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#### **LETTER**

# Cyanobacteria as indicators of water quality in Campania coasts, Italy: a monitoring strategy combining remote/proximal sensing and *in situ* data

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#### Abstract

Cyanobacterial blooms (CBs) are generally triggered by eutrophic conditions due to anthropogenic nutrient inputs to local waters (wastewater or contaminated waters). During the bloom, some species produce toxic secondary metabolites (cyanotoxins) that are dangerous for humans and animals. Here, a multidisciplinary strategy for an early detection and constant monitoring is proposed. This strategy combines remote/proximal sensing technology with analytical/biotechnological analyses. To demonstrate the applicability of this strategy, four anthropogenically-impacted sites were selected along the Campania coast of southwestern Italy, in the so called 'Land of Fires'. The sites were observed using satellite and aircraft images during summer, 2015. Algal community composition was determined using spectrophotometric analysis for the detection of the cyanobacterial pigment phycocyanin (PC). Complementary metagenomic analysis revealed the taxonomic presence of cyanobacteria belonging to genera associated with strong eutrophic conditions. Key elements of this strategy are the combination and integration of applying different methodological approaches such as the parallel and combined use of satellite, aerial and in-situ data, the simplified multispectral image indexing and classification for a truly efficient method in detecting early blooms of cyanobacteria. The effectiveness of the strategy has been validated also by the specific taxa of cyanobacteria found in the examined areas that confirm the assumption that cyanobacterial blooms may serve as useful bioindicators of degraded water quality in coastal ecosystems. To our knowledge this is the first time that the presence of cyanobacteria has been observed in water bodies along the Campania coast.

#### 1. Introduction

Cyanobacteria are the most ancient group of photosynthetic microorganisms exhibiting the widest range of physiological adaptations for colonizing diverse ecological niches; their ability to develop specific physiological mechanisms allows them to adapt to various environmental stressors. Understanding the factors that regulate cyanobacterial biodiversity, indeed, represents a

challenging research topic. Paradoxically, cyanobacteria can be both beneficial and harmful to humans. For instance, they are able to produce bioactive metabolites that may serve as potential leads in drug discovery (Teta *et al* 2013, Esposito *et al* 2015), while in some instances, also release potent neurotoxins that are harmful or even lethal to humans (Teta *et al* 2015).

The suitability of cyanobacteria for assessing water quality in various aquatic environments has

been discussed for many years (Soltani et al 2012). Unicellular cyanobacteria such as *Synechococcus* spp. and Prochlorococcus spp. have been found to dominate open ocean subtropical and tropical oligotrophic ecosystems. In contrast, a high relative abundance of other coastal cyanobacterial species have been reported in response to eutrophic conditions. This phenomenon arising from excess nutrient input is usually of anthropogenic origin, resulting from a myriad of sources including: municipal wastewater discharges, run-off of fertilizers and manure spread from agricultural areas (WHO 1999), organic pollution, and increasing delivery of fecal coliforms to coastal water bodies (Loza et al 2013). Physiological tolerances or ecological ranges of specific cyanobacterial species can differ depending on taxon-specific cellular requirements (Mateo et al 2015). Under specific conditions of light, nutrients and temperature, cyanobacteria can bloom producing a green mat that covers the surface of water bodies. As a result of eutrophication, the occurrence of CBs has increased during the last few decades. CBs produce a strong oxygen depletion in surface waters, that can result in dead zones and fish kills. In addition, during the bloom some cyanobacteria species produce toxic secondary metabolites (cyanotoxins) that may also cause fish mortality and public health risks ranging from mild skin irritations to severe illness. Therefore, monitoring bloom occurrences, composition, frequency and intensity provides important indicators of degraded water quality (Richardson et al 2014). Satellite remote sensing techniques allow the monitoring of algal and cyanobacterial blooms over large spatial and temporal scales, modelling, predicting (Cracknell et al 2001) the formation and progression of bloom events and assessing related environmental parameters. The most commonly used satellite's ocean colour sensors measure the amount of light reflected from the ocean's surface at specific wavelengths. Each satellite contains sensors with a multitude of bands (multispectral) that cover a range of wavelengths. Therefore, the identification of the spectral bands that are most useful in the detection and monitoring of bloom events is required (Heffner 2008). Satellite measurements are especially useful for algal bloom monitoring because of the unique spectral absorbance/reflectance characteristic of photosynthetic pigments. Chlorophyll and carotenoid pigments contained within algal cells are routinely used to assess algal biomass and phylogenetic composition and distribution in aquatic ecosystems. Similarly, phycobiliproteins can be used to detect the presence of cyanobacteria in water samples. Phycobiliproteins are water-soluble photosynthetic accessory pigments that serve as the major light-harvesting pigments in cyanobacteria. Although phycoerythrin is the dominant accessory pigment in marine open ocean

cyanobacterial species, phycocyanin (PC) rich cyanobacteria are widespread in both freshwater and coastal marine ecosystems. PC shows a distinctive reflectance profile, which significantly differs from Chl-a. Some of the multispectral sensors like Sea-viewing Wide Field-of-View Sensor (SeaWiFS), Landsat Enhanced Thematic Mapper Plus (ETM+), Moderate Resolution Imaging Spectroradiometer (MODIS), Advanced Land Imager (ALI) do not provide the resolution for distinguishing prokaryotic cyanobacteria from other eukaryotic algal species as these sensors are not able to detect PC (present primarily only in coastal cyanobacterial spp.). Medium Resolution Imaging Spectrometer (MERIS) bands 6 and 7 allow detecting PC features only if the concentration of cyanobacteria is sufficiently high (Kutser et al 2006). Thus, MERIS can be used in identifying cyanobacteria if they are present in relatively high abundance (Shi et al 2015). Therefore, this tool is not applicable at an early stage of a bloom (Vincent et al 2004). The spatial resolution of these sensors may also cause problems. Even the 30 m spatial resolution of Hyperion may be too coarse compared to the spatial distribution of some cyanobacterial blooms (Kutser 2009). In addition, cloud cover often makes it difficult to acquire good satellite images.

In the Italian province of Campania, coastal waters are optically complex (Morel and Prieur 1977) due to increased optical interference from atmospheric aerosols and the backscattering from optically active constituents, including phytoplankton, suspended solids and coloured dissolved organic matter (CDOM). In addition, microbial biomass may not be solely dominated by cyanobacteria. A versatile approach that can distinguish factors related to the local ecology, watershed hydrology, land use and anthropogenic impact is clearly warranted. In this study, we propose a hierarchical monitoring strategy combining remote and proximal sensing and traditional in situ aquatic monitoring. The remote sensing approach has been integrated with the use of specific indices for extracting information related to the cyanobacteria bloom. For in situ monitoring, analytical spectrophotometric analyses for determination of cyanobacterial pigment content have been integrated with the metagenomic analysis of the DNA isolated from the water samples to estimate biomass and taxonomically characterize the cyanobacterial population. Four different areas along the Campania coast, differing by location and salinity, but all extremely impacted by anthropogenic pressure, were selected as a test bed for this research.

It's noteworthy that only a few reports have documented the occurrence of cyanobacterial blooms in Campania region (Ferranti *et al* 2008) and none of them in the coastal waters; moreover, to our knowledge, this is the first time that the blooms are used as diagnostic bioindicators of degraded water quality.



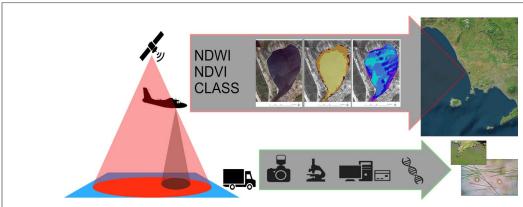


Figure 1. Monitoring strategy based on a hierarchical approach, combining remote/proximal and in situ analytical/biotechnological data

#### 2. Materials and methods

In this study we used a hierarchical monitoring approach ranging from remote/proximal sensing to analytical *in situ* methods for the detection of cyanobacteria (figure 1).

#### 2.1. Remote and proximal sensing

Algal and cyanobacterial blooms are often noticeable within the visible and non-visible areas of the electromagnetic spectrum (EM). Consequently, by using remote and proximal sensing data, it is possible to detect and track bloom development in coastal, fresh and marine waters.

In this study the remote/proximal sensing approach includes data from satellite and aerial platforms. Specific objectives of our study were prioritized to include accomplishing the following tasks. First, our major objective was to maximize the sensitivity of detecting certain surface properties. Ideally, such responses should be linearly related to allow both ease of scaling and use over a wide range of surface conditions. Second, to facilitate making consistent spatial and temporal comparisons from different environments we normalized or reduced various potential biases including, effects due to sun angle, viewing angle, the atmosphere, topography, and instrument signal to noise ratios. Lastly, we endeavored to ensure that the measurements were linked to surface processes or specific parameters (e.g. a biophysical parameter such as LAI, biomass, or APAR; Jensen 2007).

Remote sensing research is often integrated with the development of algorithms able to perform functions such as calculating phototrophic biomass (Mishra and Mishra 2014).

As in previous studies (Lega and Endreny 2016), the satellite remote sensing data were obtained from the Landsat 8 operational land imager sensor (OLI) to create Normalized Difference Vegetation Index (NDVI) images for the area. NDVI index, first applied by Rouse *et al* (1973), identifies the photosynthetic affinity or 'greenness' of the vegetation through the

reflective properties of the chlorophyll and mesophyll layers within the plants in the NIR and red part of the EM spectrum.

The NDVI index is quantitatively defined as:

$$NDVI = (R_{NIR} - R_{RED})/(R_{NIR} + R_{RED}).$$

The principle is that phototrophs display a sharp increase in the red reflectance spectra near 700 nm (due to chlorophyll a) so the difference between  $R_{\rm NIR}$  and  $R_{\rm RED}$  is a proxy for the vegetation density. Normalization relative to  $(R_{\rm NIR} + R_{\rm RED})$  can partially remove the atmospheric effects from different measurements (Hu 2009). In the case of photosynthetically active vegetation, low red reflectance is observed along with very high NIR reflectance producing a NDVI, approaching 1. Soil is observed to have NDVI values close to zero, whilst water is normally associated with negative values.

We analysed the range of NDVI values only for the data related to the water surface, using data extraction techniques based on the NDWI (Normalized Difference Water Index, Gao 1996) defined as:

$$NDWI = (R_{NIR} - R_{GREEN})/(R_{NIR} + R_{GREEN}).$$

Typically, remote sensing studies only use satellite data. However, only other types of aerial platforms (e.g. aircraft, rotorcraft and UAV/DRONE) permit enhanced resolution and flexibility on various spatial and temporal scales. In addition, with this type of focused monitoring, algae and cyanobacteria can be detected at lower concentrations due to the increased resolution.

In this study, to perform proximal sensing activities, we used an aircraft (Rockwell Aero Commander 685) equipped with a camera containing four bands (Intergraph DMC) and housed on an inertial platform (Applanix POS/AV 510-DG) and linked to a GPS system. The flight plan of the aircraft was programmed for a specific altitude to guarantee a resolution of 10–15 cm/pixel.

To overcome problems involving lower spatial resolution and a large acquisition bandwidth associated

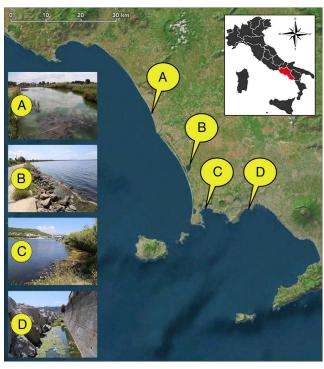


Figure 2. Map and photos of coastal sites in the Campania Province (in red) that were analyzed in this study. From North to South: (A) Agnena River, (B) Patria lake, (C) Lucrino lake, (D) Caracciolo waterfront.

with panchromatic and multispectral images respectively, we utilized a recently developed method of pan-sharpening that combines panchromatic and multispectral data to create new multispectral images with a higher geometric resolution (Belfiore *et al* 2016). The Landsat multi-spectral data were preprocessed with specific routines (Basile Giannini *et al* 2015). These data were then used to compute NDVI indices and, finally, classified. The same procedure, without an atmospheric correction routine, was used for the aircraft data.

Several classes (3 for satellite data and 5 for aircraft data) of NDVI were generated for each image to illustrate different intensities of possible photosynthetic activity at each of the sites and to highlight the bloom fronts and near field regions, as well as delineating the bloom geometry. The classes were defined by a software-based analysis that factors in the relative concentration. Specifically, the lowest concentrations fall into Class 1 and the highest concentrations fall in classes 3 and 5. The analysis of the NDVI satellite data was constrained to pixels bounded by at least 1 other pixel that was fully contained within the aquatic region. This boundary condition was set to reduce the bias associated with analyzing pixels with spectral radiation signatures derived from terrestrial regions.

## 2.2. Description of sites and sampling protocols

In situ data were collected in a similar fashion as reported in our previous coastal water sampling campaign (Lega et al 2010, Lega et al 2012). We selected four anthropogenically-impacted areas along the Campania coast for this study (figure 2), partly

covering the territory well known as the 'Land of Fires'. The name comes from the fire and/or the burial of toxic wastes by the Ecomafia that in addition to the illicit discharges of untreated urban wastewaters has caused a real environmental crisis. This region has been plagued with an increased incidence rate of diseases such as various cancers as well as malformations and other birth defects.

A short description of each selected area with some related key points is reported in the Supplementary Materials section.

Water samples from sites A-D were collected in the region where an anomaly was detected via remote/proximal sensing. Visual observation of the sites was also performed in order to pinpoint precisely the areas in which foam, high turbidity or an altered colour was visible.

At each site, hydrographic measurements of surface water temperature, salinity and pH were performed (see table 1).

# 2.3. Determination of the Cyanobacterial community composition

The presence of cyanobacteria in the water samples was at first assessed by spectrophotometric absorption detection of the cyanobacterial pigment phycocyanin (PC). All the samples were then extracted with a phosphate buffer and the crude extracts were analysed using a UV/Vis spectrophotometer. The cyanobacterial species composition was determined by 16S metagenomic analysis. Full experimental details about chemical extraction of PC and DNA isolation and metagenomic analysis are reported in



**Table 1.** Summary of samples characterization.

Code	Sample type	Site	Salinity	Temp (°C)	Microscopic observations
Al	pond water	Agnena river	10‰	29.7	spiral cyanobacteria
B1	foam	Patria lake		34.5	filamentous cyanobacteria
C1	green mat	Lucrino lake	2‰	35	filamentous cyanobacteria
D1	green mat close to the cliff	Caracciolo waterfront	3‰	28.3	filamentous cyanobacteria
D2	water close to the cliff	Caracciolo waterfront	3‰	19	_

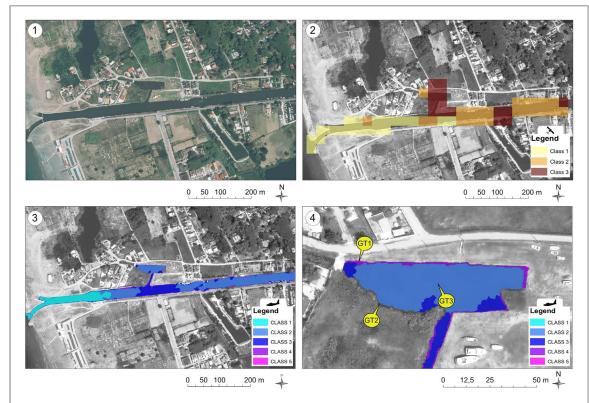


Figure 3. Agnena river (site A): (from top to bottom and left to right) 1) aerial view of the site, 2) Landsat 8 and 3) aircraft NDVI classes to reveal algal/cyanobacteria bloom, 4) detailed view of the classified spot with the evidence of ground truths.

the supplementary material available at stacks.iop. org/ERL/12/024001/mmedia.

# 3. Results

## 3.1. Remote sensing and proximal sensing

Remote and proximal data were analysed in order to highlight potential alterations of the water quality.

Visual and digital processing were carried out to detect and track bloom development in coastal, fresh and marine waters and to assess the bloom extent.

In the visual analysis, different bands of the electromagnetic spectrum in Landsat 8 and aircraft images were combined. The normalized difference vegetation index (NDVI) was obtained from all platforms that generated images and data. The results were processed andseveral classes were generated. Due to the poor spatial resolution (30 m/pixel), the satellite analysis only permitted a limited identification of the area within the bloom region. Conversely, the high resolution of the aircraft data (10–15 cm/pixel)

permitted a clear identification of the bloom. The definition of specific ground-truth areas (labelled in the pictures GT) enabled the calibration of remotesensing data and aided in the interpretation and in the analysis. Specifically, it was possible to define three characteristic targets within each site including:

- GT1, with the highest NDVI value (class 4 and 5) chosen as the sampling site;
- GT2, showing an absence of an algal bloom and therefore used as a negative control;
- GT3, used as reference value for the specific area, located in the middle of the water body with no interferences.

The GT1s, where NDVI anomalies were registered at all the sites were found in specific locations harboring CB blooms.

The first case study focused on the Agnena River (site A, figure 3), particularly on a small lake that linked with the river delta. This area suffers considerable

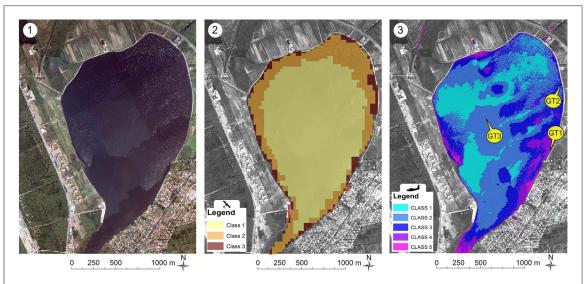


Figure 4. Patria lake (site B): (from left to right) 1) aerial view of the site, 2) Landsat 8 and 3) aircraft NDVI classes to reveal algal/cyanobacteria bloom with the evidence of ground truths.

human impact effects related to illegal discharge of wastewaters derived from a small residential area. In GT1 the presence of an unauthorized wastewater pipeline was clearly observed.

Patria (site B) is a coastal lake, linked to the sea by with a small channel that shows permanent signs of water contamination including: fish mortality, pungent odors and the presence of a white foam along the perimeter of the lake. On the lake, the GT1 area corresponded to a pipeline linked to a restaurant and was adjacent to an artificial channel that passed through a residential area on the east coast. Notably on the east side, the lake shows a semi-permanent presence of an algal mat.

In the past, the second coastal lake Lucrino (site C) was used for the breeding of mussels and even today networks, poles and other infrastructures, that reflect the stagnation of specific areas, are present. The presence of cyanobacteria was detected on the west coast (figure 5); in detail, this area is the furthest away from the connection with the sea and is in close proximity to a spa resort.

The fourth case study focused on the Caracciolo waterfront (site D); it is the principal touristic waterfront of the urban region of Naples.

The presence of cyanobacteria was detected in an area behind the breakwater that protects the water-front road and part of a pedestrian area. That specific area appeared as a tide pool, but the presence of a fig tree was noticeably (clearly, not a salt water tree) growing in the sea on a breakwater rock just beyond the walkway. The salinity value in this area was measured to be close to zero. The explanation for this seeming paradox (zero salinity in the sea) lies in the presence of an unmapped fresh water spring that flows directly behind the breakwater. In addition, it was also observed that the presence of a nearby pipe coming from a spillway that drains the public sewer from storm water overflow, also contained freshwater.

## 3.2. Water sampling

Water samples A1, B1, C1, and D1 were collected in the GT1 area of sites A, B, C, and D, respectively. Additionally, sample D2 was collected in the GT2 area of site D (Caracciolo waterfront, figure 6). Sample D2 was chosen as a negative control for our monitoring strategy validation, as both microscopic observation and aircraft NDVI values did not reveal the presence of cyanobacteria. Various chemical and physical properties of the aquatic bodies where samples were taken are summarized in table 1. There was no clear cut statistical correlation among the hydrographical variables measured. The presence of cyanobacterial species was observed in warm (>19 °C) brackish waters. The measurement of ancillary variables such as nutrient concentrations and DOC levels might have been revealing and should be made in all future studies.

In addition, light microscopic observations of the analysed samples revealed the presence of filamentous and spiral shaped cyanobacteria (figure 7).

# 3.3. Spectrophotometric analysis for determination of PC content

The presence of PC in all the field samples was investigated. Pigments from all the samples were extracted and the crude extracts were analyzed using a UV/Vis spectrophotometer. They all showed the characteristic absorption peak at 620 nm (figure 8), corresponding to the absorption peak of PC from *Spirulina* sp. In the same spectra, peaks attributable to Chl-*a* were also detected, by comparison with the UV/Vis spectra of Chl-a from *Spirulina*.

# 3.4. 16S rRNA metagenomic analysis

The cyanobacterial community inhabiting samples A1–D1 and the negative control D2 was probed using a cultivation-independent approach. Metagenomic DNA was isolated from ~20 mg sediment

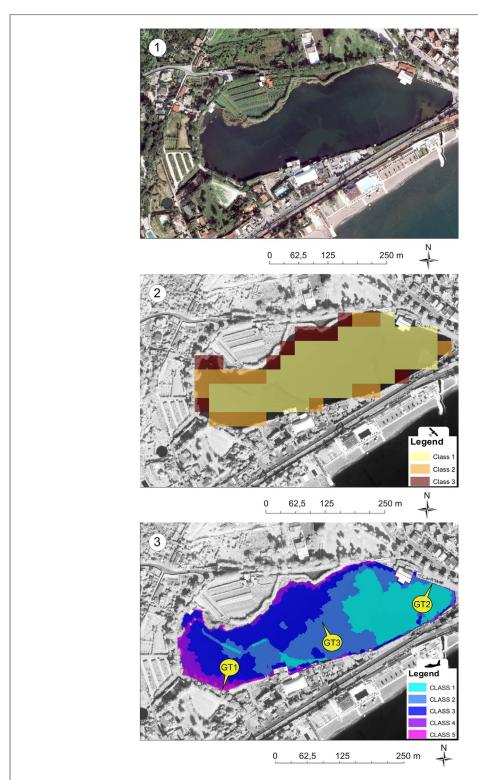


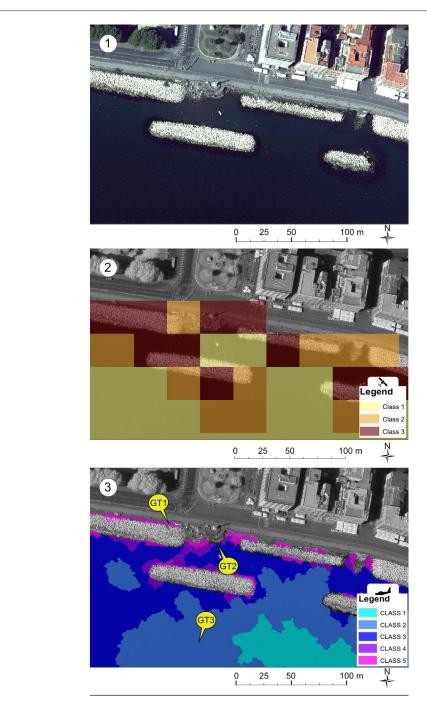
Figure 5. Lucrino lake (site C): (from top to bottom) 1) aerial view of the site, 2) Landsat 8 and 3) aircraft NDVI classes to reveal algal/cyanobacteria bloom with the evidence of ground truths.

of each sample and used as a template for PCR amplification with 16S cyanobacterial specific primers CYA106F and CYA781R(a). A 16S rRNA amplicon library was prepared for each sample with the TOPO TA cloning kit (Invitrogen, CA, USA) and plasmids from randomly chosen clones (52 clones overall) were extracted and single-read sequenced.

Eighteen unique partial 16S rRNA gene sequences were found at a dissimilarity threshold

≤2%. The rRNA sequences were analyzed using the RDP (Ribosomal Database Project) Seq Match tool and BLASTn searches, and 12 sequences were shown to belong to the Cyanobacteria group. As expected, all amplicons from the metagenome of the negative control D2 were non-cyanobacterial rRNA sequences.

The cyanobacterial rRNA sequences were assigned to the genera *Leptolyngbya* (6 strains), *Geitlerinema* 



**Figure 6.** Caracciolo waterfront (site D): (from top to bottom) 1) aerial view of the site, 2) Landsat 8 and 3) aircraft NDVI classes to reveal algal/cyanobacteria bloom with the evidence of ground truths.

(1 strain), *Pseudoscillatoria* (1 strain) and *Spirulina* (1 strain) based on the top matching sequences from BLASTn searches (figure 9). Three sequences could not be assigned because they lacked significant similarities with any known cyanobacterial species.

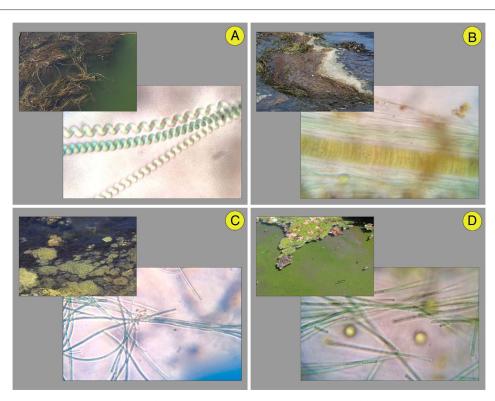
The taxonomic assignment of cyanobacterial sequences retraces the same clade formation observed in 16S rRNA neighbour joining and minimum evolution trees, inferred using either maximum composite likelihood or p-distance methods. The neighbour joining tree of the 16S cyanobacterial rRNA fragments, built using the maximum composite likelihood approach, is reported in figure S1 in supplementary material.

# 4. Discussion

Our study focused on part of the Campania region coastline, in the province of Naples and Caserta, the so-called 'Land of Fires'. Verde *et al* (2013) analysed the environmental degradation of this heavily-impacted region. The malfunction or absence of wastewater treatment systems, the intensive livestock activities and the unlawful waste disposal methods at this site resulted in significant eutrophication and presumably the presence of microbiological bioindicators.

The multidisciplinary strategy proposed here is based on the hierarchical monitoring of cyanobacterial





**Figure 7.** Microscopic observation of cyanobacteria in the analyzed samples. A is the sample from Agnena river, B from Patria lake, C from Lucrino lake, and D from Caracciolo waterfront.

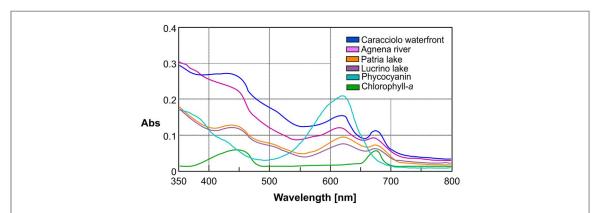
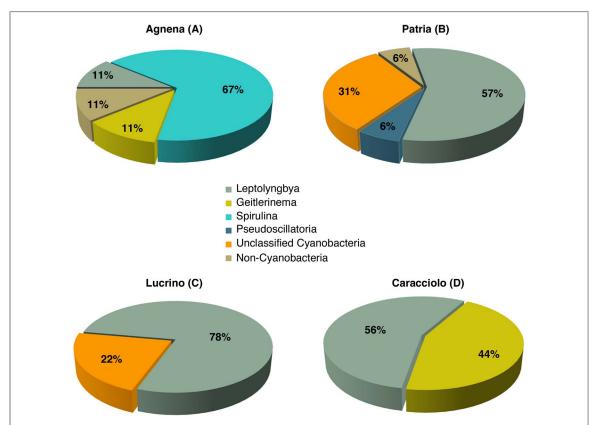


Figure 8. UV/Vis absorption spectra of the extract obtained from the analyzed samples (A, B, C, D1) and of Phycocyanin and Chlorophyll-a from Spirulina sp.

blooms by a variety of complementary approaches. Each of the applied technologies and techniques used here for environmental monitoring is by itself unable to resolve the problems connected to the early detection of cyanobacterial blooms. We propose that only through the combination and integration of applying different methodological approaches can we be truly effective in detecting early blooms of cyanobacteria. The use of aerial imagery combined with specific indices has revealed a powerful tool to relate anomalies in coastal development to environmental aquatic disturbances, and as a consequence to occurrence of cyanobacterial blooms.

Although satellites may be the instrument of choice for observing large areas, they are somewhat limited in their resolving power on finer temporal and spatial scales. Cyanobacterial blooms are indeed

dynamic, spreading rapidly over large areas where satellite spatial resolution is often too low to detect, especially during the early stages of CB blooms. Proximal sensing data, obtained in this study by the use of aircraft, highlighted the presence of anomalies in the NDVI values in all the analysed sites. The presence of cyanobacteria was confirmed by in situ analyses (spectrophotometric analysis, molecular techniques and the traditional microscopic observations). In situ sampling and spectrophotometric and molecular analyses are an essential part of the proposed procedure, in that they allow the recognition of the presence of cyanobacteria directly in field samples and provide decisive identification of developing cyanobacterial bloom species. However, the sampling activity is dramatically reduced, because sampling needs to be performed only in the



**Figure 9.** Cyanobacterial community inhabiting samples A-B-C-D1. Taxonomic profiling of the microbial community was inferred by *in silico* analysis of 16S rRNA fragments amplified from the metagenomes of the four samples.

sites identified by remote and proximal sensing. Further research to study the correlation between blooms of specific cyanobacterial species and specific pollution parameters (e.g. nitrogen to phosphorus ratios) of effluent discharge waters is clearly warranted. The specific taxa of cyanobacteria found in the examined areas confirmed the assumption that cyanobacteria could be used as bioindicators of anthropogenic pressure in aquatic systems, specifically denoting habitats that are indeed extremely polluted sites.

Previously, the only information concerning the occurrence of cyanobacteria in the Campania region denoted the presence of Aphanizomenon ovalisporum, Cylindrospermopsis raciborskii, Planktothrix rubescens in Lake Averno (Rapporto Istisan 2013, Ferranti et al 2011). In addition, a series of algal species were identified in the brackish environments of Lake Patria (Margalef 1983). To our knowledge, this study is the first that documents the presence of the aforementioned cyanobacterial species at these sites and proposes the use of them as bioindicators of a degraded and eutrophic coastal ecosystem. Leptolyngbia sp. is the most widespread species in all the analysed samples. Some species of Leptolyngbia have been associated with nutrient enriched waters and it has been reported to prefer strongly eutrophic conditions (Mateo et al 2015) and very extreme environments (Komarek 2007). The presence of the cyanobacterial species Oscillatoriales is also indicative of eutrophic conditions.

The index NDVI was successfully used to detect cyanobacterial blooms from aerial images. In fact, NDVI takes advantage of the absorption/reflectance features of Chl-a, and therefore is not expected to be able to distinguish between cyanobacterial and algal blooms. In any case, the high spatial resolution of the images from the aircraft allows us to identify with relatively high accuracy the patterns associated with the blooms. The method proposed here can therefore distinguish the typical baseline distribution of algal biomass along the coast from the episodic bloomforming presence (in our case associated to cyanobacteria) related to wastewater discharge and related freshwater plumes. However, the characteristic spectral signature of cyanobacterial PC can also potentially be used in the future to define specific absorption/ reflectance bands for the sensors carried onboard the aerial platforms, with the aim of improving the specificity of the method. Beyond a generic visualization of coastal areas, this approach can identify early signs of degrading water quality by using bioindicators and various indices of developing bloom formation. In general, the combined monitoring of bloom occurrence, composition, frequency along with physical and chemical hydrographic measurements can be useful as important indicators of degraded water quality. These measurements can support environmental impact studies by governmental officials regarding aquatic pollution problems such as determining the effectiveness of wastewater plants in preventing eutrophication impacts within specific coastal areas (Teta et al 2016).



Finally, we emphasize that the use of these specific remote sensing data products can be used in a wide range of environmental monitoring applications, including fusion of optical data with synthetic aperture radar data to detect cattle ranching (Errico et al 2015) and use of thermal imagery to monitor landfills (Lega and Napoli 2008) and detect illegal dumping (Persechino et al 2013) and other illegal activities (Lega et al 2014). In addition, remote sensing data can be strategically combined with other data layers in geographic information systems to monitor the vulnerability of cultural sites and to anticipate or predict potential environmental violations (Persechino et al 2010).

#### 5. Conclusion

We have proposed a multidisciplinary strategy for detecting potentially harmful algal blooms and for assessing degraded water quality along coastal regions. Specifically, we revealed that by utilizing a multidisciplinary approach we can target the early detection and the rapid monitoring of cyanobacterial blooms that may become important in degrading water quality in coastal ecosystems. The rapid detection of such blooms may be especially important in not only preventing the degradation of water quality, but also the deterioration of ecosystem health by the potential disruption of natural foodwebs. Early detection of these algal blooms may also prevent adverse human health effects from the release of toxins by harmful algal species. These harmful blooms may begin to proliferate under eutrophic conditions especially in a warming climate.

Key elements of this strategy are the combination and integration of applying different methodological approaches such as the parallel and combined use of satellite, aerial and in-situ data, the simplified multispectral image indexing and classification for a truly efficient method for detecting early blooms of cyanobacteria. The effectiveness of the strategy has been validated also by the specific taxa of cyanobacteria found in the anthropogenically-impacted areas along the Campania coast in southwestern Italy. To our knowledge, this is the first report regarding the presence of cyanobacteria in water bodies along the Campania coast. The results confirmed the assumption that cyanobacterial blooms may serve as useful bioindicators of degraded water quality in coastal ecosystems.

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# References

Basile Giannini M, Belfiore O R, Parente C and Santamaria R 2015 Land surface temperature from landsat 5 TM images: comparison of different methods using airborne thermal data *J. Eng. Sci. Technol. Rev.* **8** 83–90

Belfiore O R, Meneghini C, Parente C and Santamaria R 2016 Application of different Pan-sharpening methods on WorldView-3 images ARPN J. Eng. Appl. Sci. 11 490–6

Boutte C, Grubisic S, Balthasart P and Wilmotte A 2006 Testing of primers for the study of cyanobacterial molecular diversity by DGGE *J. Microbiol. Meth.* 65 542–50

Cracknell A P, Newcombe S K, Black A F and Kirby N E 2001 The ABDMAP (algal bloom detection, monitoring and prediction) concerted action *Int. J. Remote Sens.* 22 205–47

Di Meo T, Onora G, Cocozziello B and Lione E 2009 ARPAC— Relazione sullo stato dell'ambiente in Campania 2009

Errico A et al 2015 Detection of environmental hazards through the feature-based fusion of optical and SAR data: a case study in Southern Italy Int. I. Remote Sens. 36 3345–67

Esposito G, Teta R, Miceli R, Ceccarelli L S, Della Sala G, Camerlingo R, Irollo E, Mangoni A, Pirozzi G and Costantino V 2015 Isolation and assessment of the in vitro anti-tumor activity of smenothiazole A and B, chlorinated thiazole-containing peptide/polyketides from the Caribbean sponge, Smenospongia aurea *Mar. Drugs* 13 444–59

Ferranti P, Nasi A, Bruno M, Basile A, Serpe L and Gallo P 2011
A peptidomic approach for monitoring and characterising peptide cyanotoxins produced in Italian lakes by matrix-assisted laser desorption/ionisation and quadrupole time-of-flight mass spectrometry *Rapid Commun. Mass Spectrom.* 25 1173–83

Ferranti P, Fabbrocino S, Cerulo M G, Bruno M, Serpe L and Gallo P 2008 Characterisation of biotoxins produced by a Cyanobacteria bloom in Lake Averno using two LC-MSbased techniques *Food Addit. Contam.* A **25** 1530–7

Gao B-C 1996 NDWI A normalized difference water index for remote sensing of vegetation liquid water from space Remote Sens. Environ. 58 257–66

Heffner L 2008 The use of remote sensing and GIS technology in harmful algal bloom research (www.edc.uri.edu/nrs/classes/nrs409/509\_2008/Heffner.pdf)

Hu C 2009 A novel ocean color index to detect floating algae in the global oceans Remote Sens. Environ. 113 2118–29

Jensen J R 2007 Remote Sensing of the Environment: an Earth Resource Perspective 2nd edn (Upper Saddle River, NJ: Prentice Hall)

Komarek J 2007 Phenotype diversity of the cyanobacterial genus Leptolyngbya in the maritime Antarctic *Polish Polar Rese.* **28** 3211–31

Kutser T 2009 Passive optical remote sensing of cyanobacteria and other intense phytoplankton blooms in coastal and inland waters *Int. J. Remote Sens.* 30 4401–25

Kutser T, Metsamaa L, Vahtmae E and Strombek N 2006 Suitability of MODIS 250 m resolution band data for quantitative mapping of cyanobacterial blooms *Proc. Estonian Acad. Sci. Biol. Ecol.* 55 318–28



- Lega M and Endreny T 2016 Quantifying the environmental impact of pollutant plumes from coastal rivers with remote sensing and river basin modelling *Int. J. Sustain. Dev. Plann.* 11 651–62
- Lega M, Ferrara C, Persechino G and Bishop P 2014 Remote sensing in environmental police investigations: aerial platforms and an innovative application of thermography to detect several illegal activities *Environ. Monit. Assess.* 186 8291–301
- Lega M, Ceglie D, Persechino G, Ferrara C and Napoli R M A 2012 Illegal dumping investigation: a new challenge for forensic environmental engineering WIT Trans. Ecol. Environ. 163 3–11
- Lega M, Kosmatka J, Ferrara C, Russo F, Napoli R M A and Persechino G 2012 Using advanced aerial platforms and infrared thermography to track environmental contamination *Environ. Forensics* 13 332–8
- Lega M, D'Antonio L and Napoli R M A 2010 Cultural Heritage and Waste Heritage: advanced techniques to preserve cultural heritage, exploring just in time the ruins produced by disasters and natural calamities WIT Trans. Ecol. Environ. 140 123–34
- Lega M and Napoli R M A 2008 A new approach to solid waste landfills aerial monitoring WIT *Trans. Ecol. Environ.* **109** 193–9
- Loza V, Perona E and Mateo P 2013 Molecular fingerprinting of cyanobacteria from river biofilms as a water quality monitoring tool Appl. Environ. Microbiol. 79 1459–72
- Margalef R 1983 Algas de laguna salobre mediterranea (Lago Patria, Napoles) *Collectanea Botanica* **14** 305–15
- Mateo P, Leganés F, Perona E, Loza V and Fernández-Piñas F 2015 Cyanobacteria as bioindicators and bioreporters of environmental analysis in aquatic ecosystems *Biodivers*. Conserv. 24 909–48
- Messing J 1983 New M13 vectors for cloning *Methods Enzymol*. **101** 20–78
- Mishra S and Mishra D R 2014 A novel remote sensing algorithm to quantify phycocyanin in cyanobacterial algal blooms *Environ. Res. Lett.* 9 114003
- Morel A and Prieur L 1977 Analysis of variation in ocean color *Limnol. Oceanogr.* 22 709–22
- Persechino G, Lega M, Romano G, Gargiulo F and Cicala L 2013 IDES project: an advanced tool to investigate illegal dumping WIT Trans. Ecol. Environ. 173 603–14
- Persechino G, Schiano P, Lega M, Napoli R M A, Ferrara C and Kosmatka J 2010 Aerospace-based support systems and interoperability: the solution to fight illegal dumping WIT Trans. Ecol. Environ. 140 203–14
- Rapporto Istisan 11/35 Pt.1, 2013 Cyanobacteria in water for human consumption Guidelines for risk management

- Richardson L L, Stanić D, May A, Brownell A, Gantar M and Campagna S R 2014 Ecology and physiology of the pathogenic cyanobacterium roseofilum reptotaenium *Life* 4 968–87
- Rouse J W, Haas R H, Schell J A and Deering D W 1973 Monitoring vegetation systems in the Great Plains with ERTS *Third ERTS Symp.* NASA SP-351 I 309–17
- Sacchi F, Molisso A, Pacifico M, Vigliotti C, Sabbarese D and Ruberti D 2014 Late-Holocene to recent evolution of Lake Patria, South Italy: an example of a coastal lagoon within a Mediterranean delta system *Global Planet. Change* 117
- Shi K, Zhang Y, Li Y, Li L, Lv H and Liu X 2015 Remote estimation of cyanobacteria-dominance in inland waters *Water Res.* **68** 217–26
- Soltani N, Khodaei K, Alnajar N, Shahsavari A and Ashja Ardalan A 2012 Cyanobacterial community patterns as water quality Bioindicators *Iran. J. Fish. Sci.* 11 876–91
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods *Mol. Biol. Evol.* 28 2731–9
- Teta R, Della Sala G, Mangoni A, Lega M and Costantino V 2016 Tracing cyanobacterial blooms to assess the impact of wastewaters discharges on Coastal areas and Lakes *Int. J. Sustain. Dev. Plann.* 11 804–11
- Teta R, Della Sala G, Glukhov E, Gerwick L, Gerwick W H, Mangoni A and Costantino V 2015 Combined LC-MS/ MS and molecular networking approach reveals new cyanotoxins from the 2014 cyanobacterial bloom in Green Lake, Seattle Environ. Sci. Technol. 49 14301–10
- Teta R, Irollo E, Della Sala G, Pirozzi G, Mangoni A and Costantino V 2013 Smenamides A and B, chlorinated peptide/polyketide hybrids containing a dolapyrrolidinone unit from the Caribbean sponge Smenospongia aurea. Evaluation of their role as leads in antitumor drug research Mar. Drugs 11 4451–63
- Verde R, Vigliotti M, Prevedello L, Sprovieri M and Ruberti D 2013 An integrated approach to environmental quality assessment in a coastal setting in Campania (Southern Italy) *Environ. Earth Sci.* 70 407–24
- Vincent R K, Qin X, McKay R M L, Miner J, Czajkowski K, Savino J and Bridgeman T 2004 Phycocyanin detection from LANDSAT TM Data for mapping cyanobacterial blooms in Lake Erie Remote Sens. Environ. 89 381–92
- WHO 1999 Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management eds I Chorus and J Bartram (E and FN Spon)