Kill•Spill

Integrated Biotechnological Solutions for Combating Marine Oil Spills

# Deliverable D7.5

# Report on the effects of the new products on benthic assemblages

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## 1 About this deliverable

This document was prepared by EcoTechSystems s.r.l. (EcoTS) and the National University of Ireland Galway (NUIG) to describe the results obtained by field tests performed using innovative products developed in the Kill•Spill project. In particular, in the following paragraphs, the impacts of 2 of the more promising products (i.e. Oxygel and Sophorolipids) on abundance and biodiversity of marine macrozoobenthos are be reported and discussed, to provide data about actual field application.

## 2 Design protocols

In studies of accidental oil spills, systematic evaluation of impacts is hindered by the unforeseeable aspects of accidents and logistical constraints in getting to the site and making measurements (Longpré et al. 1997). Similar issues arise with field trials of remediation treatments, an objective of KillSpill. To maximize the potential information from field trials of the ecological impacts of remediation treatments, a number of recommendations can be made to guide the design of experiments. From the experimental design principles outlined above these recommendations are that a control-impact-remediation study should have (in order of importance from essential to less essential):

A) Replicates should be taken at each combination of treatment and time
B) Ameasurement of the communities should be made before the remediation treatment is applied
C) Repeated surveys should be made after the intervention
D) Control sites should be replicated
E) Treatment and/or impact sites should be replicated

The latter points relate to beyond-BACI and fully replicated designs, approaches that it is widely recognised are hard to apply in opportunistic situations.

The response variable is assumed to be a count of the ecological assemblage (benthic, pelagic or both) at the different sites. As co-variables may help interpreting the observations (Parker and Wiens 2005) these should also be measured where possible (e.g., hydrocarbon content, salinity, grain size distribution).

As the expectation is that opportunities for replicated control-impact-remediation sites will be limited, an experimental protocol for an extended BACI design is outlined below as a general protocol to guide and standardize field trials.

### 2.1 Summary of field tests

In the following paragraphs are described the experimental designs of each field experiment performed during the project. Two field experiments have been performed, dedicated to field testing of 2 products having different chemical characteristics, origins, type of action and destination. Oxygel, the first product tested, is a gel releasing oxygen over time. Its remediating action is based on the capability of shift a contaminated environment from reducing to oxidant, thus stimulating the microbial degradation of oil. The field test of Oxygel has been performed in Gela (Italy). The second tested product was the stock version of Sophorolipids, a microbial biosurfactant with low ecotoxicity, designed to treat contaminated waters. The field tests of Sophorolipids have been performed in Ancona (Italy). Both experiments have been designed according with site features and characteristics of the test product, maintaining the deliverable objectives and overall structures described above.

#### *2.1.1 Gela field experiment*

The oxygen releasing gel Oxygel, provided by Biorem Engeneering, was tested in the field exploiting the real oil spill occurred in Gela the 4th of June 2013. The spill was originated from the ENI refinery (specifically from the topping plant N.1) and impacted the Gela River and the surrounding coastal area. The larger fraction of oil was immediately removed from the coast by authorities; however, a small amount of impacted sands remained buried in the field due to coastal sedimentary dynamics.

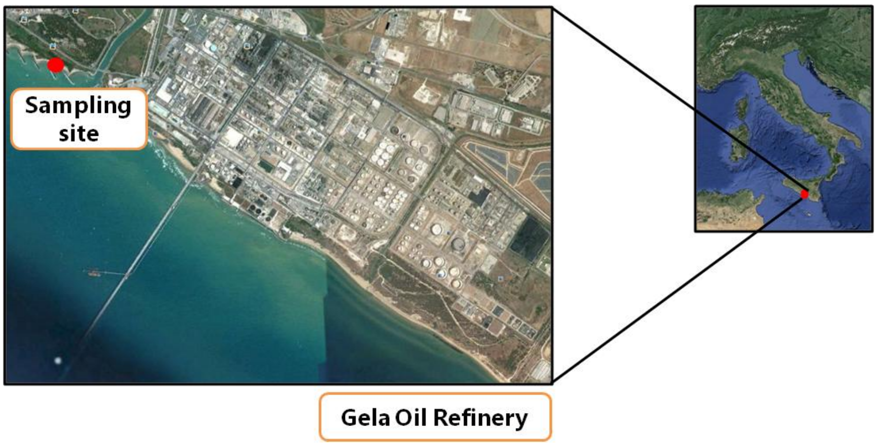


**Figure 1 Location of Gela study site.**

The field experiments have been performed in 3 stations located in an intertidal area 500 m north-west of the Gela River mouth.

The effect of Oxygel (hereby named Treatment) in oil contaminated sediments was compared to a couple of controls: a positive control (presence of oil contamination) and a negative control (absence of contamination). Treatment, Positive control and Negative Control were represented by 3 stations, named KSG1, KSG2 and KSG3 respectively (Figure 2).

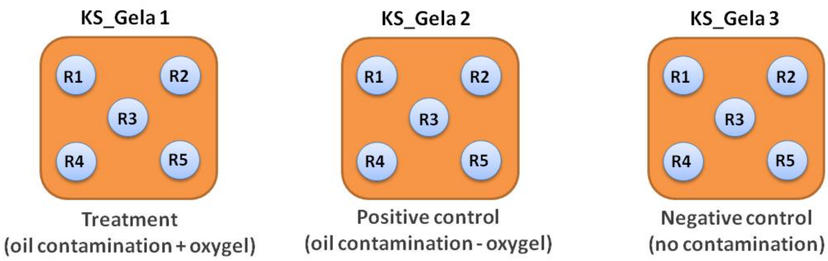


**Figure 2 Schematic representation of Gela sampling stations.**

At each station, the following physic-chemical features of sediments have been studied in the first 30 cm below the sediment surface:

- pH;
- Redox potential;
- Concentration of Dissolved Oxygen (DO).

Additionally, the abundance and biodiversity of macrozoobenthos was studied in each station in 5 replicates (R1 to R5).

The field experimentations have been authorized in October and 2 sampling campaigns have been performed as follows:

- 1st sampling campaign (T0): 7/10/2013
- 2nd sampling campaign (T1): 13/11/2013

During the first campaign, the background conditions of sediments have been measured, together with the collection of sediments samples for the quali-quantitative determinations of macrozoobenthos. At the end of the first campaign, an aliquot of oxygel (ca. 1000 mL) was buried at ca. 24 cm depth below the sediment surface at the Treatment station. During the second campaign, the same measurements and sediment collection have been performed for a proper comparison of results.

### *2.1.2 Ancona field experiment*

Sophorolipids, provided by Actygea and synthesized on the basis of molecule provided by the University of Ulster, were tested in the touristic port of Ancona. The experiment was performed by simulating a small scale oil spill in a confined volume of water and sediments inside a mesocosm. A schematic representation of experimental mesocosm is provided in Figure 3. Such device was subdivided in four experimental chambers (1.5 $m^3$ each) for parallel experiments, made in stainless steel with plexiglas walls to avoid modifications to the amount of light, wavelength and photoperiod, and is characterized by open top and bottom to allow water-atmosphere, water-sediment exchanges.

The mesocosm was deployed into the water, close to a jetty, and ensured to bottom sediments by means of ballasts.

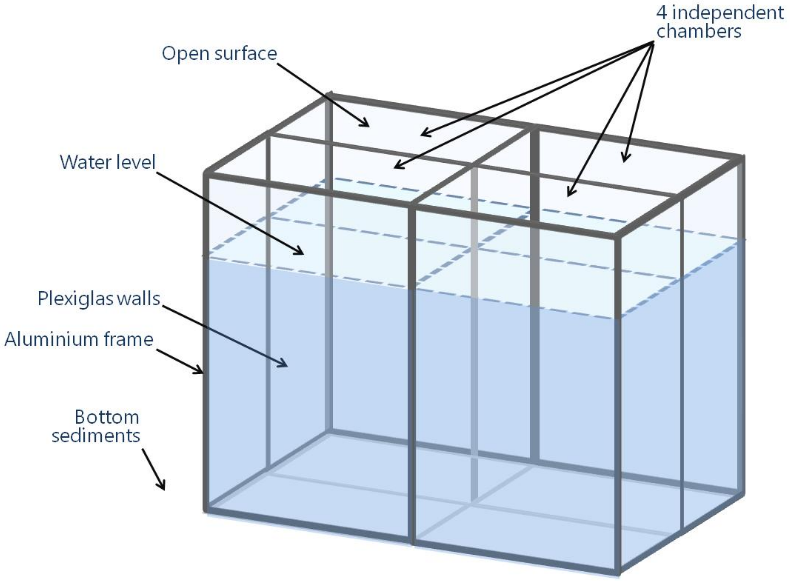


**Figure 3 Schematic representation of Ancona experimental mesocosm.**

The four chambers were used to set up a factorial experiment to test the individual impacts of Oil, Sophorolipids and the mixture Oil+Sophorolipids on macrozoobenthic communities (Figure 4).

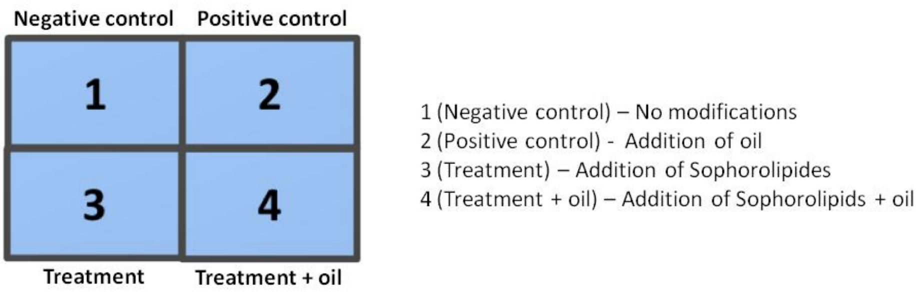


**Figure 4 Subdivision of Ancona mesocosm and use of the four experimental chambers.**

The final objective of the experiment was to disentangle the effect of oil and of an environmental friendly surfactant on the marine biota during an offshore oil spill.

The oil selected for the spill simulation was a Basrah Light, provided by British Petroleum. It was chosen as a representative of low viscosity oils. We selected a low viscosity oil to allow a better homogenization of the oil in the water column, minimizing its tendency to attach on mecosm walls and reducing the modifications to light provision. The oil was used to produce an oil slick of ca. 0.05 mm thickness on water surface in systems 2 and 4.

The experiment had an overall duration of 5 days. Along the experiment, the main physico-chemical parameters of seawater (temperature, conductivity, turbidity, dissolved oxygen, turbidity) were daily monitored by means of a multi-parametric probe Seabird 19 plus. The daily monitoring included also the concentration of hydrocarbons in seawater. In particular, the more common hydrocarbons involved in oil contamination were monitored: aliphatic hydrocarbons C>12, Polyciclic Aromatic Hydrocarbons, Benzene, Toluene, Ethilbenzene and the three Xylene isomers. The sampling of seawater for the hydrocarbon monitoring was performed daily by means of a Niskin bottle.

### 2.2 Analytical parameters and methodologies

As reported in the previous paragraphs, in the field were measured the main physic-chemical parameters of the sediments (as pH, Redox potential and DO concentration) by means of portable probes. The collected samples were analyzed in the lab for quali-quantitative studies of macrozoobenthos. The systematic classification of macrozoobenthos was operated at the lowest systematic level as possible. In all the samples collected, the following biotic indexes have been determined: i) total number of individuals), ii) number of species (N), iii) index of specific diversity (d, Shannon & Weaver 1949), iv) dominance index (j, Simpson, 1949), v) species richness (H' Margalef, 1958), vi) evenness (λ, Pielou 1966).

# 3 Results and discussion

## 3.1 Ecocompatibility of Oxygel

### *3.1.1 Physic-chemical features of sediments*

In Figure 5 are displayed vertical profiles of pH, Eh and DO concentrations measured during the second campaign, after 36 days from the Oxygel burial in station KSG1 (Treatment).

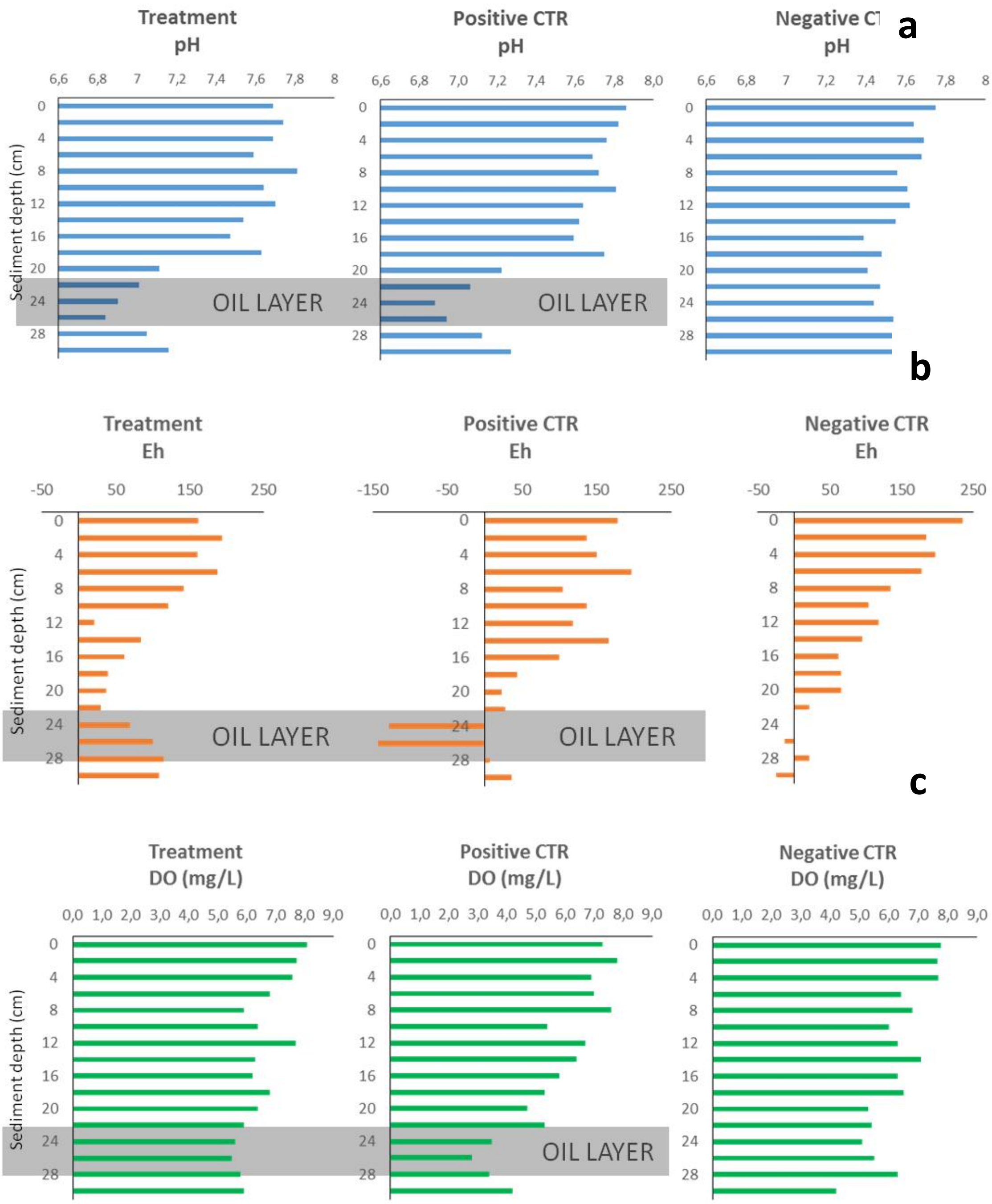


**Figure 5 Vertical profiles of pH, Eh and DO (panel a, b c respectively) measured at the second sampling campaign.**

The release of O2 didn't produce evident effects in the pH of sediments, which remained basically dependent by the presence of oil (panel a). Conversely, the Redox potential and O2 concentrations were deeply affected by the presence of Oxygel, but effects were substantially limited to a zone between 22 and 30 cm under the sediment surface, where the Oxygel was placed. The treatment apparently caused a shift of Redox potential (panel b), from negative to positive values, indicating that sediments turned from reducing to oxidant conditions. The same effect, even less evident was observed for O2 concentrations, slightly higher in the treated than in untreated sediments.

### *3.1.2 Abundance and biodiversity of macrozoobenthos*

The abundance of benthic fauna found in Gela sediments is displayed in Figure 6. The site showed low number of individuals, mainly due to the high hydrodynamism typical of the intertidal zones. The global reduction of abundances found during the second campaign is probably linked to seasonal variability, which typically shows decreases in this period of the year especially in shallow waters and intertidal zone, more exposed to weathering, waves and tidal currents.

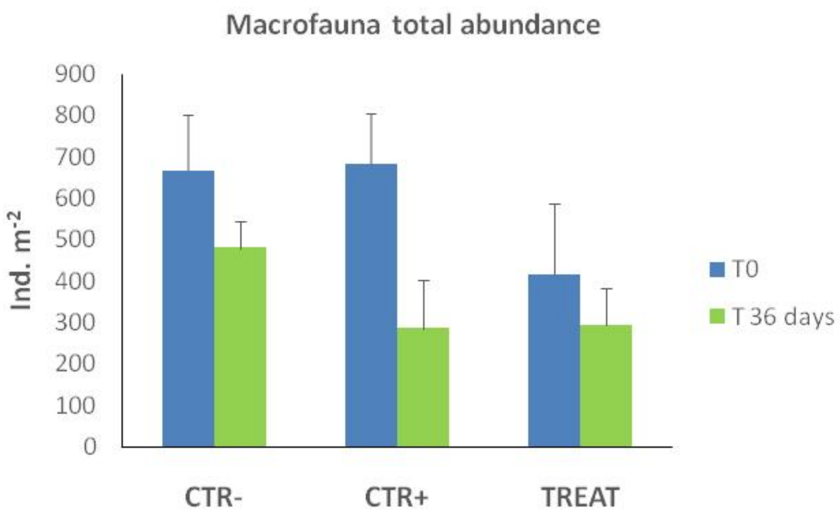


**Figure 6 Abundance of macrobenthic fauna in the 3 stations in the 1st and 2nd campaign at Gela site**

During the second campaign, the abundances found in the Positive Control and in the Treatment are significantly lower than those found in the Negative Control. However, considering the percentage of decrease from the first to the second campaign of each station, it appears that only in the Treatment and Negative Control the difference, thus present, is not statistically significant.

The list of species found in collected sediments is reported in Table 1 and biodiversity index are illustrated in Table 2.

**Table 1 List of species found in sediments of Gela**

| Phylum | Class | Order | Species |
|---|---|---|---|
| Mollusca | Bivalvia | | *Modiolus adriaticus* |
| Mollusca | Bivalvia | | *Donax sp.* |
| Mollusca | Bivalvia | | *Irus irus* |
| Mollusca | Gastropoda | | *Pseudotorinia architae* |
| Mollusca | Gastropoda | | *Volvulella sp.* |
| Artropoda | Crustacea | Amphipoda | *Apherusa sp.* |
| Artropoda | Crustacea | Amphipoda | *Cylichna cilindracea* |
| Artropoda | Crustacea | Isopoda | *Cirolana borealis* |
| Echinodermata | Ophiuroidea | | *Amphiura chiajei* |
| Anellida | Polychaeta | | *Glycera sp.* |
| Anellida | Polychaeta | | *Syllidae* |
| Anellida | Polychaeta | | Sabellida |
| Anellida | Polychaeta | | Polychaeta n.d. |
| Platyhelminthes | Turbellaria | | Turbellaria |

The extreme characteristics of the marine environment in the study area (coarse sediments, hydrodynamicity etc.) are reflected on the reduced number of species found and in the low biodiversity. This can be easily visualized by the dominance index (j), which shows the prevalence of one species on the whole amount of individuals, especially in the CTR+ in both periods.

**Table 2 Biodiversity indexes in the 2 sampling periods in Gela sediments.**

| | | N | d | J | H' | λ |
|---|---|---|---|---|---|---|
| T0 | CTR+ | 7 | 2.06 | 0.96 | 2.24 | 0.22 |
| | CTR- | 8 | 1.44 | 0.87 | 1.75 | 0.34 |
| | TREAT | 5 | 0.62 | 0.72 | 0.72 | 0.68 |
| T1 | CTR+ | 5 | 1.87 | 0.96 | 1.92 | 0.28 |
| | CTR- | 1 | - | - | 0.00 | 1.00 |
| | TREAT | 2 | 1.44 | 1.00 | 1.00 | 0.50 |

This is confirmed also by the macrozoobenthos community structure. The presence of oil contamination and heterogeneity of substrate can be related to the great differences found between stations. The Positive Control, particularly, showed a community dominated by polychaetes, whereas in the other 2 stations, the dominant group was bivalves (from 67 to 70% of total). After the second campaign, the community structure changed in all stations; however, no specific patterns were recognized. The observed changes could be related more to the seasonal and spatial variability than to the presence of Oxygel.

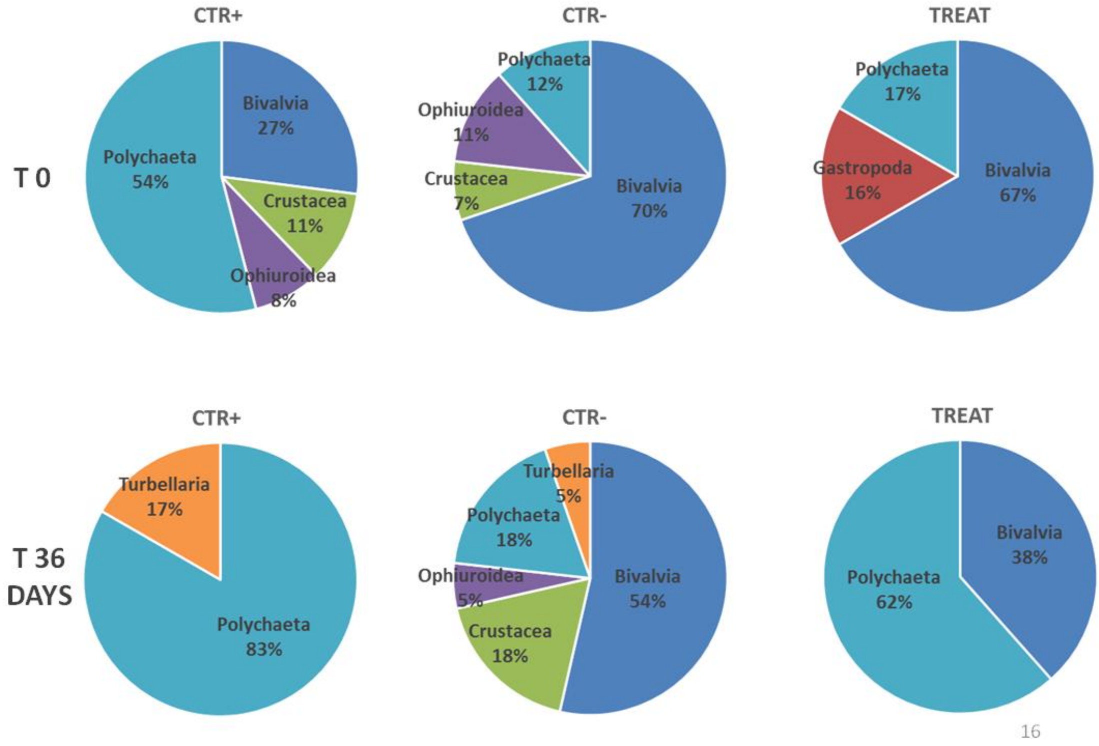


**Figure 7** **Community structure of macrozoobenthos at the 3 stations in the 1st and 2nd campaign at Gela site**

### 3.2 Ecocompatibility of Sophorolipids

The different environmental conditions to induced in the 4 experimental chambers were reflected in macrozoobenthos abundance and composition. In Figure 8 are reported the total abundance of macrozoobenthos. The possible effects linked to the presence of the mesocosm was investigated in system CTR-: the absence of significant differences with T0 suggests that, in the short term, the presence of mesocosm doesn't impact the total abundances. The more impacted systems was CTR+, where the presence of oil reduced the macrobenthos abundance of 70% if compared with the abundance found in T0 and in the untreated system (CTR-). Such reduction is probably due to the sinking of oil along the water column and its deposition on bottom sediments. The deposition of oil interferes dramatically with respiration and feeding of macrozoobenthos, causing rapidly the death of organisms. The use of Sophorolipids (system Treat) didn't produce noticeable effects in terms of total abundance of macrozoobenthos, as differences with CTR- and T0 were not statistically significant. The use of Sophorolipids on oil probably reduced the amount of oil reaching the sediment surface, causing less mortality on benthic organisms. This was confirmed by the reduced traces of oil observed on sediment surface and on samples collected. In this system, the mortality of macrobenthos was only of 35% compared with T0.

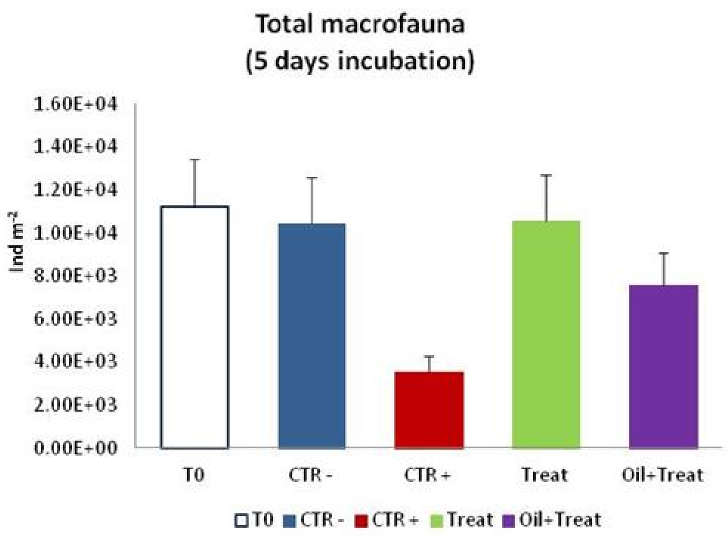


**Figure 8 Total abundances of macrozoobenthos (background conditions and after incubations)**

The background community structure of macrozoobenthos is shown in Figure 15. The taxa found and relative abundances are typical of coastal sediments, with a prevalence of molluscs (mainly bivalves) and polychaetes and a low contribution of crustaceans. The total abundance of macrozoobenthos found in background samples was 565 individuals $m^{-2}$.

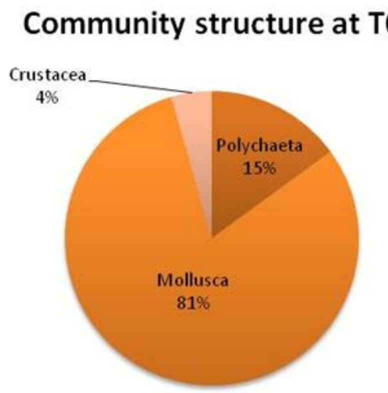


**Figura 9 Macrozoobenthos community structure (background conditions)**


**Figura 10 Three of the more representative species for molluscs, polychaetes and crustaceans found in sediment samples at T0**

The evolution of macrozoobenthos in sediments inside the mesocosms is represented in Figure 17. The original structure is basically the same in the CTR-, where we observed a very low mortality (mainly due to molluscs). A similar evolution was observed in the "Treatment" system, where the differences from the original structure were due mainly to a slight reduction of polychaetes. In both these systems, however, the differences observed in terms of relative abundance of taxonomic groups were not significant.

More evident changing was observed in presence of oil. The introduction of oil contamination caused a high mortality of all the macrozoobenthic taxa actually present in sediments. Molluscs represented the more impacted taxon, as their relative abundance changed from 81 to 74% of total specimens. Even though the relative abundance of Polychaetes and crustaceans slightly increased, their total abundances were dramatically reduced (more than 70% on average).

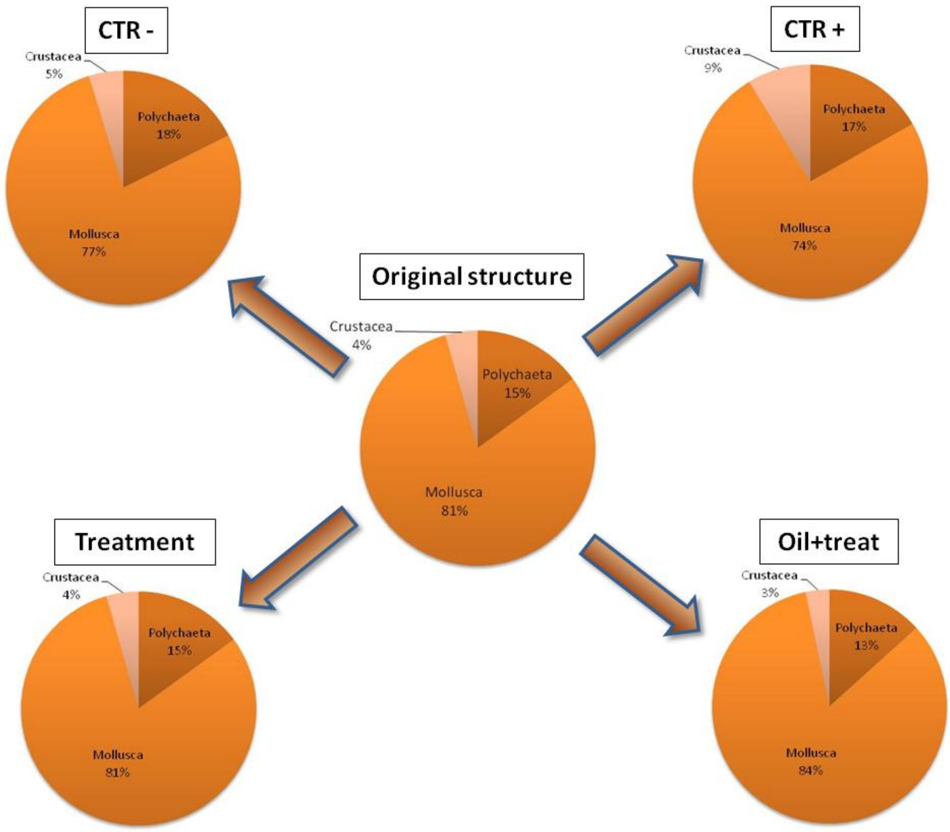


**Figura 11 Evolution of macrozoobenthos community structure after 5 days of incubation**

The community structure in the "Oil+treatment" system was also impacted, but the changing (in terms of relative abundances) was basically limited to crustaceans.

## 4 Conclusions

From the data collected, it evident the highly negative effect of oil on macrozoobenthos abundance and biodiversity. The effect of oil on this biotic component is probably due to sinking and deposition on surface sediments, causing a rapid depletion of oxygen available for life. Together with oxygen depletion, a probable major role could be played by oil toxicity, which can rapidly have lethal effects on organisms.

The use of Oxygel resulted in a visible oxygenation of subsurface sediment layers and increase of the reduction potential, providing a more favourable environment for the survival and reproduction of benthic macroinvertebrates. This result confirm what suggested by specific studies on effects of sediment oxygenation and abundance of macrobenthos (Woulds et al., 2007). Specifically, the absence of a statistically significant reduction of total amount of macrofauna between the two sampling periods in Gela could be an indication of such contribution. Such result was linked to a slight increase of biodiversity in the treated sediments, especially in terms of evenness.

The use of Sophorolipids resulted not only in a less severe mortality of benthic organisms, but also in a less evident changing of the community structure. The preservation of ratios between taxa could be an indication of a less severe impact of the oil contamination on the ecological community, preserving the original ecologic functions.

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