

Article

Dumping of Dredge Spoil in the Pelagic Habitat: Focus on Trophic Status, Phytoplankton Diversity Responses and Generation of Blooms

Ioanna Varkitzi ^{1,*} , Alexandra Pavlidou ¹ , Maria Pantazi ², Eleni Rousselaki ¹, Georgios-Angelos Hatiris ³ , Eirini Gratsia ², Vasilios Kapsimalis ¹  and Kalliopi Pagou ¹

¹ Institute of Oceanography, Hellenic Centre for Marine Research HCMR, P.O. Box 713, 19013 Anavyssos, Greece; aleka@hcmr.gr (A.P.); erousel@hcmr.gr (E.R.); kapsim@hcmr.gr (V.K.); popi@hcmr.gr (K.P.)

² Institute of Marine Biological Resources and Inland Waters, HCMR, 576A Vouliagmenis Ave., 16452 Argyroupoli, Greece; mpantazi@hcmr.gr (M.P.); e.gratsia@hcmr.gr (E.G.)

³ Hydrobiological Station of Rhodes, HCMR, Aquarium Square, 85131 Rhodes, Greece; gahatiris@hcmr.gr

* Correspondence: ioanna@hcmr.gr

Abstract: This study presents the impacts of dredge spoil dumping in the pelagic habitat during a 27-month monitoring survey in eastern Mediterranean coastal waters (Saronikos Gulf, Aegean Sea), with a focus on changes in trophic status and eutrophication levels, phytoplankton diversity and bloom dynamics. A number of environmental parameters and phytoplankton metrics were significantly influenced by the dumping operations, specifically phytoplankton diversity indices (number of species, Diatoms:Dinoflagellates ratio) and total abundance, Chlorophyll-a, light transmission, dissolved oxygen and inorganic nutrients, N:P ratio, and the Eutrophication Index (a metric for trophic status assessment). Phosphates started to increase after the first year of dumping operations, shifting the N:P ratio to values lower than 10. A similarity cluster analysis highlighted that the phytoplankton community structure during the pre-dumping and the early-dumping period was clearly discriminated from the period during and after the dumping operations. A clear shift with an increase in the Diatoms:Dinoflagellates ratio was observed immediately after the initiation of dumping operations, which maximized in the dumping site after two years of operations. Diatoms dominated the phytoplankton communities, reaching ~ 95% relative abundance in the dumping site. High biomass producers or potentially toxic diatom species proliferated forming blooms. *Pseudo-nitzschia multiseriis* was the most frequent potentially toxic species. A multivariate analysis (RDA) highlighted that among a suite of phytoplankton metrics plotted against stressors relevant to dumping, the Eutrophication Index, Chlorophyll-a, the diversity index Diatoms:Dinoflagellates ratio and the abundance of the potentially toxic diatom *P. multiseriis* emerged as the most suitable to reflect the responses of phytoplankton communities to dumping. Dredge spoil dumping at sea poses pressures to ecosystem components addressed by the European Marine Strategy Framework Directive (MSFD) monitoring programs. In such a context, this study further supports the role of phytoplankton diversity and blooms as sensitive monitoring elements for the environmental status assessment and dumping management in coastal waters.

Keywords: dredging operations; dumping of dredge spoil; phytoplankton diversity; harmful algal blooms HABs; eutrophication; trophic status assessment



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1. Introduction

Dredging and dumping of dredge spoil are very common practices in estuarine and coastal waters that have changed little over the past twenty years [1–3]. Over 99% of the sediment dumped at sea is locally generated from port expansion and the deepening of navigation channels [4]. Dredging is also used for purposes, such as beach nourishment or

land reclamation. In most of the northern European coastal areas, dredging and dumping are considered one of the most severe anthropogenic pressures, for instance, dumping is generally prohibited in the Baltic Sea with the exception of dredge material that has been previously processed in order to meet specific standards [5].

Dumping of dredge spoil can cause great alterations in the environment, as a consequence of the resuspension of sedimented material, and therefore, dumping operations are considered environmental pressures on the benthic and pelagic biota [6–8]. Suspended particles, released into the water column from the disposal of dredge sediments, can decrease water transparency and release nutrients and hazardous substances affecting marine organisms, e.g., inorganic nutrients, heavy metals, tributyltin (TBT) and organic contaminants [7,9,10]. The nutrients released from dumped dredge spoil may contribute to eutrophication issues, whereas increased turbidity may lead to ephemeral effects on light-dependent organisms, e.g., phytoplankton [4,11]. In fact, the impact of dredge spoil dumping on phytoplankton is considered essential for the assessment of the actual dredging effects [4], but such studies have been rather limited so far.

A reduction of 56–70% in the total phytoplankton primary production (sum of true phytoplankton and resuspended microphytobenthos) has been attributed to the deteriorated light regime in dredged estuarine ecosystems [12,13]. Heavy metals, such as manganese (Mn), nickel (Ni), copper (Cu) and lead (Pb) released from dumped dredge spoil, is considerably absorbed by phytoplankton, when bloom and dredging events coincide, and affect metal bioavailability in the water column [14]. Therefore, phytoplankton blooms are important biological sinks of heavy metals during dredging, which are prone to be transferred and biomagnified into the marine food web. Furthermore, shifts in phytoplankton community structure and the formation of Harmful Algal Blooms (HABs) have been reported in some cases [15,16], but the impacts on phytoplankton diversity remain largely unknown.

During dumping activities, increased levels of phosphorus and ammonia have been shown to cause significant disruption, resulting in decreased abundance of micro- and mesozooplankton, larvae density and fish eggs [17,18]. Dumping has been found to modify the benthic granulometry to finer-grained surface sediments and increase the sediment load in contaminants, e.g., aliphatic, polycyclic aromatic hydrocarbons and heavy metals [19–21]. A significant decline in species number and abundance of benthic macroinvertebrates almost to an azoic state was reported in locations up to 3.2 km away from a spoil-ground [20].

To the best of our knowledge, the studies reporting on the pressures of dredging and spoil dumping on phytoplankton diversity patterns and the formation of algal blooms are rather limited. In this study, we present the impacts of dredge spoil dumping operations on the pelagic habitat, with a focus on: (i) how the trophic status and eutrophication levels are affected, (ii) if and how the phytoplankton diversity responds, and (iii) what kind of HABs are generated during monitoring surveys for over 2 years (27 months) in a coastal area of eastern Mediterranean Sea (Central Aegean Sea, Saronikos Gulf). A set of environmental parameters (light transmission, dissolved inorganic nutrients, dissolved oxygen, temperature, salinity) and phytoplankton metrics (Chlorophyll-a, total abundance, phytoplankton diversity indices and bloom dynamics) were regularly monitored before, during and after the dumping operations. Special attention was given to the species that can form Harmful Algal Blooms (HABs) because of their role in coastal areas and our scarce knowledge of them in dumping sites. Through a multidimensional perspective, the present study attempts to designate the main drivers and stressors that affect the trophic status and the phytoplankton dynamics in the perturbed environment of a marine dumping area.

2. Materials and Methods

2.1. Study Area and Sampling

The study area is a shallow marine coastal area (max depth 77 m) in the Saronikos Gulf (Eastern Mediterranean, Greece), located in the vicinity of the waterfront of the Athens metropolitan area (Figure 1). The site received dumped sediments from large-scale

dredging operations in the lower course of the Kifissos river and its estuary. Along the main course of the river, artificial stone embankments are placed for most of its length and a motorway has been constructed over it, forming a covered canal of 9 km before the river mouth [21]. After dredging, the cross-section of the lower river reach was enlarged, increasing water discharges up to 1400 m³/s, in order to manage the flushing episodes during intense rainstorms.

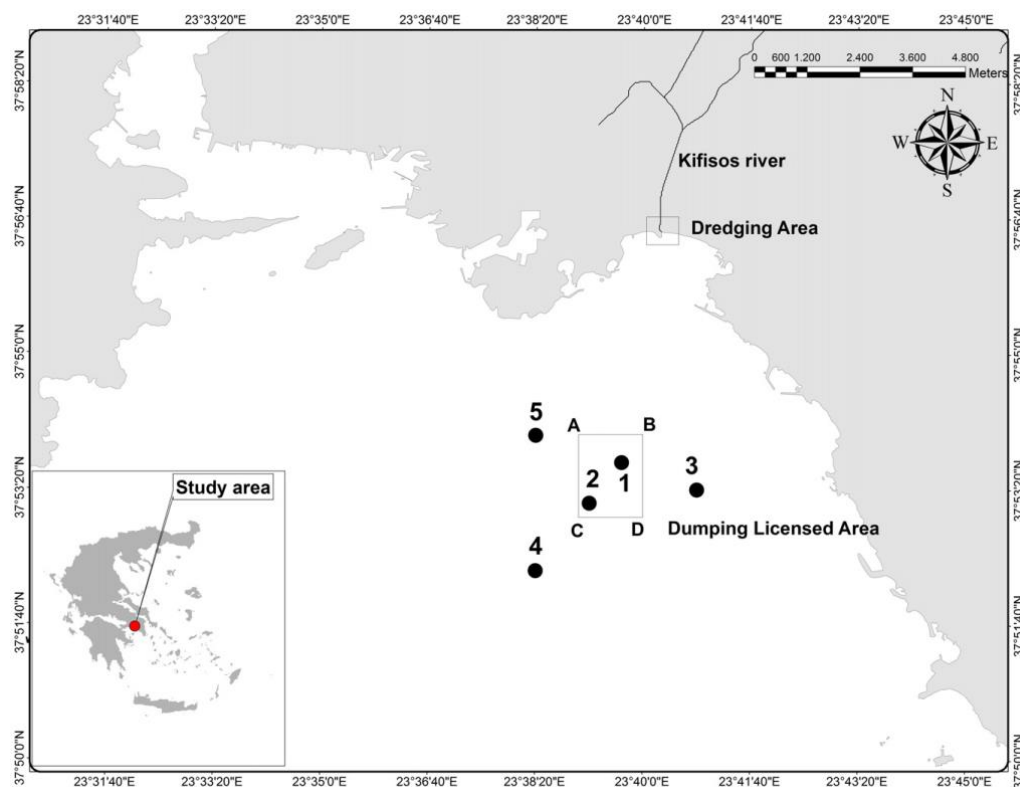


Figure 1. Map of the study area with the licensed area for the dumping of dredge spoil (rectangle ABCD). The sampling stations with numbers 1 to 5 represent stations DSS1 to DSS5, which were located within the dumping site (DSS1 and DSS2) and in the nearby site (DSS3, DSS4 and DSS5).

The dredging of the Kifissos estuary resulted in the production of significant amounts of sediments, which were licensed to be dumped further seaward in a designated coastal sea area of 1 nmi² surface (Figure 1). The sediments from the area around the river mouth were mostly fine-grained (with muddy sand, sandy mud and mud) and a total of ~700.000 m³ of dredged material were dumped with a mean monthly discharge of 33.333 m³ [20]. The dumping operations lasted from May 2010 to December 2011.

The coastal area of the licensed dumping site and the adjacent area were monitored before, during and post dumping for 27 months (April 2010 to June 2012). The area was also studied systematically during the previous years, thus the baseline status of the area was well known and totally coincided with the findings of the sampling in April 2010, just before the beginning of the dumping operations (e.g., [22–24]). A network of five stations (DSS1 to DSS5; Table 1) was monitored monthly during that period with the HCMR R/Vs ALCYONE and AEGAEON. Two stations (DSS1, DSS2) were located at the sediment disposal area (rectangle ABCD in Figure 1) and three stations (DSS3, DSS4 and DSS5) in the adjacent area.

2.2. Analyses of Environmental and Phytoplankton Parameters

Physical parameters of the water column (temperature T_{ITS90} in deg °C, salinity S and light transmission LT in %) were sampled monthly (with minor exceptions). Their vertical profiles at each station were recorded with SeaBird Electronics Inc. profilers (Models 19

and 9), with a sampling rate of 2 scans s^{-1} and descent rate $\sim 0.5 \text{ m s}^{-1}$. LT was measured with a Chelsea-Seatech Transmissometer attached on the SBE profilers and emitting at 660 nm; from LT value at any depth, the beam attenuation coefficient (BAC in 1 m^{-1}) was calculated using the formula: $BAC = -\ln(LT [\text{decimal}]) \times z^{-1}$, where z is the instrument's path length in m (0.250 m). Given that any increase in the amount of suspended matter in the water column causes a decrease in LT and consequently an increase in BAC , these two parameters can be used to trace the plume resulting from dumping operations.

Table 1. Sampling stations with coordinates and depths during the monitoring survey.

Sampling Stations	Latitude (N)	Longitude (E)	Depth (m)	Site
DSS1	37°53'40"	23°39'40"	66	Dumping site
DSS2	37°53'10"	23°39'10"	69	Dumping site
DSS3	37°53'20"	23°40'50"	50	Nearby site
DSS4	37°52'20"	23°38'20"	77	Nearby site
DSS5	37°54'00"	23°38'20"	71	Nearby site

All seawater samples were collected with oceanographic bottles monthly. For dissolved nutrient analyses, seawater samples of 100 mL were collected in polyethylene bottles, aged with 10% HCl. All the analyses were performed at the certified by EN ISO/IEC 17025:2005 (366-2) biogeochemical laboratories of HCMR, using standard methods. Nitrate, nitrite and silicate concentrations in seawater were analyzed with a SEAL nutrient autoanalyzer III [25–27]. Ammonium and phosphates in seawater were analyzed with a UV-VIS Perkin Elmer 20 Lambda spectrophotometer, [28] for ammonium and [26] for phosphate. The analyses of Dissolved Oxygen (DO) were performed on board immediately after sampling according to the Winkler method, modified by [29]. For the assessment of the trophic status and the ecological quality in coastal waters, the Eutrophication Index (EI) by [30] was computed, on the basis of nitrates, nitrites, ammonia, phosphates and Chlorophyll-a concentrations, and the extrapolated five-step quality scale was used.

The phytoplankton community structure and biomass (Chlorophyll-a as a biomass proxy) were studied in the area at depths close to the surface (2 m below) and the seafloor (2 m above). Seawater samples of 1500 mL were collected monthly for Chlorophyll-a analysis with oceanographic bottles. The samples were filtered immediately on board through GF/F filters according to the method of [31]. Seawater samples were also collected every three months for the analysis of phytoplankton diversity (species identification and abundance) with light inverted microscopy [32].

2.3. Statistical Analysis

A set of phytoplankton indices, i.e., number of species (S), Shannon H' diversity, Pielou J' evenness, etc., were computed with PRIMER software. The Bray–Curtis similarity index was also computed with PRIMER, using the group average method and $\log + 1$ transformation of data. Non-parametrical analyses, such as hierarchical agglomerative clustering or Cluster analysis, multidimensional scaling analysis, or MDS and SIMPER analysis were performed with PRIMER, on the basis of the Bray–Curtis similarity matrix according to [33,34].

In order to test the responses of phytoplankton and environmental parameters to dumping pressure, multiparametric statistical routines were performed with the R language and environment for statistical computing (R Core Team 2021). One-way ANOVA was applied using biological and environmental parameters as dependent variables and dumping period, pressure and site as independent variables. In the cases where normality and homogeneity could not be succeeded after transformation, the Kruskal–Wallis test was applied instead. Post-hoc tests of Fisher's LSD or Dunnett's T3 were used according to variance homogeneity test results. Normality was tested by means of Shapiro–Wilk test

and transformations (Ln) were applied where needed. Homogeneity of variance was tested using Levene's test. Statistical significance was set to $p < 0.05$.

Redundancy Analysis (RDA) was performed in order to visualize the relationship between phytoplankton and environmental parameters [35]. Redundancy Analysis (RDA) is a direct extension of regression analysis to model multivariate response data, combining regression with principal component analysis [36]. Before implementing RDA, variables not dimensionally homogeneous were centered on their means and standardized. Additionally, the distribution of each variable was examined, and where necessary, transformations were applied in order to linearize the relationships and reduce the effects of outliers. Variance inflation factor (VIF) was used in order to measure the extent of multicollinearity among the explanatory variables [37]. Finally, it was checked that the system was not overdetermined (i.e., the number of explanatory variables was less than the number of sites in the data matrix). The abundances of the most frequent species of *Pseudo-nitzschia* were pooled to the genus level in order to reduce the number of zeros and were log-transformed.

3. Results

The distributions of environmental parameters are presented in Figure 2a–j. Temperature and light transmission showed maximal levels in summer and minimal in winter, whereas salinity presented narrow margins between 37.8 and 39.1 (Figure 2a–c). Dissolved Oxygen (DO) concentrations were increasing in cold months and decreasing in warm months, as expected (Figure 2d). However, the lowest DO levels were observed at the dumping site (September 2011). Oxygen saturation was also estimated in order to examine if organic matter decomposition affected the DO in the water column. Oxygen saturation levels were usually higher than 80%, except for the dumping site (72.7% at DSS2, June 2011) and the nearby site (73.5% at DSS4 in September 2011) during the warm period.

Nutrient concentrations showed temporal and spatial variations (Figure 2e–j). Phosphates (PO_4) ranged below $0.15 \mu\text{M}$, but they started to increase after one year of dumping operations. Consequently, the N:P ratio decreased to less than 10 after the first year of dumping. Silicates (SiO_4) and nitrates (NO_3) were peaking mostly during the warm months (June to September) with maximum values at 3.78 and $1.11 \mu\text{M}$, respectively. The Si:N ratio followed a similar distribution. Ammonium (NH_4) did not surpass $1.00 \mu\text{M}$, while it was decreasing during the warm months.

The distributions of Chlorophyll-a (Chl-a) and phytoplankton abundance during the dumping monitoring survey are presented in Figure 3a–c. Chl-a peaked during late winter–spring (February–April) at all sites and phytoplankton abundance showed a similar distribution pattern. The Chl-a peak was shaped in the surface layer after the first year of dumping operations ($1.181 \mu\text{g L}^{-1}$ in March–April 2011), whereas after two years of dumping operations the Chl-a peak was shaped near the seabed ($0.967 \mu\text{g L}^{-1}$ in February 2012).

The Diatoms:Dinoflagellates ratio was increased after the initiation of dumping operations thanks to the increase in diatoms, and maximal levels were reached in the dumping site after two years of dumping (Figure 3d). Lower ratio levels in spring 2011 were due to higher dinoflagellate numbers. The potentially harmful algal bloom species, consisting of high biomass producers or potentially toxic phytoplankton (*Pseudo-nitzschia multiseriata*, *Chaeroceros affinis*, *Alexandrium* spp.), were present throughout the monitoring period (Figure 3e). They peaked significantly during the dumping operations in spring, shaping the distribution of the phytoplankton total abundance. The Eutrophication Index (EI as a metric for trophic status assessment) appeared to be driven by the Chl-a distribution mostly (Figure 3f). However, in autumn months, EI was driven mostly by the nutrients' availability, e.g., ammonium at all sites (October–November 2010) and silicates, nitrates and phosphates at the dumping site (September 2011). EI designated an overall moderate to good trophic status in the study area (0.28–0.41 range of EI average values for the whole study area per sampling event).

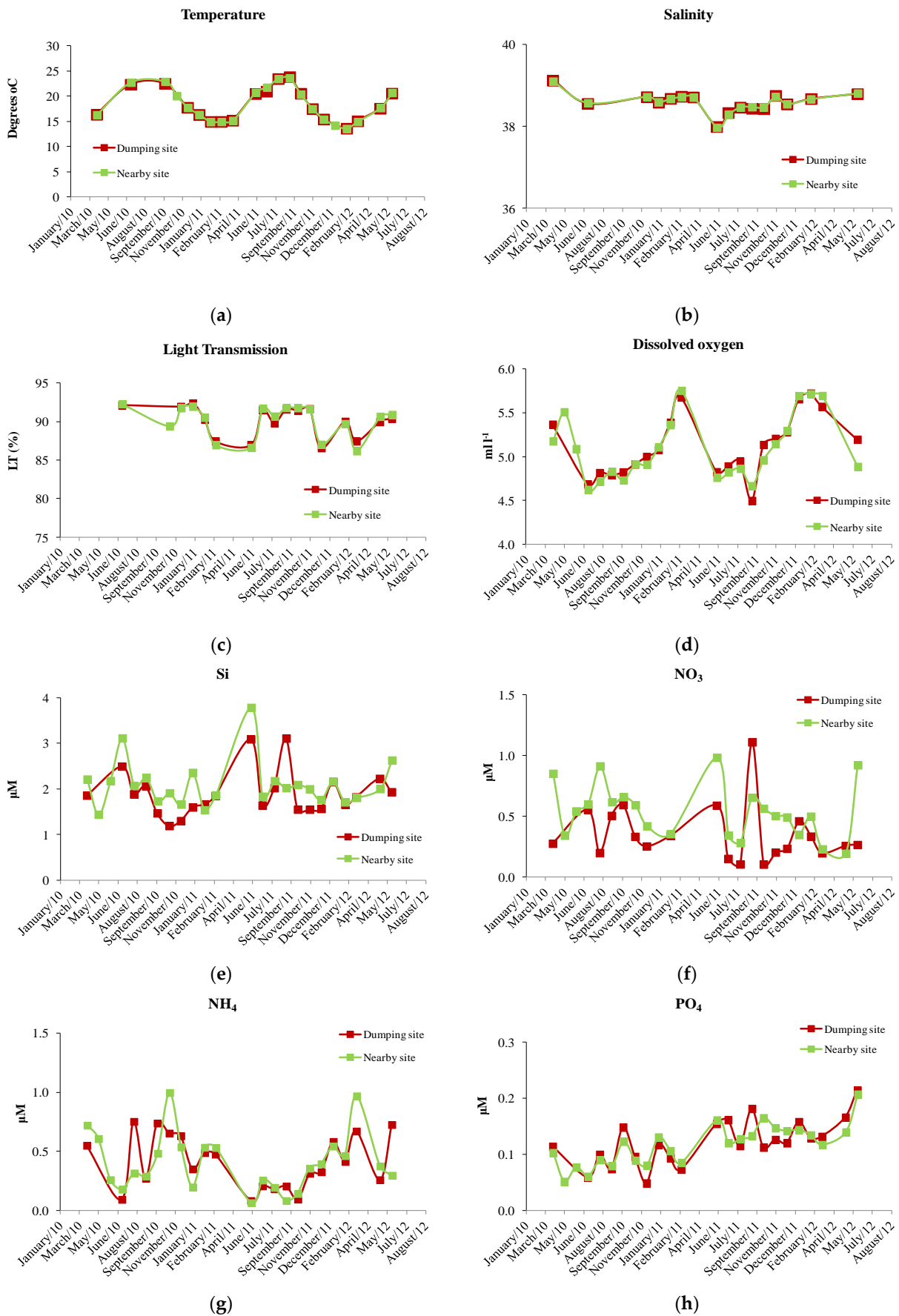


Figure 2. Cont.

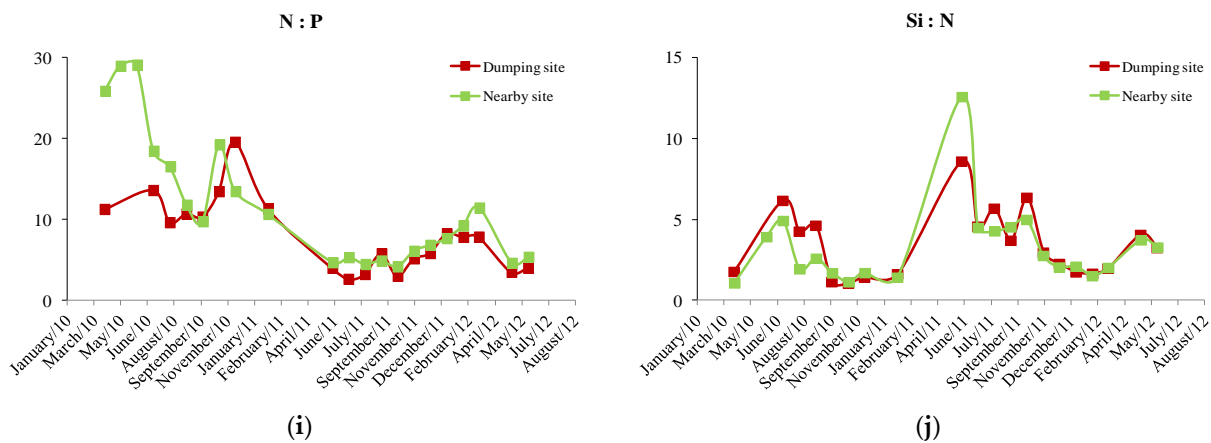


Figure 2. Distribution of temperature (a), salinity (b), light transmission (c), dissolved oxygen (d), silicates (e), nitrates (f), ammonium (g), phosphates (h), N:P ratio (i) and Si:N ratio (j) during the monitoring survey for the dredge spoil dumping in coastal waters ('Dumping site' is DSS1, DSS2 and 'Nearby site' is DSS3, DSS4, DSS5). Values are averaged from surface and bottom.

A more detailed composition of the first five most dominant species, their abundances and their relative contributions (%) to the whole phytoplankton community during the monitoring survey are presented in Supplementary Table S1. Diatoms were by far the dominant phytoplankton group in most cases, reaching relative abundances of almost 95% (October 2010 at the dumping site). Diatoms of the genera *Pseudo-nitzschia*, *Leptocylindrus*, *Chaetoceros*, *Skeletonema*, *Thalassiosira* and *Lauderia* were among the most dominant taxa. Actually, the highest Chl-a and total abundance values in spring 2011 were attributed to a bloom of the potentially toxic diatom *Pseudo-nitzschia multiseries* which was distributed throughout the study area, ranging from 69.500 to 184.200 cells/L. Nanoflagellates were the second most dominant phytoplankton group. Another peak of phytoplankton abundance (October 2010) was attributed to a bloom of nanoflagellates (198.090 cells/L) in the dumping site, whereas this was not coupled with a Chl-a peak.

In order to examine the evolution of the phytoplankton community structure over the three periods before, during and after the dumping operations, a cluster analysis was performed. In the Cluster graph (Figure 4), the phytoplankton community structure shaped one cluster for the periods of pre-dumping and early-dumping from April to October 2010, which was clearly separated from the rest. During and after the dumping operations, some of the autumn and winter months formed one cluster, whereas the spring and summer months formed two other separate clusters.

The Pearson's correlation coefficient, as well as the Spearman's correlation coefficient (for the cases where normality was not achieved: Dissolved Oxygen, Temperature, Light transmission and Salinity), were calculated (at a 0.05 significance level) in order to examine the associations among the physicochemical and the biological parameters over the whole study area (Figure 5). The Shannon diversity index H' and the Eutrophication index EI were mostly correlated with nitrogen, Diatoms:Dinoflagellates ratio with N:P ratio, potentially harmful species abundance with Si:N ratio, relative abundance of *Pseudo-nitzschia* with light transparency, Chl-a and total phytoplankton abundance with dissolved oxygen.

In order to have a deeper look into some representative parameters for the pelagic environment and phytoplankton communities, we tested statistically (one way ANOVA) if they were affected by the dumping activity (dumping site and nearby site) and/or the sampling period (before, during and after the dumping operations). The results of these comparisons are presented in Supplementary Table S2. Most environmental and phytoplankton parameters were significantly influenced by the dumping operations ($p < 0.05$), showing differences between the dumping pressure and/or the dumping period, e.g., Chl-a, total phytoplankton abundance, number of phytoplankton species, Diatoms:Dinoflagellates ratio, nitrates, phosphates, N:P ratio, silicates, the EI index, etc.

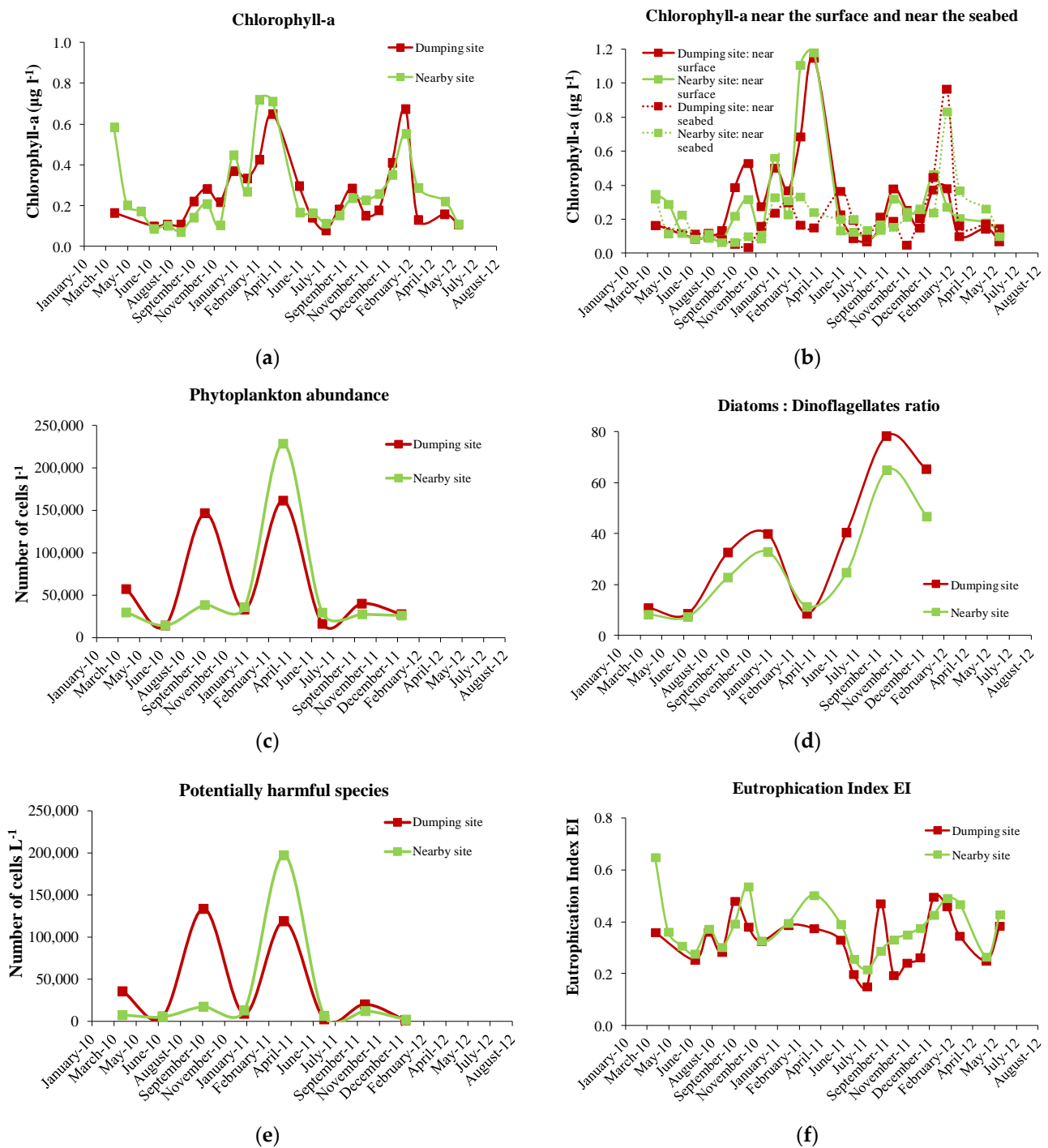


Figure 3. Distribution of phytoplankton parameters during the monitoring survey for the dredge spoil dumping in coastal waters ('Dumping site' is DSS1, DSS2 and 'Nearby site' is DSS3, DSS4, –DSS5): Chlorophyll-a (a), Chlorophyll-a near the surface and near the seabed (b), phytoplankton abundance (c), Diatoms:Dinoflagellates ratio (d), potentially harmful species (e) and Eutrophication index EI (f). Values are averaged from surface and bottom.

A deeper insight into the combined effect of the most significant environmental factors on the variation of phytoplankton community structure and diversity was attempted through the multivariate Redundancy Analysis (RDA) method (Table 2, Figure 6). Based on the Variation Inflation Factor (VIF), correlated predictors were removed, thus the environmental factors included in the model were light transmission, temperature, salinity, dissolved oxygen, dissolved nutrients (NO_3 , NO_2 , NH_4 , PO_4 , SiO_4), N:P and Si:N ratios as

explanatory variables. All values of VIF were below 10, indicating that multicollinearity will not influence the model [38]. From the available suite of nine phytoplankton parameters (Chl-a, total abundance, abundance of HAB species, relative abundance of HAB species, *Pseudo-nitzschia* multiseries abundance, and the phytoplankton diversity indices: Number of species S' , Shannon H' , Pielou J' Diatoms:Dinoflagellates ratio) and the EI, the model which better assessed the effect of the explanatory matrix on the response matrix was the one including as response variables the EI, the Diatoms:Dinoflagellates ratio, Chl-a and *P. multiseries* abundance. *Pseudo-nitzschia* was selected as the most frequent and abundant phytoplankton taxon, also among the potentially harmful species.

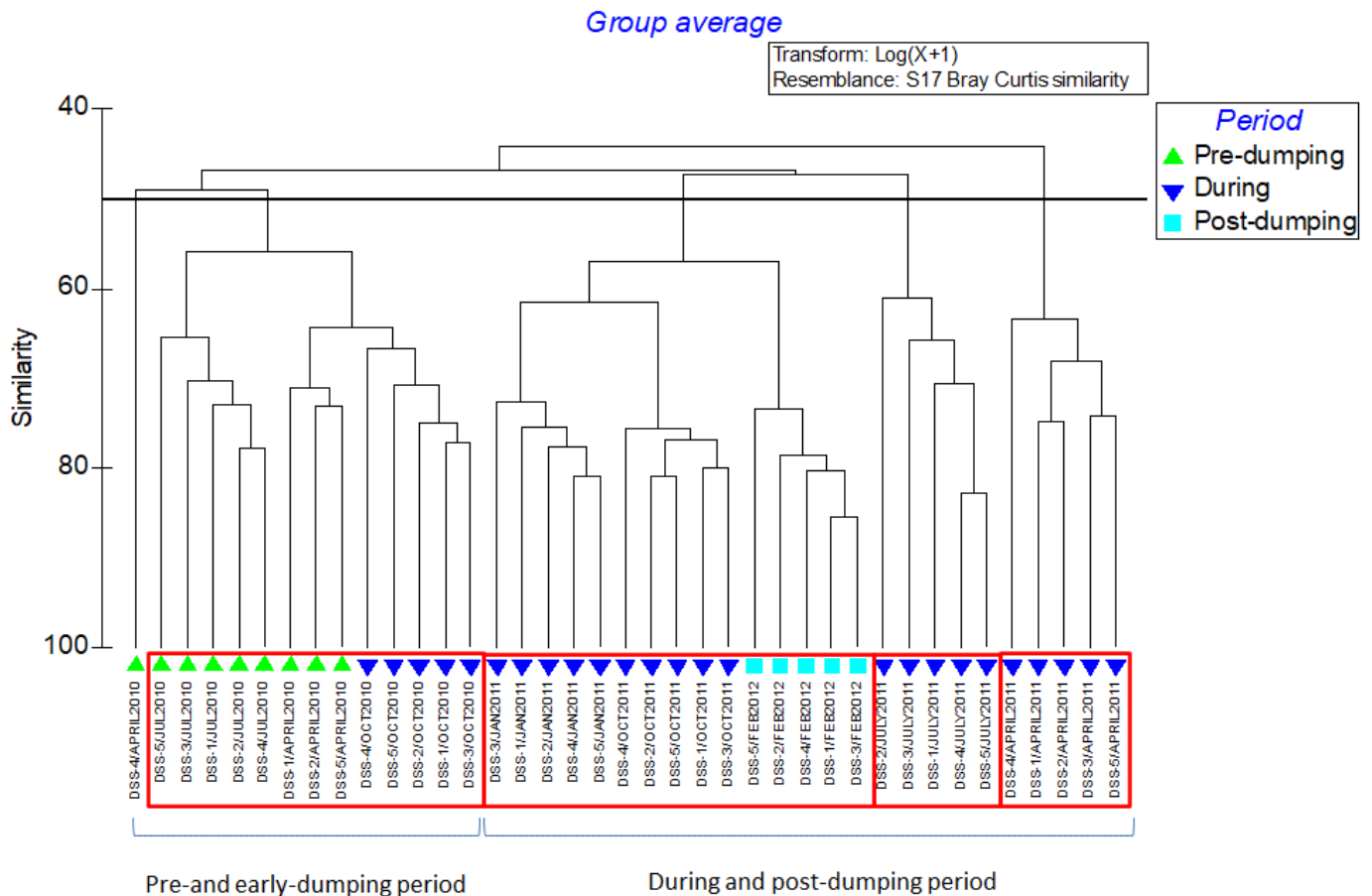


Figure 4. Cluster analysis of the phytoplankton community structure (data of species abundance, cells L^{-1}) in the sampling stations based on the different months of samplings. Data were log ($x + 1$) transformed. The similarity levels (% on the y-axis) are presented among the sampling stations (on the x-axis). The dashed line indicates the 50% similarity level above which the similarity level is considered statistically important ($p < 0.05$).

In Figure 6 the first and second axes (RDA1 and RDA2) represented 75.55% of the total variation in the multivariate system. On the first axis, the response variables were explained primarily by the Eutrophication Index (EI) and secondarily by Chl-a. On the second axis, the response variables were explained primarily by the Diatoms:Dinoflagellates ratio and secondarily by *P. multiseries* abundance (Table 2). The most influential environmental variables on the first axis were the NH_4 , Si:N ratio, NO_3 , N:P ratio, salinity and light transmission, together with phosphates (PO_4), silicates (SiO_4), salinity and dissolved oxygen on the second axis (Table 2). Light Transmission ($p = 0.001$), Salinity ($p = 0.001$), Dissolved Oxygen ($p = 0.007$), PO_4 ($p = 0.001$), NO_3 ($p = 0.001$) and NH_4 ($p = 0.005$) were found to be statistically significant explanatory variables (at 0.05 or 0.01 or 0.001 significance levels). The model was statistically significant ($p = 0.001$) and the first two RDA axes were

also found to be statistically significant: RDA1 ($p = 0.001$) and RDA2 ($p = 0.013$) at either 0.001 or 0.05 significance levels.

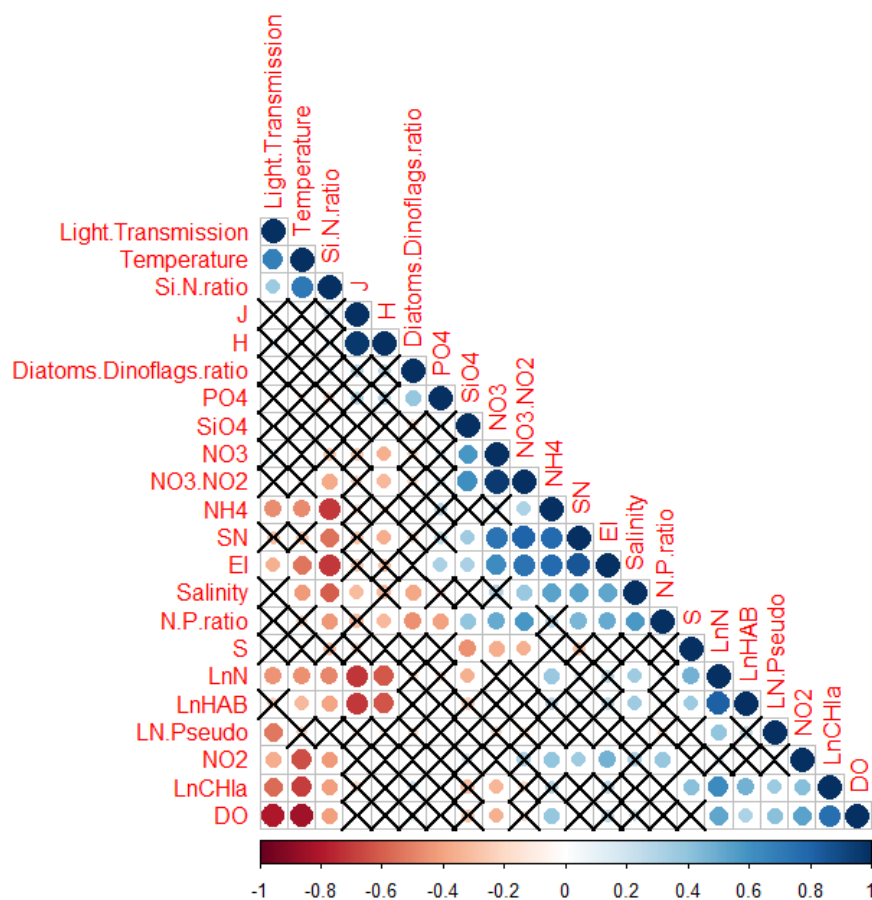


Figure 5. Corplot of Pearson and Spearman correlation analysis for the associations between the physicochemical and the biological parameters over the whole study area (J Pielou index, H Shannon index, SN total phytoplankton abundance, S total number of phytoplankton species, LnHAB abundance of potential harmful species, such as Ln, LnPseudo abundance of *Pseudo-nitzschia multiseriis* as Ln, DO dissolved oxygen). Only significant correlations (at 0.05 or 0.01 significance levels) are presented in color.

Table 2. Scores of explanatory and response variables for the first two axes of the Redundancy Analysis (RDA). Statistically significant explanatory variables are indicated in bold characters.

Response Variables	RDA1	RDA2	Explanatory Variables	RDA1	RDA2
EI	2.055	−0.03134	Ammonium NH ₄ (μM)	0.8155	0.030799
			Nitrates NO ₃ (μM)	0.6931	0.050349
Chorophyll-a (Ln)	0.07744	−0.04749	Salinity	0.5360	0.478432
			Light Transmission	−0.4017	−0.045965
Diatoms: Dinoflagellates ratio (Ln)	−0.04951	− 1.321	Phosphates PO ₄ (μM)	0.3772	− 0.690978
			Dissolved Oxygen (mL/L)	0.2722	−0.102520
			N:P ratio	0.5506	0.601707
Abundance of <i>Pseudo-nitzschia multiseriis</i> (Ln)	0.03745	0.07160	Si:N ratio	− 0.7384	−0.003835
			Nitrites NO ₂ (μM)	0.4863	0.025692
			Silicates SiO ₄ (μM)	0.4836	0.068162

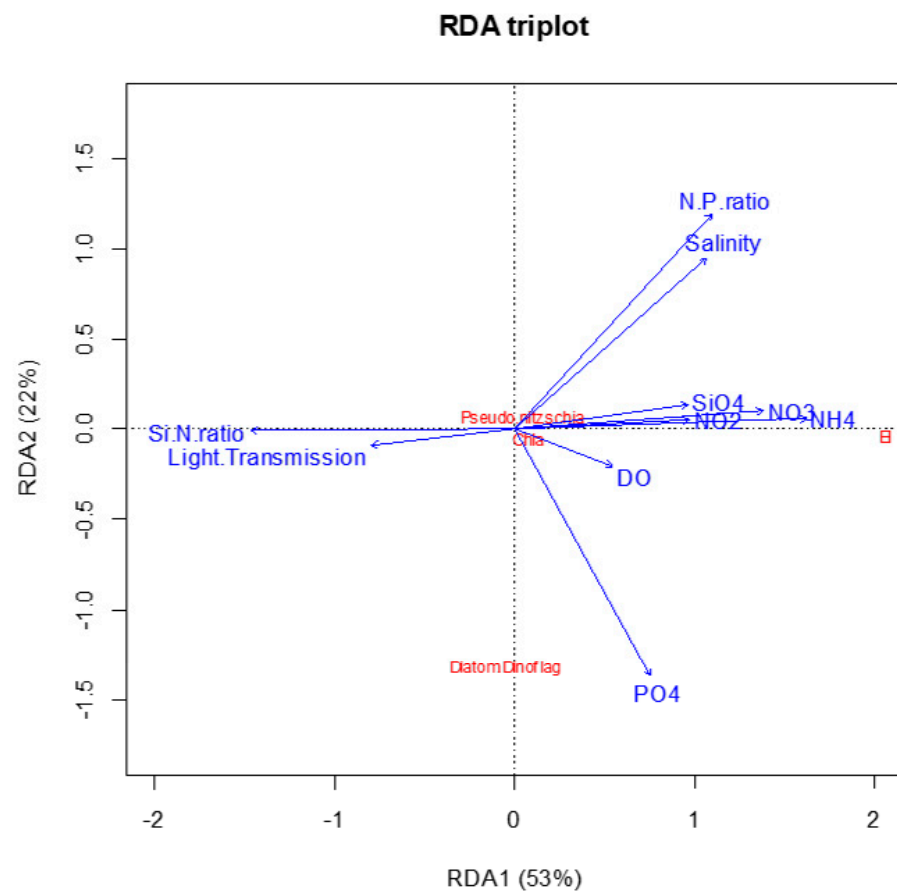


Figure 6. Triplot of Redundancy Analysis (RDA).

The RDA triplot (Figure 6) is based on Type II scaling since the correlative relationships between variables were of interest, focusing on response variables [39]. The angles between all vectors on the RDA triplot reflect their linear correlation. The approximated correlation between two variables is equal to the cosine of the angle between the corresponding vectors. The placement of the environmental variables indicates their loading on the two displayed axes, whereas the location of the response variables indicates how strongly a response variable is associated with an environmental variable. Regarding the first axis (RDA1), it can be observed that the explanatory variables NH_4 , Si:N, NO_3 , salinity and N:P present a heavy loading on it. Accordingly, the response variable Eutrophication Index indicates a strong positive association with NH_4 and NO_3 , and a strong negative association with Si:N, with respect to RDA1. Concerning the second axis (RDA2), the explanatory variables PO_4 , N:P and salinity pose a heavy loading on it, whereas the response variable Diatoms:Dinoflagellates ratio, indicates a strong negative association with N:P and salinity and a positive association with PO_4 , with respect to RDA2.

4. Discussion

Although a substantial amount of research has been conducted, some effects of the disposal of dredge sediments are rather poorly studied, e.g., the impact on phytoplankton which is considered essential for the monitoring of dredge spoil dumping [4]. The resuspension of sediments and the subsequently increased turbidity is expected to enhance the light attenuation; this factor might not be critical in hindering phytoplankton productivity in dumping areas according to [40]. However, the persistent turbidity plume has been found to reduce phytoplankton productivity in large-scale continuous extraction activity [41].

Sediment resuspension is known to be an important source of phosphate and nitrogen in shallow estuarine systems [13,40,42]. In the present study, phosphates started to increase after one year of dumping, dragging the N:P ratio to levels below 10, whereas the lowest

DO concentrations and saturation levels were observed near the seabed in the dumping site. Furthermore, a number of environmental and phytoplankton related parameters were significantly influenced by the dumping operations. The inorganic nitrogen and phosphorus, N:P ratio, silicate, light transmission and DO, Chl-a, total phytoplankton abundances and the number of species differentiated significantly over the gradient of dumping pressure and/or dumping period, according to the ANOVA outcomes. The multiparametric Eutrophication Index (EI) also showed sensitivity to the gradient of dumping pressure in the area.

In the Bahía Blanca estuary, Argentina, dumping operations since the early 1990s have been enriching the water column with suspended sediments [43,44]. Turbidity, dissolved nitrite, nitrate and phosphate concentrations have shown increasing trends there. The combined effect of the reduced river runoff and the input of new nitrogen and phosphate into the Bahía Blanca estuary have rendered the resuspension of nutrients from bottom sediments an important source for phytoplankton growth, especially during the low river flow period [45]. According to our Pearson and Spearman correlation analysis, several phytoplankton indicators, such as the Shannon diversity index H' , Diatoms:Dinoflagellates ratio, potentially harmful species abundance and the Eutrophication Index EI, were correlated mostly with nitrogen or its ratios (N:P, Si:N).

In previous studies from estuarine systems, dredging had no impact on the seasonal variability of phytoplankton biomass, reported as Chl-a values [14,46–48]. It is suggested that this was probably related to the fact that turbidity and light attenuation did not reach limiting levels for phytoplankton growth throughout the sampling period [47]. In the present study, phytoplankton total abundance and Chl-a (as a proxy of phytoplankton biomass) demonstrated the expected late winter/early spring surface peaks in the whole area. However, after two years of dumping operations, the Chl-a maxima were shaped near the seabed rather than near the surface. Chl-a and total phytoplankton abundance were mostly correlated with DO, whereas *Pseudo-nitzschia* relative abundance was correlated with light transparency (Spearman correlation). Overflows of high-concentration disposed sediments are known to produce turbid plumes at the water surface which can later be dispersed over several tens of kilometers under the combined conditions of oceanic and atmospheric forcings (e.g., currents, tide, winds) [49].

During the pre-dumping and the early-dumping period in the present study, the phytoplankton community structure was clearly discriminated from the period during and after the dumping operations, according to the similarity cluster analysis. According to the multivariate analysis findings (RDA), the most representative component of the phytoplankton communities against stressors relevant to dumping was the Diatoms:Dinoflagellates ratio, which was mostly associated with phosphates, N:P ratio and salinity. A significant increase in the Diatoms:Dinoflagellates ratio emerged during the second year of dumping operations due to the proliferation of diatoms. Diatoms are known to outcompete other phytoplankton groups by growing fast and forming blooms when nitrogen and silicon become available [50–52]. This ecological advantage may lie in their siliceous cell walls, which require less energy to synthesize compared to the organic cell walls of dinoflagellates [53]. Furthermore, their high affinity for nitrates and the formation of nutrient storage vacuoles make diatoms more competitive under pulsed nutrient supply regimes [54,55]. High biomass blooms of diatoms have been found to proliferate for short periods even in the open oligotrophic waters of Greece (Aegean Sea, Eastern Mediterranean), when conditions are favorable [56].

Diatoms clearly dominated the phytoplankton communities reaching approximately 95% of relative abundance in the studied dumping site (*Pseudo-nitzschia*, *Leptocylindrus*, *Chaetoceros*, *Skeletonema*, *Thalassiosira* and *Lauderia* among the most dominant taxa). These high diatom numbers were probably favored by the release of silicon and nitrogen from the dredge spoil, whereas they maximized the Diatoms:Dinoflagellates ratio after two years of dumping operations. A clear shift in phytoplankton community composition towards the dominance of the small diatom *Thalassiosira minima* at >80% of the total

phytoplankton abundance in summer has been documented in the Bahía Blanca Estuary, Argentina [57]. The diatoms *Actinocyclus normanii*, *Chaetoceros affinis*, *Skeletonema costatum*, *Thalassiosira* spp. have also been found to comprise a substantial part of the plankton diatom communities in the brackish waters of the Klaipėda Strait under extensive dredge spoil dumping activities [58]. The release of resting stages into the water column is considered a common mechanism for the establishment of diatom blooms [59,60]. For example, the resuspension of resting spores of the *Chaetoceros* genus has been suggested as a potential trigger factor for winter diatom blooms in the dredged Bahía Blanca Estuary [61]. On coasts with rapid shifts in sedimentation patterns, dumping can also impact surf zone diatoms [62] or increase benthic to pelagic diatom ratios [63].

Our knowledge of species that form Harmful Algal Blooms (HABs) at dumping sites is rather scarce so far. A previous study reported an extensive HAB event with *Prymnesium parvum* (Prymnesiophyceae) resulting in hypoxic conditions and fish kills in the Zandvlei Estuary (South Africa) that is subjected to dredging activities [16]. In the present study, high biomass producers or potentially toxic species of phytoplankton were present throughout the sampling period and peaked significantly during the dumping operations in spring. In many cases, there was just one species exceeding 50% of the total phytoplankton abundance even in the summer months. *Pseudo-nitzschia multiseries* were the most frequent potentially toxic species present in all sites and throughout the sampling period. Furthermore, the abundance of *P. multiseries* emerged as the second most representative component of the phytoplankton community structure against the dumping stressors, according to the multivariate analysis (RDA). A bloom of *P. multiseries* (up to ~185,000 cells/L) generated a peak of Chl-a and total abundance after one year of dumping. The dominance of *P. multiseries* and other potentially toxic *Pseudo-nitzschia* species has been documented also in other perturbed coastal areas of Greece, such as the Maliakos Gulf and Thermaikos Gulf [64–66]. In addition to the competitive advantages of diatoms described above, *P. multiseries* is known to have also a ferritin that facilitates blooming after iron inputs even in nutrient-limited oceanic regions, linking iron uptake to substantial metabolic reactions, such as photosynthesis, nitrate assimilation, the urea cycle and carbohydrate synthesis [67,68].

Other frequent species mostly in the dumping site were the potentially harmful diatoms *Chaetoceros affinis* and *Leptocylindrus minimus*. The potentially toxic dinoflagellates of the genus *Alexandrium* were also present at low concentrations at the dumping and some nearby stations. In a dumping area of Izmir Bay in Turkey, dredge sediments' dumping operations were considered to favor the growth and reproduction of the red tide flagellates *Heterosigma* cf. *akashwo* and *Gymnodinium* cf. *mikimotoi* [69]. In our study, an autumn bloom of nanoflagellates (~200,000 cells/L in October 2010) occurred a month after the dumping operations started, but this was not followed by a Chl-a increase. This might be attributed to the heterotrophic character of some nanoflagellates that could enable them to temporarily proliferate on the organic matter released by the disposed of sediments.

Sediment resuspension at dumping sites is known to release significant organic matter amounts in seawater [70], which suggests a potentially important alternative carbon and nutrient pool for phytoplankton communities with heterotrophic and mixotrophic capabilities, such as nanoflagellates and numerous HAB species [71,72]. Model results from a dredged estuary further support this linkage by indicating a relationship between elevated total phosphorus availability and the increased abundance of Dinophyceae and Prymnesiophyceae [16]. Organic resources released by dredging have been suggested to favor also the proliferation of heterotrophic prokaryotes and the dominance of larger photosynthetic picoeukaryotes (≥ 2.5) over the small photosynthetic prokaryotes (≤ 1 μm) [15] thanks to their mixotrophic potential [73]. The expansion of toxin-producing cyanobacteria blooms caused by the transport of contaminated inland waters to estuarine and coastal marine waters, is also attracting high interest [74]. Among the toxins produced by cyanobacteria (cyanotoxins), microcystins are the most common with effects on the pelagic habitats and

phytoplankton [75]. In this sense, dredge spoil dumping can also cause this particular environmental problem, which needs further studies.

The Diatoms:Dinoflagellates ratio index was the most important parameter to explain the responses of phytoplankton diversity against the dumping pressures in the present study (multivariate RDA analysis). Previous research has shown that phytoplankton diversity indices are sensitive to pressures and currently under investigation for reliable environmental assessments in the framework of the European Marine Strategy Framework Directive implementation (MSFD, 2017/848/EC), although more effort is recommended for testing on a wide spatial scale to cover wider gradients of natural and anthropogenic pressures [76–79]. In a recent study, a set of eight diversity indices was tested against different anthropogenic pressure levels within a common data set of phytoplankton communities (structure and abundance) from the Adriatic, Ionian and Aegean Seas [80]. Most of the tested diversity indices were able to distinguish between the highest level of impact and the rest of the impact categories. These indices maintained the distinction between two levels of subsequently dichotomized impacts (no impact to low impact vs. high impact) across latitudinal and longitudinal gradients.

There is some proof till now about the remediation of dumping sites in coastal areas with high resilience thanks to the coastline morphology and hydrological conditions. Such a case is a dumping site in Suape Bay, Brazil, where Chl-a and dissolved inorganic nutrients did not show significant changes in their patterns. Some authors suggested that the environment was influenced by strong hydrodynamism and greater penetration of coastal waters after the opening of some reefs, causing greater water renewal, and therefore, lower disturbance by the dumping process [6]. In any case, apart from dredging and dumping of spoils, many other human activities co-occur in coastal areas, such as aquaculture, offshore oil and gas facilities, wind, wave and tidal installments [81]. In this respect, phytoplankton is considered an important ecosystem component for marine monitoring and coastal management, due to the increased probability of overlap between high productivity patches and maritime developments in coastal waters [82].

5. Conclusions

In this study, we tried to elucidate the impacts of dredge spoil dumping operations on the pelagic habitat and the phytoplankton responses in a case study from Eastern Mediterranean coastal waters. Phytoplankton diversity metrics and the trophic status reflected the dumping pressure. The generation of blooms was favored in this perturbed environment, supporting the dominance of potentially toxic diatoms. Phytoplankton is, therefore, suggested as a sensitive tool for the monitoring and management of dredging operations. Follow-up data would be quite informative to collect over an extended period of time in order to determine any long-term effects of dumping pressure.

The implementation of the MSFD in European marine waters is expected to influence anthropogenic activities, such as dredging for navigation and new constructions (harbors, breakwaters, bridges, tunnels and wind farms), land reclamation and coastal protection, sediment management and sand mining, the laying of cables and pipelines, etc. In this context, dredging and sediment disposal at sea are posing pressures against a variety of ecosystem components addressed by the MSFD monitoring programs, such as sea-floor integrity [23] and plankton diversity [75], with phytoplankton as a key biological element for the monitoring of the marine environment.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14152343/s1>, Table S1: Five most dominant phytoplankton species, their abundances (cells 10^3 L^{-1}) and their relative contribution (%) to the total phytoplankton community (in descending order) in the dumping site and the nearby site. In bold characters, the relative contributions above 50%; Table S2: One-way ANOVA with the biological and physicochemical parameters (as dependant variables) which presented significant differences (values in bold with statistical significance at $p < 0.05$) versus the dumping period and the site (as independent variables).

Only parameters with significant differences are presented (Chl-a, N total phytoplankton abundance, S number of phytoplankton species).

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