Small MICROplastics (<100 µm) bioindicaToRs in the changing ArctiC EnviRonment (MICROTRACER)

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1. Summary of the Microtracer project

The Arctic Ocean is undergoing several transformations in relation to global climate change. Small microplastics (SMPs) or nanoplastics (NPs) carried by marine aerosol may settle in the land ice and be released to inland waters with ice melting. As sea ice extent reduces, MPs may enter the region following ocean transport and increasing shipping and fishing activities with implications on Arctic biota, human health, and socioeconomic issues related to the exploitation of marine resources. First analyses on amphipods collected in Ny-Ålesund confirmed the presence of SMPs. Nevertheless, the threat posed by SMPs/NPs to polar biota and regional human health is not fully understood. The Arctic marine environment supports productive food webs, vulnerable to pollutants and pathogens; multiple antibiotic-resistant bacteria have been found in Arctic regions. Antibiotic-resistant bacteria, pathogens, and pollutants (e.g., SMPs/NPs) can affect wildlife and humans with severe socioeconomic consequences and costs.

The research activities will be structured into 7 main work packages (WP).

-WP1 Project Coordination;

-WP2 Sampling of biota and sediments in different sites of the Ny-Ålesund area (e.g., sites at Kongsfjorden, sites at Krossfjorden, and Ny-Ålesund). At least two common Arctic marine species of Gammaridae, Amphipoda, and other invertebrate species will be sampled;

-WP3 Small MPs and NPs in biota and sediments;

-WP4 MPs-associated microbiota and pathogens;

-WP5 Chemical characterization of biota and sediments and pollutants on MPs;

-WP 6 Biomarkers application;

-WP7 Dissemination

2. Aims of the Project and Period of the study

The MICROTRACER project aims at the following:

-a) quantifying and identifying according to polymeric nature MPs and NPs in sediments and biota (i.e., Amphipoda and other invertebrates) to verify their suitability as bioindicators;

-b) identifying markers of MPs and NPs' impacts on biota. As ingested MPs can trigger molecular, cellular, or physiological effects in the studied species, biomarkers could serve as early indicators of biological responses to ingested MPs and related pollutants. MPs uptake could reduce organisms' energy assimilation and modify their feeding rate. Body biochemical composition will be evaluated, together with the effects of MPs ingestion on energy metabolism (called cellular energy allocation), as a sensitive physiological indicator of stress that could reduce resource allocation for growth, reproduction, and survival;

-c) assessing body burden (e.g., organic pollutants and trace elements) of bioindicators and pollutants adsorbed on MPs;

-d) determining the abundance, diversity, and metabolic activity of bioindicators' microbiota and its changes due to MPs ingestion or adsorbed pollutants. In the selected bioindicators, water, and sediments, the quali-and quantitative analysis of MPs-and NPs-attached microbiota (plastisphere) will be performed, together with the search for potential pathogens (E. coli, Enterococcus spp., Vibrio spp.). Antibiotic-resistant bacteria will also be searched.

Funded by the Arctic Research Program (PRA), MICROTRACER (2022-2024) is coordinated by the Institute of Polar Sciences (ISP) of the Italian National Research Council (CNR).

1. Participant Institutions

Coordination:

Dr. Gabriella Caruso (CNR, Institute of Polar Sciences-ISP, Messina), PI (Principal Investigator).

Research Units (RU)- Leaders and Participants:

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RU 2: Dr. Valentina Iannilli (RU Leader), Dr. Claudia Trotta, Dr. Francesca Lecce (Lab of Biodiversity and Ecosystem Services, ENEA, Rome).

RU 3: Prof. Sara Bogialli (RU Leader), Prof. Moreno Meneghetti, Dr. Lucio Litti (Dept. of Chemistry, University of Padua, Padua).

RU 4: Prof. Marco Oliverio (Ru Leader), Dr. Andrea Setini (Dept. of Biology and Biotechnologies, Sapienza University of Rome, Rome).

Field scientists:
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The MICROTRACER project has a Research in Svalbard (RiS) ID n. 11919 A1.
Take a look at the RiS website: (https://www.researchinsvalbard.no/project/f6e50000-7757-d25d-4af6-08d9e43b4340/project-info).

3. Samples collection and preliminary treatment

Prior to the first Arctic Microtracer field activities, all the project participants discussed, approved, and shared sampling site locations (Fig. 1 and Fig. 2, Table 1) operative protocols (OPs) to be carried out for field sampling, sample handling in the laboratory and shipping of materials and collected samples.

Below are the established OPs:

- Operative protocol for sample collection (sediment and amphipods) for bacteriological/toxicological analysis (CNR-ISP, Messina/Venice Lab);
- Operative protocol for biota collection and following determination of MPs (CNR-ISP, Venice Lab);
- Operative protocol for biota collection and RNA analysis (Enea, Rome Lab).

4. Field Activities

The fieldwork activity occurred from 28th August to 26th September. Two field scientists were involved in sampling both marine sediment samples and biota samples collected at several sites. During the Microtracer campaign in Ny-Ålesund, samples were treated and stored at the Italian Arctic Station "Dirigibile Italia".

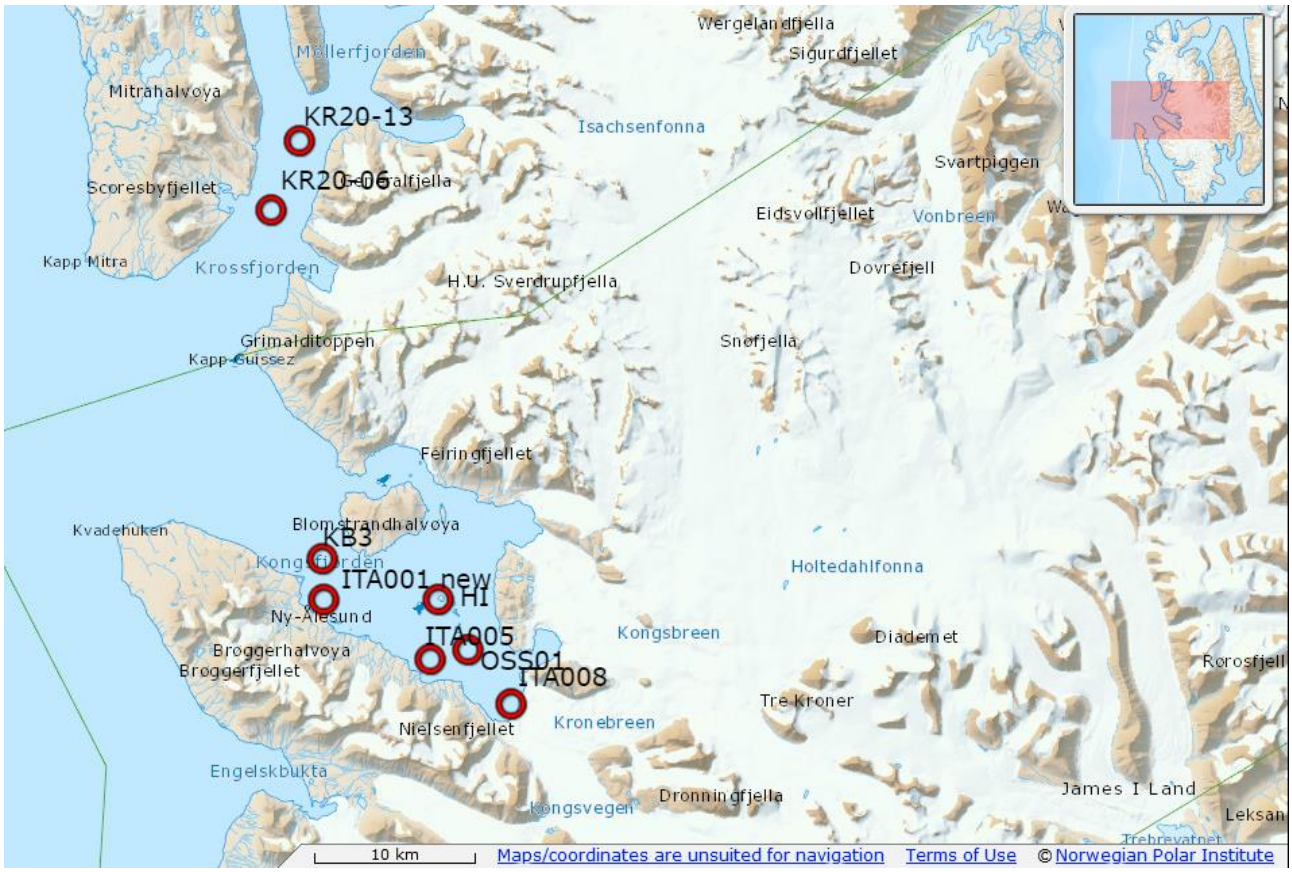

***Figure 1** – Locations of sediment sampling sites in Kongsfjorden and Krossfjodern. Sediment samples were collected with the grab on board MS Teisten (Kings Bay) or manually (HI) on the coast (https://toposvalbard.npolar.no/).*

Figure 2 *– Locations of Amphipods sampling sites in Kongsfjorden. Sampling was carried out with a small hand net from the coast. Each point was sampled at two different times to obtain a second pool. SI and HI sites were reached with the NPI Polar Circle (https://toposvalbard.npolar.no/).*

Table 1 *– Summary of the sediment and biota sampling sites with date and time, type of sampling, coordinates, depth (for the grabbed sediment samples), and additional notes related to the weather conditions and other sampling information.*

2022 Sampling Date (dd/mm)	Time (hh:mm)	ID Station	Fieldwork name	Latitude (°N)	Longitude (°E)	Depth (m)	Notes
31/08	13:14	***KR20-13***	SEDIMENT	79°11.34’	11°47.5’	381	Krossfjorden Wind around 4-5 m/s Low sea state
	14:30	***KR20-06***	SEDIMENT	79°08.99’	11°43.25’	379	
02/09	14:06	***ITA005***	SEDIMENT	78°54.21’	12°15.58’	~32	Kongsfjorden Close to the glacier Weather similar to 31/08
	15:07	***ITA008***	SEDIMENT	78°52.82’	12°29.88’	~55	
06/09	09:24	***ITA001 new***	SEDIMENT	78°56.027’	11°56.367’	~60-70	Closest to Ny-Ålesund Cloudy Wind around 4-5m/s The depth sensor was broken – depth was calculated manually

2022 Sampling Date (dd/mm)	Time (hh:mm)	ID Station	Fieldwork name	Latitude (°N)	Longitude (°E)	Depth (m)	Notes
	10:39	***KB3***	SEDIMENT	78°57.375’	11°55.670’	> 300	
09/09	15:27	***OSS01***	SEDIMENT	78°54.561	12°22.025’	~77	Rainy Flat sea No wind
14/09	16:05 – 16:30	***HT*** *(Harbour Teisten)*	AMPHIPODS	78°55’42,7’’	11°56’12.3’’	/	Small hand net used to move the algae under the harbor
15/09	09:50 – 10:34	***SI*** *(Storholmen Island)*	AMPHIPODS	78° 56.136’	12° 13.766’	/	Cloudy No wind Low tide
	10:55 – 11:25	***HI*** *(Observasjons Holmen Island)*	AMPHIPODS	78° 56.217’	12° 16.290’	/	Sampling around algae in rocky areas
21/09	09:35 – 10:15	***SI 2nd pool*** *(Storholmen Island)*	AMPHIPODS	78° 56.136’	12° 13.766’	/	Sunny Wind 8 m/s from south/east Flat sea
	10:45 – 11:30	***HI 2nd pool*** *(Observasjons Holmen Island)*	AMPHIPODS	78° 56.217’	12° 16.290’	/	
	10:38	***HI*** *(Observasjons Holmen Island)*	SEDIMENT (manually)	78° 56.217’	12° 16.290’	From the coast, 10-15 cm	

2022 Sampling Date (dd/mm)	Time (hh:mm)	ID Station	Fieldwork name	Latitude (°N)	Longitude (°E)	Depth (m)	Notes
24/09	14:00 – 14:45	***HT 2nd pool*** *(Harbour Teisten)*	AMPHIPODS	78°55'42.7''	11°56'12.3''	/	Cloudy Light rain Less windy than the previous days (it reached even 20 m/s)

5. Sampling of sediments

Sediment samples were collected on board the MS-Teisten, Kings Bay research vessel (Fig. 3a). Samples were collected at seven points across Krossfjorden (2 sites) and Kongsfjorden (5 sites - one close to Ny-Ålesund). On board, the activities were coordinated within both the AFG (Arctic Field Grant) project "Dynamics of anthropogenic pollutants in the Arctic fjord environment" (RiS ID n. 11872) and the FIKO project (Freshwater input in the Kongsfjorden – RiS ID n. 10578) of CNR-ISP Bologna working group. This team collected/launched three moorings in the two fjords and sampled sediments to analyse some organic contaminants. Sediment samples were taken with a Van Veen grab (size 1000 cm^2 - Fig. 3b). only one sample was collected directly from the coast with a spoonbill (HI Station 2nd pool – Fig. 3c).

(a) (b) (c)

Figure 3 – *Sediment sample collected on board of MS Teisten (a) with a Van Veen grab (Kings Bay) (b); sediment sample collected from the coast with a stainless-steel spoonbill – Samples were put into an aluminium container and pre-processed in "Dirigibile Italia" laboratories (c).*

A sensor measured the rope's length and the point's depth. Depth, coordinates, time, and weather conditions were signed for each sampling point. Since it was not possible to take the sample from the holes in the upper part of the grab, it was opened directly on the deck (covered by aluminium foils).

A superficial portion of the sediment was put in an aluminium container with a stainless steel spoonbill and then covered with aluminium foils to avoid external contamination. Nitrile gloves were used during this procedure. The aluminium containers were maintained in a cold styrofoam box until their arrival in the laboratory, where they were partially processed.

6. Laboratory activity for sediment and biota

Sediment samples were pre-processed at the Italian Arctic "Dirigibile Italia" research station. Each activity was conducted wearing a cotton lab coat, face mask, and nitrile gloves. The area was cleaned before the lab activity. Each sample was divided into different aliquots using a stainless-steel spoon (Fig. 4 and Table 2):

- Sterile plastic containers (100 mL) for bacteriological analysis. Stored at -20°C
- Sterile glass jars (about 100-200 mL) for bacteriological analysis. Stored at -20°C – Once finished, samples were preserved in two sterile plastic falcon tubes (50 mL).
- Sterile Falcon tubes (50 mL) for the organic component. Stored at -20°C
- Sterile plastic containers (100 mL) for bacteriological analysis. Stored at +5°C
- Sterile glass container (about 100-200 mL) for bacteriological analysis. Stored at +5°C
- Decontaminated glass container (about 200 mL) for microplastic analysis. Stored at +5°C.

(a) (b) (c)

***Figure 4** – Pre-processing of sediment samples divided in different aliquots (a); examples of samples subdivision for the +5°C and -20°C aliquots (b, c).*

A few organisms were found in the sediments, mostly worms, divided into 50 mL Falcon tubes and then frozen at -20°C (stations KR20-13, KR20-06 and KB3). All the samples were logged, closed with parafilm, and stored until shipping. Sediment samples for MPs analysis were carried to Venice on the way back.

7. Sampling activity for Arctic Marine organisms

The second topic of the fieldwork activities was sampling Amphipoda and other Arctic organisms. Two samplings were carried out around the coasts of the Loven Islands, the first on September 15th and the second on September 21st. These sites (HI - Obs-Holmen Island; SI - Storholmen Island) were re-sampled in a second expedition to have an additional pool at the same site. The NPI Polar Circle (Zodiac inflatable boat) was used to reach these sites. The third sampling site (HT – Harbour Teisten) was reached on Ny-Ålesund pier, an area rich in algae, the typical habitat of these organisms (Fig. 5). Again, two pools at the same site were taken, one on the 14th and the other on the 24th of September.

Sampling activity was performed using a small hand net (Fig. 5). Plastic containers and bottles were used to check and transport the samples to the laboratory. At each point, about 20-25 hatches were performed to grab some organisms and then these were transferred into a plastic container. The salty water with all the organisms was poured into a plastic bottle to be pre-processed in the laboratory. During this procedure, nitrile gloves and the containers were first washed and decontaminated.

(a) (b)

***Figure 5** – Sampling of amphipods from the harbor (HT) (a) and the Loven Islands (SI and HI) (b) – A small hand net was used to collect these invertebrates, mainly present in rocky and algal areas.*

8. Laboratory activities

Once at the "Dirigibile Italia" station laboratories, the organisms were divided into three aliquots (Fig. 6):

- RNA-later aliquot: one individual (at maximum 3, if too small) in Eppendorf with RNA-later. Then frozen at -20°C.
- TOX: one individual (at maximum 3, if too small) in Eppendorf and then stored at -20°C. Each organism was first dried on a sheet of paper towels.
- MPs: more than 10 individuals in small glass bottles (25 mL) with 80% ethanol for microplastics analysis. These organisms were also divided according to the dimension (i.e., small/medium – big).
- Taxonomy: the remaining organisms were put into another glass bottle of 25 mL with 80% ethanol for the taxonomy. The ethanol was changed one/two times before the departure from Ny-Ålesund.

Different species of amphipods were found during sampling. During this preliminary phase, they were not divided by species/genus except for the Caprellidae, which are more easily recognizable than the Gammaridae or Ischyrocerus (Table 3). Each Eppendorf vial and glass bottle were named, closed with parafilm, and stocked within hermetic zip plastic bags at -20°C. TOX and RNA-later samples will be shipped to Rome, while those for MPs and Taxonomy were delivered directly to Venice on the return journey.

(a) (b) (c)

***Figure 6** – Pre-processing of amphipods samples; plastic containers where the salty water was poured before the allocation of the organisms (a); amphipods in glass bottles for microplastics analysis (b); single organism in Eppendorf for the RNA-later aliquot (c).*

9. Critical points encountered during the survey

Samples of sediments and amphipods were correctly collected. Unfortunately, few organisms were caught within the sediment samples. Most of the individuals were worms, but no other species were caught up, and the abundance was still inadequate.

Sediment sampling activities on the MS-Teisten during the first weeks of September had no difficulties due to the weather. It was not too windy, and it hardly rained.

The weather was more uncertain in the second half of September, and the wind was very strong for some days. Therefore, the number of sampling points that could be reached by boat was reduced. In addition, a high transport of sea ice from the glaciers made it impossible to reach the target points for sampling amphipods. The Loven Islands and the harbor of Ny-Ålesund, in contrast, were more easily accessible.

10. Data availability

Data are still not available, but sediment and biota samples shipped to Italy will then be processed in Messina, Rome, and Venice laboratories. Here below, two tables (Table 2 and Table 3) related to the pre-processing phases carried out at “Dirigibile Italia” laboratories (Ny-Ålesund, Svalbard Islands).

***Table 2** – Summary of the different aliquots of sediment samples collected by grab or manually - One part was divided for bacteriological analysis, another one for the organic component, and finally, the last part for microplastics analysis - Where found, biota was also isolated (*When the sterile jars ran out, two 50 mL Falcon tubes were used to replace them).*

2022 Sampling date (dd/mm)	**ID Sampling site**	**Bacteriology**		**Sediment for organic component** *Falcon tube (50 mL)*	**Biota in sediment** *Falcon tube (50 mL)*	**Microplastics** *Glass container (100 mL)*
		PP-container (100 mL)	*Glass container (100 mL)*			
31/08	***KR20-13***	-20 °C	-20 °C	-20 °C	6/7 worms (-20 °C)	1 Sediment sample
		5 °C	5 °C			
	KR20-06	-20 °C	-20 °C	-20 °C	4 worms (-20 °C)	1 Sediment sample
		5 °C	5 °C			1 Water field blank (from aluminium tray)
02/09	***ITA005***	-20 °C	-20 °C	-20 °C	/	1 Sediment sample
		5 °C	5 °C			1 Water field blank (from aluminium tray)
	ITA008	-20 °C	-20 °C	-20 °C	/	1 Sediment sample
		5 °C	5 °C			

2022 Sampling date (dd/mm)	**ID Sampling site**	**Bacteriology**		**Sediment for organic component** *Falcon tube (50 mL)*	**Biota in sediment** *Falcon tube (50 mL)*	**Microplastics** *Glass container (100 mL)*
		PP-container (100 mL)	*Glass container (100 mL)*			
06/09	***ITA001 new***	-20 °C	-20 °C	-20 °C	/	1 Sediment sample
		5 °C	5 °C (*)			1 Water field blank (from aluminium tray)
	KB3	-20 °C	-20 °C (*)	-20 °C	1st Falcon with 4 worms; 2nd Falcon with 2 worms.	1 Sediment sample
		5 °C	5 °C (*)			
09/09	***OSS01***	-20 °C	-20 °C	-20 °C	/	1 Sediment Sample
		5 °C (*)	5 °C (*)			
22/09	**HI 2nd pool**	-20 °C	-20 °C	-20 °C	/	1 Sediment Sample
		5 °C (*)	5 °C (*)			

Table 3 – Table of amphipods sampling sites and related samples processed in the laboratory - For each aliquot (TOX, RNA-later, Taxonomy, and MPs), the exact numbers of organisms per Eppendorf (2 mL) or glass container are reported (*if not specified one organism per each Eppendorf).

Sample date (dd/mm)	ID Sampling site	Type	TOX	RNA-later	MPs (n° and size of organisms)	Taxonomy
14/09	**HT** (Harbour Teisten)	Other Amphipods	7 Eppendorf*	6 Eppendorf	1 big About 8 small-medium	/
15/09	**SI** (Storholmen Island)	Caprellidae	2 Eppendorf		1	/
		Other Amphipods	8 Eppendorf	8 Eppendorf	1 big > 10 small-medium	/
	HI (Obs-Holmen Island)	Caprellidae	10 Eppendorf (1 with 2 organisms)	10 Eppendorf	>10	/
		Other Amphipods	9 Eppendorf (some have more than 1 organism)	8 Eppendorf (some have more than one organism)	>10	/
21/09	**SI 2nd pool** (Storholmen Island)	Other Amphipods	10 Eppendorf with 2 organism;	14 Eppendorf with 1 organism;	20 small	17 organisms

Sample date (dd/mm)	ID Sampling site	Type	TOX	RNA-later	MPs (n° and size of organisms)	Taxonomy
			1 Eppendorf with 2 small organisms; 1 Eppendorf with 3 organisms	1 Eppendorf with 2 organisms	17 medium 1 big	
21/09	**HI 2nd pool** (Obs-Holmen Island)	Other Amphipods	1 Eppendorf with 2 small organisms; 7 Eppendorf with 1 organism each	1 Eppendorf with 2 small organisms; 7 Eppendorf with 1 organism each	3 big 10 small-medium	6 organisms
24/09	**HT 2nd pool** (Harbour Teisten)	Other Amphipods	19 Eppendorf with 1 organism;	18 Eppendorf with 1 organism; 1 Eppendorf with 2 small organisms	25 medium 10 big	8 organisms

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