Kill•Spill

Integrated Biotechnological Solutions for Combating Marine Oil Spills

Deliverable D7.6

Report on the effects of the new products on the planktonic food webs

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1 About this deliverable

This document was prepared by EcoTechSystems s.r.l. (EcoTS) to describe the results obtained by field tests performed with the aim of assessing the environmental impact of one among the more promising products developed by Kill•Spill consortium for oil dispersion, namely the stock version of Sophorolipids. This test was performed particularly on abundance and biodiversity of marine phytoplankton and zooplankton, and integrated the few existing data about the actual impact of crude oil and eco-compatible surfactants on biological communities in real systems.

2 Design protocols

2.1 Field test methodology

The tested product was the stock version of Sophorolipids, a microbial biosurfactant result of the scale-up production performed by Actygea srl on the basis of the original product provided by the University of Ulster. Sophorolipids obtained very good performances in terms of ecotoxicity, displaying very low EC50 values with all the target species tested in laboratory assays perfomed in Task 7.1 of Kill Spill project.

The field tests of Sophorolipids were performed 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 inFigure 1. 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.

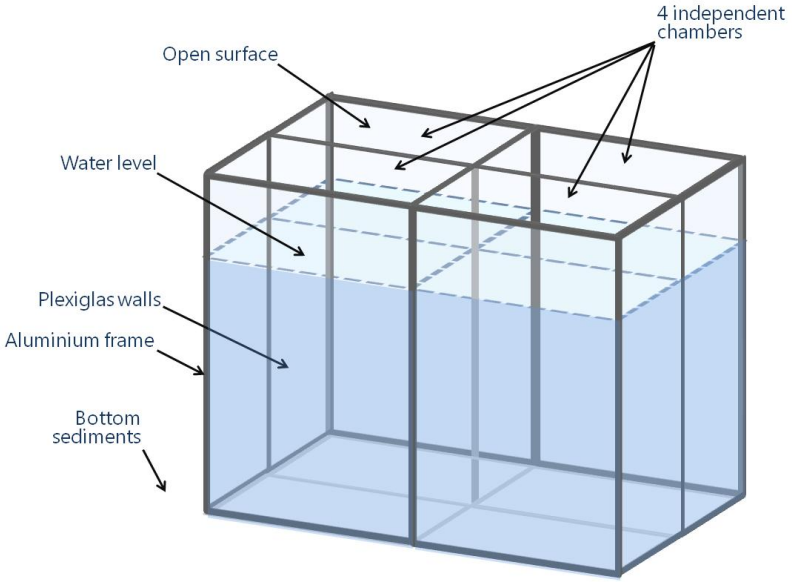

Figure 1 Schematic representation of Ancona experimental mesocosm.

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 2).

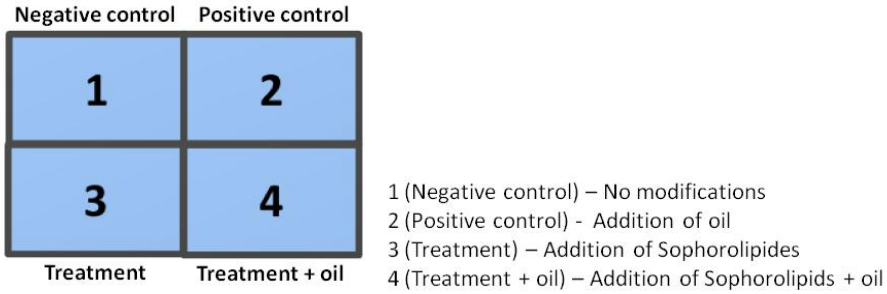

Figure 2 Subdivision of Ancona mesocosm and use of the four experimental chambers.

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. During 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 (PAH), Benzene, Toluene, Ethylbenzene and the three Xylene isomers (BTEX). The sampling of seawater for the hydrocarbon monitoring was performed daily by means of a Niskin bottle lowered and closed at mid depth into the water column. The same device was used for the daily collection of seawater samples for the quali-quantitative determinations of phytoplankton. One liter of sample was collected at each sampling. Each sample was fixed by 2 mL of Lugol solution and transferred to the laboratory for analyses.

Zooplankton was collected lowering a small zooplankton net (NHBS, 150 µm mesh) vertically into each chamber up to the seabottom and recovering it. Once recovered, the net was washed from the outside to allow the collection of all organisms in the collector. The sample was then transferred in PE containers and fixed with Accustain formalin free fixative.

2.2 Laboratory methodologies

The chemical determinations in seawater samples were performed according with the following table.

Table 1 Analytical methods and detection limits of chemical determinations in seawater

Variable	Method	Detection limit
Aliphatic Hydrocarbons C>12	EPA 3510C	0.02 mg/L
PAH	EPA 8270 D	0.1 µg/l
BTEX	EPA 5030B, APHA 6220	0.0001 mg/l

Abundance and taxonomical composition of phytoplankton were obtained by using Utermöhl method (Hasle, 1978). Collected samples were manually homogenized to obtain representative subsamples from the storage bottle. Subsamples were obtained by filling sedimentation chambers with a known volume of sample (100 ml). Quali-quantitative analyses were performed by observation of counting chambers under an inverted microscope. Phytoplankton was counted and identified dividing organisms in four main groups, namely Diatoms, Dynoflagellates, Coccolitophorides and Others.

The laboratory determinations of zooplankton were performed splitting the samples by means of a Plankton Splitter into an appropriate number of aliquots, depending on the observed concentration of organisms. Each aliquot was used for zooplankton enumeration and identification. Analyses have been performed under STEMI 2000 Stereomicroscope (magnification 6,5-50X). Results have been expressed as number of individuals m^{-3}.

3 Results and discussion

In Table 2 are reported the background characteristics of seawater, collected at mid depth inside the mesocosm after its deployment. These conditions reflected the typical hydrological conditions of coastal waters of North Adriatic in autumn (Artegiani et al., 1997).

Table 2 Background physical and chemical conditions of Ancona touristic port

Ancona touristic port Background conditions	
Temperature	24.03°C
Salinity	37.15 PSU
pH	8.26
Transparency	1.8 m
Fluorescence	1.38 µg L^{-1} chl a
Dissolved Oxygen	4.82 mg L^{-1}
Aliphatic hydrocarbons C>12	b.d.l.
Total PAH	b.d.l.
BTEX	b.d.l.

*b.d.l.: below detection limit

The addition of oil resulted in an immediate change of physicochemical parameters of seawater inside the mesocosm, with a reduction of dissolved oxygen and fluorescence. Such reduction increased in the first hours after the beginning of the experiment, reaching minimum values after 2 days. In the next hours up to the end of the experiment, the DO concentration and the fluorescence showed a stabilization on values lower than the background values. Specifically, the DO stabilized on ca. 3.7 mL L^{-1} (23% lower than the background concentration); the fluorescence stabilized on ca. 0.45 µg L^{-1} chlorophyll a (68% lower than the background concentration).

Figure 3 shows the temporal evolution of DO and fluorescence in the four experimental systems. The reduction of values in presence of oil is evident also if Sophorolipids are added, even though slightly lower. It is to be remarked that a decrease of Dissolved Oxygen over time was detected also in the negative control (ca. 6%), probably due to the physical isolation of the water mass inside the chambers.

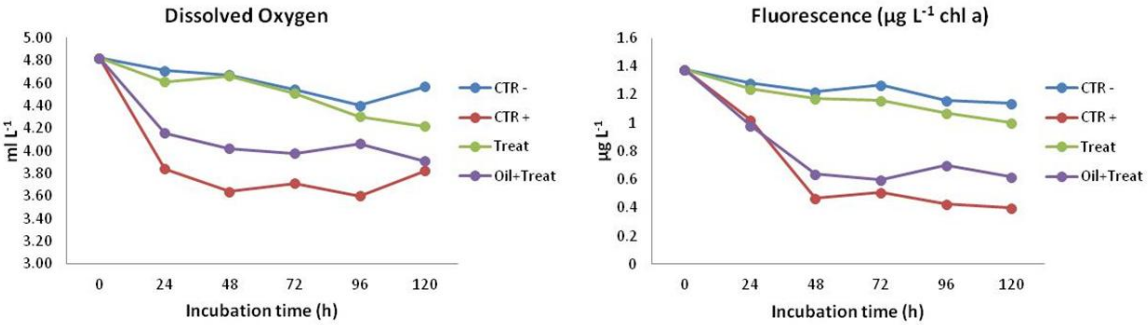

Figure 3 Temporal evolution of Dissolved Oxygen (left panel) and fluorescence (right panel) in the four experimental systems

The effect of oil affected not only the temporal trends, but also the vertical profiles of DO and fluorescence. The Figure 4 shows the concentrations of DO and fluorescence after 48h of incubation along the water column. For DO, it is evident the great discrepancy between the two systems without oil (CTR- and Treat) and systems in which the oil was added (CTR+ and Oil+treat). In particular, it is evident that the homogeneous distribution of oxygen over the vertical profile in absence of oil turns into a less homogeneous, decreasing pattern from the surface to the bottom, indicating a reduced oxygen exchange with the atmosphere that can't compensate the oxygen consumption in lower water layers.

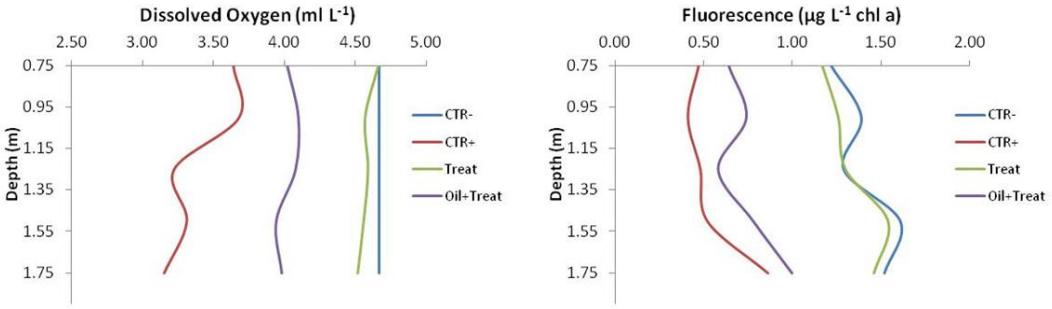

Figure 4 Vertical profiles of Dissolved Oxygen (left panel) and fluorescence (right panel) in the four experimental systems

The phytoplanktonic community at background conditions, together its evolution along the 5 days of incubation, are illustrated in Figure 5.

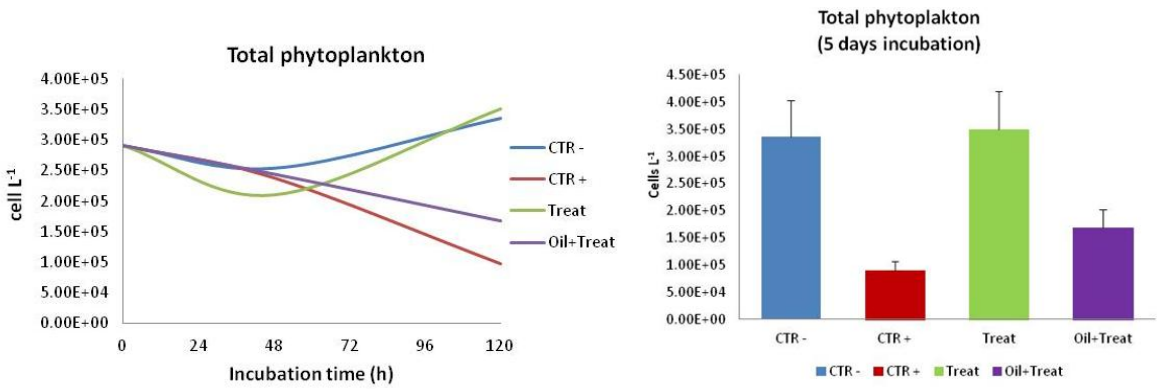

Figure 5 Total abundances of phytoplankton (background conditions and temporal evolution)

The temporal evolution of total phytoplanktonic abundances in the untreated system (CTR-) showed, after an initial decrease (first 24-48h), a slight increase due to a possible increase of nutrients and temperature inside the experimental system. Differences in terms of total abundances from T0 and T120h were not statistically significant. A very similar trend was followed by the "Treat" system (in which only Sophorolipids were added): also in this system, after a decrease of total abundances observed in the first 48 hours (27%), the total abundances reached values slightly higher than the starting abundances (difference not statistically significant). The modifications to phytoplanktonic community (increase of cell size and abundance) and the formation of an algal biofilm on plexiglas walls, led us to stop the experiment.

Data collected from systems in which the oil was added suggest a high impact of oil contamination on phytoplanktonic community. In these systems, the total abundances decreased dramatically along all the incubations, reaching the minimum value of 1.02 x 10^5 cells L^{-1} in the CTR+ (67% decrease). Such high mortality could be due to the toxic effect of oil, but also to the physical sequestration of cells performed by oil during its sinking along the water column. The use of Sophorolipids as surfactants slightly limited the mortality of phytoplankton in the experimental systems, limiting the cell loss to ca. 50%. Such effect is probably due to the reduction of oil sinking along the water column, limiting the physical removal and confining the toxic effects to the boundary of the system, where the oil firstly accumulated after treatment.

The qualitative analyses performed on samples collected at mid depth inside the mesocosms allowed to study the community structure of phytoplankton. The background conditions are represented in Figure 6

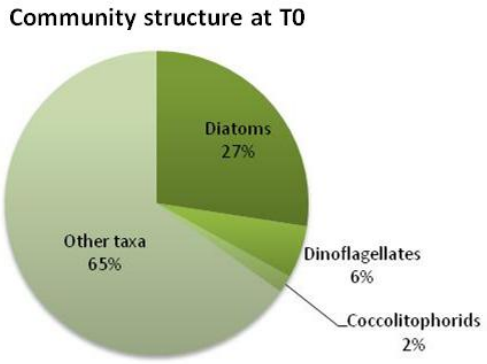

Figure 6 Total abundances of phytoplankton (background conditions)

The diatoms represented ca. 27% of the total phytoplankton, whereas dinoflagellates and coccolitophorids represented a minor fraction (6 and 2%, respectively). The community was largely dominated by phytoflagellates, which (together with other taxonomic groups) have been reported as "other taxa"and represented 65% of the total amount of the collected phytoplankton.

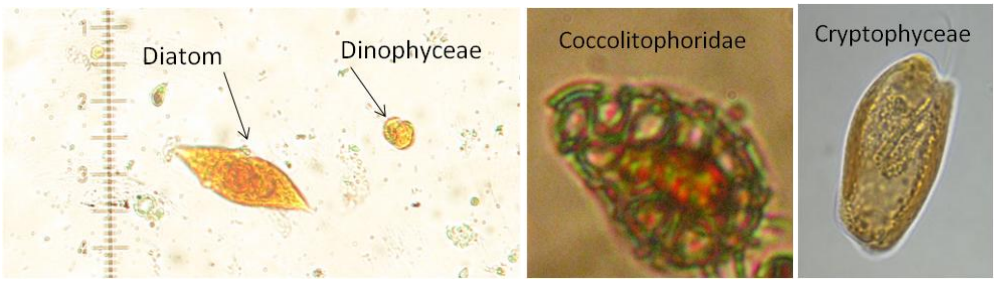

Figure 7 Representative specimens of the more common phytoplanktonic groups found in seawater samples.

In Figure 8 are represented the temporal evolution of the 4 experimental systems. The community structure of phytoplankton was not affected by the confinement due to mesocosm in the short time, since the relative abundances of the main taxonomic groups did not show major variations along the incubations and at the end of the experiment (CTR-). The use of Sophorolipids led to very similar results: also in this case, no significant differences were found comparing the background community structure and the community structure after 5 days of incubation, suggesting no effects on the planktonic community caused by Sophorolipids.

Significant effects were found in both systems in which the oil was added. In the positive control (oil without addition of Sophorolipids) diatoms represented the more impacted taxon, decreasing their relative abundance from 27 to 14% of the total. The use of Sophorolipids, thus resulting in an increase of the relative abundance of dinoflagellates, reduced the loss of diatoms to 20%. Diatoms are widely recognized as particularly sensitive to environmental pollution (Walsh et al., 1985), and are used as biological indicators of water quality (Hellawel 2012). Diatoms have been successfully used to monitor several disturbances (as eutrophication, Smol and Douglas, 1996; Wu, 1999). Because of their short generation time, diatoms might be better early detectors of environmental deterioration and recovery than invertebrates and fish (Cattaneo et al., 2004). Diatoms respond to perturbation at the community level through shifts in dominant taxa, but also at the individual level. For these reasons, the better preservation of diatom community, together with the reduction of phytoplankton loss, observed in the system treated with Sophorolipids, suggest that these molecules could represent a valid product for environmental recovery.

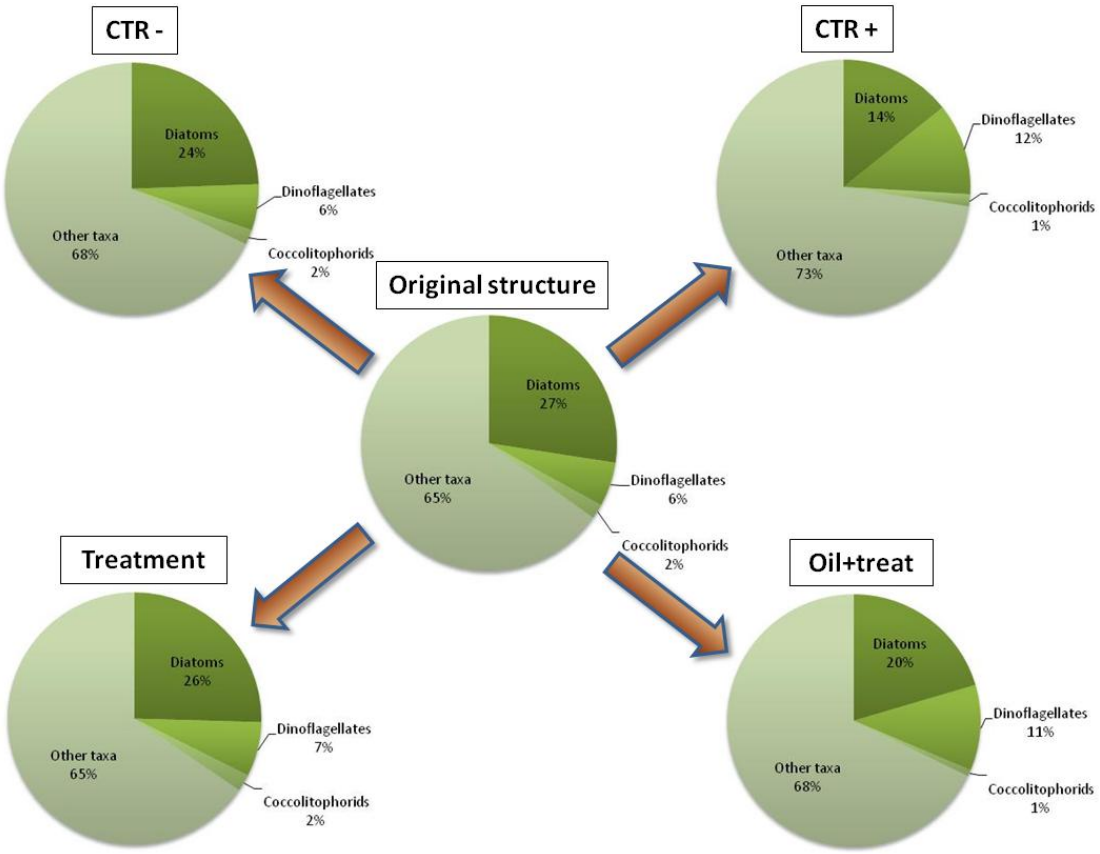

Figure 8 Evolution of phytoplankton community structure after 5 days of incubation

The autochtonous zooplanktonic community in the study site at the background is shown in Figure 9. The planktonic community was divided into microzooplankton and mesozooplankton and represented as cumulative amount of the main groups.

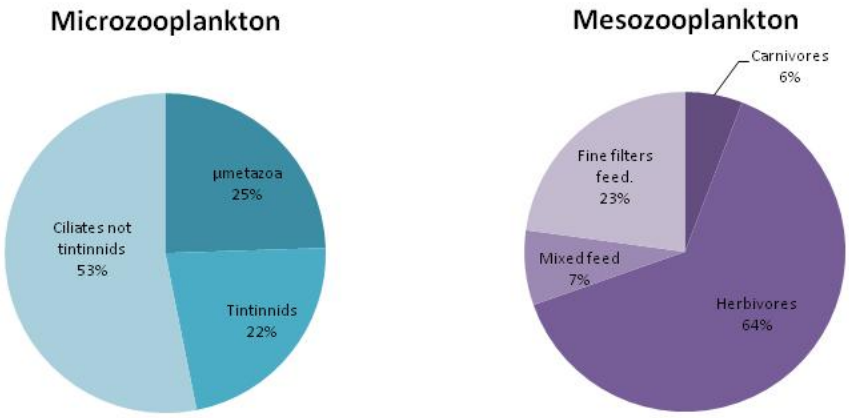

Figure 9 Relative importance of the dominant groups in zooplanktonic community (background conditions)

The microzooplankton showed an abundance ca. 2 orders of magnitude higher than the mesozooplankton (1.43 x 10^6 ind. m^{-3} vs 1.46 x 10^4 ind. m^{-3}, respectively). Among microzooplankton, the Ciliates not classified as tintinnids represented the more abundant group (ca. 53% of total organisms). Among mesozooplankton, the most abundant group was represented by Herbivores, with the huge amount of *Clausocalanus spp.* These results fall in the range of previous studies performed in North Adriatic (e.g. Mozetic et al., 1998; Fonda-Umani et al. 1992; Franco & Michelato, 1992).

In Figure 10 the temporal variations of main ecological groups of microzooplankton and mesozooplankton are shown.

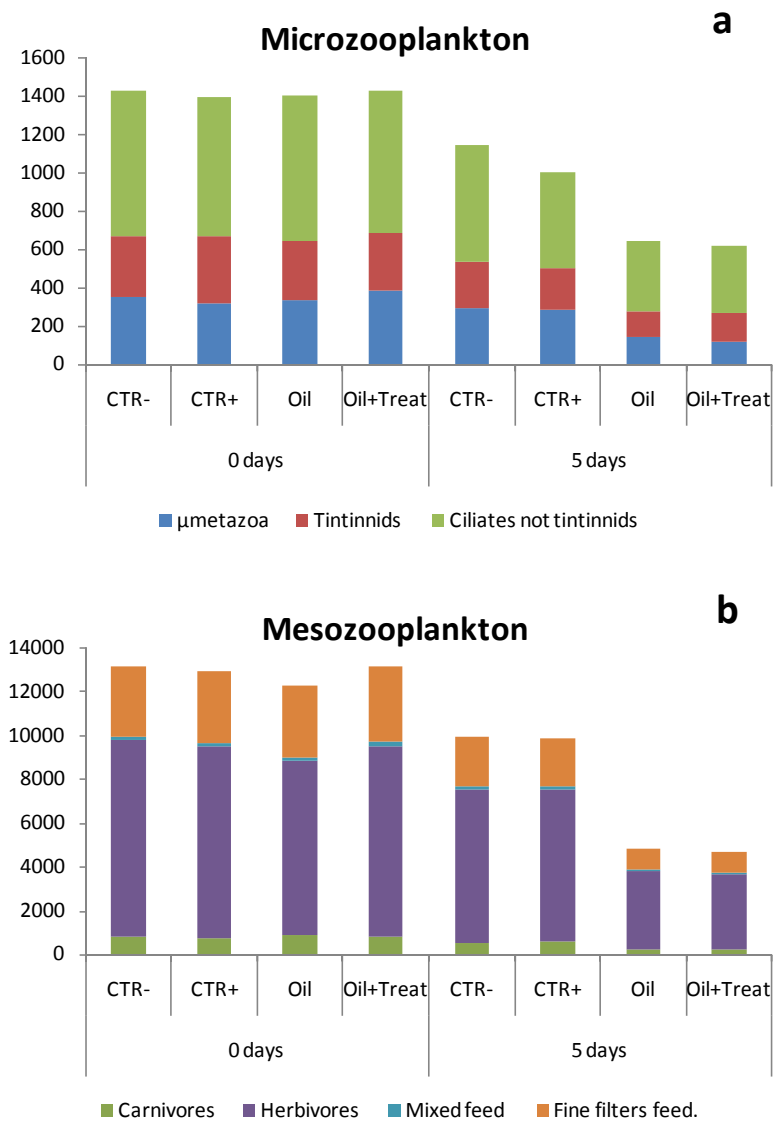

Figure 10 Temporal variation of main ecological groups of microzooplankton (panel a) and mesozooplankton (panel b) at the start of experiment (0 days) and after 5 days of incubation.

The confinement of the tested ecosystem is probably a reason for the general decrease observed for all groups of micro-and mesozooplankton: such decrease ranged from 20 to 24% for microzooplankton (Ciliates not tintinnids and Tintinnids, respectively) and from 22 to 33% for

mesozooplankton (herbivores and carnivores, respectively). All the differences were statistically significant ($p>0.01$ in all cases).

The addition of oil to experimental systems was the main cause of loss of organisms displayed by Oil and Oil+Treat Systems. In the Oil system, the mortality along the 5 days of experiments (calculated on the basis of actual abundance of organisms in the CTR- system at day 5) was, on average, 45% for microzooplankton 54% for mesozooplankton. Among microzooplankton groups, the most impacted were the micrometazoans, which abundance decreased of 52% respected to the untreated system. The most impacted group of mesozooplankton was that of carnivores, which abundances were reduced of 62% at the end of the experiment. No significant differences were observed between Oil and Oil+Treatment systems, suggesting that the addition of sophorolipids at these concentrations does not play a role in the preservation of zooplanktonic community.

4 Conclusions

The results obtained by mesocosm incubation represent some of the few data available about the impact of light crude oil on phyto- and zooplanktonic community obtained in a real marine system confined by a mesocosm. The results provided here are also the only data available about the impact of a biosurfactant (i.e. sophorolipids) on a marine planktonic community.

About the ecocompatibility of sophorolipids, it is clear from the results that, at the concentrations used, these molecules do not exert any noticeable impact on planktonic communities. Only minor differences were observed in terms of total abundances and community structure between the two control systems, basically due to the slight heterogeneity of natural planktonic communities.

Whereas the quali-quantitative data at the background fell in the typical ranges of surface waters of Adriatic Sea, the addition of crude oil and sophorolipids produced significant variations on both phytoplanktonic and zooplanktonic communities. The addition of crude oil caused important decreases of total planktonic abundances, on average higher than 50%. As reported before, this decrease can be due to the combined effect of toxicity and physical sequestration of organisms from the water column operated by the sinking oil.

Slight differences in the phytoplanktonic community structure were potentially induced by the addition of sophorolipids, and particularly in the percentage of diatoms. Diatoms are widely recognized as particularly sensitive to environmental pollution, and are used as biological indicators of water quality. The better preservation of diatom community in presence of Sophorolipids, together with the reduction of phytoplankton loss, observed in the system treated with Sophorolipids, suggest that these molecules could represent a valid product for environmental recovery.

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