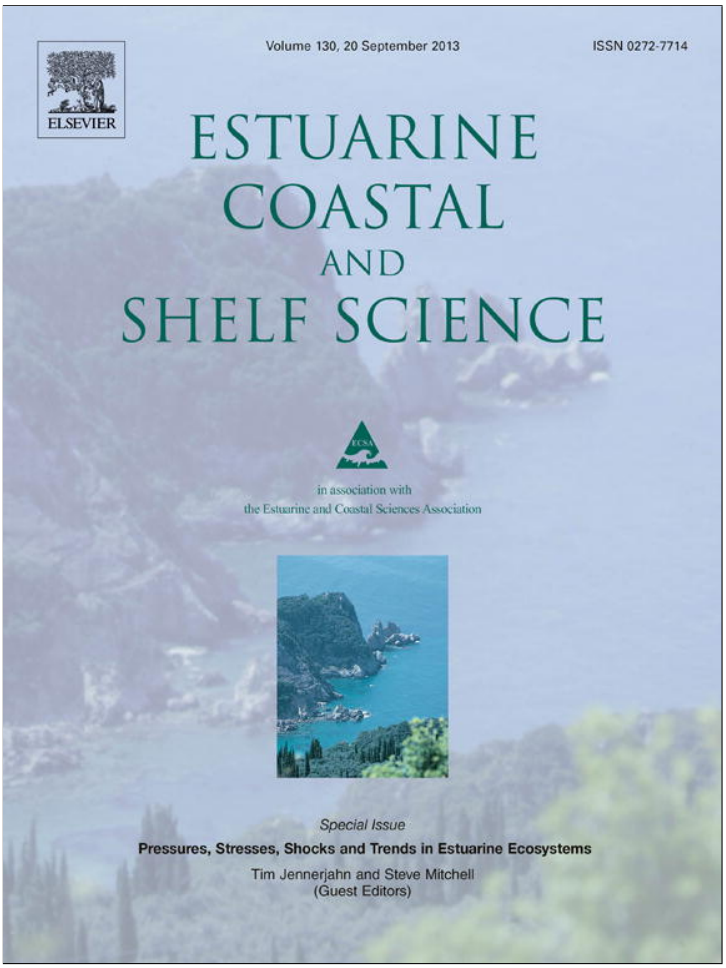

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Impact of contaminated-sediment resuspension on phytoplankton in the Biguglia lagoon (Corsica, Mediterranean Sea)

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ABSTRACT

In shallow human-impacted systems, sediment resuspension events can result in pulsed exposures of pelagic organisms to multiple contaminants. Here, we examined the impact of the resuspension of contaminated sediment on phytoplankton in the Biguglia lagoon (Corsica, Mediterranean Sea), by conducting an *in situ* microcosm experiment over a 96-h period. Natural phytoplankton was exposed to elutriates prepared from a contaminated-sediment resuspension simulating process, and its functional and structural responses were compared with those of non-exposed phytoplankton. The elutriates displayed moderate multiple contamination by trace metals and PAHs. Our results show that elutriate exposure induced both functional and structural phytoplankton changes. Elutriates strongly stimulated phytoplankton growth after 24 h of exposure. They also enhanced phytoplankton photosynthetic performance during the first hours of exposure (up to 48 h), before reducing it toward the end of the experiment. Elutriates were also found to slightly stimulate Bacillariophyceae and conversely to slightly inhibit Dinophyceae in the short term. Additionally, they were found to stimulate phycocyanin-rich picocyanobacteria in the short term (8–48 h) before inhibiting it in the longer term (72–96 h), and to inhibit eukaryotic nanophytoplankton in the short term (8–48 h) before stimulating it in the longer term (72–96 h). Sediment resuspensions are thus likely to have significant effects on the global dynamics and functions of phytoplankton in contaminated coastal environments.

1. Introduction

Coastal lagoons are critical transition zones that provide essential ecosystem services including shoreline protection, water quality improvement, fisheries resources, and habitat and food for a wide range of species (Levin et al., 2001). Their estimated economic value is substantial (22,832 USD ha^{-1} $year^{-1}$; Costanza et al., 1997). More than 40% of the world's population live within 100 km of the coast, and an even larger population is predicted to do so within the next two decades (Duxbury and Dickinson, 2007; Martinez et al., 2007). Hence, coastal lagoons are increasingly subject to anthropogenic pressures. The chemical contamination of the environment originating from human activities, notably through domestic, industrial and agricultural wastes, poses an increasing threat to these systems (Levin et al., 2001).

Sediment is traditionally viewed as a sink for contaminants. However, when disturbed, moved or relocated, sediment-bound contaminants can be remobilized in the water column (Latimer et al., 1999; Saulnier and Mucci, 2000) and thereby become bioavailable to pelagic organisms. Sediment can therefore act as a substantive source of contaminants (Burton and Johnston, 2010; Roberts, 2012), and sediment resuspensions can be viewed as pulsed environmental disturbances (Roberts, 2012). Owing to their shallowness and location at the land–sea interface, coastal environments such as lagoons can experience frequent and significant sediment resuspension events. A range of processes, both natural (e.g., wave action, tidal currents, bioturbation and storm surge) and anthropogenic (e.g., dredging, shipping and trawling; Olsen et al., 1982) can generate sediment resuspensions. Many of the processes that result in sediment resuspensions are expected to

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increase in severity and frequency due to climate change and growing human pressure on coastal environments (Schiedek et al., 2007; Roberts, 2012). However, the effects of resuspension of contaminated sediment on pelagic organisms remain underresearched and have not been fully incorporated into ecological risk assessment for relevant management of coastal ecosystems (Roberts, 2012).

While accounting for only 1–2% of the total photosynthetic carbon biomass, phytoplankton contributes to approximately 45% of primary production on Earth (Field et al., 1998), and therefore plays a major role in biogeochemical cycles and in the functioning of marine ecosystems. As a result of its position at the basis of numerous food webs, any alteration of its structure and physiological activities may trigger a cascade of impacts throughout the entire ecosystem. Understanding how phytoplankton responds to contaminants is thus of primary importance in forecasting the ecological consequences of the chemical contamination of the environment and in targeting priority management and restoration efforts in aquatic systems. Several studies have reported detrimental effects of contaminants on phytoplankton (e.g., see reviews of DeLorenzo et al., 2001; Pinto et al., 2003; Torres et al., 2008). However, as highlighted by some authors (e.g., Stachowski-Haberkorn et al., 2008; Laviale et al., 2010), most earlier research lacks environmental realism. The main reason for this is that past experiments were mainly based on laboratory investigations typically performed under controlled conditions, from single-contaminant tests, and without taking into account biotic and abiotic factors. Yet consideration of the environmental context has been shown to be essential to achieving a comprehensive understanding of the biological effects of contaminants (for reviews see Clements and Rohr, 2009; Clements et al., 2012). Phytoplankton responses to contaminants can, for example, be influenced by species interactions (Leboulanger et al., 2001; Ortmann et al., 2012), grazing pressure (Munoz et al., 2001), temperature (Bérard et al., 1999), water chemistry (Knauer et al., 2007), light exposure history (Guasch and Sabater, 1998; Laviale et al., 2010) or contamination exposure history (Dorigo et al., 2004; Serra et al., 2009). In addition, in its natural environment, phytoplankton is exposed to contaminant mixtures and, depending on the mixture, additive, synergistic or antagonistic effects may result from contaminant interactions (DeLorenzo and Fleming, 2008; Knauert et al., 2009; Relyea, 2009).

In this study, we examined the impact, over a 96-h period, of contaminated-sediment resuspension on phytoplankton in a Mediterranean lagoon (Biguglia lagoon, Corsica). For this purpose, we used *in situ* immersed microcosms, as these have been shown to be realistic experimental systems to assess the effects of contaminants on natural phytoplankton (de la Broise and Palenik, 2007). Phytoplankton was exposed to contaminated-sediment elutriates prepared from a sediment resuspension simulating process. The effects of elutriates on phytoplankton were assessed through comparisons of phytoplankton structural and functional variables among microcosms (control and elutriate-treated) and over time.

2. Materials and methods

2.1. Study area

With a surface area of 14.5 km^2, the Biguglia lagoon (42° 37′ N, 9° 27′ E; Fig. 1) is the largest wetland of Corsica. It is linked to the Mediterranean Sea through a narrow channel to the north and through the Fossone canal to the south (Fig. 1).

The prevailing winds are the Libeccio (south–west) and the Sirocco (south-east). As the Biguglia lagoon is very shallow (average depth 1.2 m), winds exceeding 5 m s^{-1} provide enough energy to stir up the top layer of the sediment (Mouillot et al., 2000). Such winds frequently occur in the study area (frequency: ≈15%; Mouillot et al., 2000) and result in significant sediment resuspension.

The Biguglia lagoon is of marked biological interest and has accordingly been included in the RAMSAR list (list of wetlands of international interest) since 1990. Its watershed lies to the south–west of the city of Bastia and covers an area of 180 km^2 that includes four towns with a population of approximately 35,000 in winter and around twice that number in summer. For the past four decades, human settlement and associated activities (particularly tourism, agriculture and industrial activities) have been rapidly increasing all around the lagoon (GEOMORPHIC, 2003). They currently pose substantial threats to the water quality and the ecological equilibrium of the lagoon.

2.2. Sediment elutriate preparation and contaminant analysis

Sediment and water samples were collected in the Biguglia channel (Fig. 1), an area located close to urban, industrial and agricultural zones. Sediment was extracted using a Van Veen grab, and sieved (2 mm mesh) to eliminate fauna and plant/wood fragments. Water was collected with a bucket and immediately filtered (200 μm mesh net) to remove the largest fragments and organisms. Three sediment sub-samples were stored in glass bottles, at 4 °C (cf. US EPA's storage recommendations: e.g., US EPA, 1994, 2003), until contaminant analysis. Another sediment subsample was added to the filtered water (1:4 *w*/*v* ratio) and stirred for 8 h to mimic sediment resuspension, according to the method of Geffard et al. (2002). After an 8 h settling period, the overlying solution was siphoned off and filtered down to 0.2 μm (successive filtrations: 200 μm mesh net, 20 μm mesh net, 3 μm pore-size Whatman glass fiber filters, and 0.2 μm pore-size Nucleopore membrane filters) to eliminate microorganisms. The solution obtained formed the contaminated-sediment elutriate. One part of this elutriate was diluted with 0.2 μm filtered water to prepare a half-diluted contaminated-sediment elutriate.

Contaminant analyses of sediment and elutriates were performed using standardized methods at the Laboratory of Rouen (COFRAC accredited laboratory, France; http://www.laborouen.com). They included the following chemical groups: trace metals (8), organotins (3), polycyclic aromatic hydrocarbons (PAHs; 16), pesticides (58), phenols and derivatives (23), polybromodiphenyl ethers (10), halogenated solvents (4), aromatic solvents (16), and chloroalkanes (sum of C_{10}–C_{13}). In all, 139 individual substances, listed as priority pollutants in several Directives (e.g., European Water Framework Directive 2000/60/EC), were analyzed.

2.3. Microcosm experiment

The *in situ* microcosm experiment ran from July 11 to 15, 2011. Twelve 3.5 L glass bottles (Schott-Duran) were used to make microcosms: control (four replicates); elutriate-treated (CSE, four replicates), and half-diluted elutriate-treated (CSE½, four replicates). At the sampling station (Fig. 1), 16.8 L of water was collected within the 0–80 cm water surface layer and filtered to 200 μm; the 200 μm filtration eliminated large debris and most zooplankton, thus minimizing grazing effects during incubation. A volume of 1.4 L of this 200 μm filtered water was poured into each of the twelve microcosms; 1.4 L of elutriate and 1.4 L of half-diluted elutriate were then added to the CSE and CSE½ microcosms, respectively, and 1.4 L of 0.2 μm filtered water (previously collected at the sampling station) was added to each of the control microcosms to obtain the same sample dilution as the elutriate-treated microcosms. All the microcosms contained 0.7 L of air (20% of the total volume), bringing

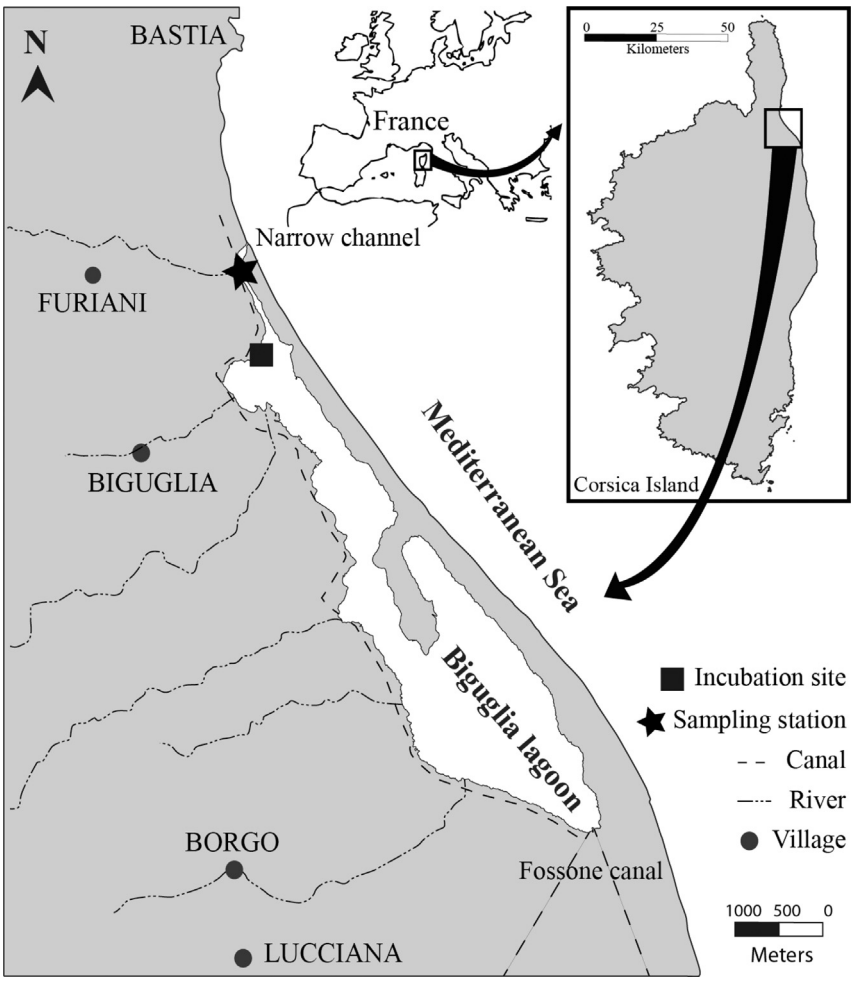

Fig. 1. Localization of the sampling station and the incubation site in the Biguglia lagoon (Corsica, France).

the system close to the natural environmental conditions (de la Broise and Palenik, 2007).

The Control and CSE½ microcosms were enriched with NH_4^+ to obtain similar concentrations in all microcosms at the beginning of the experiment. We considered only ammonium enrichment in our experiment, as nitrogen is likely to be the most limiting factor for phytoplankton in the Biguglia lagoon. This lagoon is mesotrophic/eutrophic (Garrido, 2012; Orsoni and Baldi, 2013), and several previous studies (e.g., Downing et al., 1999; Souchu et al., 2010) have shown that phytoplankton growth is mostly limited by nitrogen in nutrient-rich waters.

The microcosms were incubated at 40 cm depth in the lagoon (incubation site; Fig. 1) to mimic the average *in situ* environmental conditions of temperature and irradiance within the ≈80 cm water surface layer. The temperature and the photosynthetically available radiation were continuously measured (temperature data logger HOBO ProV2, Onset; light intensity recorder MkV/L, Alec Electronics). Samples were collected from each microcosm at the beginning of the experiment (700 mL), and after 8 h (100 mL), 24 h (100 mL), 48 h (300 mL), 72 h (100 mL) and 96 h (the remaining 1.5 L) of incubation, for subsequent analyses. Salinity (85 DO/Conductivity Instrument, YSI) and pH (pH-meter SB70P, SYMPHONY) were measured at each sampling time within the microcosms. Nutrient analyses were performed on samples collected at the beginning of the experiment, at the Laboratory of Rouen. Phytoplankton analyses of samples are detailed below.

2.4. Analysis of phytoplankton biomass and photosynthetic efficiency

Biomass (concentration of all antenna pigments that transfer absorbed energy via chlorophyll *a* to the photosynthetic reaction centers) and photosynthetic activity efficiency of samples were evaluated with a pulse-amplitude-modulated fluorometer (PHYTO-PAM Phytoplankton Analyzer; Heinz Walz GmbH, Effeltrich, Germany). Samples were kept in the dark during processing. A 2 mL aliquot was introduced into the measuring chamber and allowed to stabilize in modulated (non-actinic) light (ML) for 2 min. The maximum photosystem II (PSII) quantum yield (F_v/F_m) was determined using the equation: $F_v/F_m = (F_m - F_o)/F_m$ (Kitajima and Butler, 1975). The very low level of energy supplied by ML induces the initial intrinsic fluorescence, F_o, which represents the fluorescence when all PSII reaction centers are open and potentially available for electron transport (i.e., photosynthesis). F_m, the maximal fluorescence was measured after application of a saturating pulse of light (wavelength peak: 655 nm; intensity: 4000 μmol photons m^{-2} s^{-1}, duration: 200 ms). The effective (or operational) quantum yield of PSII (Φ_{PSII}) was determined by: $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty et al., 1989). F_s, the light-adapted constant fluorescence level, was measured under actinic light (≈1000 μmol photons cm^{-2} s^{-1}), and F_m', the light-adapted maximal fluorescence level, was recorded during five consecutive saturating pulses given at 30 s intervals (first pulse applied after 60 s of light exposure) and averaged.

2.5. Discrimination of phytoplankton taxonomic groups by fluorometry

A multiwavelength fluorometer (FluoroProbe, bbe-Moldaenke, Kiel FRG) was used to identify and quantify the biomass (in eq. Chl*a*) linked to the main phytoplankton taxonomic groups that occurred in the samples (Chlorophyceae, Cyanobacteria, Bacillariophyceae plus Dinophyceae, and Cryptophyceae; see methods in Beutler et al., 2002; Leboulanger et al., 2011). Measurements were made from 25 mL of dark-adapted samples continuously stirred in a 25 mL glass cuvette. Data were recorded for 60 s and averaged.

2.6. Phytoplankton analysis by microscopy

At the beginning of the experiment, 50 L of water was collected in the sampling station, filtered, concentrated using an Apstein net (20 μm mesh net; final volume: 100 mL), fixed (formaldehyde, 2.5% final concentration) and stored in the dark for microscopic examination. At the end of the incubation, samples from each replicate microcosm were pooled ($\approx$1 L), fixed with acid Lugol's iodine solution (5%, final concentration), and stored in the dark for microscopic examination.

Sub-samples of 20 mL were poured into settling chambers for sedimentation (Utermöhl, 1958; AFNOR, 2006). Identification, to the lowest possible taxonomic level, and quantification of phytoplankton were carried out using a phase-contrast inverted microscope (CKX31, OLYMPUS). To ensure that the error in the estimate of cellular abundance remained within the limits of ±10%, a minimum of 400 cells were counted when possible (Lund et al., 1958; Uehlinger, 1964).

2.7. HPLC pigment analysis

Samples (up to 200 mL) were filtered through 25 mm Whatman GF/F glass fiber filters (pore size 0.7 μm); and the filters were then stored at −80 °C until analysis. Pigment extraction was carried out in methanol by sonication (Jeffrey et al., 1997). After 30 min freezing at −20 °C, extracts were filtered on GF/F filters to remove debris. Pigment identification and quantification were performed by high-performance liquid chromatography (HPLC, C8 reverse phase column; Waters Alliance 2695), according to Zapata et al. (2000).

2.8. Flow cytometry analysis

Samples (1.4 mL) were fixed with 0.2 μm filtered formaldehyde (37%) and stored at −80 °C. Analyses were performed at the Regional Flow Cytometry Platform for Microbiology (PRECYM; http://precym.com.univ-mrs.fr) of the Mediterranean Institute of Oceanography, using a FACSCalibur flow cytometer (488 nm Argon laser and 635 nm diode laser; Becton Dickinson). For each particle (cell) analyzed, two light scatter intensities were collected: the forward angle light scatter, related to the size of the particles, and the right angle light scatter (or side scatter), related to their shape and granularity. Additionally, four fluorescence intensities were collected: FL1, green fluorescence (centered on 530 nm), FL2, orange fluorescence (centered on 585 nm), FL3, red fluorescence (>650 nm), and FL4, far red fluorescence (induced by the 635 nm diode laser, centered on 661 nm). In this study, FL2–FL4 signals were related to phycoerythrin, chlorophyll and phycocyanin contents of the cells, respectively. To focus on phytoplankton, the trigger signal was set up on the red signal (FL3) induced by the 488 nm laser beam. Filtered synthetic seawater (successive filtrations through 0.8 and 0.2 μm porosity Acropak filters) was used as sheath fluid. The flow rate of the sheath fluid was set to "High" ($\approx$100 μl min^{-1}), and the flow rate of the samples to "Low" ($\approx$12 μl min^{-1}) or "High", depending on the particle richness of samples. We used 2 μm beads (Fluoresbrite YG, Polyscience) and TruCount beads (Becton Dickinson) as internal standards and to control the flow rates, respectively. Data were acquired with the Cell Quest software (Beckton Dickinson), and analyzed using the WinList software (Verity Software House, USA).

2.9. Statistical analysis

Differences in variables among treatments and according to exposure times were investigated with a repeated measures analysis of variance (ANOVA). Where ANOVA revealed significant differences, Tukey's HSD *post hoc* comparison tests were performed to determine where significant differences occurred (STATISTICA software, StatSoft). Tests were assessed at the $P = 0.05$ level.

Cluster analysis (UPGMA, percent similarity) was performed from HPLC pigment data to evaluate the similarity of the phytoplankton structure among samples (MVSP software, Kovach Computing Service, Anglesey, Wales, UK).

3. Results

3.1. Contaminant levels of sediment and elutriates

Table 1 shows the contaminant concentrations measured in sediment and elutriates.

Three chemical groups were detected in sediment: trace metals, organotins, and PAHs. Some elements displayed concentrations ranging between the sediment ERL ("effects range-low") and ERM ("effects range-medium") guideline values established by Long et al. (1995): Cr (trace metal), Cu (trace metal), Ace (PAH) and Flr (PAH). The trace metal Ni exhibited sediment concentrations above the ERM threshold value (Long et al., 1995).

In elutriates, the main substances found were trace metals (As, Cr, Cu, Hg, Ni, Pb and Zn) and PAHs (Flt, BaA, Chry, BbF, BkF, BaP, BghiP and Ind). It is likely they were released from the sediment during the sediment resuspension simulating process. The organotins MBT and DBT were also found in elutriates, but in very low concentrations.

3.2. Physico-chemical parameters

Over the course of the experiment, the water temperature varied from 29 °C to 33 °C in the incubation site, and the light irradiance varied from 0 to 535 μmol photons m^{-2} s^{-1} within the microcosms (day/night cycle). Salinity and pH were similar within all the microcosms and throughout the experiment ($\approx$24‰ and 8, respectively).

NO_3^- and NH_4^+ concentrations were similar among microcosms ([NO_3^-] in μmol L^{-1}: 0.61 ± 0.07 in Control, 0.37 ± 0.09 in CSE½, and 0.35 ± 0.14 in CSE; [NH_4^+] in μmol L^{-1}: 59.2 ± 16.0 in Control, 43.1 ± 0.4 in CSE½, and 48.3 ± 0.5 in CSE; $P \geq 0.05$). By contrast, NO_2^-, $H_3PO_4^-$ and SiO_4^{3-} concentrations were significantly higher in elutriate-exposed than in control microcosms ([NO_2^-] in μmol L^{-1}: 0.11 ± 0.01 in Control, 0.18 ± 0.01 in CSE½, and 0.24 ± 0.02 in CSE; [$H_3PO_4^-$] in μmol L^{-1}: <0.53 in Control, 1.18 ± 0.04 in CSE½, and 2.12 ± 0.07 in CSE; [SiO_4^{3-}] in μmol L^{-1}: 34.0 ± 0.4 in Control, 52.3 ± 0.5 in CSE½, and 62.8 ± 0.1 in CSE; $P < 0.05$).

3.3. Fluorescence parameters

F_v/F_m: This parameter ranged from 0.15 ± 0.01 (CSE, at the beginning of the experiment) to 0.61 ± 0.01 (CSE½ and CSE, after 48 h of incubation; Fig. 2). Over the experiment period, it did not significantly differ among microcosms, but it significantly differed among times (Fig. 2, Table 2). By contrast, considering the treatment–time interaction, this parameter was significantly higher in

Table 1
Concentrations of contaminants in sediment (mean ± SE) and elutriates. Regarding sediment, concentrations are expressed in μg g^{-1} dry wt. for trace metals, in μg Sn kg^{-1} dry wt. for organotins, and in μg kg^{-1} dry wt. for polycyclic aromatic hydrocarbons (PAHs), pesticides, phenols and derivatives, and aromatic solvents. Elutriate concentrations are expressed in μg L^{-1} for all elements, except for PAHs and organotins, which are expressed in ng L^{-1} and μg Sn L^{-1}, respectively.

		Sediment	Elutriate	Diluted elutriate
Trace metals	Arsenic (As)	5.5 ± 0.2	8.0	5.0
	Cadmium (Cd)	0.2 ± 0.0	bdl	0.1
	Chromium (Cr)	**164.7 ± 2.6**	25.4	16.8
	Copper (Cu)	**42.0 ± 1.0**	15.2	10.4
	Mercury (Hg)	0.1 ± 0.0	0.076	0.094
	Nickel (Ni)	**116.0 ± 1.0**	25.9	17.8
	Lead (Pb)	23.3 ± 0.3	6.7	3.4
	Zinc (Zn)	108.7 ± 0.9	30	25
Organotins	Monobutyltin (MBT)	4.8 ± 0.3	0.003	0.003
	Dibutyltin (DBT)	3.1 ± 0.4	0.005	0.007
	Tributyltin (TBT)	3.2 ± 0.2	bdl	bdl
Polycyclic Aromatic Hydrocarbons (PAH)	Naphthalene (Nap)	12.7 ± 6.9	bdl	bdl
	Acenaphthene (Ace)	**44.5 ± 35.9**	bdl	bdl
	Fluorene (Flr)	**20.9 ± 17.1**	bdl	bdl
	Phenanthrene (Phe)	67.8 ± 31.7	bdl	bdl
	Anthracene (Ant)	10.9 ± 4.1	bdl	bdl
	Fluoranthene (Flt)	118.6 ± 43.3	11	12
	Pyrene (Pyr)	118.5 ± 54.0	bdl	bdl
	Benzo(a)anthracene (BaA)	65.9 ± 31.8	9	9
	Chrysene (Chry)	61.9 ± 31.5	12	15
	Benzo(b)fluoranthene (BbF)*	71.3 ± 38.1	16	15
	Benzo(k)fluoranthene (BkF)*	38.0 ± 20.0	7	7
	Benzo(a)pyrene (BaP)	75.7 ± 39.2	18	17
	Dibenzo(a,h)anthracene (DahA)	7.1 ± 3.6	bdl	bdl
	Benzo(g,h,i)perylene (BghiP)*	91.5 ± 49.4	25	18
	Indeno(cd)pyrene (Ind)*	63.7 ± 33.9	15	14
Pesticides	Gamma-HCH (lindane)	0.7 ± 0.2	bdl	bdl
	p,p′-DDT	bdl	0.004	0.004
	Delta-HCHa	1.6 ± 0.2	bdl	bdl
Phenols and derivatives	Nonylphenol	300.3 ± 250.3	4.16	7.43
	4-para-nonylphenol	300.3 ± 250.3	4.16	7.43
Aromatic solvents	Benzene	8.7 ± 3.7	bdl	bdl
	Toluene	11.7 ± 6.7	bdl	bdl

Concentrations in sediment equal to or greater than the ERL ("effects range-low") value and below the ERM ("effects range-medium") value (Long et al., 1995) are in bold, and those equal to or greater than the ERM value are in bold and underlined. PAH compounds for which no effect range values are provided by Long et al. (1995) are indicated by an asterisk (*).
bdl: below detection limits.
Elements that were bdl in sediment and elutriate samples are not reported in the table: i.e., the PAH Acenaphthylene (Acy), some pesticide compounds (Linuron, Metoxuron, Methabenzthiazuron, Chlortoluron, Monolinuron, Metobromuron, Neburon, Monuron, Isoproturon, Diuron, Pentachlorobenzene, Alpha-HCH, Beta-HCH, Hexachlorobenzene, Heptachlore, Aldrin, Isodrin, Exo-Epoxy-Heptachlor, Endo-Epoxy-Heptachlor, *o,p′*-DDE, Alpha-Endosulfan, *p,p′*-DDE, Dieldrin, *o,p′*-DDD, Endrin, Beta-Endosulfan, *p,p′*-DDD, *o,p′*-DDT, Endosulfan sulfate, Acetochlor, Alachlor, Chlordane, 1,3-Hexachlorobutadiene, Desethylatrazine, Simazine, Atrazine, Terbuthylazine, Desethylsimazine, Desethyl-Terbutylazine, Dimethoate, Diazinon, Disulfoton, Methyl-Parathion, Methyl-Pyrimiphos, Malathion, Ethyl-Parathion, Ethyl-Pyrimiphos, Ethion, Fenitrothion, Ethyl-chlorpyrifos, Methyl-Azinphos, Ethyl-Azinphos, Chlorfenvinphos, Trifluralin, Tributylphosphate), some phenol and derivative compounds (2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,3-dichlorophenol, 2,6-dichlorophenol, 3,5-dichlorophenol, 3,4-dichlorophenol, 2,3,5-trichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4-trichlorophenol, 2,3,6-trichlorophenol, 3,4,5-trichlorophenol, 4-chloro-3-méthylphenol, Pentachlorophenol, 4-tert-butylphenol, 4-tert-octylphenol, 4-n-octylphenol, 4-n-nonylphenol), some aromatic solvents (o-xylene, m-xylene, p-xylene, Ethylbenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, Chlorobenzene, 4-chlorotoluene, 3-chlorotoluene, 2-chlorotoluene, 1,3,5-trichlorobenzene, 1,2,3-trichlorobenzene, Isopropylbenzene), all analyzed polybromodiphenyl ethers (pentabromodiphenyl ether, octabromodiphenyl ether, decabromodiphenyl ether, BDE 47, BDE 77, BDE 99, BDE 100, BDE 153, BDE 181, BDE 209), all analyzed halogenated solvents (1,2-dichlorobenzene, chloroform, 1,2-dichloroethane, dichloromethane) and chloroalkanes (C10-13 chloroalkanes).

treated than in control microcosms after 48 h of incubation, and was significantly lower in CSE½ than in CSE and control microcosms at the end of the experiment (after 72 h and 96 h of incubation; Fig. 2, $P < 0.05$).

Φ_{PSII}: This parameter ranged from 0.10 ± 0.01 (CSE, at the beginning of the experiment) to 0.27 ± 0.01 (CSE½, after 48 h of incubation; Fig. 2). Over the experiment period, it significantly differed among both treatments and exposure times (Fig. 2, Table 2). By contrast, considering the treatment−time interaction, this parameter was significantly higher in treated than in control microcosms after 24 h of incubation, and was significantly lower in CSE½ than in control microcosms at the end of the experiment (after 96 h of incubation; Fig. 2, $P < 0.05$).

Photosynthetic pigment concentration: This ranged from 1.27 ± 0.05 (Control, at the beginning of the experiment) to 108.91 ± 5.40 μg L^{-1} (CSE½, after 48 h of incubation; Fig. 2). Over the experiment period, significant differences were found among both treatments and exposure times (Fig. 2, Table 2). By contrast, considering the treatment−time interaction, pigment concentration was significantly higher in treated than in control microcosms after 48 h of incubation and to the end of the experiment (Fig. 2, $P < 0.05$).

3.4. Phytoplankton taxonomic groups discriminated by fluorometry

The main taxonomic groups determined with the FluoroProbe, together with their contribution to the global phytoplankton biomass, are shown in Fig. 3.

The concentrations of the total phytoplankton, the Chlorophyceae, the Cyanobacteria, and the Bacillariophyceae plus Dinophyceae significantly differed according to both treatment ($P < 0.05$) and time ($P < 0.05$). The total phytoplankton concentration ranged from 5.01 ± 0.11 (Control, at the beginning of the

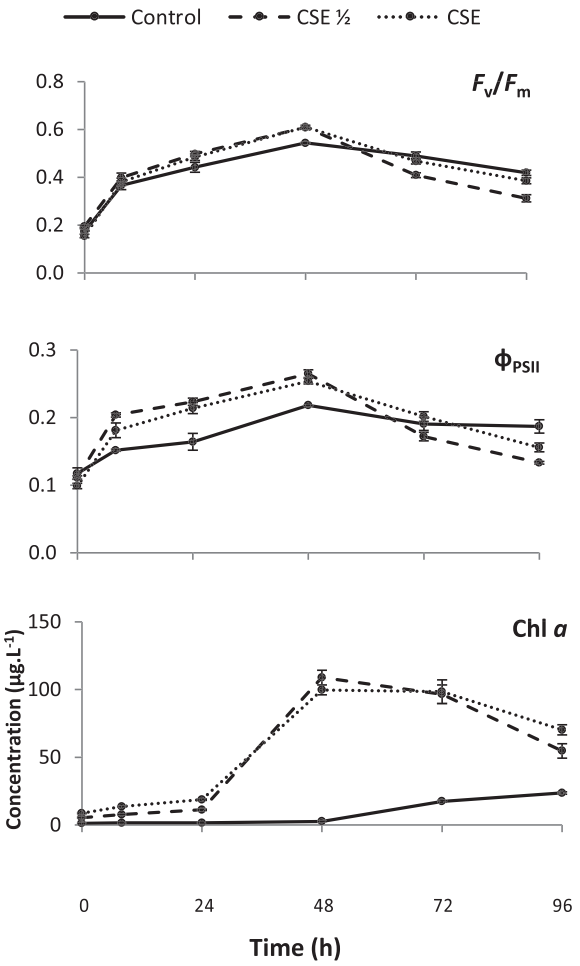

Fig. 2. Temporal evolution of the fluorescence parameters (F_v/F_m, Φ_{PSII}) and the total Chl *a* (mean ± S.E.), measured with the PHYTO-PAM analyzer, in the different microcosms (Control, CSE½ and CSE).

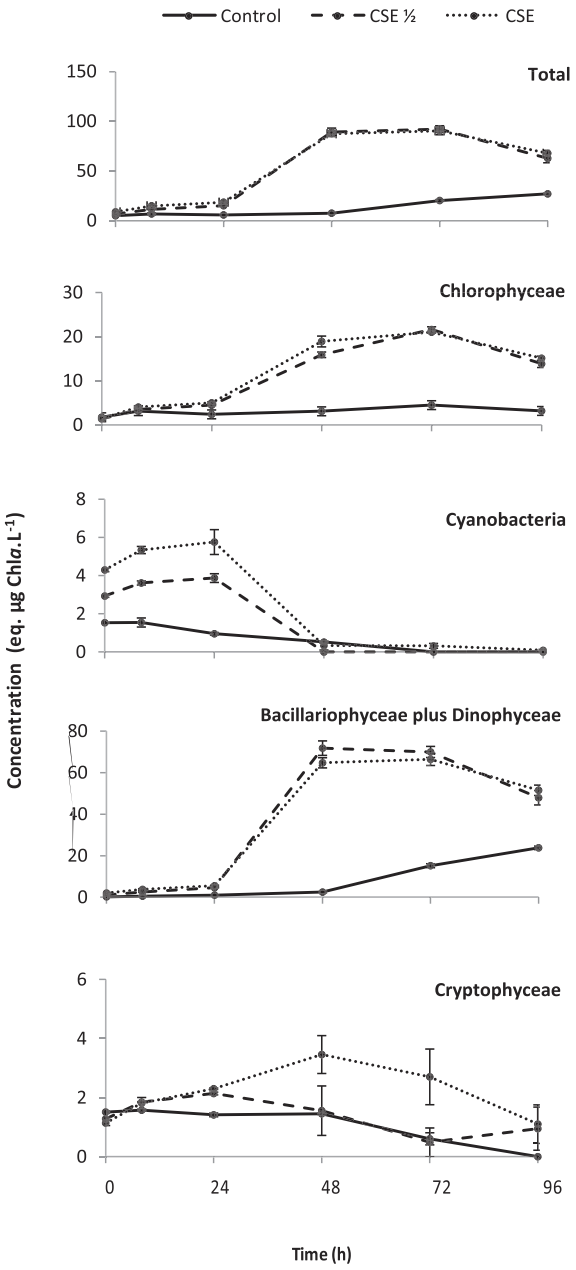

Fig. 3. Concentrations (mean ± SE; in eq. µg Chla L^{-1}) of the total phytoplankton (Total) and the main phytoplankton taxonomic groups (Chlorophyceae, Cyanobacteria, Bacillariophyceae plus Dinophyceae, and Cryptophyceae) in the different microcosms (Control, CSE½ and CSE) and over time. Data obtained with the FluoroProbe.

experiment) to 92.10 ± 3.47 eq. µg Chl*a* L^{-1} (CSE½, after 72 h of incubation). The Chlorophyceae concentration ranged from 1.38 ± 0.04 (CSE½, at the beginning of the experiment) to 21.63 ± 0.63 eq. µg Chl*a* L^{-1} (CSE½, after 72 h of incubation). The Cyanobacteria concentration ranged from 0 (Control, after 72 h and 96 h of incubation; CSE½, after 48 h and 72 h of incubation) to 5.76 ± 0.65 eq. µg Chl*a* L^{-1} (CSE, after 24 h of incubation). The concentration of the "Bacillariophyceae plus Dinophyceae" group ranged from 0.21 ± 0.03 (Control, at the beginning of the experiment) to 71.82 ± 3.48 eq. µg Chl*a* L^{-1} (CSE½, after 48h of incubation). By contrast, for the Cryptophyceae concentration, no significant difference was observed among either treatments or times ($P \geq 0.05$).

Table 2
ANOVA results for differences in fluorescence parameters (A: F_v/F_m; and, B: Φ_{PSII}) and photosynthetic pigment concentration (C).

	df	MS	*F*	*P*
A − F_v/F_m				
Treatment	2	0.00098	0.746	0.50
Time	5	0.22844	6150.72	0.00
Treatment × Time	10	0.00586	15.79	0.00
B − Φ_{PSII}				
Treatment	2	0.001517	4.63	0.04
Time	5	0.024627	159.45	0.00
Treatment × Time	10	0.002508	16.24	0.00
C − Photosynthetic pigment concentration				
Treatment	2	13871.31	168.47	0.00
Time	5	11974.21	302.69	0.00
Treatment × Time	10	2258.31	57.09	0.00

3.5. Microscopic data

The phytoplankton from the Biguglia lagoon was dominated by *Prorocentrum micans* (Dinophyceae; more than 90% of total counts). Two other Dinophyceae species were also present (*Alexandrium* sp. and *Protoperidinium* sp.; <3%). A few undetermined species of Bacillariophyceae and Cyanobacteria was also found.

At the end of the experiment, the Control and the CSE microcosms were still dominated by Dinophyceae species (mainly

P. micans and *Protoperidinium* sp.), whereas CSE½ microcosms were co-dominated by Dinophyceae (mainly *P. micans* and *Protoperidinium* sp.) and Bacillariophyceae (mainly *Diatoma* sp. and *Navicula* sp.) species.

3.6. HPLC pigment data

The UPGMA cluster analysis, performed from the HPLC pigment data, revealed structural differences of the phytoplankton community between control and treated microcosms (Fig. 4). Whereas the structure of the phytoplankton community displayed a close similarity among the treated microcosms (92% and 94% of similarity after 48 h and 96 h of incubation, respectively; Fig. 4), it exhibited less similarity between the control and the treated microcosms (79% and 86% of similarity after 48 h and 96 h of incubation, respectively; Fig. 4).

Using the method described in Vidussi et al. (2001), diagnostic pigments (peridinin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, alloxanthin, and zeaxanthin) were used to estimate the relative abundances of the principal taxonomic groups composing the communities (Dinophyceae, Bacillariophyceae, Prymnesiophyceae, Cryptophyceae, and Cyanobacteria, respectively) after 48 h and 96 h of incubation. The structural differences revealed by the UPGMA analysis after 48 h of incubation thus appear to be mainly linked to the Bacillariophyceae group, which was more abundant in elutriate-treated ($\approx$70% of total) than in control microcosms (40% of total), and to Dinophyceae which was, by contrast, relatively less abundant in elutriate-treated ($\approx$4% of total) than in control microcosms (20% of total). Regarding the structural differences revealed by the UPGMA analysis after 96 h of incubation, Bacillariophyceae and Dinophyceae also appeared to be the main explanatory groups; both were slightly less abundant in elutriate-treated (Bacillariophyceae: 57% of total; Dinophyceae: 8% of total) than in control microcosms (Bacillariophyceae: 66% of total; Dinophyceae: 11% of total). Other unidentified phytoplankton groups may also explain the structural differences observed between the elutriate-treated and the control microcosms (relative abundance of unidentified groups in elutriate-treated and control microcosms, respectively: $\approx$30% and 20%).

3.7. Flow cytometry data

Picophytoplankton was differentiated from nanophytoplankton through the forward scatter signal (FSC; $FSC_{picoplankton} < FSC_{2\ \mu m\ beads}$; $FSC_{nanoplankton} > FSC_{2\ \mu m\ beads}$).

Despite significant noise probably induced by debris from sediment elutriates, six phytoplankton groups were distinguished. Picoplankton cells with low FL3 red fluorescence (produced by Chl*a*), no FL2 orange fluorescence (induced by phycoerythrin), and a bright FL4 red fluorescence (induced by phycocyanin) were classified as phycocyanin-rich picocyanobacteria (Fig. 5), as in the study of Bec et al. (2011). A *Synechococcus*-like cyanobacteria group was discriminated by its low FSC ($<FSC_{2\ \mu m\ beads}$) and high orange fluorescence (phycoerythrin-rich cells). An unidentified group of cells exhibited very low FL3 red fluorescence, no FL2 orange fluorescence, high side scatter signal (SSC), but low FSC (unidentified picoplankton group). A eukaryotic nanophytoplankton group was spotted by its high FSC ($>FSC_{2\ \mu m\ beads}$), high FL3 red fluorescence intensity, and absence of FL2 fluorescence. Additionally, two minority groups (only found in a few samples) were detected: a picophytoplankton group (characterized by low FSC, low FL3 red fluorescence that was however brighter than for cyanobacteria, and no FL2 orange fluorescence) and an unidentified group (resolved only once, characterized by low FSC, intermediate FL3 red fluorescence between cyanobacteria and nanophytoplankton, and no FL2 fluorescence).

Fig. 6 shows the concentrations, in the different microcosms and over time, of the four main groups identified by flow cytometry (R1–R4). Globally, over the experiment period, the concentrations of phycocyanin-rich picocyanobacteria and eukaryotic nanophytoplankton were significantly higher in elutriate-treated than in control microcosms ($P < 0.05$). However, it is noteworthy that according to the time, the concentrations of these groups did not always significantly differ between treated and control microcosms; whereas the concentrations of the phycocyanin-rich picocyanobacteria were significantly higher in treated than in control microcosms during the first hours of the experiment (after 8 h, 24 h and 48 h of incubation, $P < 0.05$), they were similar at the end of the experiment (after 72 h and 96 h of incubation, $P \geq 0.05$). Moreover, whereas the concentrations of the eukaryotic nanophytoplankton were similar among microcosms during the first hours of the experiment (until 24 h of incubation, $P \geq 0.05$), they were significantly higher in treated than in control microcosms during the last hours of the experiment (after 48 h and 72 h of incubation, $P < 0.05$). The concentrations of the *Synechococcus*-like cyanobacteria and of the unidentified picoplankton group were globally similar among microcosms and throughout the experiment (only few significant differences were found in the treatment–time interaction analysis).

4. Discussion

The contaminant concentrations measured in the sediment of the Biguglia lagoon point to multiple contamination by trace metals and

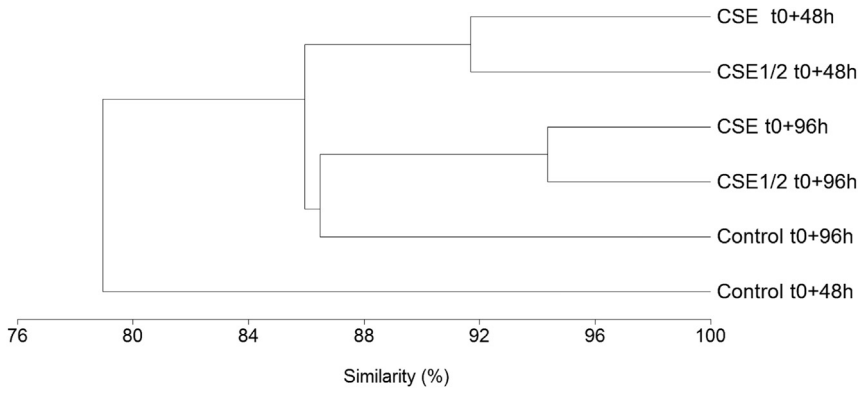

Fig. 4. UPGMA dendrogram showing structure similarity (%) among phytoplankton communities (initial; Control, CSE½, and CSE microcosms, at t0 + 48 h and t0 + 96 h). This dendrogram was obtained from the HPLC pigment data.

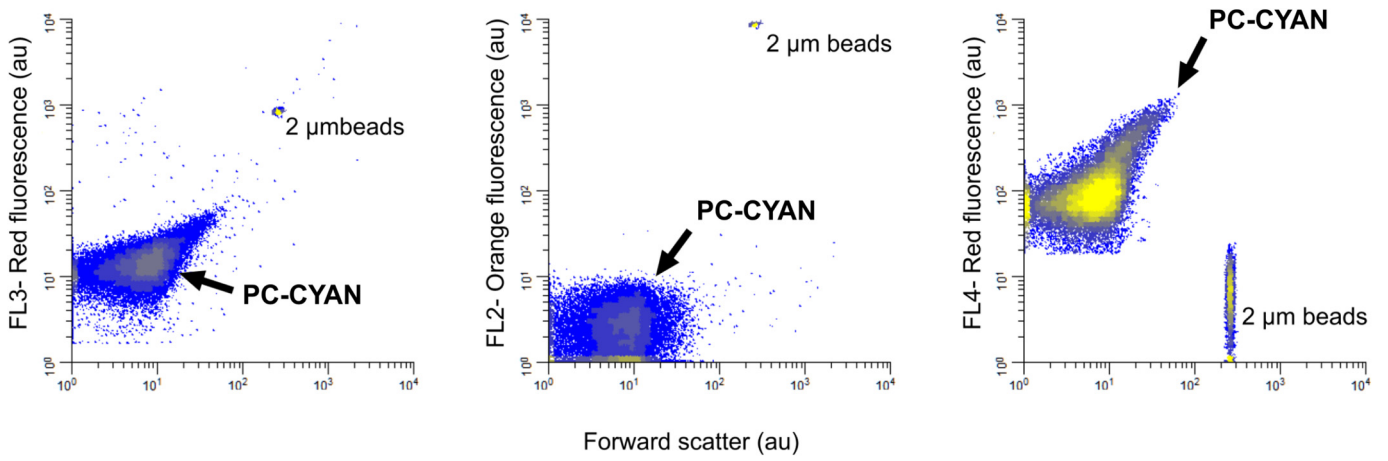

Fig. 5. Cytograms showing the phycocyanin-rich picocyanobacteria group (PC-CYAN) composed of small-size cells ($<FSC_{2\ \mu m\ beads}$) that exhibited a weak orange fluorescence signal after excitation by the 488 nm laser beam (signal related to phycoerythrin content) and a high red fluorescence intensity after excitation by the 635 nm diode laser (signal related to phycocyanin content). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PAHs. Compared with other lagoons in the world (see Accornero et al., 2008), the sediment trace metal concentrations measured in this lagoon are in the range of those found for industrial and densely populated areas such as the Gulf of Lion (South of France), thus suggesting a moderate/high metal contamination of the Biguglia lagoon (the Ni contamination appears particularly high). With a total PAH concentration of 869 µg kg^{-1}, the PAH contamination of the sediment of the Biguglia lagoon can be qualified as moderate from the levels assigned by Baumard et al. (1998). According to the guideline values established by Long et al. (1995; i.e., ERL and ERM values), the metal and PAH contamination of the sediment of the Biguglia lagoon is likely to induce adverse effects on biota. With concentrations ranging between the ERL and ERM values, the trace metals Cr and Cu, and the PAHs Ace and Flr are considered as able to cause occasional adverse biological effects (ERL_{Cr} = 81 and ERM_{Cr} = 370 µg g^{-1}; ERL_{Cu} = 34 and ERM_{Cu} = 270 µg g^{-1}; ERL_{Ace} = 16 and ERM_{Ace} = 500 µg kg^{-1}; ERL_{Flr} = 19 and ERM_{Flr} = 540 µg kg^{-1}). The trace metal Ni, with a concentration above the ERM threshold value (ERM_{Ni} = 51.6 µg g^{-1}), is considered as able to cause frequent adverse biological effects.

The sediment resuspension simulating process resulted in metal- and PAH-enriched elutriates. Globally, the multi-contamination of the obtained elutriates was characterized by a mixture of the trace metals As, Cr, Cu, Ni, Pb, and Zn, and the PAHs Flt, BaA, Chry, BbF, BkF, BaP, BghiP, and Ind. Also, together with contaminants, some nutrients were released into the water column during the sediment resuspension simulating process. Thus to make a reliable evaluation of the impact of contaminants on phytoplankton, we enriched the Control and the CSE½ microcosms with NH_4^+ so that this nutrient was at similar concentrations in all the microcosms at the beginning of the experiment, as nitrogen was likely to be the most limiting element for the phytoplankton occurring in the Biguglia lagoon (cf. Section 2.3).

The results obtained with the FluoroProbe and the PHYTO-PAM instruments were consistent, and point to growth stimulation of the elutriate-exposed communities. Yet several previous research reports describe decreases in the phytoplankton biomass after exposure to chemicals: e.g., the trace metal Zn (for concentrations similar to ours; Rochelle-Newall et al., 2008), the herbicide paraquat (concentrations tested: 10 and 40.5 µg L^{-1}; Leboulanger et al., 2011), and crude oil (for concentrations ≥2.28 mg L^{-1}; Huang et al., 2011). Nevertheless, other studies have reported, as in our study, increases in the phytoplankton biomass after chemical exposure (e.g., the herbicide diuron at concentrations of 2 and 11 µg L^{-1}, Leboulanger et al., 2011; crude oil at low doses, Huang et al., 2011), or no phytoplankton biomass change (e.g., the insecticide fenitrothion at concentrations of 10 and 100 µg L^{-1}; Leboulanger et al., 2011).

Among the main chemical compounds that were detected in elutriates, and hence that were likely to induce effects on phytoplankton, were essential micronutrient metals (Cu, Ni and Zn). Unlike the non-essential trace metals (e.g., As, Cd, Hg, and Pb) that may interfere with the proper functioning of enzymes and associated cofactors, the essential micronutrient metals are involved in protein structure stabilization, facilitate electron transfer reactions and/or catalyze enzymatic reactions (Torres et al., 2008). For example, Cu, Fe, and Zn are known to play key roles in photosynthetic electron transport within thylakoids (Raven et al., 1999). However, even though some trace metals are essential for metabolic and biological processes, they may also induce adverse effects above threshold levels. A recent study on phytoplankton showed that Co, Ni, Cu, and Zn exposure resulted in growth stimulation at low levels (e.g., for Cu: from 0.06 to 31.8 µg L^{-1}), but had opposite effect at higher levels (e.g., for Cu: ≥63.5 µg L^{-1}; Chakraborty et al., 2010). The biomass increase we observed for elutriate-exposed phytoplankton could thus result from stimulation by the essential metals occurring at low levels in elutriates (Cu, Ni, and Zn).

The maximum F_v/F_m values recorded in control and elutriate-treated microcosms (0.54 and 0.61, respectively; values recorded after 48 h of incubation) indicate a good physiological state of the phytoplankton communities at that time (Maxwell and Johnson, 2000). Surprisingly, with a maximum of 0.27, Φ_{PSII} values suggest a moderate or even a poor physiological state of the phytoplankton, whatever the incubation conditions (control or treated; Maxwell and Johnson, 2000). Such large differences between the F_v/F_m and Φ_{PSII} values were also reported in a previous study conducted on the phytoplankton of the Biguglia lagoon (Garrido et al., 2013).

The temporal evolution of the F_v/F_m and Φ_{PSII} fluorescence parameters reveals slight differences in the phytoplankton photosynthetic performance among microcosms. The overall pattern observed was a slight increase in the photosynthetic performance of the elutriate-exposed phytoplankton in the short term (8–48 h), and the opposite trend (i.e., slight decrease) in the longer term (72–96 h). This suggests that elutriate exposure may induce changes in the light-utilization efficiency by the phytoplankton. In the short term, elutriates may stimulate phytoplankton photosynthetic activity; in the longer term, they may, by contrast, inhibit

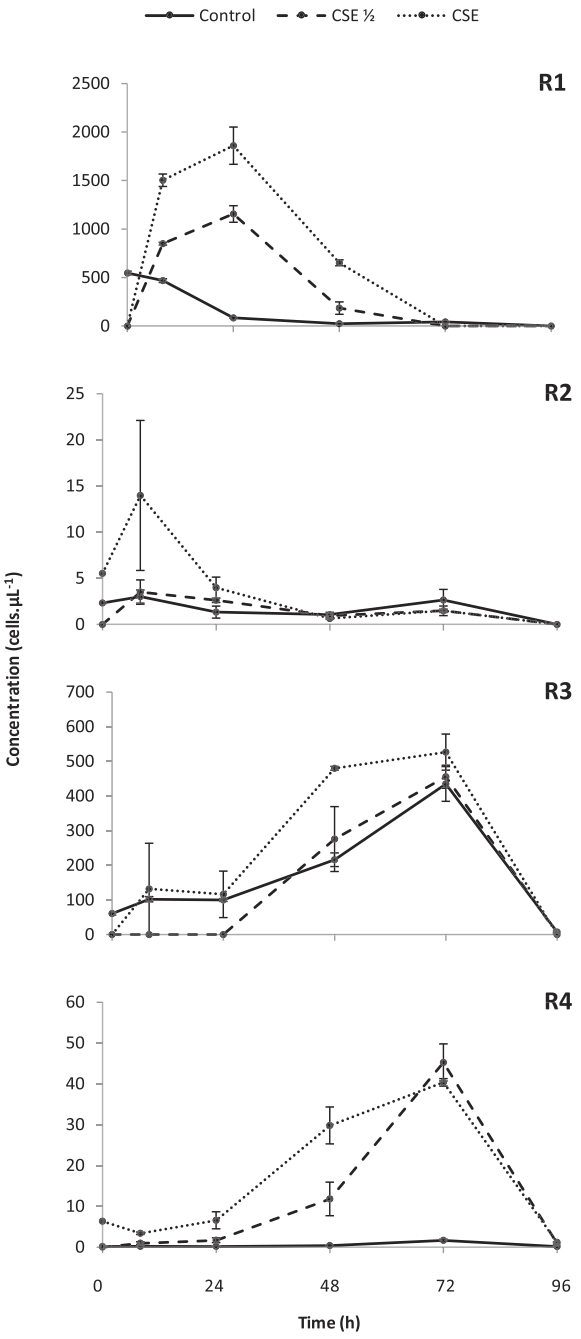

Fig. 6. Dynamics of the four main clusters identified by flow cytometry (R1: phycocyanin-rich picocyanobacteria; R2: *Synechococcus*-like cyanobacteria; R3: unidentified picoplankton group; R4: eukaryotic nanophytoplankton) in the different microcosms (Control, CSE½ and CSE; mean ± SE; in cells μL^{-1}).

it. Sediment resuspensions could thus be beneficial to phytoplankton when brief, while detrimental when they last longer. Most of the previous ecotoxicological research conducted on algae and aquatic plants has reported decreases in the photosynthetic activity after chemical exposure (e.g., Macinnis-Ng and Ralph, 2004; Pérez et al., 2006). Such results, generally explained by damage induced by contaminants on the photosynthetic apparatus, are consistent with our observations for long-term elutriate exposure (72–96 h). The slight stimulatory effects that elutriates induced on the phytoplankton photosynthetic activity in the short term may be explained by possible deployment of defense mechanisms by phytoplankton cells to maintain high their photosynthetic performance. It has been demonstrated that microalgae can employ a variety of biochemical strategies to cope with chemical toxicity before any damage occurs (Torres et al., 2008). For example, Morelli et al. (2009) showed that after exposure to metal-contaminated elutriates, diatoms are able to synthesize phytochelatins, cysteine-rich polypeptides involved in metal chelation mechanisms (Grill et al., 1985; Cobbett, 2000), before physiological disruptions occurred. Furthermore, it cannot be excluded that the stimulation observed at short term after elutriate exposure could also result from nutrient enrichment, as together with contaminants some nutrients were released into the water column during the sediment resuspension simulating process.

In the present study, we used three complementary methods (HPLC, microscopy and flow cytometry) to detect changes in the structure of the phytoplankton communities because, as highlighted by Stachowski-Haberkorn et al. (2009), none of these methods alone can provide a full overview of structural changes likely to occur after chemical exposure.

The initial phytoplankton community, which was collected in the Biguglia lagoon, was dominated by Dinophyceae, mainly by the Dinophyceae species *P. micans*.

Elutriate exposure induced slight changes in the structure of the phytoplankton community in the short and longer terms (after 48 h and 96 h of incubation, respectively). In the short term, it appeared to slightly stimulate Bacillariophyceae, and conversely to slightly inhibit Dinophyceae. In the longer term, it appeared to slightly inhibit Bacillariophyceae and Dinophyceae while stimulating other unidentified phytoplankton groups.

Additionally, elutriate exposure seemed to stimulate phycocyanin-rich picocyanobacteria in the short term (after 8 h of incubation and up to 48 h of incubation) before inhibiting it in the longer term (after 72 h and 96 h of incubation), and to inhibit eukaryotic nanophytoplankton at short term (from incubation time and up to 48 h of incubation) before stimulating it in the longer term (after 48 h and 72 h of incubation). Similar reverse responses within the "pico-nanophytoplankton" compartment were observed after exposure of marine phytoplankton to a fungicide (Opus at 100 $\mu g\ L^{-1}$; decrease in the prasinophyte-like populations and increase in *Synechococcus* populations; Stachowski-Haberkorn et al., 2009).

5. Conclusion

In this study, we examine the impacts of contaminated-sediment resuspension on phytoplankton in a Mediterranean lagoon. We show that although contaminated-sediment resuspension induces releases of toxic chemicals into the water, it can quickly (within few hours) and strongly stimulate phytoplankton growth. In the short term, it can enhance the phytoplankton photosynthetic performance, before reducing it in the longer term. This suggests that brief sediment resuspension events could be beneficial to phytoplankton communities, while longer events could be detrimental to this important autotrophic component. The adverse effects of toxic chemicals could thus be offset by nutrients that are also released in the water during resuspension events. Additionally, we show that sediment resuspension can modify the phytoplankton community structure, particularly impacting the small phytoplankton cells. It may stimulate phycocyanin-rich picocyanobacteria in the short term before inhibiting it in the longer term, and inhibit eukaryotic nanophytoplankton in the short term before stimulating it in the longer term. Our findings therefore suggest that, in contaminated coastal environments, sediment resuspensions are likely to have significant effects on the global dynamics and functions of phytoplankton.

Acknowledgments

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