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Seasonal distribution of ultraphytoplankton and heterotrophic prokaryotes in relation to abiotic variables on the north coast of Sfax after restoration

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ABSTRACT

The Taparura project was set up to restore the north Sfax coast (Tunisia) by shutting down the northern phosphate plant responsible for chronic pollution and uncontrolled phosphogypsum dumping. The restoration effect on coastal ultraphytoplankton (<10 μm) and heterotrophic prokaryotes was investigated using conventional flow cytometry over four successive seasons during 2009–2010. Cell concentrations were generally higher than values reported for the open sea, both in the western and eastern Mediterranean basins. One striking point was that chl *a* concentration on the north Sfax coast was unchanged after restoration but was still one order of magnitude higher than in the Gulf of Gabès. Restoration of pH, following the shutdown of the phosphate processing plants on the north coast, appeared to reach normal levels for seawater during the study, whereas seawater acidification persisted on the south coast where plants are still in operation. The largest ultraphytoplankton biomass was from an unknown cell group, whose identity and role needs to be established.

1. Introduction

The Sfax coast, located to the south eastern Mediterranean Sea, has one of the main ports in the Gulf of Gabès and is emerging as an important and rapidly-developing industrial centre in Tunisia. The coastal area has been subsequently subjected to an increase in industrial activities (Tayibi et al., 2009) and anthropogenic pollution (Hamza-Chaffai et al., 1995, 1996, 1997) mainly from the phosphate-treating plants which have been developed along the coast. The Taparura project was launched in 2008. It is part of an environmental policy and management programme aimed at tackling the pollution threatening the Sfax beaches and coastal waters (Hamza-Chaffai et al., 1997; Ben Brahim et al., 2010; Abdennadher et al., 2012). The Taparura project involved the clean-up of a 400 ha zone, removing 4.3 million m^3 of polluted soil including 1.7 million m^3 of phosphogypsum. The phosphogypsum, was untreated and stockpiled along the coastline, 6 m above sea level in an uncontrolled landfill covering an area of more than 150 ha. Phosphogypsum is an industrial by product formed in the manufacture of phosphoric acid from natural phosphate rock using the wet process (Tayibi et al., 2009). In order to identify the impact of the phosphate-treating plant and the effect of the pollution generated on water quality, a preliminary investigation (Rekik et al., 2011) was conducted before the coastal area restoration. This study addressed the summer spatial distribution of zooplankton and its links with microphytoplankton and ciliates, using taxa enumeration techniques. Following the clean-up phosphogypsum and the restoration of the area, this larger survey programme has taken place. It has included the investigation over four seasonal sampling campaigns covering abiotic variables, microphytoplankton and zooplankton (Rekik et al., 2013b). Microorganisms rapidly react to environmental changes, it was therefore important to conduct a post-restoration survey at the single-cell level of the plankton community using a size spectrum comprising heterotrophic prokaryotes, autotrophic picophytoplankton and nanophytoplankton. In this paper, we address the restoration impact on the microbial assemblage distribution along the Sfax north coast over four successive seasons during 2009 and 2010, using flow cytometry.

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2. Materials and methods

2.1. Study site and sampling strategy

The study area in the zone 34°43′–34°46′ N and 10°46′–10°49′ E covering the north coast of Sfax (Tunisia), a city with a dry climate (average precipitation: 210 mm) under the influence of hot southerly winds (Sirocco). The city coastline (50 km) stretches from the Sidi Mansour area in the north, to Chaffar in the south. The Sfax north coast (12 km) extends from the phosphate treatment plant (NPK), near the harbour, to Sidi Mansour, a zone characterised by its geomorphologic polygon (Fig. 1).

A grid of sampling points was defined to characterise the coastal water (abiotic features) and its microorganisms (ultraphytoplankton and heterotrophic prokaryotes) in order to assess the impact of the restoration along the north-coast, looking at the distance from the coast and also considering in parallel the surface and the water sediment interface. Indeed, the shallowness of the coastal waters could generate differences between samples from the surface and the water–sediment interface, deposits on the bottom or sediment resuspension generating conditions different from those at the surface. Fig. 1, shows the 18 stations that where sampled over the continental shelf in waters whose depth ranged from 0.5 to 7.0 m. During four successive seasons, autumn and winter in 2009, and spring and summer in 2010, seawater samples were collected at both the surface and water–sediment interface with a Van Dorn-type closing bottle.

2.2. Physical and chemical factors

Temperature, salinity and pH were measured immediately after sampling using a multi-parameter kit (Multi 340 i/SET). The concentration of suspended matter was determined by measuring the dry weight of the residue after filtration of 0.5 dm^3 of seawater with a Whatman GF/C membrane filter. To determine nutrient concentration, subsamples (120 cm^3) were preserved (−20 °C, in the dark) immediately upon collection. Nutrient (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, Si $(OH)_4$, total nitrogen (T-N) and total phosphate (T-P)) concentrations were determined with a Bran and Luebbe type 3 autoanalyser. The N/P ratio was calculated as DIN (DIN, dissolved inorganic nitrogen = $NO_2^- + NO_3^- + NH_4^+$) to DIP (DIP, dissolved inorganic phosphorus = PO_4^{3-}) ratio.

2.3. Flow cytometry

Subsamples (1.8 cm^3) for determining heterotrophic prokaryotes and autotrophic piconanoplankton abundances were immediately fixed with 0.2 cm^3 of a 20% (w/v) borate-buffered formalin solution, pH 7.2, and then frozen in liquid nitrogen. They were stored at −80 °C in the laboratory until analysis by flow cytometry which was conducted at the Regional Flow Cytometry Platform for Microbiology (PRECYM, <https://precym.mio.univ-amu.fr/>) at the Mediterranean Institute of Oceanography (MIO) of Marseille (France). Frozen samples were transferred in dry ice to MIO where they were analysed with a FACS Calibur flow cytometer (Becton Dickinson). Samples were thawed at room temperature, homogenised and directly analysed for autotrophic pico- and nanophytoplankton. For analysis of heterotrophic prokaryotes, a subsample of 300 mm^3 was incubated for 15 min in the dark with a 10 mm^3 SYBR Green solution (Sigma, commercial solution diluted 1000 times) to stain the nucleic acids and distinguish unambiguously the heterotrophic prokaryotes (green fluorescence) from inorganic particles, detritus and free DNA (Marie et al., 2000). The SYBR Green family dyes are widely used to stain nucleic acids of aquatic bacteria and in particular marine bacteria (Lebaron et al., 1998). SYBR Green is the dye of choice due to its high fluorescence yield when bound to nucleic acids (<http://www.invitrogen.com>). Data were collected and stored in list mode with the Cell QUEST PRO software (Becton Dickinson), and analysed with the SUMMIT software package (DAKO). Cell groups were resolved on the basis of the cell optical properties regarding scatter and fluorescence. To isolate heterotrophic prokaryotes from overlapping small autotrophic cells, the latter were removed on the basis of their red fluorescence. Cell sorting was conducted with the InfluxTM Mariner flow cytometer cell sorter (Becton Dickinson) of PRECYM.

2.4. Scanning electron microscopy

Sorted samples were filtered onto 0.2 μm nuclepore filters (13 mm diameter), dehydrated through successive ethanol baths,

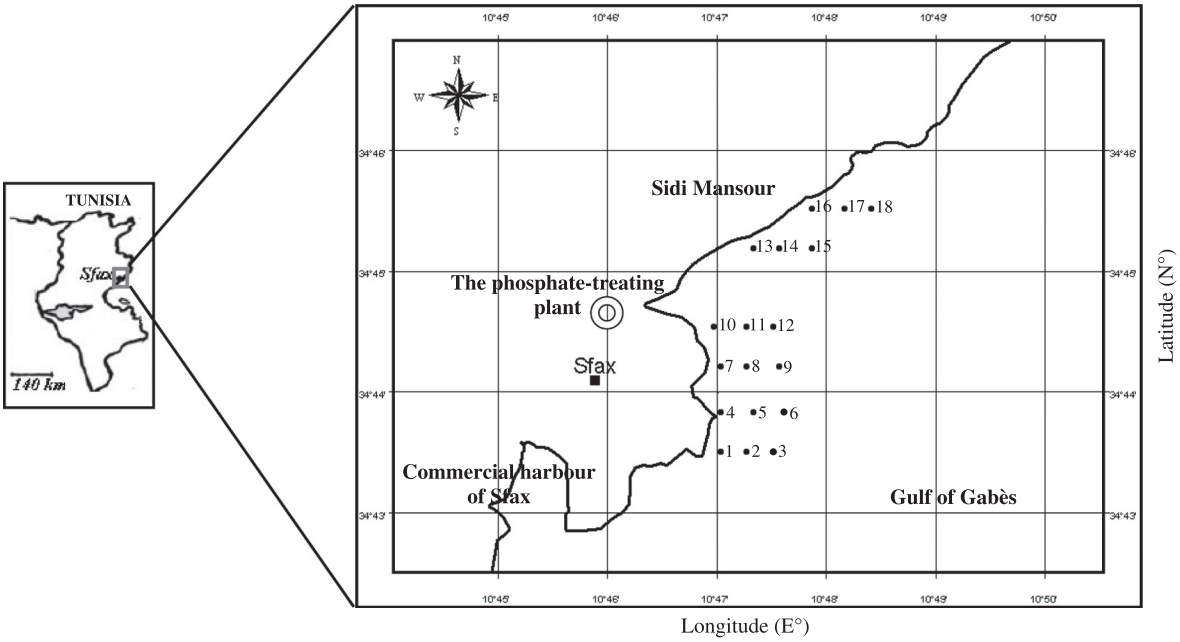

Fig. 1. Location of sampling stations on the north coast of Sfax.

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