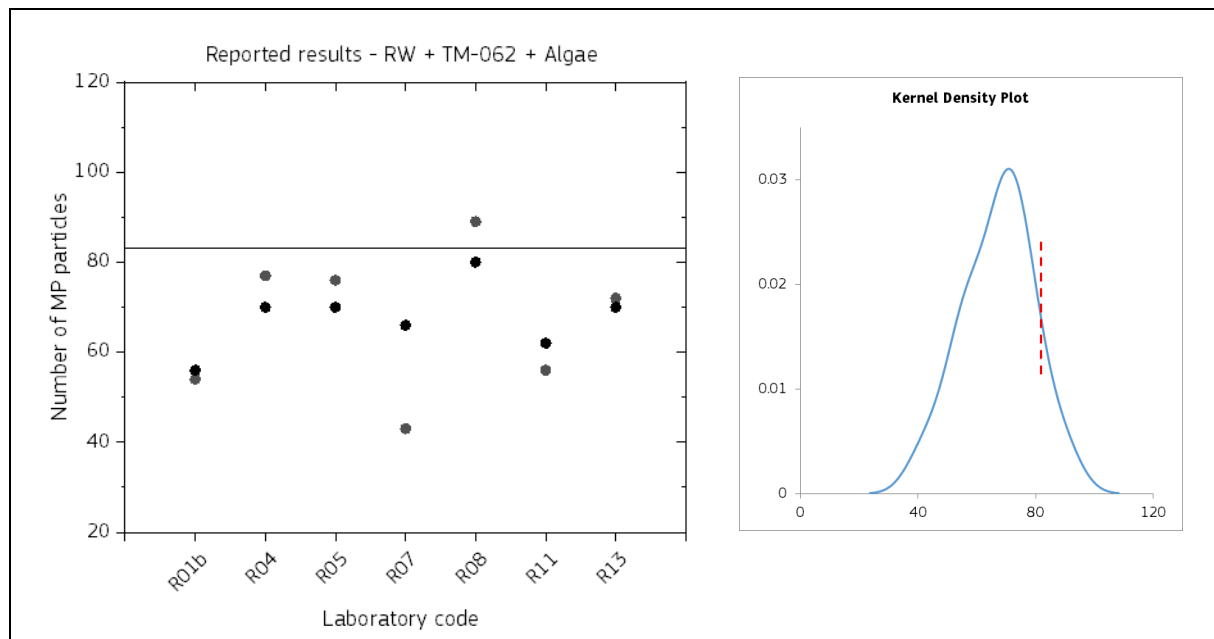
	Recovery	s_r	S_b	S_R	(particles)
Grand average	62.3	0.779	18.1	5.3	18.8
Assigned value	83	75%	29.0%	8.5%	30.2%

Where s_r : repeatability standard deviation; s_R : reproducibility standard deviation

Source: JRC

Annex 6.3. Results reported for S4: RW + TM-062 + Algae

Lab.Code	1 st sample	Result 1	2 nd sample	Result 2
R01b	RT-C-234	54	RT-C-368	56
R04	RT-C-235	77	RT-C-259	70
R05	RT-C-370	76	RT-C-372	70
R07	RT-C-225	43	RT-C-229	66
R08	RT-C-255	89	RT-C-366	80
R11	RT-C-251	56	RT-C-261	62
R13	RT-C-242	72	RT-C-367	70



ANOVA calculation for Sample 4 (RW + TM-062 + Algae)

		ANOVA				
	df	SS	MS	Variance	s	F
(laboratories) g	7					
(replicates) m	2					
between	6	1528.8	254.80	101.01	10.050	4.82
Within	7	369.5	52.785	52.785	7.265	3.86
total	13	1898.3	146.025	127.40	11.2879	
		Recovery	s_r	S_b	S_R	
Grand average	67.2	0.840	7.3	10.1	12.4	(particles)
Assigned value	83	81%	10.8%	15.0%	18.5%	

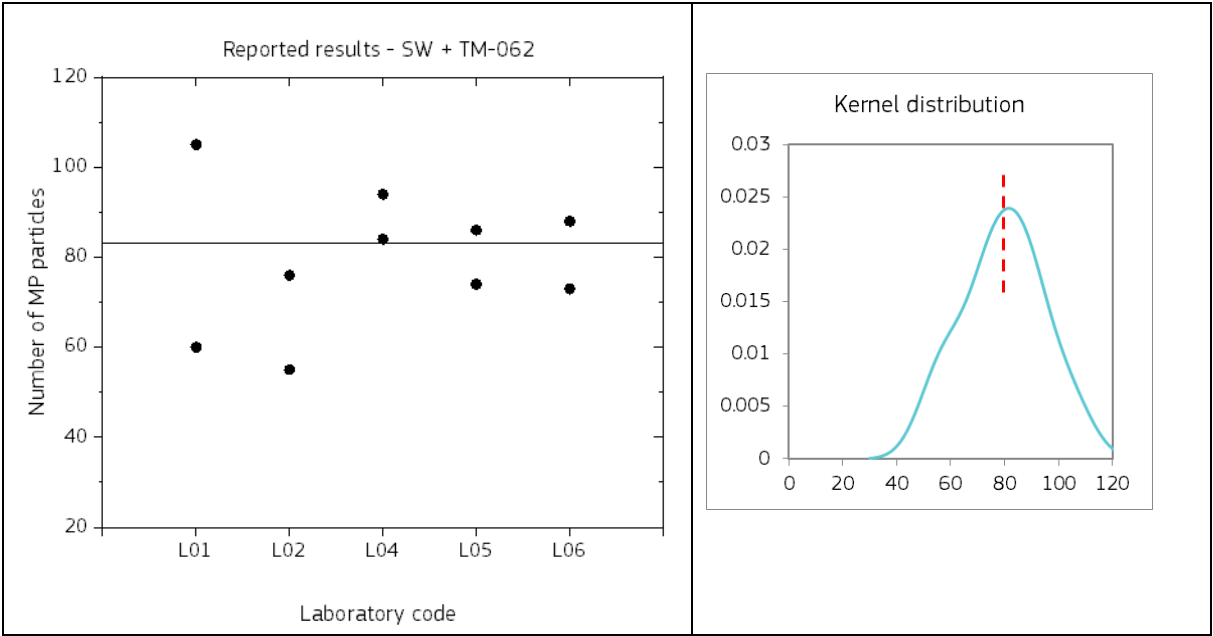
Where s_r : repeatability standard deviation; s_R : reproducibility standard deviation

Source: JRC

Annex 7. Results reported by the laboratories of the Scientific Alliance

Annex 7.1. Results reported for S1: SW + TM-062

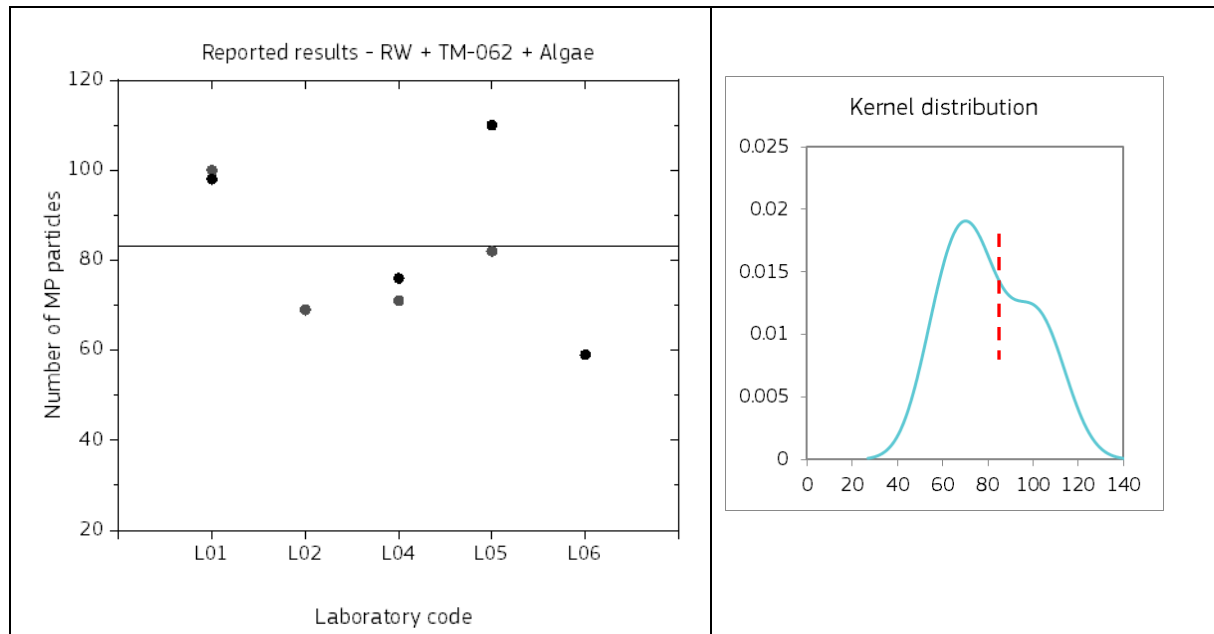
Lab.Code	1 st sample	Result 1	2 nd sample	Result 2
L01	ILC-A-118	60	ILC-A-071	105
L02	ILC-A-119	76	ILC-A-166	55
L04	ILC-A-158	84	ILC-A-167	94
L05	ILC-A-165	74	ILC-A-175	86
L06	ILC-A-178	73	ILC-A-125	88



Source: JRC

Annex 7.2. Results reported for S4: RW + TM-062 + Algae

Lab.Code	1 st sample	Result 1	2 nd sample	Result 2
L01	ILC-B-249	100	ILC-B-237	98
L02	ILC-B-125	69	ILC-B-245	lost
L04	ILC-B-217	71	ILC-B-231	76
L05	ILC-B-214	82	ILC-B-260	110
L06	ILC-B-224	59	ILC-B-254	59



Source: JRC

Annex 8. Results reported by Member State laboratories

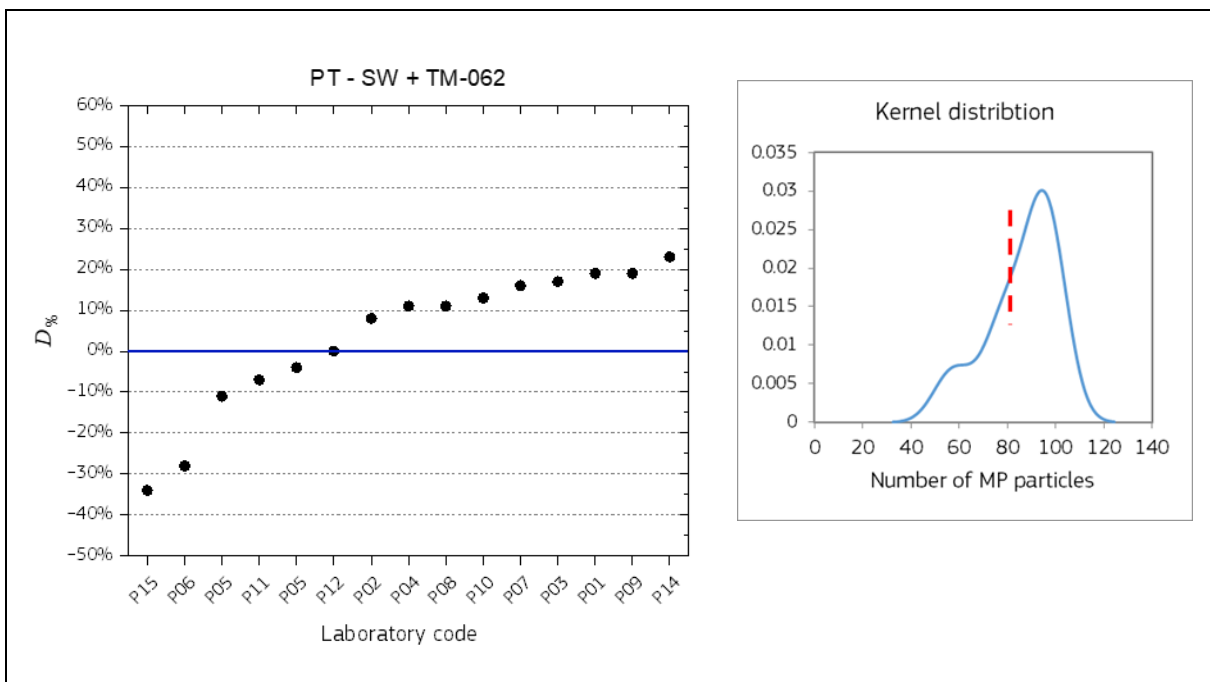
The individual performance of the laboratories participating in to the proficiency testing round was expressed in terms of $D\%$ (calculated in accordance with ISO 17043:2023 [12] and ISO 13528:2022 [13]) as

$$D\% = 100 \frac{(x_i - x_{pt})}{x_{pt}},$$

where ' x_i ' is the measurement result reported by a participant and ' x_{pt} ' is the assigned value.

Annex 8.1. Results reported for S1: SW + TM-062; for which $x_{pt} = 83$ MP particles.

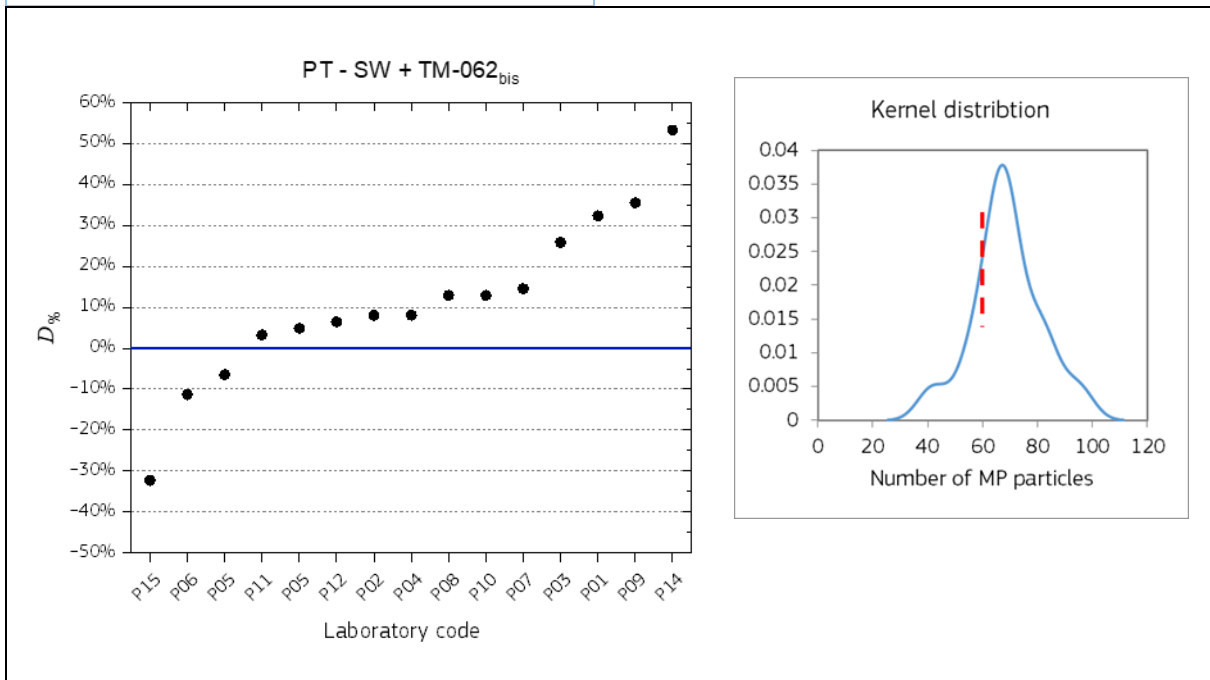
Lab	Sample	x_i	D%
P01	PT-A-213	99	19 %
P02	PT-A-121	90	8 %
P03	PT-A-207	97	17 %
P04	PT-A-252	92	11 %
P05	PT-A-173	74	-11 %
P05	PT-A-096	80	-4 %
P06	PT-A-185	60	-28 %
P07	PT-A-247	96	16 %
P08	PT-A-153	92	11 %
P09	PT-A-086	99	19 %
P10	PT-A-005	94	13 %
P11	PT-A-195	77	-7 %
P12	PT-A-196	83	0 %
P14	PT-A-197	102	23 %
P15	PT-A-122	55	-34 %



Source: JRC

Annex 8.2. Results reported for S2: SW + TM-062bis; for which $x_{pt} = 62$ MP particles.

Lab	Sample	x_i	D%
P01	PT-B-015	70	13 %
P02	PT-B-023	70	13 %
P03	PT-B-008	82	32 %
P04	PT-B-012	64	3 %
P05	PT-B-018	55	-11 %
P05	PT-B-010	66	6 %
P06	PT-B-031	67	8 %
P07	PT-B-044	67	8 %
P08	PT-B-003	65	5 %
P09	PT-B-006	78	26 %
P10	PT-B-032	95	53 %
P11	PT-B-001	42	-32 %
P12	PT-B-037	71	15 %
P14	PT-B-029	84	35 %
P15	PT-B-025	58	-6 %



Source: JRC

Annex 9.a Additional information as provided by the Scientific Alliance

Lab code	L01	L02	L04	L05	L06
Accredited method?	No	No	No	No	No
Whole sample used for analysis?	Yes	Yes	Yes	Yes	Yes
After transfer, rinse collected for analysis?	Yes	Yes	Yes	Yes	Yes
Filter type used	PTFE	PTFE	GF/A filters	Platinum vapourisation-deposition-coated polycarbonate filter membranes	Steel
Filter diameter (mm)	47	25	47	47	35
Mesh size (µm)	10	10	1.6	30	10
Specify analytical technique used		µFTIR	ATR-FTIR	Nicolet in10 infrared microscope (FT-IR)	Bruker Lumos FTIR
Describe the experimental procedure for identification	µFTIR analysis	FTIR spectra compared to library	Several particles of each colour were measured using ATR-FTIR	<ul style="list-style-type: none"> • Digestion of org. matter with NaClO • Incubation 24 h at 45 °C, 100 rpm. • Digestates filtered onto platinum-coated membranes using glass Büchner apparatus. • Filters transferred to Nicolet in10 FT-IR microscope stage. • Polymer identification via GEPARD software. • Spectral matching against custom Micro-litter library 	
Specify analytical technique used		µFTIR particle Wizard	Multispectral imaging using VideometerLab4	<ul style="list-style-type: none"> • GEPARD underestimated particle integrity; larger particles (> 500 µm) frequently split into multiple selections. • Result: overestimation of particle number. • Software performs better for 50–300 µm particles. • Automatic colour recognition inadequate under darkfield illumination. 	Leica DMS 1000 Optical Microscope
Describe the experimental procedure for quantification	Two Chemical digestions with KOH and H2O2, each for 48h Filtration 10µm PTFE Nile Red staining for 15 min's Fluorescent microscopy of		Microplastics analysed with VideometerLab 4 (multispectral imaging system). Strobed LED illumination; up to 19 wavelengths (365–970 nm).	GEPARD: reliable chemical identification per particle. Particle counting unreliable; multiple selections per particle. Optical mosaics printed (A3); particles counted manually with	

Lab code	L01	L02	L04	L05	L06
	the whole filter for quantification of the MPP		Spectral data on chemical and physical properties. Analysis and measurement via VideometerLab software (v3.26.3.0).	recorded chemical ID. Indicates need for software improvement for efficient quantification of larger (> 500 µm) microplastics.	
Sample 1 code	ILC-A-118	ILC-A-119	ILC-A-158	ILC-A-165	ILC-A-178
Sample 2 code	ILC-A-071	ILC-A-166	ILC-A-167	ILC-A-175	ILC-A-125
Sample 3 code	ILC-B-249	ILC-B-125	ILC-B-217	ILC-B-214	ILC-B-224
Sample 4 code	ILC-B-237	ILC-B-245	ILC-B-231	ILC-B-260	ILC-B-254
Type of polymers identified in Sample 1	A; PE B; PP C; Epoxy	PE;PP	PE, PP	PE;PP	PE; PP
Type of polymers identified in Sample 2	A; PE B; PP C; Epoxy	PE;PP	PE, PP	PE;PP	PE; PP
Type of polymers identified in Sample 3	A; PE B; PP C; Nylon	PE;PP	PE, PP	PE;PP	PE; PP
Type of polymers identified in Sample 4	A; PE B; PP	None (sample lost)	PE, PP	PE;PP	PE; PP
Total number of MPP (Sample 1)	60	76	84	74	73
Total number of MPP (Sample 2)	105	55	94	86	88
Total number of MPP (Sample 3)	100	69	71	82	59
Total number of MPP (Sample 4)	98	0	76	110	59
Nb of MPP (1000-4999* µm *Feretmax) in Sample 1	0	0	2	0	
Nb of MPP (1000-4999* µm *Feretmax) in Sample 2	0	0	3	3	
Nb of MPP (1000-4999* µm *Feretmax) in Sample 3	PP: 2	0	0	1	
Nb of MPP (1000-4999* µm *Feretmax) in Sample 4	PP: 5		2	1	
Nb of MPP (300-999 µm) : 1	PE/PP: 58	55	82	71	
Nb of MPP (300-999 µm) : 2	PE/PP: 93 Epoxy: 3	50	88	81	
Nb of MPP (300-999 µm) : 3	PE/PP: 94	53	71	76	
Nb of MPP (300-999 µm) : 4	PE/PP: 91		74	102	
Nb of MPP (100-299 µm) : 1	PE: 1 Epoxy: 1	21	0	3	
Nb of MPP (100-299 µm) : 2	PE: 1 Epoxy: 5	5	3	2	
Nb of MPP (100-299 µm) : 3	PP: 1 Nylon: 2	16	0	5	
Nb of MPP (100-299 µm) : 4	PE: 2		0	4	
Nb of MPP (50*-99 µm *Feretmin): Sample 1	0	0	0	2	
Nb of MPP (50*-99 µm *Feretmin): Sample 2	Epoxy: 3	0	0	0	
Nb of MPP (50*-99 µm *Feretmin): Sample 3	1	0	0	6	
Nb of MPP (50*-99 µm *Feretmin): Sample 4	0		0	6	
Meas. uncertainty (MU) : 1	NA				

Additional Comments/Observations

L01	<p>Epoxy was only found in ILC-A-118 and in ILC-A-071 (seawater).</p> <p>A procedural blank was taken along the process and here no MPP were identified.</p> <p>ILC-A-118 contains a lower amount of MPP then the other 3 samples.</p> <p>PE/PP = total MPP's for both polymers (spiked particles in different colours). Blue/Red = PP, Green/white = PE. When needed we can provide you the exact amount of MPP per type of polymer.</p>
L02	ILC-B-245 sample was lost during analyses. No measurement could be done
L04	<p>The particles were very electrostatic, trying to stick to every surface, not behaving like the particles in the environment.</p> <p>Some of the particles were stuck together - it was hard to tell if it was one particle at first and it broke apart, or if there were two</p>
L05	<p>I notice that many particles with a Feret_{min} of 50-99 have a Feret_{max} of 100-299. In these cases, I have recorded them in both rows. I hope ok.</p> <p>The greater number of small particles in the samples subject to digestion may indicate fragmentation during 24 hour digestion/agitation?</p> <p>Let me know if a more detailed method is required</p>
L06	

Source: JRC

Annex 9.b Additional information as provided by the EM-PT-2025 participants

Lab code	Accredited method	entire sample used for analysis?	Bottle rinsed and rinse analysed?	Filter type used	Filter diameter (mm)	Mesh size (µm)
P01	No	Yes	Yes	Metal mesh; Glass fiber filter	47	1.2
P02	No	Yes	Yes	Sartorius 1289 cellulose	55	8-12
P03	No	Yes	Yes	Stainless steel	35	300 + 10
P04	No	Yes	Yes	PC membrane filter, Whatman Nuclepore	47	12
P05	No	Yes	Yes	Whatman Membrane Filters	47	0.45
P05	No	Yes	Yes	Whatman Membrane Filters	47	0.45
P06	No	Yes	Yes	METALLIC SIEVES	200, 200, 200, 200, 100	5000, 1000, 300, 100, 25
P07	No	Yes	No	PTEG Gold-Coated Membrane	25	0.8
P08	No	Yes	Yes	Stainless steel wire mesh	47	100
P09	No	Yes	Yes	Microfiber glass paper filter	47	1,2
P10	No	Yes	Yes	metal filter	47	100
P11	No	Yes	Yes	Whatman GF/B	47	1.00
P12	No	Yes	Yes	Glass fibre	47	0.7
P14	No	Yes	Yes	Whatmann glass µfiber (GF/D)	47	2.7
P15	No	Yes	Yes	sterile white MCE (Mixed cellulose ester) black gridded membrane filter	47	0.45

Lab code	Analytical technique for identification	Experimental procedure for identification	Analytical technique for quantification	Describe the experimental procedure for quantification
P05			Stereomicroscopes OLYMPUS SZX10 equipped with a digital camera Luminera. INFINITY ANALYZE software to measure the longest dimension of each item (mm).	Sample processing under laminar flow bench (HN14) with fibre-free (Tyvek) lab coats. Airborne contamination monitored using blank filters. Particles matching blank features excluded. One fibre excluded in PT-A-096 (transparent, 1570 µm) and one in PT-B-018 (green, 530 µm).
P01	Filtration, Digestion, sieving, filtration	Sample filtered on metal mesh, transferred to beaker and digested (KOH:NaClO, 48 h, 40 °C). Sieved at 300 µm; < 300 µm fraction discarded. > 300 µm fraction filtered onto glass fibre filter.	Stereo-microscope, measurement, FTIR-ATR	Glass filter examined under stereo-microscope; particles photographed. Size, colour and shape recorded. Subset analysed by FTIR-ATR for polymer identification..
P02	Nile Red / fluorescence microscope AND µRaman spectroscopy	Samples were transferred into beakers, digested with H2O2 30 % (resulting in 10 % in suspension), transferred via cellulose filters (Sartorius 1289) with ethanol, stained in suspension and	Nile Red/FL microscopy and Raman spectroscopy	Particles identified, counted and measured by fluorescence microscopy (Zeiss K5/7 MAT, 5×). Polymer composition determined by Raman spectroscopy (DRX2xi Thermofisher) for all particles > 100 µm

Lab code	Analytical technique for identification	Experimental procedure for identification	Analytical technique for quantification	Describe the experimental procedure for quantification
		transferred onto Anodisc filters (aluminium oxide, 47 mm, 0.2 µm);		and a subset of smaller particles.
P03	Visual microscopy, fluorescence microscopy, FTIR ATR	Visual microscopy for morphology, colour and size classification. Nile Red staining and fluorescence microscopy to support identification of plastic particles and quantification. FTIR-ATR for polymer identification of representative particles > 300 µm (method validation). No µFTIR imaging for 20–300 µm fraction; focus on > 300 µm particles in relatively clean seawater matrix.	Visual microscopy in combination with fluorescence microscopy	Visual counting, supported by (automated) counting of particles from fluorescence image
P04	Micro-Fourier Transform Infrared Spectroscopy (µFTIR)	After visual counting, selected particles were transferred from the PC membrane to a diamond compression cell. Polymer composition determined by µFTIR (transmission mode); spectra matched against a reference library.	Light microscopy with digital imaging	Samples filtered onto PC membrane filters. Filters examined by light microscopy with digital imaging. Microplastics visually counted and sized using image analysis software.
P06	Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR)	ATR-IR was used to identify the polymers of each individual particle.	Stereoscope	Sequential sieving (coarse to fine). Each size fraction collected in pre-weighed Petri dishes, dried, oxidised (H ₂ O ₂), re-dried and re-weighed. Microplastics counted by two analysts under stereomicroscope..
P07	Microplastics analysed using Agilent 8700 LDIR (Laser Direct Infrared) chemical imaging system. Automated counting and polymer identification by IR spectroscopy; spectra matched against Agilent Microplastics Starter Library 2.1. Particle size range: 50–5000 µm.	Samples were directly filtered through a gold-coated filter diameter 25 mm, mesh 0.8 µm under vacuum and then analysed without further manipulation. A filter blank was analyzed prior to the sample.	Microplastics analysed using Agilent 8700 LDIR system. Automated counting and polymer identification by IR spectroscopy; spectra matched against Agilent Microplastics Starter Library 2.1. Size range: 50–5000 µm. Results accepted at ≥ 85 % match, according to instrument criteria.	Samples were directly filtered through a gold-coated filter diameter 25 mm, mesh 0.8 µm under vacuum and then analysed without further manipulation. A filter blank was analyzed prior to the sample
P08	ATR-FTIR	Suspected particles handpicked under stereomicroscope; analysed by ATR-FTIR	Fluorescence microscopy	Particles on filter stained with Nile Red (10 µg/mL in EtOH); 30 min at 50 °C. Excess dye rinsed with

Lab code	Analytical technique for identification	Experimental procedure for identification	Analytical technique for quantification	Describe the experimental procedure for quantification
		(PerkinElmer Spectrum Two, diamond crystal). Spectra recorded 550–4000 cm^{-1} (8 scans). Compared with FLOPP, FLOPP-e and in-house libraries. Identification accepted for HQI > 0.9.		EtOH; air-dried. Examined by fluorescence stereomicroscopy (Leica MZ10 F, GFP LP filter). Fluorescent particles photographed; maximum diameter measured (Las X).
P09	Direct visual sorting was done under a stereomicroscope LEICA S9i.	Filters examined with specified magnification. Only non-organic items (no cellular structure) counted as microplastics.	Direct counting	The items were counted according to colour and typology in an ad hoc generated statistic.
P10	Nile red fluorescent microscopy	Samples digested following Löder et al. (2017): 10 % SDS, protease, 35 % H_2O_2 and chitinase. Filtered onto 2–3 GFF filters. Filters stained with Nile Red (1.0 mg/mL).	Fluorescent particles were count with an automated Leica-LASX program	Dyed microplastics imaged by epifluorescence microscopy (blue excitation, 525 nm emission; Leica M205 FCA, 0.78 \times). Image analysis with LAS X (v3.9.0). Exposure 200 ms. Particles > 20 μm and grayscale \geq 40 classified as plastics. Size expressed as Feret maximum.
P11	Visual identification and FT-IR spectroscopy	Filters examined under dissecting microscope. Suspected microplastics tested mechanically and thermally using tweezers and needles.	Total count and classification of individual MPs according to their shape, color, and composition.	Classification followed the Guidance for Monitoring Marine Litter in European Seas. Shape and colour recorded visually for each item. \geq 10 % of MPs characterised by FT-IR.
P12	Visual identification by stereomicroscopy (ZEISS SteREO Discovery V8, Axiocam 208, Labscope v3.4). Polymer composition confirmed by ATR-FTIR spectroscopy.	Suspected particles pre-selected by stereomicroscopy (ZEISS SteREO Discovery V8), isolated with tweezers and analysed by ATR-FTIR. Spectra recorded 4000–400 cm^{-1} (4 cm^{-1} resolution, 32 scans). Library matching (OMNIC; > 15,000 spectra); acceptance \geq 70 % match with manual verification.	Microplastics quantified by stereomicroscopic visual counting; image analysis used for sizing and classification.	Samples filtered onto glass fibre filters; examined by stereomicroscopy (ZEISS SteREO Discovery V8). Suspected particles counted and measured (length, width) using Labscope v3.4. Results reported as particles per sample. ATR-FTIR confirmation performed on a subset (15 randomly selected particles per sample) for time efficiency.
P14	Microscopic analysis using a stereo microscope (Olympus SZX10) combined with a digital camera (Olympus SC180)	Samples filtered onto GF/D Whatman glass fibre filters (47 mm, 2.7 μm) using glass vacuum system. Bottles and equipment rinsed with ultra-pure water (18.2 M Ω); rinsates filtered. Filters transferred to covered Petri dishes, dried at 45 $^\circ\text{C}$, sealed	Particles manually counted by stereomicroscopy (Olympus SZX10) with digital camera (Olympus SC180).	Sent samples and bottle-rinse filtrates analysed; results summed. Particle length and width measured during counting. Size classification based on largest dimension.

Lab code	Analytical technique for identification	Experimental procedure for identification	Analytical technique for quantification	Describe the experimental procedure for quantification
		and stored in desiccator. Microscopic analysis performed for particle counting.		
Lab code	Code Sample 1	Code Sample 2	Type(s) of polymer identified in Sample 1	Type(s) of polymer identified in Sample 2
P01	PT-A-213	PT-B-0015	Polypropylene; Polyethylene	Polypropylene; Polyethylene
P02	PT-A-121	PT-B-023	PP (57%), PE (34 %), PET, PA, PS	PP (58%), PE (32 %), PET, PA, PS
P03	PT-A-207	PT-B-008	PE (Green, White), PP (Blue, Red)	PE (White), PP (Blue, Red)
P04	PT-A-252	PT-B-012	PE, PP	PE, PP
P05	PT-A-096	PT-A-173	None	None
P05	PT-B-018	PT-B-010	None	None
P06	PT-A-185	PT-B-031	Polyethylene; Polypropylene	Polyethylene; Polypropylene
P07	PT-A-247	PT-B-044	Polyethylene (PET); Polypropylene (PP); Polyethylene Terephthalate (PET), Polyamide (PA), Rubber	Polyethylene (PET); Polypropylene (PP); Polyethylene Terephthalate (PET)
P08	PT-A-153	PT-B-003	PP; PE	PP; PE
P09	PT-A-086	PT-B-006	3	2
P10	PT-A-005	PT-B-032	none	none
P11	PT-A-195	PT-B-001	Polypropylene; Polyethylene	Polypropylene; Polyethylene
P12	PT-A-196	PT-B-037	Polyethylene, polypropylene	Polyethylene, polypropylene
P14	PT-A-197	PT-B-029	None	None
P15	PT-A-122	PT-B-025		

Lab code	Total number MPP: Sample 1	Total number MPP: Sample 2	1000-4999* μm *Feret _{max} : 1	1000-4999* μm *Feret _{max} : 2	300-999 μm : 1	300-999 μm : 2	100-299 μm : 1	100-299 μm : 2	50*-99 μm *Feret _{min} : 1	50*-99 μm *Feret _{min} : 2
P01	99	70	4	38	95	30	0	2		
P02	90	70	4	31	76	29	5	4	5	6
P03	97	82	0	42	89	39	8	1	0	0
P04	92	64	3	30	87	33	2	1	0	0
P05	80	74								
P05	55	66	19	25	36	41				
P06	60	67	2	12	58	55	0	0	0	0
P07	96	67	7	28	68	20	6	9	15	10
P08	92	65	1	21	91	44	0	0	0	0
P09	99	78								
P10	94	95	9	40	60	26	9	13	16	16
P11	77	42								
P12	83	71	4	33	79	38	0	0	0	0
P14	102	84	15	39	82	43	5	2	0	0
P15	55	58								

Lab code	Measurement uncertainty	Method of MU estimation : 1
P01		
P02	N = 5	1: LOD from 3 blank samples LOD(mean+3*s.d.) >100 μm = 5 (exclusively fibres/filaments); LOD (50-99 μm)=10 2: LOD>100 μm = 5 (exclusively fibres/filaments); LOD (50-99 μm)=10
P03		
P04		
P05		
P05		
P06	± 4 or ± 1	different researcher
P07	.	Information regarding the uncertainty has not yet been investigated
P08		
P09		
P10		
P11		
P12	84.0 \pm 1.2 68.7 \pm 2.4	Measurement uncertainty estimated for total microplastic count. Each sample counted three times (n = 3). SD calculated; standard uncertainty u = SD/ $\sqrt{3}$. Expanded uncertainty U = k*u (k = 2; \approx 95 % confidence). Results reported as mean \pm U.
P14		
P15		

Additional Comments/Observations

P01	Polypropylene = blue & pink particles ; Polyethylene = transparent & green particles																																																						
P02	PET, PA and PS have only been identified in small numbers (all except for two fibres PA in PT-B and one fibre PET in PT-A) were fragments <100 µm). The polymer composition of particles <100 µm has only been identified on a random subset; sporadically particles adhered to each other, and it was difficult to separate whether it is one single particle or two to three adhered to each other. Some particles <100 µm seemed to be broken from larger particles.																																																						
P03	We submit only data for the analyses of the second replicate without ultrasonic treatment. During handling of the first replicate, we realised that the particles were quite fragile to 1-minute ultrasonic treatment, because they seemed to disintegrate into multiple smaller particles that were picked up at 10 µm filter applied below the 300 µm filter. For the first replicate, we also applied a chemical digestion procedure with NaOH that we normally use for analysing MP in a dirty environmental sample matrix, followed by filtration before using ultrasonic treatment to get all collected particles into a suspension before a second filtration step that we will use for the final quantification of MP in the sample.																																																						
P04	Quantification was based on the total number of particles per filter (0,1 L of filtered sample) and not normalized to the volume of 1 L of 1m3.																																																						
P06	The polymer types observed were Polyethylene for white and green particles and polypropylene for blue and red particles. Green was only observed in sample PT-A-185 and not in PT-B-031. However, in PT-B-031, in the fraction 100-299 µm, organic matter was observed but after oxidation with hydrogen peroxide, these particles were no longer visible (they were oxidized). The total mass of the microplastics in the samples were between 20 and 10 mg. Another interesting observation was that blue particles after oxidation change colour and became white. Thank you for the interesting experience.																																																						
P07	<p>We observed Polyethylene (PE) and Polypropylene (PP) as the major plastics in both samples. Polyethylene Terephthalate (PET), Polyamide (PA), and rubber were observed as minority components, and in some cases, as fibres. We are uncertain whether these plastics come from the spiking process or the water matrix. Unfortunately, the matrix blank was not available for the analysis.</p> <p>The reported number of microplastics corresponds to the content in the samples, that is, in the 0.1 L.</p> <p>The following tables summarize the number of plastics found by size and type</p> <p>Table1: Polyethylene (PET) results</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Size</th> <th>Sample ID</th> <th>50-99</th> <th>100-299</th> <th>300-999</th> <th>1000-4999</th> </tr> </thead> <tbody> <tr> <td>300-1000</td> <td>PT-A-247</td> <td>3</td> <td>-</td> <td>25</td> <td>2</td> </tr> <tr> <td>300-5000</td> <td>PT-B-044</td> <td>4</td> <td>4</td> <td>7</td> <td>15</td> </tr> </tbody> </table> <p>Table2: Polypropylene (PP) results</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Size</th> <th>Sample ID</th> <th>50-99</th> <th>100-299</th> <th>300-999</th> <th>1000-4999</th> </tr> </thead> <tbody> <tr> <td>300-1000</td> <td>PT-A-247</td> <td>4</td> <td>2</td> <td>43</td> <td>5</td> </tr> <tr> <td>300-5000</td> <td>PT-B-044</td> <td>5</td> <td>5</td> <td>13</td> <td>13</td> </tr> </tbody> </table> <p>Table 3 other polymers</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Size</th> <th>Sample ID</th> <th>50-99</th> <th>100-299</th> <th>300-999</th> <th>1000-4999</th> </tr> </thead> <tbody> <tr> <td>300-1000</td> <td>PT-A-247</td> <td>4(PET)</td> <td>4(PET)</td> <td>1(PA)</td> <td>3(rubber)</td> </tr> <tr> <td>300-5000</td> <td>PT-B-044</td> <td>1 (PET)</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>	Size	Sample ID	50-99	100-299	300-999	1000-4999	300-1000	PT-A-247	3	-	25	2	300-5000	PT-B-044	4	4	7	15	Size	Sample ID	50-99	100-299	300-999	1000-4999	300-1000	PT-A-247	4	2	43	5	300-5000	PT-B-044	5	5	13	13	Size	Sample ID	50-99	100-299	300-999	1000-4999	300-1000	PT-A-247	4(PET)	4(PET)	1(PA)	3(rubber)	300-5000	PT-B-044	1 (PET)			
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P08	Results of polymer type VS size are also available as well as results for samples PT-A-95 and PT-B-016																																																						
P14	A smaller bottle would be better for this sample volume to reduce carry-over loss to the filter.																																																						

Source: JRC

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