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ORIGINAL RESEARCH ARTICLE

Reflectance spectra classification for the rapid assessment of water ecological quality in Mediterranean ports

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Summary Ports are open systems with direct connection to the sea, therefore any potential impact on port waters may have implications for the health of adjacent marine ecosystems. European WFD addressed ports in the category of Heavily Modified Water Bodies (HMWBs) and promoted implementation of protocols to monitor and improve their ecological status. TRIX index, which incorporates the main variables involved in the trophism of marine ecosystems (Nitrogen, Phosphorus, Chlorophyll *a*, Dissolved Oxygen), is widely utilized in European coastal areas to evaluate trophic status. The relationships between the variables involved in TRIX computation, particularly Chlorophyll *a* concentration, and water spectral reflectance provides an alternative method to evaluate the quality and ecological status of the port water. Hyper-spectral (380—710 nm) water reflectance data were recorded by a portable radiometric system in five ports from the Western and Eastern Mediterranean Basin. The spectral distance between samples was measured by two metrics using both the original and reduced spectra and was implemented within a hierarchical clustering algorithm. The four spectral classes that emerged from this operation were statistically analysed versus standard water quality descriptors and phytoplankton community features to evaluate the ecological significance of the information

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obtained. The results indicated a substantial coherence of different indicators with more than 60% of the total TRIX variability is accounted for by the proposed classification of reflectance spectra. This classification is therefore proposed as a promising Rapid Assessment Technique of ports water ecological quality, which can serve as an effective monitoring tool for sustainable management of ports.

1. Introduction

Over the last century, Mediterranean ports have rapidly developed through the increasing establishment of commercial and tourism-related maritime networks. At the same time, the development of maritime activities is generating a large environmental impact that cannot always be controlled, nor even timely assessed. Ports are open systems with direct connection to the sea, therefore any potential impact on port waters may have implications for the health of adjacent marine ecosystems.

The European Community has built a system of Framework Directives and Policies, such as the Water Framework Directive 2000/60/EC (WFD), Marine Strategy Framework Directive 2008/56/EC (MSFD) and Integrated Maritime Policy IMP COM (2013) 133, encouraging the implementation of standards and protocols to monitor and improve the ecological status of the marine environment by promoting sustainable procedures. Ports are addressed by the WFD in the category of Heavily Modified Water Bodies (HMWBs) being the result of physical alterations by human activity and not meeting a "good ecological status" (GES). However, only a few ports are currently managing their maritime activities in an environmentally benign way and there is a lack of harmonization regarding port environmental criteria among the Mediterranean neighbouring countries. Even though water quality management experiments are under investigation in some European ports (Ondiviela et al., 2012) and in spite of an urgent necessity, the development of fast, reliable and common monitoring methods, widely known as Rapid Assessment Techniques (RATs), is still required.

Among them, the TRIX index, which incorporates the main variables (Nitrogen, Phosphorus, Chlorophyll *a*, Dissolved Oxygen) involved in the trophic processes of marine ecosystems, was proposed as a synthetic indicator of coastal seawater trophic status (Vollenweider et al., 1998). TRIX was adopted by the Italian legislation (D. Lgs 152/99 — Annex 1) to classify the coastal waters in four classes. TRIX was then revised to meet the requirements of WFD (Pettine et al., 2007) and was applied to other Mediterranean, Atlantic and Northern Sea coastal water bodies (Cabrita et al., 2015; Primpas and Karydis, 2011, and references therein) and it was recommended as a synthetic metric for a common eutrophication monitoring strategy in Mediterranean countries (UNEP, 2007).

Nevertheless, the acquisition of all the variables involved in TRIX requires *in situ* measurements, collection of samples and laboratory analyses, which are time-consuming and expensive, especially if a long term and spatially complete monitoring scheme is required. Among these variables, Chlorophyll *a* concentration (Chl-*a*), as a universally accepted indicator of phytoplankton biomass, is the most adequate biological parameter to evaluate the trophic status of aquatic ecosystems and the risk of anomalous blooms, particularly in coastal waters (Boyer et al., 2009; Van de Bund and Poikane, 2015) and it can be therefore accepted as a suitable indicator of overall water quality (Caroppo et al., 2013; Uusitalo et al., 2016).

Chl-*a* is one of the main Light Attenuating Substances (LAS: phytoplankton estimated as Chl-*a*, Non-Algal Particles (NAP) of which in most cases the Total Suspended Matter (TSM) is a proxy, and the Chromophoric Dissolved Organic Matter - CDOM) whose presence affects the spectral reflectance of both coastal and open sea waters. The dependence of seawater spectral reflectance on optically active organic and inorganic LAS, both dissolved and suspended, was highlighted and early established by Morel and Prieur (1977). These authors proposed a classification of marine waters based on the characteristics of reflectance spectra, introducing the differences between Case 1 and Case 2 waters. In Case 2 waters, which are dominant in coastal and port areas, the variability of LAS proportions leads to a strong heterogeneity in optical features, which makes the characterization of these waters dependent on the knowledge of local specific conditions (Eloranta, 1978; IOCCG, 2000; Kaczmarek and Wozniak, 1995; Vertucci and Likens, 1989).

The estimation of the main ecological characteristics of coastal and port waters by the acquisition and processing of optical data is therefore theoretically justified, however operationally complicated (Behrenfeld and Boss, 2006; IOCCG, 2000; Nourisson et al., 2013, 2016; Ouillon and Petrenko, 2005; Reinart et al., 2003). Several hierarchical supervised and unsupervised classification methods were implemented to discriminate and classify reflectance spectra based on differences in shape and magnitude (Eleveld et al., 2017; Gonzalez Vilas et al., 2011; Moore et al., 2014; Palacios et al., 2012; Shen et al., 2015; Shi et al., 2014; Spyrakos et al., 2018; Vantrepotte et al., 2012). These techniques have produced good results even in optically complex waters. In particular, Eleveld et al. (2017) developed an algorithm to define optical lacustrine water types through a cluster analysis of *in situ* hyperspectral reflectance spectra.

The present work aims at applying a similar approach to overcome the above-mentioned problems related to the assessment of port seawater quality by means of standard methods including the TRIX index. In particular, a hierarchical classification of hyperspectral reflectance spectra acquired in the waters of five Mediterranean ports was performed taking into account the use of different spectral metrics. This spectral classification was then analysed in relation to water ecological quality obtained using the TRIX index and other descriptors such as Chl-*a*, nutrient concentrations, phytoplankton composition and LAS. A protocol based on the measurement of reflectance spectra is thus proposed as an alternative and/or complementary to existing techniques for the rapid assessment of water ecological quality in Mediterranean ports.

2. Material and methods

2.1. Study areas and sampling stations

The present work was carried out within the MAPMED project (Chatzinikolaou et al., 2018; MAPMED, 2015) with the aim of developing standardized monitoring protocols for Mediterranean port environments. During this project, several of the biological and environmental variables most frequently used for water quality assessment were measured, to evaluate water quality in Mediterranean ports. The ports surveyed were: Port of Cagliari (Sardinia, Italy) (C), Port of El Kantaoui (Sousse, Tunisia) (E), Port of Heraklion (Crete, Greece) (H), Porto Marina (Alexandria, Egypt) (M) and Port of Viareggio (Tuscany, Italy) (V) (Table 1, Fig. 1; MAPMED, 2015). The last port was surveyed after the project end. The choice of these ports can be considered representative of most Mediterranean ports, where various activities of different impact occur.

The port of Cagliari is both a touristic and commercial port. The port of El Kantaoui hosts mainly tourism activities and medium-small leisure boats. The port of Heraklion is similar to that of Cagliari as for activities but smaller in size. Porto Marina is an artificial marina for leisure activities; the port of Viareggio is both a tourism port and yacht maintenance and construction port (Fig. 1; Table 1; MAPMED, 2015). In both ports of Viareggio and Cagliari freshwater inputs from inland water bodies can affect the trophic and ecological conditions. The port of Viareggio is crossed by the Burlamacca Channel, receiving the eutrophic waters from the Massaciuccoli Lake and the inland network of the channel which may transfer farming and urban wastewaters (Autorità di Bacino del fiume Serchio, 2010; Cabassi et al., 2017; Lastrucci et al., 2017). The port of Cagliari receives water from the brackish Molentargius Pond complex in the eastern side and from the Santa Gilla canal in the north-western side (Fumanti and Cavacini, 2002).

Seasonal samplings were carried out in the ports of Cagliari, El Kantaoui and Heraklion in winter (February—March 2012), spring — beginning of tourism season (May—June 2012), and summer — end of tourism season (September 2012). In Porto Marina, only one sampling took place in winter (December 2013) and in the port of Viareggio one sampling was performed in summer (July 2014).

In each port, the sampling stations were identified according to their spatial arrangement and uses (Table 1). In the following text the stations are referred to with an alphanumeric code: the first number (on the left) refers to the

Table 1 Coordinates and details of the sampling stations in the five Mediterranean ports of the study.

Stations	Latitude	Longitude	Water depth	Details on use
Cagliari (Sardinia, Italy)				
C1	39°2′2.58″N	29°7′24.12″E	7.8 m	Leisure boats
C2	39°12′20.46″N	29°1′27.55″E	4.5 m	Intermediate station
C3	39°12′27.12″N	29°0′20.95″E	8.3 m	Passenger ships
C4	39°12′25.98″N	29°0′9.43″E	13.5 m	Cargo ships
C5	39°11′52.94″N		11.4 m	Port entrance
El Kantaoui (Sousse, Tunisia)				
E1	35°53′38.64″N	10°35′53.16″E	2.5 m	Leisure boats
E2	35°53′34.44″N	10°35′58.92″E	4.0 m	Intermediate station
E3	35°53′34.65″N	10°36′4.44″E	3.2 m	Port entrance
Heraklion (Crete, Greece)				
H1	35°20′36.32″N	25°8′9.93″E	3.7 m	Leisure boats
H2	35°20′37.56″N	25°8′27.54″E	11.3 m	Intermediate station
H3	35°20′44.70″N	25°8′40.87″E	19.5 m	Passenger ships
H4	35°20′42.70″N	25°8′52.28″E	10.5 m	Cargo ships
H5	35°20′48.72″N	25°9′7.94″E	19.0 m	Shipyard
H7	35°20′50.82″N	25°9′17.88″E	7.0 m	Port entrance
Porto Marina (Alexandria, Egypt)				
M1	30°49′23.48″N	29°2′2.58″E	2 m	Internal station
M2	30°49′33.02″N	29°1′27.55″E	2.5 m	Port entrance
M3	30°49′40.52″N	29°0′20.95″E	5 m	Leisure boats
M4	30°49′37.06″N	29°0′9.43″E	4.5 m	Leisure boats
Viareggio (Tuscany, Italy)				
V3	43°51′51.40″N	10°14′45.90″E	5.2 m	Intermediate dock
V6	43°51′40.70″N	10°14′16.40″E	3.8 m	Outer dock
V7	43°51′34.10″N	10°14′1.20″E	5 m	Outside the port
V9	43°52′1.40″N	10°15′24.70″E	2.2 m	Inner dock in the channel

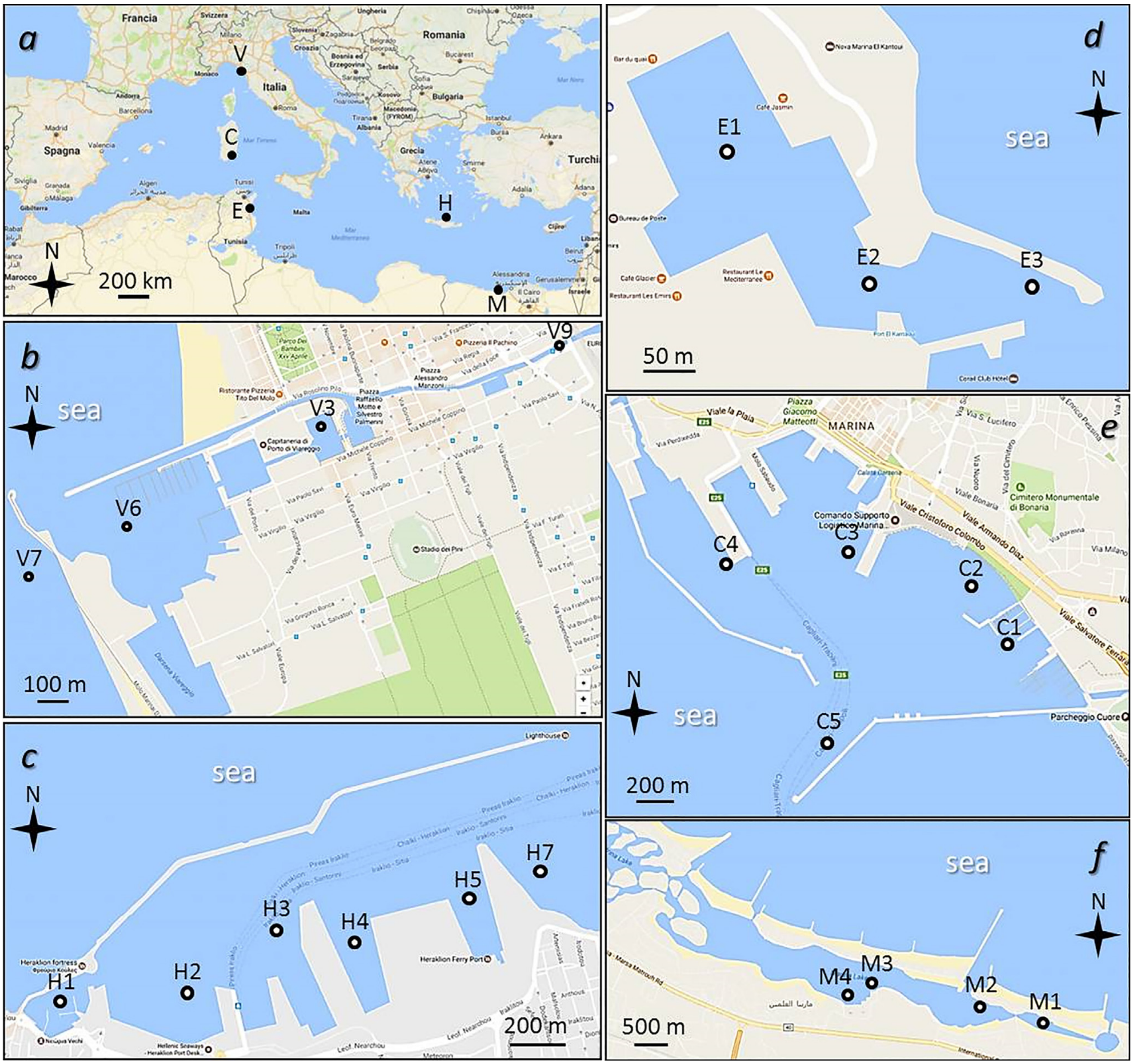

Figure 1 Location of the five study sites in the Mediterranean Sea (a). Sampling stations are indicated for each port (further details on location and uses are reported in Table 1). Port of Viareggio (V), Tuscany, Italy (b); Port of Heraklion (H), Crete, Greece (c); Port of El Kantaoui (E), Sousse, Tunisia (d); Port of Cagliari (C), Sardinia, Italy (e); Port of Porto Marina (M), Alexandria, Egypt (f). *Source*: © Google Earth v. 7.1.2.2041.

sampling campaign (1 — February—March, 2 — May—June, 3 — September, 4 — December, 5 — July); the letters refer to the port: C — Cagliari, E — El Kantaoui, H — Heraklion, M — Porto Marina, V — Viareggio; the numbers to the right refer to the sampling stations (Table 1 and Fig. 1).

2.2. In situ measurements

At each station (Table 1; Fig. 1), seawater temperature, salinity, pH, Dissolved Oxygen concentration (DO) and percent of saturation (DO%) were measured from aboard of a small boat using a WTW 3420 multi-meter at Cagliari, El Kantaoui, Heraklion and Porto Marina, while the same parameters were measured with a multi-probe Hydrolab Idroprobe at Viareggio. Seawater optical properties, such as hyperspectral downwelling and upwelling irradiance (E_d, E_u; 380—710 nm; 1 nm interval) were measured just above the water surface and at 30 cm depth.

The optical measurements were also taken from aboard a small boat by a portable radiometric system (PUMS, Portable Underwater Mini-Spectroradiometer), composed of a diode-array spectrometer (AvaSpec-2048, Avantes), with a 350—900 nm grating and 50 or 600 μm optical fibres with SMA connections, that allowed the cosine collector (CC-UV/VIS) to be extended away from the boat and a battery pack as detailed in Nourisson et al. (2016). Irradiance spectra were visualized and acquired using the software AvaSoft 6.1. Light measurements were taken in port areas free from the presence of shading ships or other structures, around noon, with calm waters and reduced cloudiness conditions, to avoid strong fluctuations of the underwater radiant flux (Kirk, 2011). The PUMS was always used on the side of the boat facing the sun, to minimize shade effects by the boat. Irradiance Reflectance spectra (R) were calculated as the ratio between E_u and E_d measurements at 30 cm depth.

Irradiance reflectance is not the first choice optical property to be used for sea colour remote sensing applications which are preferably based on remote sensing reflectance. However, under optimal solar lighting conditions, irradiance reflectance represents a proxy, even for Case 2 waters, which can be also measured with fewer uncertainties (Loisel and Morel, 2001; Mobley, 2018).

2.3. Seawater and phytoplankton laboratory analyses

Three replicate samples of surface seawater were collected from each station with thermally insulated plastic carboys, and used for chemical analyses of inorganic nutrients, Chlorophyll *a* concentration (Chl-*a*), Total Suspended Matter (TSM) and Chromophoric Dissolved Organic Matter (CDOM). A sample of 1 L of seawater was filtered from each replicate sample using pre-combusted (300°C for 1.5 h) Whatman GF/F filters (47 mm). Both filtered water (500 mL) and filters were stored in a freezer (−20°C) and analysed for inorganic nutrients (DIN = NO_2 + NO_3 + NH_4; DIP = PO_4,) and Chl-*a*, respectively. The analyses for NO_2, NO_3 and PO_4 were performed according to Strickland and Parsons (1972); for NH_4 the Ivančić and Degobbis (1984) method was used. Chl-*a* and phaeopigments concentration were determined according to the fluorometric methods of Yentsch and Menzel (1963) and Arar and Collins (1992).

To determine TSM concentration, water volumes ranging from 500 to 1000 mL (depending on the amount of suspended substances) were filtered from each station using 47 mm GF/F pre-combusted and weighted glass-fibre filters (Whatman). The filters were preserved at −20°C for a maximum of two weeks, then they were oven-dried and weighted again, to assess TSM concentrations [mg L^{-1}] as described in Strickland and Parsons (1972), modified for salt waters (Van der Linde, 1998).

Another seawater sample of 100 mL from each station was filtered through pre-combusted 47 mm GF/F fibreglass filters (Whatman), collected in dark glass bottles, fixed with sodium-azide (NaN_3) at a final concentration of 0.0002 mg L^{-1} (Ferrari et al., 1996) and stored at 3°C for a maximum of two weeks. To calculate the CDOM absorption spectra, these samples were re-filtered through a 0.2 μm PC membrane (Nuclepore) and placed in quartz cuvettes with an optical path of 10 cm. Absorbance measurements (Shimadzu UV-2501-PC spectrophotometer) were carried out against freshly distilled water (MilliQ grade) and NaN_3 at the same concentration of the sample. The resulting values were converted in absorption values. Spectra were interpolated in the range of 380—710 nm by an exponential function and nonlinear least-squares method (Stedmon et al., 2000). The interpolated CDOM absorption values [m^{-1}] at 440 nm, $a_{\mathrm{CDOM}}(440)$, were used as an estimate of CDOM concentration.

For phytoplankton analyses, a sample of 250 mL of seawater from each station was fixed with neutralized formaldehyde (final concentration 1%) and stored in dark glass bottles. Subsamples of variable volumes were observed under an invertoscope (Zeiss IM35, ph. c., 40×) after sedimentation, following standard methods (Zingone et al., 2010). Phytoplankton abundances and composition were analysed and related to Chl-*a* for 38 samples; microscope observations are lacking for samples 3C1, 3C2, 2C5, V9 and the samples from Porto Marina.

2.4. Data processing

The trophic index TRIX (Vollenweider et al., 1998) is commonly defined by a linear combination of the logarithms of four environmental variables: Chlorophyll *a* concentration (Chl-*a*) in μg L^{-1}, Oxygen as absolute percent deviation from saturation (aDO%), total Nitrogen (N) and total Phosphorus (P), in μg L^{-1}. The TRIX index can, therefore, be calculated using the following equation:

$$\mathrm{TRIX} = (\log \mathrm{Chla}*|\mathrm{aDO}\%|*N*P)-(-1.5))/1.2. \tag{1}$$

The scale factors 1.5 and 1.2 in equation (1) are based on an extended dataset for evaluating long-term trends and spatial trophic patterns in Italian northern Adriatic (Vollenweider et al., 1998) and Tyrrhenian coastal waters (Giovanardi and Vollenweider, 2004; Penna et al., 2004). TRIX score ranges from 0 to10 covering four trophic states: 0—4 high quality and low trophic status — "High"; 4—5 good quality and moderate trophic status — "Good"; 5—6 moderate quality and high trophic status — "Moderate", and 6—10 degraded quality and very high trophic status — "Poor". In the present study, since no data for total Nitrogen (N) and total Phosphorus (P) were available, the $\mathrm{TRIX}_{(\mathrm{DIN,DIP})}$ index was used instead, where DIN and DIP data replaced total N and total P respectively. The $\mathrm{TRIX}_{(\mathrm{DIN,DIP})}$ index is one of the alternative indices recommended by Vollenweider et al. (1998).

The considered water quality descriptors (DO%, DIN, DIP), including the concentration of LAS (Chl-*a*, CDOM, TSM), phytoplankton communities and TRIX were first subjected to exploratory statistical analysis, calculating data averages and standard deviations. In addition, the correlation coefficients between the measured variables were calculated (considering together all the data collected) and their significance with the *z*-transformation and *t*-test (Sokal and Rohlf, 1995). Two levels of statistical significance/probability were utilized — significant $0.01 < P < 0.05$ (*) and highly significant, $P < 0.01$ (**).

2.5. Classification of the reflectance spectra

The simplest method to assess the information content of the available reflectance spectra on seawater composition and TRIX would be the application of supervised regression or classification techniques (Morrison, 2004). These techniques are capable of building complex multivariate predictive models, whose stability, however, is strongly dependent on the training data used, which should theoretically be representative of all possible environmental conditions examined.

This limits the generalization capacity and exportability of the techniques when the number of training samples is small in relation to the variety of environmental situations to take into consideration.

An alternative unsupervised method was therefore preferred, based on the grouping of the reflectance spectra into a reduced number of clusters, which could be informative about seawater parameters and TRIX. The grouping of the reflectance spectra was obtained by applying a hierarchical clustering method, which can efficiently reveal spectra similarities, allowing for straightforward identification of the optimum number of classes (clusters). The hierarchical clustering was carried out using Ward's (1963) minimum variance method, which is based on a classical sum-of-squares criterion, minimizing within-group dispersion at each binary fusion (Murtagh and Legendre, 2014).

Two metrics were used to compute the spectral distances among samples: the classical Euclidean (EU) and Spectral Angle (SA) distances. The EU is the most conventional measure of spectral distance and indicates the total difference between spectra. In contrast, the SA distance is based on the cosine of the angle between standardized spectra and measures the similarity in shape between the two reflectance vectors, without detecting spectral amplitude differences (Maselli et al., 2009).

In order to reduce data redundancy and make the spectra comparable to those of satellite imagery, EU and SA metrics were calculated after aggregating the original spectra, composed of 330 1-nm bands (from 380—710 nm), into spectra of 61 5-nm bands. Another trial was performed to assess the possibility of using more reduced spectra, which would permit the use of simpler data collection devices. Following Lee and Carder (2002), Lee et al. (2007, 2014) and Harvey et al. (2015), the aggregated spectra were therefore further reduced to 12 bands reproducing the spectral configuration most suitable for marine studies, which was defined for the MODIS and MERIS sensors (Table 2). The hierarchical clustering with the two spectral metrics was then applied to both aggregated spectra.

The efficiency of the cluster analyses performed was assessed by comparing the cluster averages of the examined seawater parameters to the original values. This operation was carried out by linearly regressing the cluster descriptor averages against the measured descriptor values. The percentages of total parameter variance accounted for by the cluster averages, which are equivalent to the determination coefficients (R^2) of the regressions, were used as an indicator of the method efficiency (Sokal and Rohlf, 1995).

Table 2 Wavelengths [nm] of the 12 bands selected for the applied spectral sampling, with indication of the reference sensor.

Band number	Wavelength	MODIS	MERIS
1	412	X	X
2	443	X	X
3	469	X	
4	488	X	X
5	531	X	
6	547	X	
7	555	X	X
8	645	X	
9	667	X	X
10	678	X	X
11	700		
12	710		X

3. Results

3.1. Water quality descriptors

The mean concentration of LAS and standard water quality descriptors are shown in Table 3 for each port. Viareggio had the highest values of Chl-*a*, TSM, CDOM, DIP and DIN, the lowest of DO% and, consequently, the highest value of the trophic index $TRIX_{(DIN,DIP)}$ (7.23). In Cagliari, the mean concentrations of Chl-*a*, TSM, CDOM, DIN and DIP were also high, DO% was oversaturated and TRIX was 5.44. In both ports of El Kantaoui and Heraklion, the mean Chl-*a* ranged between 0.8 and 0.9 $\mu g\ L^{-1}$, DO% between 87 and 94%, and $TRIX_{(DIN,DIP)}$ was for both ports 2.36; however, some differences in the concentrations of LAS were present: high CDOM at El Kantaoui and high TSM at Heraklion. On the other hand, the lowest mean values of Chl-*a*, CDOM, DIP and DIN, along with the lowest $TRIX_{(DIN,DIP)}$ value (0.93), were recorded at Porto Marina.

In addition, some seasonal fluctuations were observed at the ports of Cagliari, El Kantaoui and Heraklion. The most important were: (i) a strong spring increase of Chl-*a* reaching the mean value of 14.28 mg m^{-3} at Cagliari; (ii) an increase of CDOM in spring and summer up to 0.43 m^{-1} and a doubling of the average DIP and DIN concentrations in summer at El Kantaoui; (iii) the highest average concentration of TSM (4.44 mg L^{-1}) in winter at Heraklion. The maximum of phytoplankton mean cell density was recorded in the waters of Viareggio (Table 3). Spring maxima were evident in Cagliari and El Kantaoui, where the seasonal fluctuation varied from 10 in February to 10^3 cells mL^{-1} in May—June. Heraklion showed the lowest mean of 62.3 cells mL^{-1} with a maximum around 10^2 cells mL^{-1} in September and the least seasonal variation.

The statistical inter-relations between water quality descriptors were characterized by the respective correlation coefficients (Table 4). The correlations were significantly positive between $TRIX_{(DIN,DIP)}$ and Chl-*a*, CDOM, DIP and DIN. Phytoplankton abundances were well correlated with Chl-*a* ($N = 38$; $r = 0.76^{**}$). No significant correlation was observed between TSM and DO%, while CDOM was highly significantly correlated with DIP, DIN and $TRIX_{(DIN,DIP)}$.

3.2. Reflectance spectra classification

The two dendrograms obtained by applying cluster analyses with EU and SA distances to the 12 bands sampled spectra are shown in Fig. 2a and b, respectively. In both cases, the levels of dissimilarity of the samples are indicated by the height on the *y*-axis. The dendrograms obtained when using the spectra of 61 5-nm bands had lower levels of dissimilarity but a virtually identical configuration and are therefore not shown. The examination of these dendrograms led us to the identification of four clusters as optimal for the present dataset. The use of greater numbers of clusters produced null or

Table 3 Mean concentrations of water quality ecological descriptors and $TRIX_{(DIN,DIP)}$ index ± standard deviation for each port.

	Chl-*a* [mg m^{-3}]	TSM [mg L^{-1}]	CDOM [m^{-1}]	DO%	DIP [μg L^{-1}]	DIN [μg L^{-1}]	Cell density [cells mL^{-1}]	$TRIX_{(DIN,DIP)}$
Cagliari	8.66 ± 6.66	3.51 ± 1.28	0.26 ± 0.15	121.6 ± 28.6	41.28 ± 54.1	239.8 ± 216.5	988 ± 1203	5.44 ± 1.35 "Moderate"
El Kantaoui	0.90 ± 0.55	1.85 ± 0.44	0.20 ± 0.09	86.9 ± 17.1	1.75 ± 1.78	10.34 ± 10.16	531 ± 768	2.36 ± 1.48 "High"
Heraklion	0.77 ± 0.43	2.98 ± 1.40	0.06 ± 0.03	99.4 ± 3.6	0.60 ± 0.47	57.0 ± 77.70	62.3 ± 49.8	2.36 ± 0.60 "High"
Porto Marina	0.38 ± 0.03	2.54 ± 0.51	0.09 ± 0.05	98.8 ± 1.5	0.41 ± 0.06	4.33 ± 0.77	n.a.	0.93 ± 0.64 "High"
Viareggio	9.34 ± 4.02	3.57 ± 4.02	0.82 ± .65	36.0 ± 15.6	430.98 ± 413	347.4 ± 187.7	2473 ± 1433	7.23 ± 1.39 "Poor"

Table 4 Correlations between water ecological quality descriptors.

	TSM	CDOM	DO%	DIP	DIN	$TRIX_{(DIN,DIP)}$
Chl-*a*	0.59**	0.58**	0.02	0.51**	0.67**	0.80**
TSM		0.36*	−0.01	0.33*	0.38*	0.44**
CDOM			−0.59**	0.97**	0.72**	0.71**
DO%				−0.62**	−0.29	−0.31*
DIP					0.66**	0.63**
DIN						0.82**

* Significant correlation ($0.01 < P < 0.05$).
** Highly significant correlation ($P < 0.01$).

marginal increases in the information content regarding the examined seawater quality descriptors.

The four clusters obtained using the two metrics partitioned the sampled stations differently. In the case of the EU distance, the spectra were mostly grouped according to their amplitude, while the use of the SA distance produced clusters that were uniform in spectral shape. Accordingly, the two partitions accounted for the fractions of seawater quality descriptors variance differently (Fig. 3). In the case of the EU distance, quite low percentages of variance were obtained for most quality descriptors, which indicated a correspondingly low information content of the four spectral clusters. The use of SA distance increased the information content of the four clusters for all water quality descriptors with the exception of TSM, for which the low R^2 was further decreased. The increase in R^2 was particularly evident for Chl-*a* and $TRIX_{(DIN,DIP)}$, where at least 60% of the total variance was explained by the four clusters.

3.3. Main features of the spectral classes obtained

The original and normalized spectra of the four clusters identified using the SA distance are shown in Fig. 4. The normalization, which was carried out by dividing the spectra to their maximum value, removes the spectral amplitude variations that are automatically disregarded by this metric, providing an effective visualization of the internal homogeneity of the four clusters. The first reflectance spectral class SC1 contained 22 spectra prevalently coming from the stations of Heraklion (Fig. 4a and b). The main features of this class were the blue-green maxima and the widespread in their values (almost 8 times). The maxima of these spectra were at 500, 535 and 560 nm. A steep fall was evident between the maximum and 600 nm; then the reflectance spectra slightly decreased down to the minimum of about 700 nm. In this band (600—700 nm), the typical features linked with the presence of phytoplankton biomass, such as the minimum at 670 nm due to maximum red absorption and the maximum around 683 nm due to Chl-*a* fluorescence, were generally absent or just hinted in some spectra. Another important feature of the reflectance spectra of SC1 was the smooth decrease from the maximum to the blue end at 400 nm; in this spectral band the influence of CDOM absorption and even the absorption of the NAP (Non-Algal Particles) were predominant.

The second reflectance spectral class (SC2) was composed by 13 spectra (Fig. 4c and d) and the majority came from the port of El Kantaoui. These spectra had maxima shifted toward higher wavelength being mainly positioned at 565—570 nm and had a slightly lower spread of the maxima (about 6 times) with respect to SC1. In the red band, a more evident minimum at 670 nm and a maximum around 685 nm were present.

The third reflectance spectral class (SC3) was composed by four reflectance spectra (Fig. 4e and f), two from El Kantaoui and two from Cagliari, characterised by low spread of the maxima and the presence of a narrow maximum in the green at 565—570 nm and much lower values in the blue band, as compared to SC1 and SC2. In addition, the minimum at about 665 nm and the maximum at about 685 nm were clearly due to the red absorption and fluorescence emission

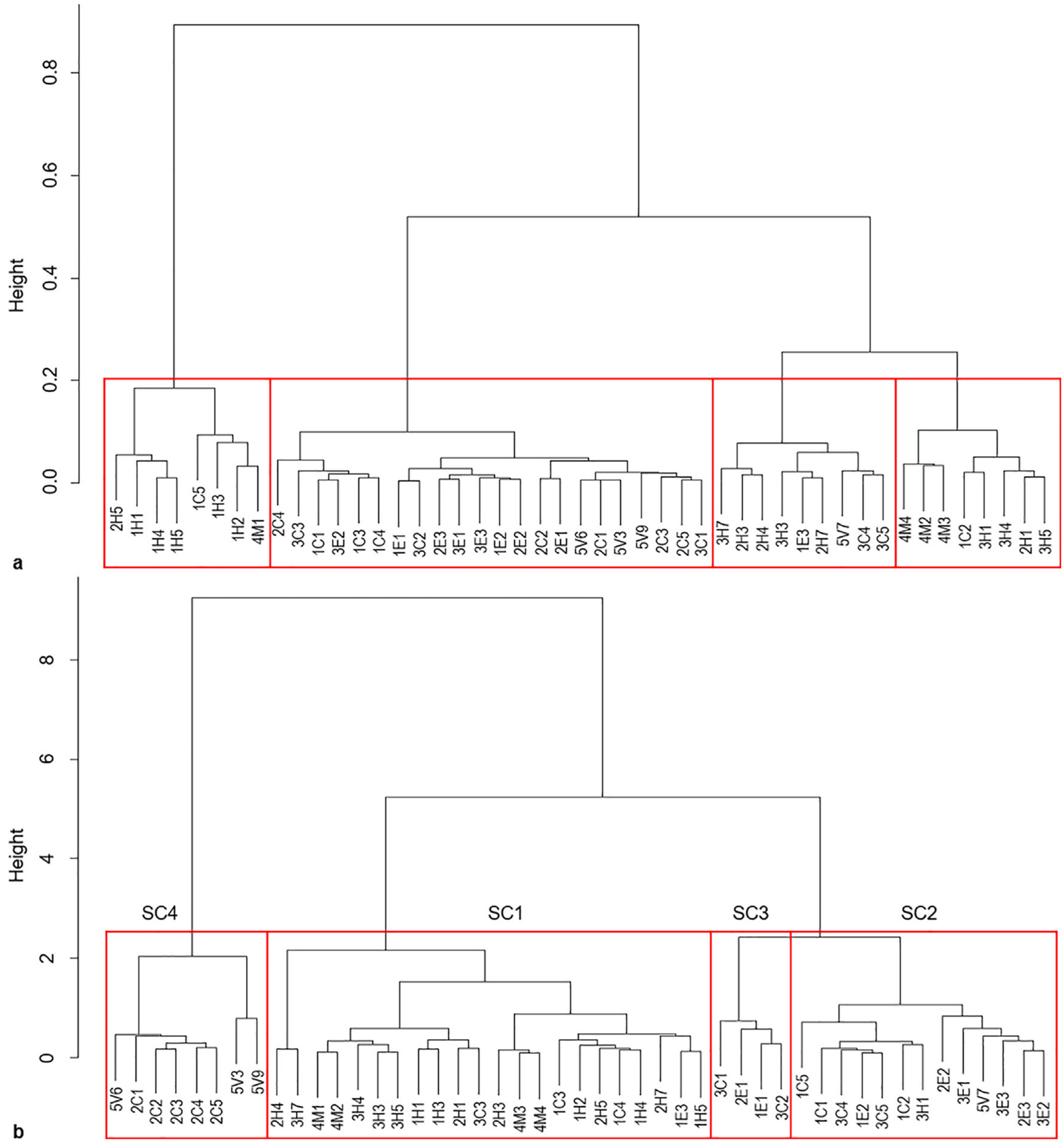

Figure 2 Dendrograms obtained by applying Euclidean (EU) (a) and Spectral Angle (SA) (b) distances to 12 bands reflectance spectra. The boxes indicate the divisions into four classes, which were identical to those obtained using 61 5-nm bands. In (b) the name of the four SA identified classes are reported. The codes refer to the samples: campaign (1—5, February—March, May—June, September, December, July); port (V — Viareggio, C — Cagliari, H — Heraklion, E — El Kantaoui, M — Porto Marina); and station inside each port (1—7).

of Chl-*a*. In optically complex waters these features are also related to the combined effect of scattering and absorption, as evidenced by Gitelson (1992) and subsequent studies (Dall'Olmo and Gitelson, 2006; Gitelson et al., 2008).

In the fourth reflectance spectral class (SC4), composed by eight spectra (five from Cagliari and three from Viareggio), the previously distinct features appeared more pronounced (Fig. 4g and h). The maximum at about 565 nm was narrower, the values in the blue were closer to zero and the minimum observed at 665 nm was sharper, as well as the structures up to 685 nm.

The main characteristics of the four identified SCs mainly depended on the concentration of LAS. In SC1, mean Chl-*a* (Fig. 5a) was 0.80 mg m^{-3}, the lowest value among the four classes analysed. In addition, this class had the lowest mean CDOM concentration (0.065 m^{-1}), while TSM concentration was 2.78 mg L^{-1} and appeared dominant in comparison to the other two LAS (Fig. 5a). In SC2, mean Chl-*a* and CDOM increased slightly up to 2.98 mg m^{-3} and 0.21 m^{-1}, respectively, while the mean concentration of TSM was about the same as in SC1 (Fig. 5a), thus LAS, being more balanced. SC3 showed a further increase in the mean Chl-*a*, which raised up

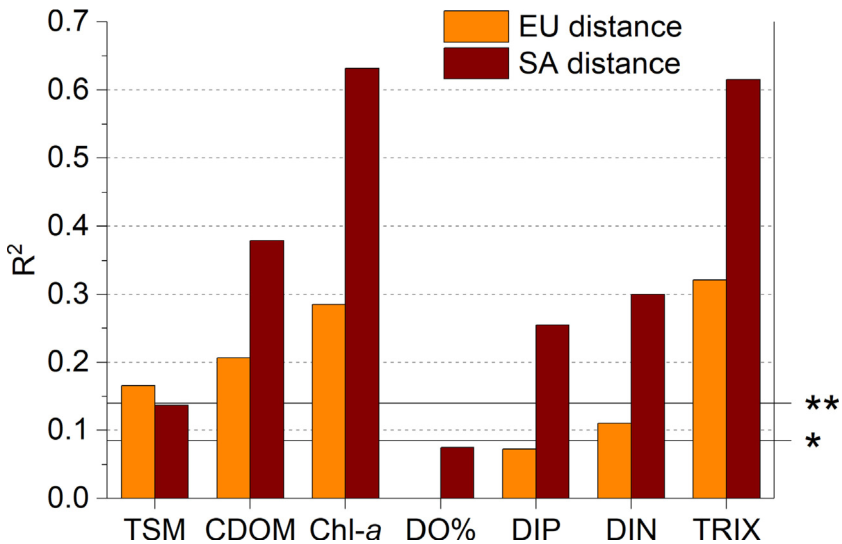

Figure 3 Fractions of seawater quality descriptor variances accounted for the four clusters identified by applying cluster analyses with EU and SA distances to the sampled spectra (Fig. 2a and b). Line with: * significant correlation level ($0.01 < P < 0.05$); ** highly significant correlation level ($P < 0.01$).

to 6.55 mg m^{-3}, a slight increase of CDOM (0.267 m^{-1}) and a concentration of TSM similar to those of SC1 and SC2 (Fig. 5a). SC4 was characterized by the highest mean values of LAS among all spectral classes. Chl-*a* reached 13.10 mg m^{-3}, CDOM 0.577 m^{-1} and TSM 3.88 mg L^{-1} (Fig. 5a).

The mean values of the standard water quality descriptors for each spectral class (Fig. 5b) clearly highlighted consistency with the proposed optical classification. Although the mean DO% remained close to 100% in all classes, the mean concentrations of DIP and DIN increased from SC1 to SC4, in which the nutrients reached the highest values. Similarly, the average $TRIX_{(DIN,DIP)}$ index increased from 2.1 in SC1 to 7 in SC4. The water quality based on $TRIX_{(DIN,DIP)}$ index hence ranged from "High" in SC1 and SC2, to "Good" in SC3 class and "Poor" in SC4.

When phytoplankton communities were grouped on the basis of the four spectral clusters identified (SC1—SC4, Fig. 2b, Fig. 4), several diversifying features were observed (Table 5). Mean phytoplankton density (Table 5) increased from a minimum in SC1, mainly consisting of the Heraklion samples, to the highest value in SC4 (Cagliari and Viareggio samples). In SC1, the total abundance was more equally distributed among the different taxonomic phytoplankton groups than in the other spectral classes and the presence of coccolithophores was almost exclusive. At the same time, a common trend could be detected from SC1 to SC3 (Table 5): an increase of diatoms dominance due to the blooms of coastal waters typical genera (*Skeletonema* spp., *Chaetoceros* spp.), followed by a reduction of all other phytoplankton groups and a decrease of diversity. In SC4, the contribution of the group "Others" was greater, incrementing taxonomic diversity, but, while in the other spectral classes this category mainly comprised Cryptophytes, Prasinophytes and other nanoflagellates, in SC4 Cyanobacteria (*Merismopedia* sp.), Chlorophytes (*Scenedemus* spp., *Monoraphidium* spp.), Euglenoids and Chrysophytes (*Ollicola vangoorii*, nanoflagellates) were the dominant taxa.

4. Discussion

The five ports included in our survey are representative of a sufficiently broad spectrum of such environments in the Mediterranean coastal areas. The tourism-linked uses of the surveyed ports suggested the implementation of seasonal samplings in relation to the tourism activity pressures, at least in three of the selected ports, Cagliari, Heraklion and El Kantaoui. The other two ports were not sampled seasonally and were included in the dataset only to facilitate comparisons. The results constitute a suitable starting basis to evaluate the proposed optical methodology for water quality assessments in HMWBs. For port areas, the literature on water quality assessment is limited. Ondiviela et al. (2012) recommended setting specific standards for HMWBs, depending on the activities that are carried out in the areas and specific water flows. The presented work is the first one implemented for Mediterranean ports which can be used to specifically take in account HMWBs.

4.1. Water quality classification based on the reflectance spectra

Although the waters of the ports considered in the present study constitute a fairly broad and representative range of environmental conditions, a limited number of bio-optical classes (four) was sufficient to properly summarize their spectral variability. Other authors, with a wider spectrum of environmental and bio-optical conditions, have resorted to a much greater number of classes to fully represent the spectral characteristics of the measured reflectance (Eleveld et al., 2017; Moore et al., 2009; Spyrakos et al., 2018).

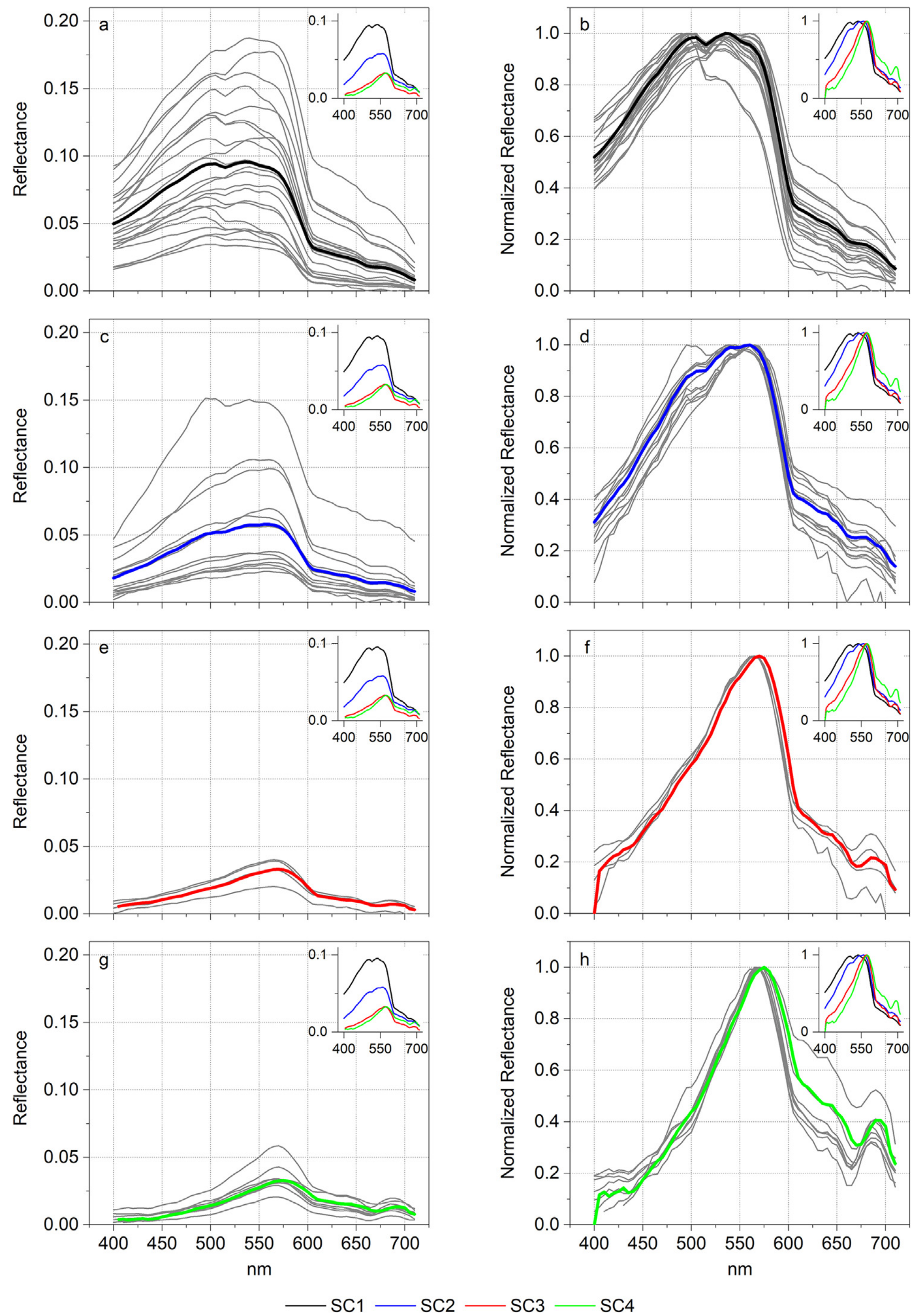

Figure 4 (a) Class 1 (SC1) SA clustering reflectance spectra; (b) SC1 maximum normalized spectra. (c) Class 2 (SC2) SA clustering reflectance spectra; (d) SC2 maximum normalized spectra. (e) Class 3 (SC3) SA clustering reflectance spectra; (f) SC3 maximum normalized spectra. (g) Class 4 (SC4) SA clustering reflectance spectra; (h) SC4 maximum normalized spectra. The mean reflectance spectra of the four SC are reported for comparison both in the upper right panel and together with the spectra of each group.

Moreover, the number of four clusters is in accordance with the four states under which the $TRIX_{(DIN,DIP)}$ index is usually interpreted (Vollenweider et al., 1998).

In Case 2 waters and in particular those of ports that optically can be placed among the most complex ones, the interpretation-classification of the reflectance spectra in terms of LAS contributions and water quality descriptors might be problematic. For this reason two approaches have been used: EU and SA metrics. The clusters obtained in this study using the EU distance were mainly related to amplitude variations of the sample spectra and were consequently poorly informative on most water quality descriptors.

Spectral amplitude variations, in fact, are mainly related to water clarity, i.e. to the concentration of LAS components whose dynamics are usually de-correlated with the other water quality descriptors in coastal waters (Vantrepotte

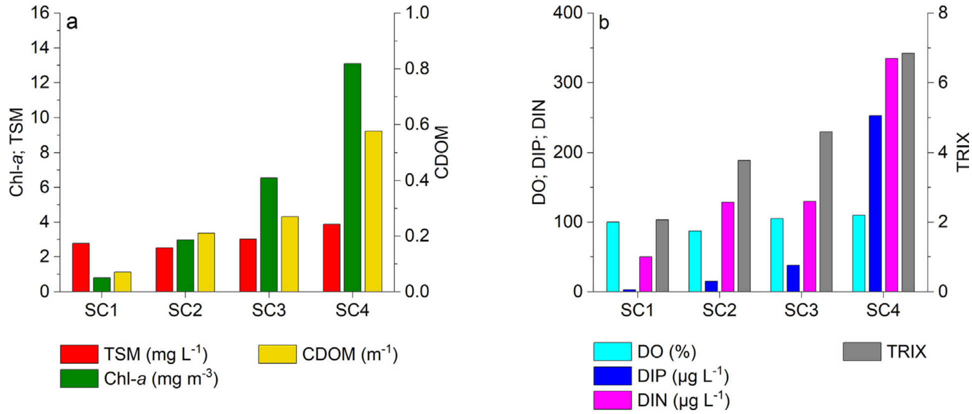

Figure 5 (a) Mean concentration of LAS, and (b) mean concentrations of DO%, DIP, DIN and $TRIX_{(DIN,DIP)}$ index in each of the four spectral classes obtained using SA clustering (Fig. 2b, Fig. 4a—h).

et al., 2012). Among these components, the most important is the concentration of TSM, which is poorly correlated with $TRIX_{(DIN,DIP)}$ (Table 5 and Fig. 3). In addition, these variations are affected by possible noise, for example due to possible inaccuracies during the spectral measurements (Craig et al., 2006). This may explain the poor performance of this metric in differentiating the $TRIX_{(DIN,DIP)}$ levels of the examined samples.

The alternative use of the SA metric eliminates the differences due to amplitude variations of the sample spectra, thus emphasizing the differences due to shape variations (Maselli et al., 2009). In addition, the SA procedure does not require any *a posteriori* transformation of the reflectance spectra which can modify their original shape. The effectiveness of the classification obtained using SA distances is evident also from a simple qualitative analysis of the normalized spectra, as their spectral features were very similar within each class (Fig. 4). According to Maselli et al. (2009), SA variations are mainly related to differences in absorption and mostly in Chl-*a* and to a lesser extent to CDOM and TSM. This has particular importance in Case 2 waters (Gordon and Morel, 1983; IOCCG, 2000), such as those of the examined ports, which are characterized by high concentrations of complexly interacting LAS. The direct effect of Chl-*a* in the determination of $TRIX_{(DIN,DIP)}$, as well as the indirect effect due to the statistical association of Chl-*a* and CDOM with the other components of the $TRIX_{(DIN,DIP)}$ index (DIP, DIN; Table 4) explain the observed higher performance of the SA metric in discriminating classes informative on seawater ecological quality. This is in accordance with recent studies on this subject, which demonstrated that normalized spectral reflectances perform better than the original values for the classification of complex Case 2 waters and dark lake waters where absorption is dominant (Ficek et al., 2012; Shalles, 2006; Spyrakos et al., 2018), while this is not the case in lakes with high diffusion due to TSM load (Eleveld et al., 2017).

Table 5 Mean total cell densities and mean relative abundances [%] of the different phytoplankton groups in the four spectral classes.

	SC1	SC2	SC3	SC4
Density [cells mL^{-1}]	53.8	567.0	872.0	2910.5
Diatoms [%]	36.13	80.17	93.70	70.24
Dinoflagellates [%]	24.85	2.34	3.29	0.91
Coccolithophores [%]	13.30	0.83	0.00	0.06
Others [%]	25.72	16.66	3. 01	28.80

The choice of an unsupervised clustering method to produce a spectral classification informative on seawater quality descriptors and $TRIX_{(DIN,DIP)}$ was mainly justified by the necessity for a strong generalization capacity, which could not be obtained by applying supervised methods to the limited number of samples available. A similar choice has been recently made also by other authors, who applied unsupervised cluster analysis to the optical classification of Chinese spectrally complex waters (Shen et al., 2015; Spyrakos et al., 2018) and global lake waters (Eleveld et al., 2017). The hierarchical clustering method currently chosen allows a straightforward representation of the cluster similarities and a simple identification of the optimum partitioning. In particular, Ward's agglomerative criterion allows finding compact, spherical clusters, which is important in the present case, where the desired aggregations should be as much as possible internally homogeneous. Actually, the identified clusters were internally homogeneous in shape and accounted for at least 60% of the total $TRIX_{(DIN,DIP)}$ variance, even when using a relatively small number of spectral bands (Fig. 4). In fact, the optimal classification was obtained through the cluster analysis of spectra with only 12 bands; the use of the whole spectral range with many more bands did not lead to a significant improvement in the results. The clear distinction of the four spectral classes identified suggests that the proposed method may be applicable also to spectra sampled in even fewer bands.

4.2. Ecological soundness of the four spectral classes

The application of the proposed optical classification methodology determined a meaningful re-ordering of the sampled stations with respect to the ports and seasonal samplings by optimizing their association and providing a snapshot of ecological water quality. At a first level, the stations of each of the five ports were grouped preferentially in a particular optical class. Some stations, however, were not grouped together within the same port, likely due to their seasonal or local peculiarities in LAS concentrations. The information content of reflectance spectra currently observed is closely related to the quantity of Chl-*a*, through in vivo absorption, influenced by phytoplankton composition (Johnsen et al., 2011; Organelli et al., 2017) and secondarily to CDOM absorption.

The four spectral classes that emerged from the application of the proposed optical classification method were arranged following an increase of LAS concentrations, in particular, Chl-*a*, nutrients (especially DIP) and consequently $TRIX_{(DIN,DIP)}$ index and were also in accordance with the features highlighted in the phytoplankton communities. Phytoplankton abundance and composition varied extensively through the seasons, so that the spectral classes grouped heterogeneous communities. Despite this high variability, in SC1 phytoplankton communities of different seasons were characterized by low densities and the occurrence of Coccolithophores, indicative of a marine rather than an inland input. In SC2 and SC3 the phytoplankton densities increased, enhancing Diatoms dominance and reducing the contribution of other groups. Finally, in SC4, the dominance of Diatoms appeared reduced due to the increase of phytoplankton classes typical of more eutrophic conditions related to the input of inland waters, such as Cyanobacteria and Chlorophytes (Dembowska et al., 2018; Gökçe, 2016; Reynolds, 2006).

The distribution of the main water quality indicators in the four SCs shows that the waters belonging to the four SCs have significantly different qualities and ecological characteristics. In particular, this distribution indicated that these waters presented quality, ecological and trophic characteristics decreasing from SC1 to SC4. As clearly emerged from the $TRIX_{(DIN,DIP)}$ index, the SC4 showed ecological conditions that may be considered alarming for some features related to eutrophication, such as the high phytoplanktonic biomass, nutrient concentration and the strong over-saturation of dissolved oxygen.

4.3. Conclusions and prospects

The current study proposes a classification method of port waters (HMWBs) based on the extensive use of reflectance spectra measured *in situ* and elaborated through an unsupervised clustering algorithm and the SA metric. The proposed classification of reflectance spectra agrees with the one derived from ecological indicators, such as Chl-*a*, $TRIX_{(DIN,DIP)}$ and phytoplankton community composition observed in the five Mediterranean ports of this study. The protocol provides a synthetic view of port water quality conditions in terms of general ecological status as established by the trophic index $TRIX_{(DIN,DIP)}$ and phytoplankton biomass and composition. In addition, it provides a sufficient resolution to identify minor variability linked to seasonality and differences among sectors of the same port. This method is theoretically applicable even if prior knowledge of water quality ecological conditions is not available. Moreover, due to its potential to increase the frequency of port water monitoring, thanks to the speed and low cost of necessary measurements, the proposed method may provide an improvement over previous investigations of port ecological water quality. The proposed method, accordingly retuned, could be also utilized for the classification to all Mediterranean coastal areas at risk of eutrophication including transitional waters. As previously noted, the ecological meaning of the spectral classification obtained is dependent on the association between the examined seawater descriptors and the shapes of the respective reflectance spectra, which is direct for Chl-*a*, and statistically inferred for CDOM due to its association with DIP and DIN. Consequently, the association between $TRIX_{(DIN,DIP)}$ and spectra is mostly indirect and its stability should be confirmed by the analysis of a more extended and representative dataset across Mediterranean port areas. More specifically, the efficiency of the SA metric in different Case 2 waters remains to be verified, where the Chl-*a* and CDOM signal is strongly masked by that of TSM.

The proposed optical classification method may be applied to above water radiometry implemented by portable or automated instruments as in Hommersom et al. (2012) or Brando et al. (2016), as well as to high spatial resolution satellite imagery, such as those acquired by the Sentinel 2 Multi Spectral Instrument (MSI). In the first case, there are operational advantages in the measurement of the sea surface reflectance, which, however, is also relatively expensive and time-consuming. In the second case, clear advantages are associated to the use of images that offer particularly great potential for monitoring port water quality, thanks to their high spatial resolution (10 m), frequent revisiting time (every 5 days), routinely application of high-quality pre-processing steps and free distribution policy. On the other hand, the use of satellite imagery should face additional issues due to the limited information provided by the few available satellite spectral bands and to the effects of atmospheric disturbances.

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