LIFE sedremed
ENHANCED BIOREMEDIATION OF CONTAMINATED MARINE SEDIMENTS

LIFE20 ENV/IT/000572

LIFE SEDREMED

Bioremediation of contaminated sediments in coastal areas of ex-industrial sites

LIFE20 ENV/IT/000572

START DATE OF THE PROJECT: 1 October 2021

DURATION OF THE PROJECT: 42 months

DELIVERABLE B1.2

Sampling campaign report

DUE DATE OF DELIVERABLE: **Dicember, 2024**

ACTUAL SUBMISSION DATE: **March, 2025**

BENEFICIARY LEADING THIS DELIVERABLE: **SZN**

CONTRIBUTING BENEFICIARY: **UNIVPM, EKO, IDRA, ISO**

Index

Executive summary

This deliverable recapitulates all the different sampling activities which were performed during the LIFE SEDREMED project. The collection of environmental samples was pivotal for different actions of the project and different types of sampling were necessary for each specific tasks. The partners, led by SZN, cooperated to choose the most appropriate sampling approach and designed specific plans accordingly. Mesocosms set-up needed the collection of large quantity (≈100 kg) of superficial samples (0-50 cm), while chemical analysis required the collection of cores up to 2 meters of depth to properly characterize the state of pollution of the sediments and also microbial analysis.
Finally, ecotoxicological analysis were performed with the collection of small cores and macrofauna.

1. Definition of sampling areas

In the preparatory action, two areas (area H and area L) (Figure 1) were selected for the project activities.

Previous reports from the ABBACO project (https://www.szn.it/index.php/it/ricerca/ecologia-marina-integrata/progetti-conclusi/abbaco), showed that the area H is significantly more polluted by both hydrocarbons and metals than the area L; these data were then confirmed in the first report on the monitoring activities (see Deliverable D3.1). Also, it was noted that contamination levels were higher in the deeper levels of sediments (>1 m of depth).

SAMPLING SITE COORDINATE:

Site H (High contamination):

40° 48,350’ N

14° 10,020’ E

Site L (lower contamination)

40° 48,306’ N

14° 10,131’ E

Figure 1. Aerial view and coordinates of the two selected area for SEDREMED activities.

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Therefore, area H was employed for the collection of sediments for the running of the first mesocosms (see Paragraph 2). Then, coring activities for chemical analysis were performed in both H and L area (see Paragraph 3.1), and the results confirmed the ABBACO outcomes. After that, the technology was installed in the area H (see Deliverable B2.1) and, consequently, all the other sampling activities took place in the designed area.

2. Sampling for mesocosms set-up

Based on the ABBACO results, it was decided to collect sediment for the mesocosms from the most contaminated area H, using Van Grab Samplers (Figure 2). The sediments corresponded approximately to the first 30-50 cm of depth and they were mechanically homogenized and stored in sterile plastic bags in packs of 1,5 kg on average. Before shipping, sediments were kept at 4°C.

Figure 2. Van Grab Samplers utilization for the collection of marine sediments.

Three samplings were performed by SZN team using SZN vessels Vettoria or Phoenicia (Figure 3) according to their availability:

1) **Sampling for preparatory mesocosms**

Date: 14/07/2022 using the Vettoria.

Sampling sites: H

About 70 kg of mixed sediments were sampled and sent to IDRABEL to start mesocosms activities.

2) **Sampling for mesocosms alpha**

Date: 20/04/2023 using the Phoenicia

Sampling sites: H

About 100 kg of mixed sediments were sampled and sent to IDRABEL to set-up 3 mesocosms runs in parallel.

3) **Sampling for mesocosms omega**

Date: 14/09/2023 using the Phoenicia

Sampling sites: H

About 100 kg of mixed sediments were sampled and sent to IDRABEL to set-up 3 mesocosms runs in parallel.

Figure 3. SZN vessels utilized for the sampling activities: Vettoria on the left and Phoenicia on the right.

3. Monitoring campaign for chemical analysis

These sampling activities were performed in the framework of Action B3. To properly characterize the sediments, it was decided to collect cores down to approximately 2 meters, in order to characterize the level of pollution at different depths. The coring activities were performed by the company Deep Sea Technology (DST) using vibrocoring as a specific sampling tool (Figure 4).

Figure 4. Vibrocoring device utilized by DST for coring collection.

3.1 Preliminary coring campaign (December 2022)

The preliminary monitoring campaign was conducted by SZN with DST in December 2022 at the two potential treatment areas (H and L) described in Paragraph 1. In each selected area, two independent sediment cores, down to ca. 170 cm depth, were recovered by DST. The cores were then retrieved by SZN team (Figure 5A), opened to release the sediments (Figure 5B), and sliced into 4 different sediment layers (0-25 cm, 25-50 cm, 50-100 cm, >100 cm). These different layers were then homogenized and divided (Figure 5C) before being placed into specific vials to be shipped to specific partners. Altogether, sediment material was collected from 16 sediment layers (2 sites x 2 cores x 4 sediment depths). These samples have been analysed by various monitoring tools and were also used for laboratory assays.

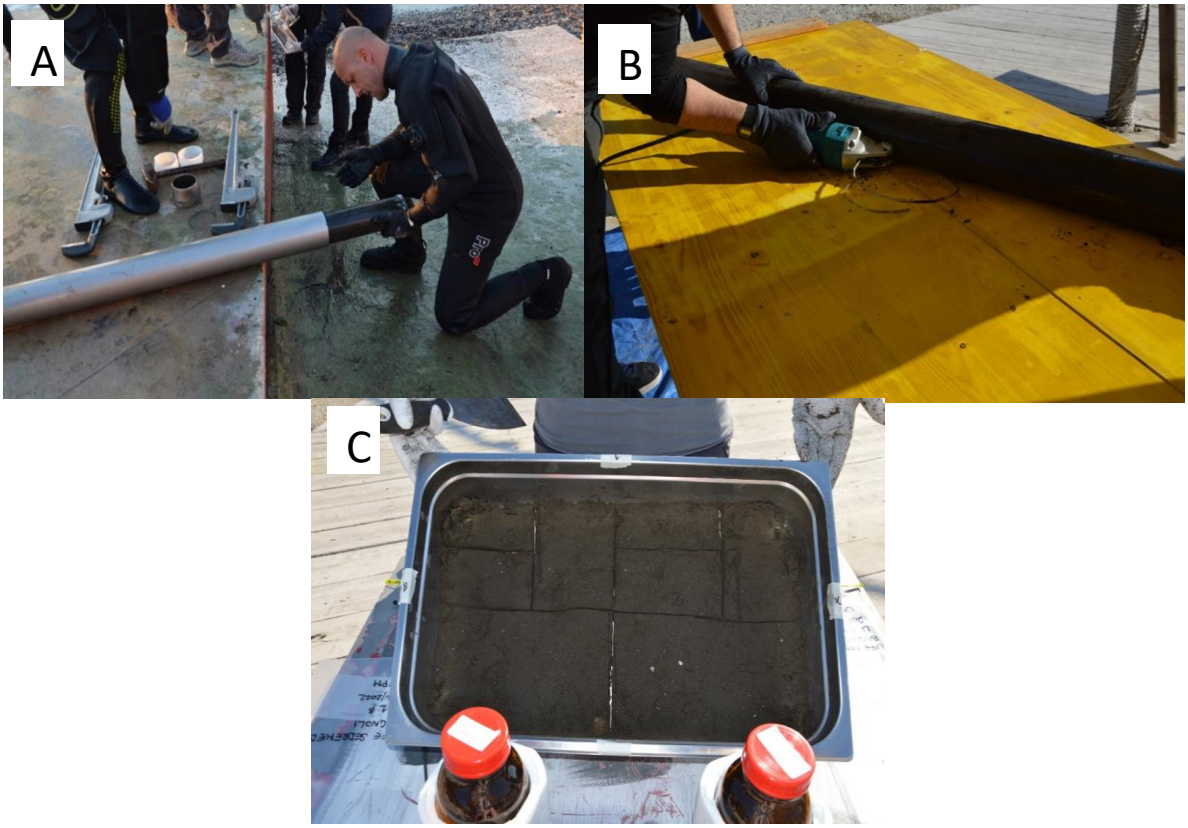

Figure 5. Different steps for the recovery of samples from marine cores. A) Recovery of the sediments from the steel core connected to the vibrocore. B) The longitudinal cut of the core in its plastic enclosure. C) The division of the homogenized samples before collection into the vials.

3.2 Coring campaign for the installation monitoring (November 2023-July 2024)

The coring approach tested in December 2022, was replicated and further implemented for the sampling activities to monitor the installation (see Deliverable B2.1). To properly monitor the installation, an appropriate monitoring strategy was designed. Specifically, in the installation area of 10 x 10 m, it was decided to collect 4 different cores: two in the Ekogrid-Idrabel (cores AI1 and AI2) area and two in the only Ekogrid areas (cores CE1 and CE2). To properly evaluate the effect of

the installation, a control area was established roughly 10 m away from the installation area, and two cores were collected (CC1 and CC2). A complete scheme of the monitoring set-up is shown in figure 6.

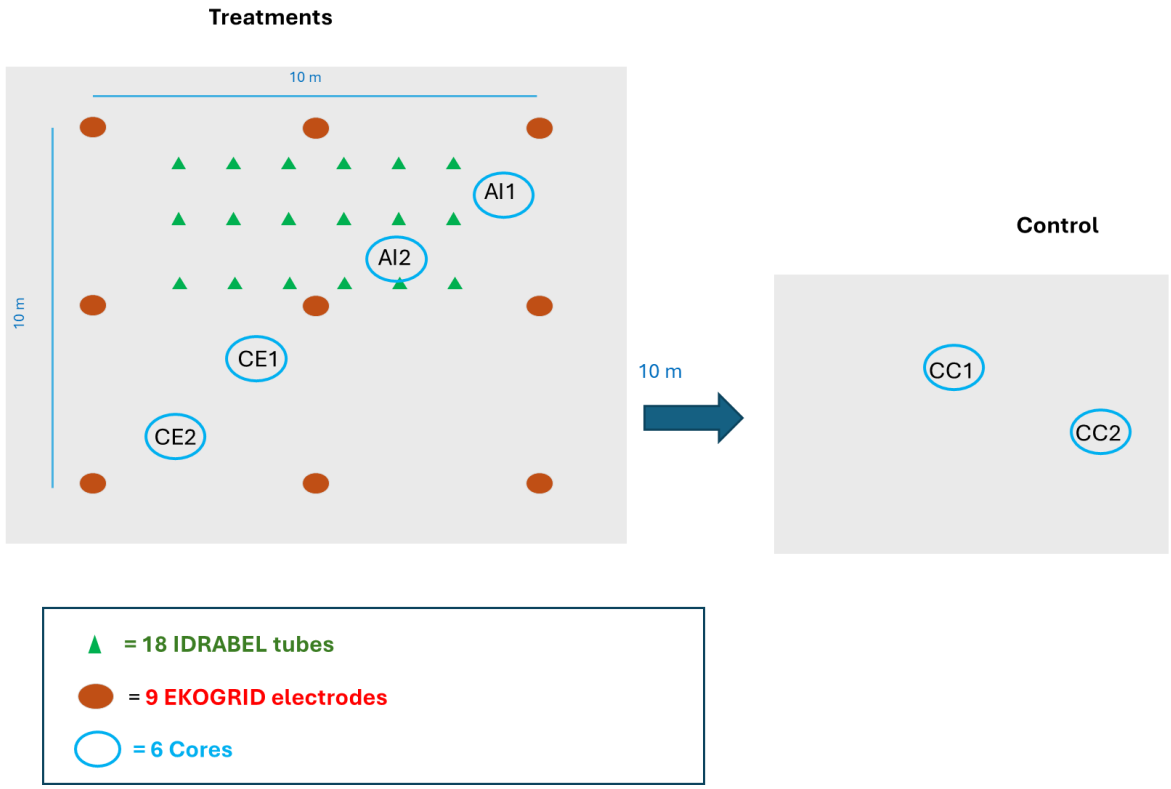

Figure 6. Schematic overview of the sampling locations of the 4 sediment cores in the pilot treatment area (AI1, AI2, CE1, CE2), where EKOGRID electrodes (red circles) and IDRABEL products (green triangles) were used, and of the 2 sediment cores in the control area (CC1, CC 2).

This time, each core was sliced into three sediment layers (0-50 cm, 50-100 cm, 100-bottom) and sediment sub-samples were used for ecotoxicological and chemical analysis. In addition, small amounts of sediments were collected at seven different layers (0-2 cm, 20 cm, 40 cm, 60 cm, 90 cm, 120 cm, 150 cm/bottom) and preserved in RNA later for molecular analysis (Figure 7).

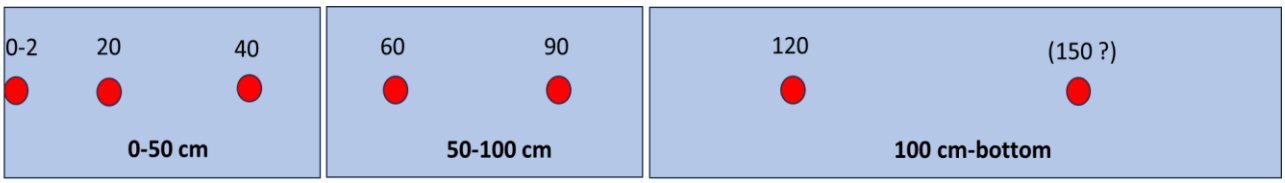

Figure 7. Schematic overview of the slicing of each long core collected from the experimental field area in the three sediment layers. The red dots correspond to the 7 layers where sediments for molecular analysis were collected.

This type of sampling was performed 3 times:

1) t0, end of November 2023 (one week before the installation);

2) t1, beginning of April 2024;

3) t2, July 2024.

Results from these samples will be available in the final report (Deliverable B3.2).

4. Monitoring campaign for ecotoxicological analysis (BACI)

UNIVPM in joint collaboration with SZN elaborated a detailed sampling strategy for investigating the potential benefits for benthic biodiversity and ecosystem functioning of the in situ remediation technologies based on a BACI (Before-After-Control-Impact) design. In particular, such design foresees the collection of replicated sediment samples in three different areas: 1) an area in which the in situ remediation technologies, in combination (TR1) or alone (TR2), were tested, 2) an adjacent area in which the same operation for the installation of the in situ technologies was done (i.e. artifact control) and 3) an other adjacent area to be used as control (free of electrodes and tubes) (Figure 8).

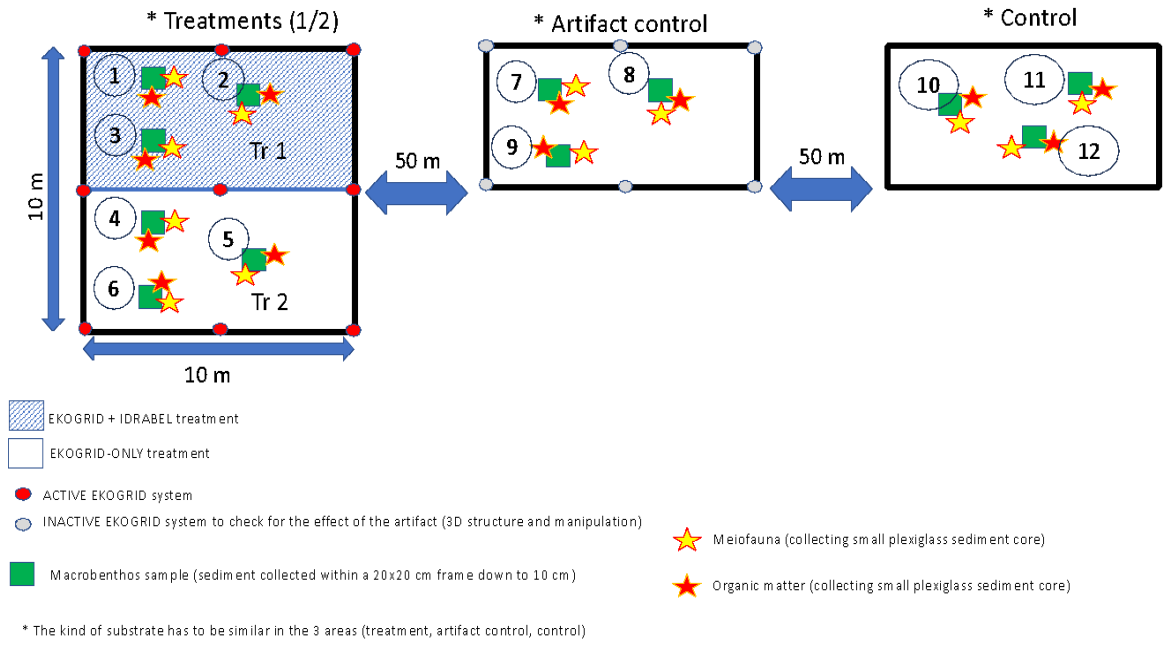

Figure 8. Schematic overview of the sampling design used for the monitoring of the potential ecosystem benefits and ecological sustainability of the selected remediation technologies.

Sediment samples (20 cm of depth) were collected in June, July, and October 2023 (Errata: in the Deliverable B3.1, it was recorded the wrong date, as the last of three took place in October rather than September) by SZN professional SCUBA divers in all of these areas, before the *in situ* remediation test; they have been collected again during the remediation test in February and September 2024 (at least for the analysis of some variables) and they have been collected also, at three different time intervals after the end of the remediation trial in December 2024, January and February 2025. In particular, for the determination of meiofaunal abundance and assemblage composition, organic matter biochemical composition, and extracellular enzymatic activities, two plexiglass cores of different diameter of sediments per site were randomly collected within the selected areas (Figure 9A). For macrofauna, samples were collected within a 20x20 cm metal frame, randomly positioned on the soft bottom within the selected areas, using a bailer down to 10 cm

within the sediment. The sediment was underwater transferred within labelled plastic bags and later sieved on board of the research vessel, Vettoria, using a 0.5 mm mesh (Figure 9B). The sieved material was transferred into 2 L plastic jars and fixed using 85% alcohol (Figure 9C).
Samples for the macrofauna determination were not collected during the two campaigns carried out during the remediation test.

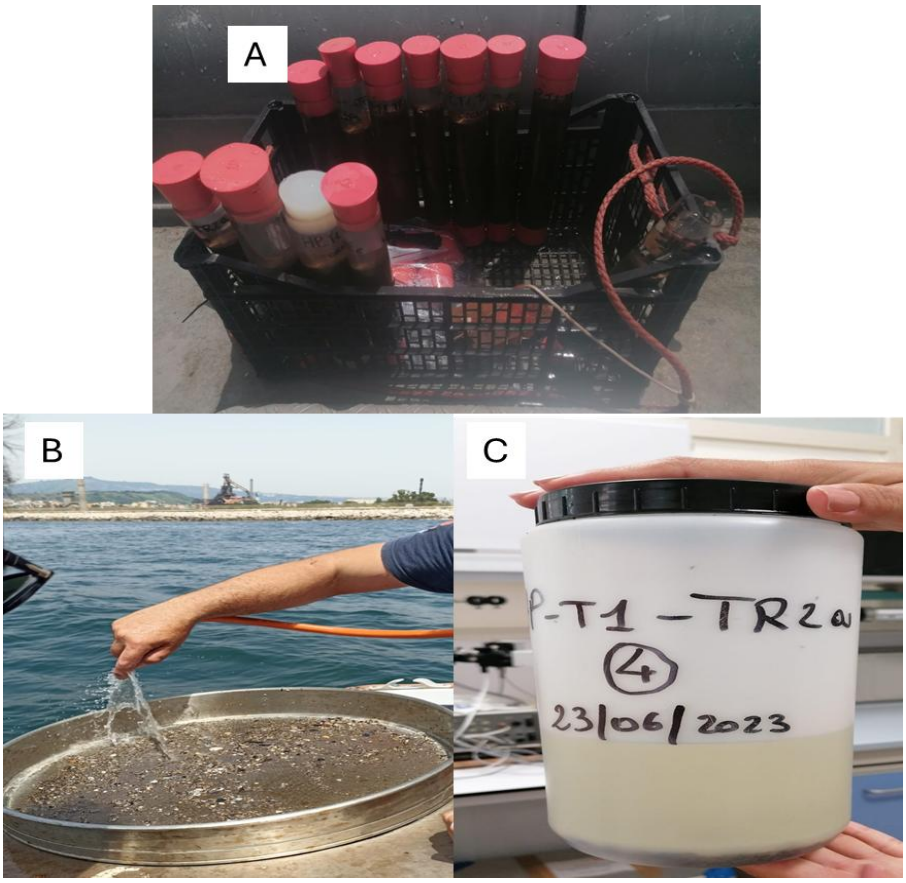

Figure 9. Different steps for the recovery of samples for ecotoxicological analysis. A) Small cores for meiofauna and chemical analysis; B) Sediments sieving for the collection of macrofauna; C) Macrofauna samples stored in 85% ethanol.