MICROPLASTICS IN SEDIMENT
Comparison of extraction and characterization methods
Case study in Marseille Bay (France)

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INTRODUCTION

Microplastic pollution has become a major concern for the protection of the marine environment worldwide. In Europe, microplastics in sediment have been selected as an indicator of environmental assessment within the framework of the MFSD and OSPAR convention (Baüerlein, et al, 2023). However, the use of this inter-directive indicator requires harmonization of extraction and identification methods. One fundamental criterion for assessing this pollution involves quantifying concentrations in the environment, which can be altered during the isolation of microplastics from in-situ samples. Indeed, the recovery rate is an important factor that identifies the accuracy, quality, and efficiency of sample processing (Dimante-Deimantovica et al, 2022). Moreover, identification techniques also play a crucial role in avoiding false positives or negatives. Given the numerous existing techniques for extracting and identifying microplastics in sediment, harmonization of protocols and methods appears essential, particularly to achieve data comparability. This is the question that the partners of the JPI Ocean Andromeda project, engaged in WP2, have attempted to address in this task.

SAMPLING CRUISE – September 2021

Figure 1 – Antedon II ship used for the cruise

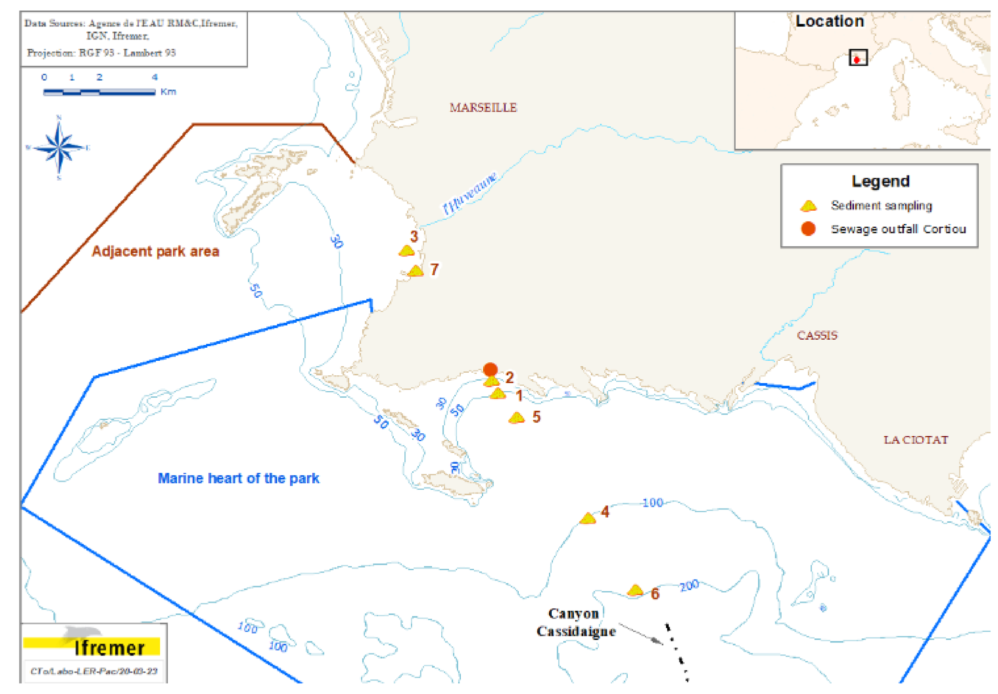

Figure 2 – Sampling sites for sediment samples in the bay of Marseille – French waters in the Western Mediterranean Sea (projection RGF93-Lambert 83)

→ **Inter-comparison exercise** : sediment taken at 10 m depth in Marseille Bay , France, northwest coast of the Mediterranean Sea

→ **In-situ samples :** MP contamination in Marseille Bay and Calanques, 7 stations sampled : 2 in the Marseille Bay and 5 in the Calanques National Park at different depths : Beach, 10m, 30m, 50m, 75m, 100m, 150m.

MATERIAL & METHODS

➢ Methods for inter-comparison exercise

Sediment sampled at a 10m depth with a Reyneck corer

CLEANING
Density separation
2 technics:
1. Saturated NaCl
2. H_2O_2 digestion/$ZnCl_2$

NEGATIVE CONTROL
6 samples, 50 g each
Target concentration:
0 items/50 g d.w

Adding artificial MPs
From CARRAT, fragments of size class 500-1000 µm

POSITIVE CONTROL
6 samples, 50 g each
Target concentration:
15 items/50 g d.w
Made by Ifremer

Extraction and characterization of MPs by each partner with their own methods

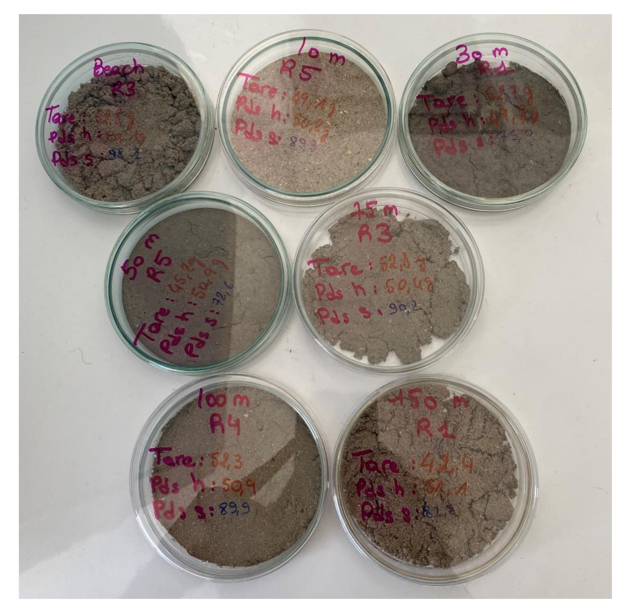

Figure 3 – Sediment samples

➢ *In situ* sediment samples : analysed by each partner with the same methods as in the inter-comparison exercise

Table 1 - Methods of extraction and characterization of microplastics

Partners	PARTNER 1 (P1)	PARTNER 2 (P2)	PARTNER 3 (P3)	PARTNER 4 (P4)	PARTNER 5 (P5)	PARTNER 6 (P6)
Identification method	Stereomicroscope visual identification using Nile Red	FTIR spectroscopy + visual verification with fluorescence microscopy	Visual identification using « hot needle test »	FTIR spectroscopy	Stereomicroscope visual identification using Nile Red	LDIR chemical identification combined with FTIR spectroscopy
Extraction method	H_2O_2/$ZnCl_2$ density extraction	H_2O_2/NaI density extraction	NaI density extraction	NaI density extraction	NaI density extraction	Saturated NaCl density extraction

PRELIMINARY RESULTS

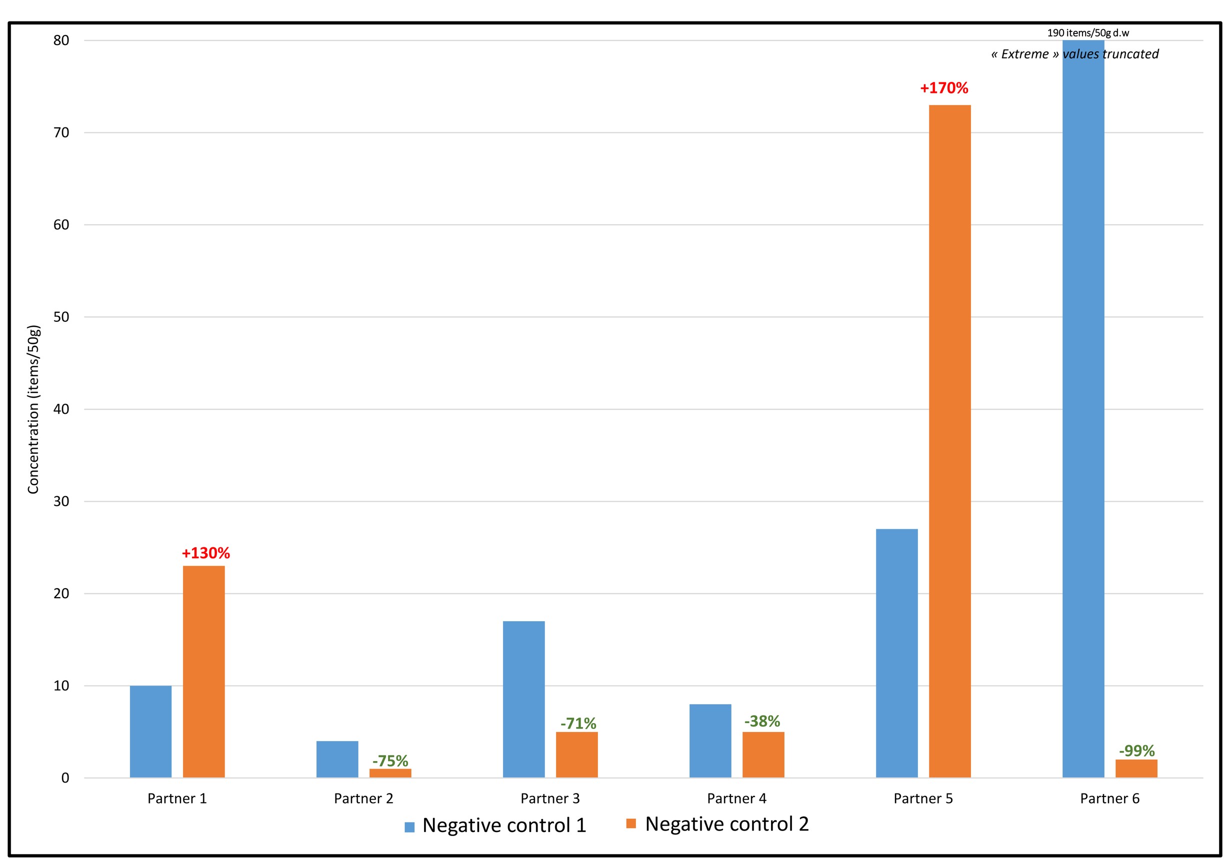

Figure 4 - Comparison of MPs concentration found by each partner in NC1 (NaCl extraction) and in NC2 (H_2O_2/ $ZnCl_2$ extraction) and their percentage of difference

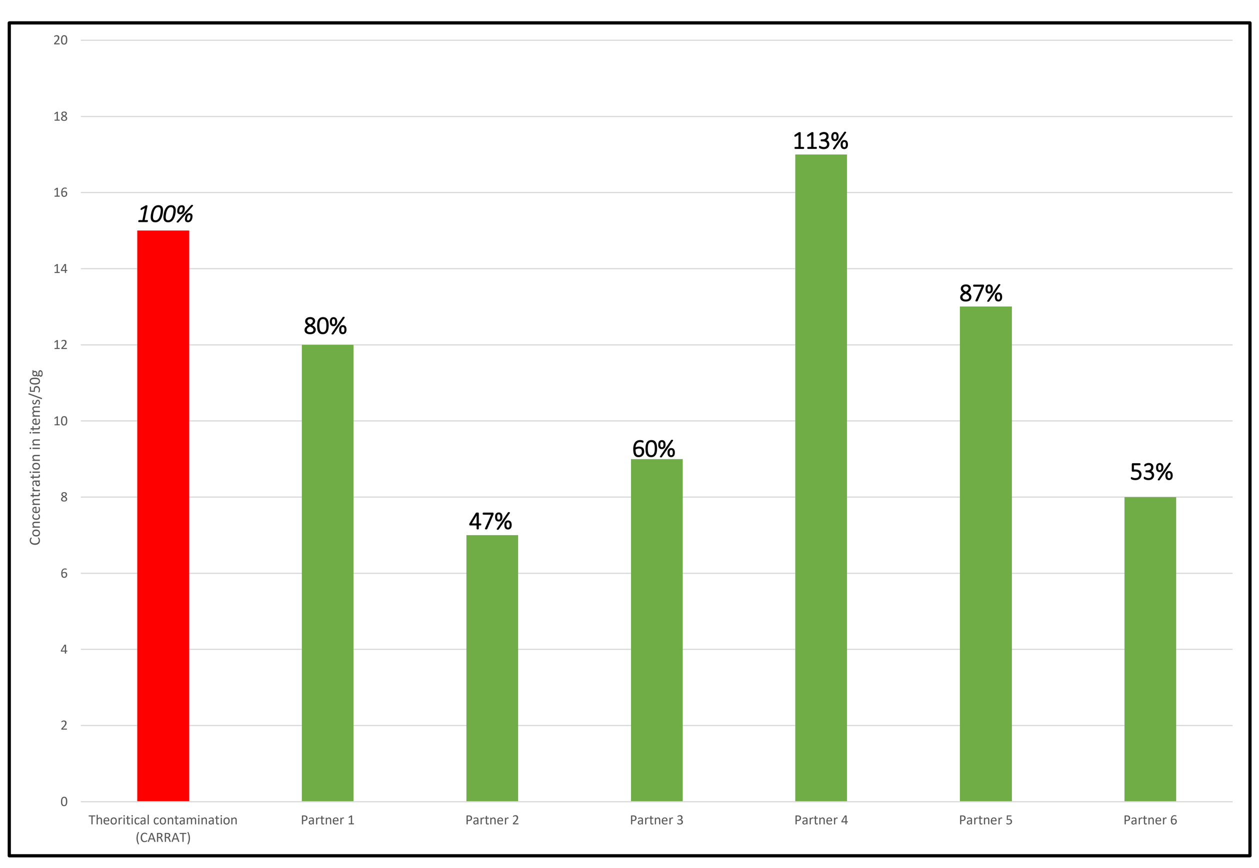

Figure 5 - MPs concentration found by each partner in Positive Control compared to the theoretical contamination (15 items/50 g d.w). % correspond to recovery rate

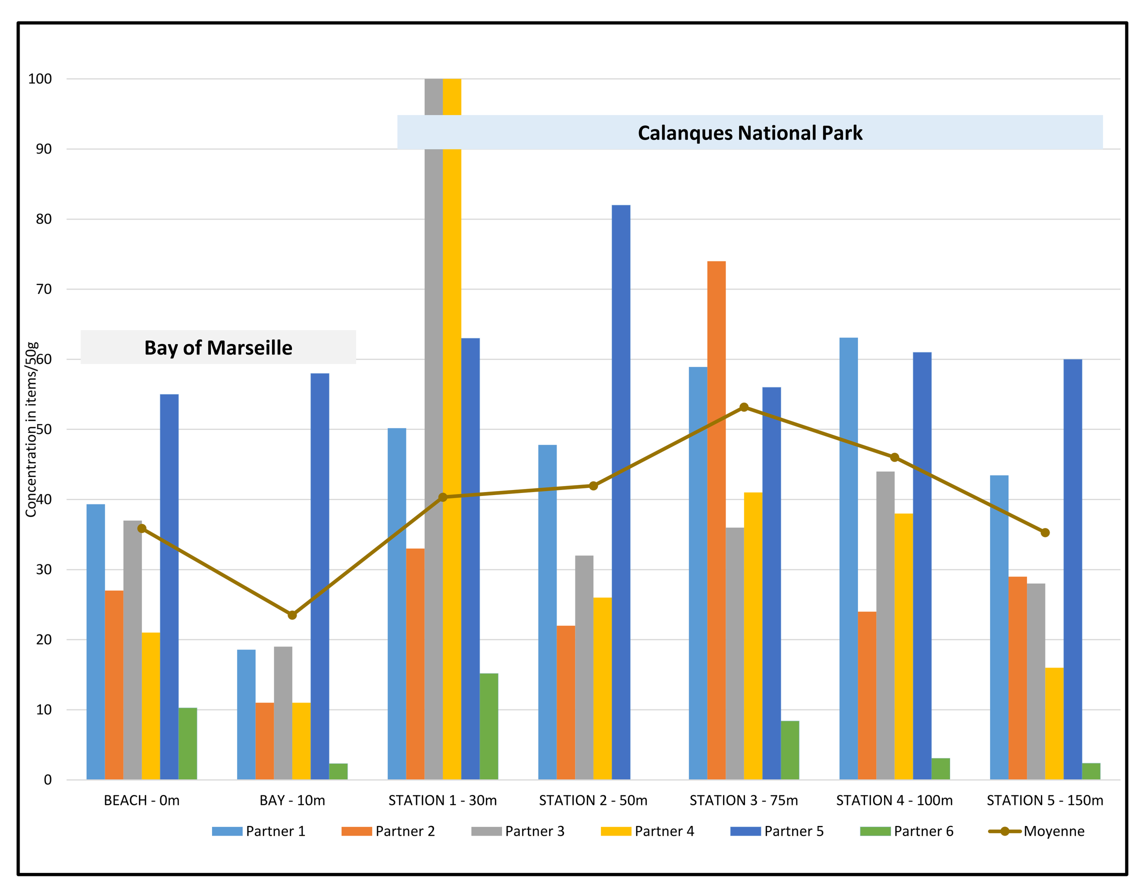

Figure 6 - MPs concentration found by each partner in in-situ samples, for all size classes and typologies.

- Although two partners found a higher recovery rate on NC 2, the use of H_2O_2 (for sediment cleaning) and of $ZnCl_2$ increased recovery rates. This method appears to be the case for most partners more effective than with NaCl.
- The digestion step (H_2O_2) improves the recovery rates.

- Nile Red technic is more effective on fragments with a size class superior to 300 µm than on other size and typologies of MPs.
- Extraction techniques exert an impact on the recovery rates and are subject to various laboratory-related factors (such as laboratory contamination or particle loss).

- MPs contamination appears in mean higher in the Calanques National Park than in the Marseille Bay, possibly due to the presence of a discharge zone for treated wastewater. More samples are needed to confirm this hypothesis.

CONCLUSION: Recovery rates range from 47% to 113%, and none of the partners achieved the exact MP concentration expected. Recovery rates are strongly influenced by the extraction techniques. Sediment cleaning can be improved with a digestion step (H_2O_2) and the use of high-density reagents such as $ZnCl_2$ or NaI. Reference identification techniques such as FTIR remain the most reliable, but the fluorochrome method remains a cost-effective and high-performance solution for fragments larger than 300 µm, and could be applied to monitoring programs such as the MFSD. In-situ samples were analyzed in parallel using the same techniques, and the results showed considerable variability. However, the average environmental signal shows larger contamination in the Cortiou Calanque (National Park) than in the highly anthropized Bay of Marseille, which would be interesting to confirm with a larger sampling plan.