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Water quality and human health: A simple monitoring model of toxic cyanobacteria growth in highly variable Mediterranean hot dry environments

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ABSTRACT

Due to population growth, urbanization and economic development, demand for freshwater in urban areas is increasing throughout Europe. At the same time, climate change, eutrophication and pollution are affecting the availability of water supplies. Sicily, a big island in southern Italy, suffers from an increasing drought and consequently water shortage. In the last decades, in Sicilian freshwater reservoirs several *Microcystis aeruginosa* and more recently *Planktothrix rubescens* blooms were reported.

The aims of the study were: (1) identify and quantify the occurring species of cyanobacteria (CB), (2) identify which parameters, among those investigated in the waters, could favor their growth, (3) set up a model to identify reservoirs that need continuous monitoring due to the presences, current or prospected, of cyanobacterial blooms and of microcystins, relevant for environmental and, consequentially, for human health.

Fifteen artificial reservoirs among the large set of Sicilian artificial water bodies were selected and examined for physicochemical and microbiological characterization. Additional parameters were assessed, including the presence, identification and count of the cyanobacterial occurring species, the measurement of microcystins (MCs) levels and the search for the genes responsible for the toxins production. Principal Component Analysis (PCA) was used to relate environmental condition to cyanobacterial growth.

Water quality was poor for very few parameters, suggesting common anthropic pressures, and PCA highlighted clusters of reservoirs vulnerable to hydrological conditions, related to semi-arid Mediterranean climate and to the use of the reservoir. In summer, bloom was detected in only one reservoir and different species was highlighted among the Cyanobacteria community. The only toxins detected were microcystins, although always well below the WHO reference value for drinking waters (1.0 μg/L). However, molecular analysis could not show the presence of potential cyanotoxins producers since a few numbers of cells among total could be sufficient to produce these low MCs levels but not enough high to be proved by the traditional molecular method applied. A simple environmental risk-based model, which accounts for the high variability of both cyanobacteria growth and cyanotoxins producing, is proposed as a cost-effective tool to evaluate the need for monitoring activities in reservoirs aimed to guarantee supplying waters safety.

1. Introduction

Due to population growth, urbanization and economic development, demand for freshwater in urban areas is increasing. At the same time, climate change, eutrophication and pollution are affecting the availability of water supplies (Yousefi et al., 2019). Unlike ground waters, surface waters are characterized by a rapid deterioration due to urbanization, agriculture and breeding practices possibly exacerbated by climate change (Salari et al., 2018; Bagherzadeh et al., 2018), and therefore they need to be treated to eliminate or reduce pollutants (Qin

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et al., 2014; Fakhri et al., 2017). Moreover, a progressive increase of toxic cyanobacteria (CB) occurrence and blooms in surface freshwaters has been reported worldwide in the last decades (Huisman et al., 2018). CB are aquatic organisms able to colonize a wide variety of habitats such as fresh and brackish waters, sea and thermal waters (Greer et al., 2016). They may reach high concentrations producing blooms with the possible production of various groups of toxic metabolites (cyanotoxins) (Buratti et al., 2017; Ferrante et al., 2013) that can lead to adverse health effects (Filippini et al., 2020; Fiore et al., 2020).

Italy is one of the European countries suffering both of the decreasing availability of water and by worsening in water quality. In 2015, every Italian citizen residing in major metropolitan areas consumed an average of 89.3 m^3 (or 245 L) of drinking water per day. Although groundwater represents the major source for drinking water in Italy, in recent years, to ensure a continuous supply, local aqueducts increased the withdrawal of surface water from natural or artificial water bodies. This situation occurred mostly in, but not limited to, the southern areas, such as in Sicily, the biggest Italian region, the fourth for population and the second for the use of freshwater for agriculture activities (ISTAT, 2016) and characterized by a dry hot climate. To face the increasing need of water, in the 1940–1960 years several water reservoirs were built up, but despite this, Sicily suffers from water shortage and periods of droughts, compared to other Italian regions.

In Italy, the phenomenon of CB blooms is mainly associated with *Planktothrix rubescens* and *Microcystis aeruginosa* which produce several variants of Microcystins (MCs), one of the numerous classes of cyanotoxins (Ferrante et al., 2016; Funari and Testai, 2008). Sicilian lakes, ranging from mesotrophic to hyper-eutrophic state, due to inputs of untreated waste waters, agricultural run-off from the surrounding basin and urban waste, were not an exception. Some of them were indeed affected by intense toxic *P. rubescens* and *M. aeruginosa* blooms until 2010 ((Naselli-Flores and Barone, 1994), when a plan to avoid the total drainage of the reservoirs and to go back towards the original hydrological conditions was implemented, but since then no systematic analysis of the situation was carried out.

Simple treatments for production of drinking waters from surface waters, could not be sufficient to eliminate extracellular toxins. Therefore, the use of this waters potentially contaminated by cyanotoxins as drinking supplying can represent a risk of exposure for human health. (Westrick et al., 2010).

So, the aims of the study were:

- identify and quantify the species of cyanobacteria (CB) present;
- identify which parameters, investigated in the waters, could favor their growth;
- set up a model to identify reservoirs that need continuous monitoring due to the presences, current or prospected, of cyanobacterial blooms and of microcystins, relevant for environmental and, consequentially, for human health.

2. Materials and methods

2.1. Physicochemical and microbiological characterization of Sicilian reservoirs

Fifteen out of 33 Sicilian reservoirs (*sensu* Lgs. D. 152/2006: “whose feeding basin is impacted by anthropic activities that could compromise the quality and having a surface of at least 1 Km^2 or with a volume of at least 5 million m^3”) were selected considering:

- geographical location (at least one per province);
- intended use (giving priority to those used for drinking purpose);
- historical memory (giving priority to those already affected by cyanobacterial blooms).

Total basins capacity and volume variability of the 15 water reservoirs are reported in Table 1. From summer 2016 to summer 2017, we carried out seasonal samplings in the various lakes. In one reservoir (#4), after detection of a cyanobacterial bloom in summer 2017, samples were collected weekly for the following month. One sampling site on the surface and one in the thermocline were identified; when possible, for the larger lakes, samples were collected from 2 to 4 surface sites.

For each sample, about 10 L of water were sampled by an immersion sampler provided with a 1 l glass bottle with automated closure. After mixing in an acid rinsed bucket, samples were divided in adequate number of aliquots and stored at 4 °C until analysis.

Investigated chemical-physical and microbiological parameters and their relative analytical methods are reported in Table 2 and were validated according to ISO Standard.

2.2. Speciation of cyanobacteria community and cell density

For CB species identification and counting, two aliquots of 50 ml were collected in Falcon® plastic tubes, one fixed with Lugol solution (final concentration 1%) and the other with formaldehyde (final concentration 4%) and stored in the dark at 4 °C until analysis. For identification, 10–50 ml Lugol fixed samples were sedimented in sedimentation chamber for 24h and observed in transmitted light on an inverted microscope (Olympus BX50) at a magnification of 200× and 400×. CB species were identified according to Komárek et al. (2014).

Cells were counted in epifluorescence microscopy. Aliquots of formaldehyde fixed samples (5–10 ml) were filtered onto 0.2 μm (25 mm diameter) black polycarbonate filters (Whatman). At least 20 fields or 200 cells/filaments/colonies were counted under green light in autofluorescence (530-550ex/590em broadband) using an epifluorescence microscope (Olympus BX51). The filaments/colonies were counted with a 10x objective, cells were counted with a 40× objective. For each sample, the number of cells per filament/colony was determined as the average number of cells counted on at least 50 colonies.

2.3. Determination of microcystins

For each sample, 1 l of water was filtered through glass filters of 1.2 μm porosity (Millipore - Darmstadt, Germany). Filters were kept at −20 °C for a night and subsequently defrosted at room temperature to help the cell lysis. Afterwards, they were inserted in a Falcon test tube and extracted twice using 15 ml methanol (LC-MS grade, Sigma Aldrich) in an ultrasonic bath for 15 min at 20 °C.

The filtered samples were extracted using a solid phase extraction SPE by Bond Elut SPE cartridge C18 (Agilent Technology - Santa Clara, USA) activated according to manufacturer's instructions and eluted with 5 ml methanol with 0.1% trifluoroacetic acid (TFA; Sigma Aldrich). The extracts were pooled in a total volume of 20 ml (concentration factor 1:50). Finally, 0.5 ml of each extract was dried under nitrogen flow and reconstituted with 0.5 ml of deionized water, produced by a MilliQ system (<18 MΩcm-1 resistivity). In the same way, an analytical blank and a quality control sample (1.0 μg/l MC-LR, Tecna s.r.l., Trieste, Italy) were extracted.

ELISA assay was performed using certified kit Microcystins-ADDA ELISA of the Abraxis LLC (Warminster, PA, 18974), according to the manufacturer's instructions. Since the reported instrumental Limit of Detection (LOD) was 0.10 μg/l and samples concentration factor was 50, the actual LOD was 2 ng/l. Samples with a MCs level >100 ng/l were diluted.

Positive samples were also analyzed by UPLC-MS/MS, to detect the following variants: YR, LW, LY, LR, LF, LA, RR, Nodularin. The methanol extracts were dried under nitrogen flow and reconstituted with 30 μl of methanol and analyzed by a Waters UHPLC-ESI-TQD Acquity system with Electro-Spray ionization and by Mass Quadrupole Mass Spectrometry. An Acquity UPLC® HSS C18 1.8 μm - 2.1 × 150mm column was used and the mobile phase consisted in water and methanol (both

Table 1
Total basins capacity and volume variability of the 15 water reservoirs.

Basin	TOTAL BASIN CAPACITY (MMC)	VOLUME (MMC) AUG-16	VOLUME (MMC) SEP-16	VOLUME (MMC) OCT-16	VOLUME (MMC) NOV-16	VOLUME (MMC) DEC-16	VOLUME (MMC) JAN-17	VOLUME (MMC) FEB-17	VOLUME (MMC) MAR-17	VOLUME (MMC) APR-17	VOLUME (MMC) MAY-17	VOLUME (MMC) JUN-17	VOLUME (MMC) JUL-17	MIN VOLUME (MMC)	MAX VOLUME (MMC)	AVERAGE VOLUME (MMC)	SD (MMC)
1	4.19	2.51	2.09	1.68	1.26	2.39	2.45	3.9	3.59	3.28	3.3	2.94	2.57	1.3	3.9	2.7	0.8
2	20.7	10.78	8.8	7.04	5.31	5.8	4.81	10.85	14.95	15.26	15.46	14.8	13.71	4.8	15.5	10.6	4.2
3	18	7	6.84	5.78	5.94	6.6	6.89	8.61	9.62	10.12	8.82	9.01	8.41	5.8	10.1	7.8	1.5
4	23.6	0.72	0.92	0.57	0.56	1.14	1.38	2.2	2.07	1.34	1.66	1.5	1.19	0.6	2.2	1.3	0.5
5	20	11.63	17.94	10	9.31	9.14	9.78	19.73	20.11	20.05	20.03	19.76	19.18	9.1	20.1	15.6	5.0
6	18	7.51	3.99	4.51	3.12	1.88	1.53	4.99	6.74	6.85	7.14	6.14	5.29	1.5	7.5	5.0	2.0
7	150.5	55.88	30.52	14.75	10.72	5.12	6.23	37.58	51.77	66.83	68.52	67.31	50.91	5.1	68.5	38.8	24.7
8	100	53.78	46.57	41.24	36.84	39.08	36.42	58.25	61.01	60.87	60.06	55.02	48.94	36.4	61.0	49.8	9.6
9	30.4	11.23	8.87	6.79	4.83	3.53	2.96	8.74	16.06	23.03	24.48	19.47	16.79	3.0	24.5	12.2	7.5
10	20.2	15.56	15.65	14.83	14.62	14.42	14.39	15.74	16.57	16.58	16.37	15.95	15.4	14.4	16.6	15.5	0.8
11	72.5	46.84	46.84	35.98	32.32	29.46	25.6	30.12	37.09	35.05	34.19	30.53	25.8	25.6	46.8	34.2	7.0
12	32.8	14.56	12.41	11.5	10.92	10.48	9.57	12.51	13.51	12.83	12.06	11.01	9.19	9.2	14.6	11.7	1.6
13	80	3.9	4.44	2.95	2.73	1.46	2.01	2.22	2.46	2.58	2.67	2.35	2	1.5	4.4	2.6	0.8
14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	34.8	24.85	24.02	22.4	21.9	22.24	21.93	30	25.65	24.71	24.8	24.07	23.15	21.9	30.0	24.1	2.2

Table 2
List of standard parameters analyzed and methods references.

Analytes	Methods
ALUMINIUM	UNI EN ISO 17294-2:2005
AMMONIUM	APAT CNR IRSA 4030/B Man 29 2003
ARSENIC	UNI EN ISO 17294-2:2005
BARIUM	UNI EN ISO 11885:2009
BOD5	APAT CNR IRSA 5120 Man 29 2003
BORON	UNI EN ISO 11885:2009
CADMIUM	UNI EN ISO 17294-2:2005
CALCIUM	UNI EN ISO 11885:2009
CHROME	UNI EN ISO 17294-2:2005
CLORIDE	UNI EN ISO 11885:2009
COD	APAT CNR IRSA 5130 Man 29 2003
CONDUCIBILITY	UNI EN 27888:1995
CUPPER	UNI EN ISO 17294-2:2005
CYANIDE	ASTM D2036A 2015
DISSOLVED OXYGEN (DO)	APAT CNR IRSA 4120/A1 Man 29 2003
FIXED RESIDUE 180°	APAT CNR IRSA 2090/A Man 29 2003
FLUORIDE	UNI EN ISO 10304-1:2009
HARDNESS	UNI EN ISO 11885:2009
IRON	UNI EN ISO 11885:2009
LEAD	UNI EN ISO 17294-2:2005
MAGNESIUM	UNI EN ISO 11885:2009
MANGANESE	UNI EN ISO 17294-2:2005
MERCURY	UNI EN ISO 12846:2013
NICKEL	UNI EN ISO 17294-2:2005
NITRATE	UNI EN ISO 10304-1:2009
NITRITE	UNI EN ISO 10304-1:2009
PAH	APAT CNR IRSA 5080 Man 29 2003
PESTICIDES	APAT CNR IRSA 5060 Man 29 2003
PH	UNI ISO 10523:2012
PHOSPHATE	APAT CNR IRSA 4110/A2 Man 29 2003
PHOSPHORUS	UNI EN ISO 15587-2:2002* + UNI EN ISO 11885:2009
POTASSIUM	UNI EN ISO 11885:2009
SALINITY	APAT CNR IRSA 2070 Man 29 2003
SELENIUM	UNI EN ISO 17294-2:2005
SODIUM	UNI EN ISO 11885:2009
SULFATE	UNI EN ISO 10304-1:2009
SURFACTANS	APAT CNR IRSA 5170 Man 29 2003
TEMPERATURE	APAT CNR IRSA 2550 B Man 29 2003
TOTAL NITROGEN	APAT CNR IRSA 4060 Man 29 2003
TURBIDITY	APAT CNR IRSA 2110 Man 29 2003
VANADIUM	UNI EN ISO 17294-2:2005
VISIBILITY	APAT CNR IRSA 2120 Man 29 2003
VOC	EPA 8260C 2006 + EPA 5030C 2003
ZINC	UNI EN ISO 17294-2:2005
COLONIES COUNTING 37°C	UNI EN ISO 6222:2001
COLONIES COUNTING 22°C	UNI EN ISO 6222:2001
COLIFORMS BACTERIA 37°C	UNI EN ISO 9308-1:2017
E. COLI	UNI EN ISO 9308-1:2017
INTESTINAL ENTEROCOCKS	UNI EN ISO 7899-2:2000

added at 0.1% with formic acid) in percentages of gradient variables during the race. The analysis was performed using the MRM acquisition method, selecting the ionic transitions from the values of m/z obtained from the analysis of suitable reference materials and shown in the Table 3.

Table 3
Cyanotoxins and their ionic transitions (m/z).

Analytes	ESI	Fragment 1	Fragment 2
Microcystin-LR	+	994.6 > 134.8	994.6 > 126.8
Microcystin-YR	+	1045.3 > 135	1045.3 > 127
Microcystin-LW	+	1025.5 > 135	1025.5 > 127
Microcystin-LY	+	1001.5 > 107	1001.5 > 134.8
Microcystin-LF	+	985.7 > 212.7	985.7 > 134.9
Microcystin-LA	+	910.6 > 134.9	910.6 > 106.9
Microcystin-RR	+	520 > 134.8	520 > 126.9
NODULARINA	+	824.8 > 134.8	824.8 > 102.9

2.4. Identification of microcystin-, cylindrospermopsin- and saxitoxin-producing cells by PCR

The presence of potentially toxic populations among the cyanobacterial species was investigated by performing qualitative PCR, looking for the presence of genes responsible for the production of cylindrospermopsin (CYN), saxitoxin (STX) and MCs. PCR was performed only on the reservoir #4, since only in this basin, during the bloom in summer 2017, cyanobacterial cells reached the adequate density to proceed with the molecular analysis. The identified species were *Raphidiopsis raciborskii* (ex *Cylindrospermopsis raciborskii*), *Microcystis aeruginosa*, *Anabaenopsis* sp., *Pseudoanabaena* sp. and *Planktothrix rubescens*. Samples were frozen at −20 °C until the DNA extraction, performed with a commercial kit according to the manufacturer's instructions (GenElute ™ Bacterial Genomic DNA Kit, Sigma). The quality of the genomic DNA was assessed spectrophotometrically by measuring the A260/A280 ratio (Eppendorf Biophotometer).

Standard cultures used as positive/negative controls for the PCR reactions were supplied by the Pasteur Culture Collection (*M. aeruginosa* PCC7806), the Culture Collection of Algae and Protozoa (*P. rubescens* CCAP 1460/3) and the Australian National Algae Culture Collection (*Dolichospermum circinale* CS-337/02, *R. raciborskii* CS-1101, *Chrisosporum ovalisporum* CS-1034 and *R. raciborskii* CS-508 non-toxic).

To verify the presence and the quality of cyanobacterial DNA, a preliminary PCR was carried out following the method by Neilan et al. (1995), generating a product of 685 bp within the phycocyanin operon of CB. Then qualitative PCRs addressed to the identification of CYN- or STX- or MC-producing cells were performed. All PCRs products were separated and visualized on 2% agarose gel electrophoresis stained with ethidium bromide.

For the cylindrospermopsin-synthetase gene, a multiplex PCR was applied for the simultaneous amplification of the rpoC1 gene of *R. raciborskii* and of the two PKS (polyketide synthase) and PS (peptide synthetase) determinants indicative of potential CYN production also in *C. ovalisporum*, according to the method by Fergusson and Saint (2003).

For the saxitoxin-synthase gene, the amplification of three potential indicators of STX production, stxA, stxG and stxS genes, was carried out applying the method by Savela et al. (2015).

Regarding the MC genes, amplification of the mcyE marker was performed, applying the methods by Rantala et al. (2006) and Vaitomaa et al. (2003).

2.5. Statistical analysis

RStudio version 1.1.453 (2009–2018 RStudio, Inc.) was used to perform the Principal Components Analysis (PCA). The PCA on the distance matrix was used to study the spatial disposition of lakes and the parameters that could influence the water quality, excluding the biological indicators. For each parameter the average value obtained from all samplings in each water body was considered. The analysis was repeated deleting some redundant parameter, until the significance did not decrease significantly. The *rda* function (regularized discriminant analysis) was used for PCA. Data were standardized before analysis and the results were displayed in a biplot distance. Sites (the water bodies) were scaled proportionally to eigenvalues. Species (chemical-physical parameters of waters) were unscaled and the weighted dispersion was equal on all dimensions. The sample size was considered appropriate for this purpose (Zuccarello et al., 2019).

Reservoirs were divided in two groups: the control group (#1, 2, 5, 6, 8, 9, 11, 12, 13, 14), where no cyanobacteria blooms did not show in last years, and the case group (#3, 4, 7, 10, 15), where cyanobacteria did show recently. For each parameter, Wilcoxon-Mann-Whitney test was performed to highlight significative differences (p-value < 0.05) among the two groups and identify one by one parameters that could be influence the blooms.

2.6. Environment risk based model to estimate the need of monitoring activities

Since the cyanotoxins presence is not included among the parameters for assessing the suitability of surface water's specific use, a simple model was developed to identify the artificial water bodies with a likelihood of CB and/or cyanotoxins occurrence. The aim was to provide the local authorities a warning tool to set up tailored monitoring and the adequate management measures to prevent possible risk of bloom and, consequentially, of exposure for population from drinking water coming from the reservoir or using it for other purposes (e.g. cooking).

The qualitative model was based on identifying environment risk ratings (i.e., from low risk to high risk) in a simplified probability-consequence matrix P/C (WHO, 2017). For each lake, the *probability* (P) (unlikely = 0–1, possible = 2, likely = 3, highly probable = 4) of occurrence of the event (i.e. a cyanotoxins concentration above a specific threshold, see below) was estimated as the sum of two factors: the presence of MC-producing CB (e.g. *M. aeruginosa* and *P. rubescens*) (no presence = 0, presence = 1, bloom = 2) and the MC levels. The latter parameter was arbitrarily set as very low (absence of cyanotoxins) = 0, low (cyanotoxins levels <1 μg/l) = 1, high (cyanotoxins levels ≥1 μg/l) = 2, considering the WHO threshold level of 1 μg/l for drinking water (WHO, 2017).

Since the more severe risk related to the presence of MCs in waters is represented to the possible human exposure and, consequentially, the human health effects, the degree of *consequence (C)*, ranging from mild = 1 to modest = 2, significant = 3 and serious = 4, was related at the risk associated to exposure to MCs assuming the daily use of drinking water from the water body affected by cyanotoxins-producing CB for long periods (chronic exposure of the population) or in case of bloom. C is expressed as the ratio between MC exposure expressed as Estimated Daily Intake (EDI) via drinking water for adults and children and the Tolerable Daily Intake (TDI) of 40 ng/kg bw/day (WHO, 2017). The level mild and modest were assigned when the ratio was <0.1 and between 0.1 and 0.99, respectively; the level significant and serious were assigned when the ratio was between 1 and 10 and > 10, respectively. For each water body, the exposure estimated as the EDI, was calculated using the following equation:

$$EDI = (C \times IR)/BW$$

where: IR is the Ingestion Rate assumed to be 2 l/day for adults and 1 l/day for children; C is the detected maximum MC concentration (ng/l) and BW is the body weight assumed to be 60 kg for adults and 10 kg for children (WHO, 2017).

Finally, the need for monitoring (M) to prevent risky exposure associated to MC occurrence corresponds to PxC, as shown in the matrix in Fig. 1. When PxC is between 0 and 2 (*unlikely*, with a *modest* C or *possible* with a *mild* C), monitoring seems not to be needed; with a value between 2 and 4 need for monitoring is considered low; between 6 and 9 monitoring is recommended; from 12 to 16 monitoring is highly recommended.

3. Results

3.1. Physicochemical and microbiological characterization of Sicilian artificial water bodies

According to the Legislative Decree 152/2006, surface waters to be used for drinking are classified in three quality classes (AI, AII and AIII, in decreasing quality ranking, thus requiring increasingly harsh treatments before water being potable) with respect to physicochemical and microbiological parameters. Our results, based on at most four seasonal samplings for each reservoir, allowed us to draw a picture of the conditions for the selected 15 Sicilian artificial water bodies. Detailed results of chemical, physical and microbiological analysis are reported in

Probability / Consequence	Unlikely (0-1)	Possible (2)	Likely (3)	Probable (4)
Mild (1)	No action	No action	Low priority	Low priority
Modest (2)	No action	Low priority	Recommended	Recommended
Significant (3)	Low priority	Recommended	Recommended	Highly recommended
Serious (4)	Low priority	Recommended	Highly recommended	Highly recommended

Fig. 1. Matrix identifying the degree of 'need for monitoring' associated to MC and CB occurrence.

Table SM1. Comparing our data with the legal thresholds, in 70% of the lakes the total coliforms parameter fell in AII and the rest was well within the AIII classes. For the fecal Streptococci, the situation was much better, being 80% of the samples within class AI and only 20% in class AII. Therefore, all the reservoirs could be used as source for drinking water regarding the microbiological status.

As for the legally binding chemical parameters, only 4 of them (sulfate, Biological Oxygen Demand in 5 days or BOD5, conductivity and Fe) exceeded the AIII thresholds, with different combination (Table 4). Accordingly, two lakes (#9, 12) would result as class AII, whereas the others would not result suitable for drinking purpose, even if #1, 2, 5, 6, 11 and 15 only for one parameter.

The exceedance of the same parameters for the selected water bodies located in different part of Sicily suggests common pressures onto the water bodies. High levels of BOD5, the most frequently no compliant parameter indicated a high organic matter content, whereas conductivity, as a measure of the salts concentration, was highly correlated to the concentration of Cl, Na, Mg and Sulfate ($n = 63$, $p < 0.001$). Fe content was highly variable between the water bodies, ranging from below the LOD up to 3587 μg/l.

Among the additional parameters measured in our survey, Total Phosphorous or TP (0.01–2.58 mg/l) and Total Nitrogen or TN (0.3–14.5 mg/l) were at levels of eu-hyper-eutrophy in all lakes; since they were analyzed on unfiltered water samples reflected also the quota related to organic particulate matter. Finally, highly variable aluminum concentrations (from 20 up to 4550 μg/l) have been found.

Table 4
Standard parameters exceeding at least once AIII thresholds in the lakes. Maximum values measured during the study period.

Lakes	BOD5[a]*	Conductivity[b]	Iron[c]*	Sulfate[d]*
1	10			
2	7.5			
3		1750		606
4	59	1354	2900	309
5		1140		
6	8			
7	24	1501		277
8	13			350
10	9	2366		431
11			3587	
13	12	1137		260
14	10	2606		1020
15				590

Thresholds of parameters for class AIII: [a]Biological Demand of Oxygen 5 days<7 mg/L; [b] = 1000 μS/cm; [c] = 1000 μg/L; [d] = 250 mg/L * = the decree allows exceptions for these parameters.

3.2. Speciation and density of cyanobacterial community

In ~60% of the lakes where CB were found, the co-presence of several species was observed (Table 5). In July 2017, a *Microcystis aeruginosa* bloom was detected in lake #4. Weekly samples, until mid-Sept, showed that the composition of the cyanobacterial community changed dramatically in a very short time. In July the bloom was dominated by *Microcystis* sp. and *Raphidiopsis raciborskii* (10^8 and 10^7 cell/l, respectively), the only two species detected. By mid-Aug these two species disappeared being fully replaced by *Anabaenopsis* sp. and *Planktothrix rubescens*, which in mid-Sept were still growing (10^7 and 10^6 cell/l, respectively) (Fig. 2).

3.3. MCs occurrence

Table 6 shows the MCs mean values by seasons for each lake. MCs presence have been screened by ELISA, in samples concentrated 50x. The values were very low, with a range of <2–728 ng/l (median 25.4) and 76% of values < 100 ng/l, never exceeding the WHO reference value for drinking waters (1.0 μg/l). When positive samples were tested with UPLC-MS/MS, very few samples were above LOD, with much lower values and a high degree of variability. Among variant only MC-LR was identified, with the exception of lake #3 in which also MC-YR was detected once.

3.4. Identification of CYN-, STX- and MC-producing cells by PCR during the bloom

The preliminary PCR within the phycocyanin operon amplified a 685 bp product in all the samples of the bloom analyzed, thus confirming the presence of cyanobacterial DNA.

No amplification of the mcyE marker was observed in the analyzed samples, suggesting that the fraction of toxin producing CB had to be very small. In the positive controls *Microcystis* PCC7806 and *P. rubescens* CCAP 1460/3, as expected, amplification of the 370 bp fragment occurred.

None of the samples was able to produce STX, as demonstrated by the lack of amplification of the fragments of 648 bp, 519 bp and 382 bp, diagnostic respectively of the StxA, StxG and StxS genes as observed in the positive control CS-337/02 (*Dolichospermum circinale*).

Also, the CYN-producing genes were absent from the CB community. Indeed, no amplification of the ps and pks toxicity markers was observed in the samples, while the formation of the respective amplification products was confirmed in the two positive controls, CS-1101 *R. raciborskii* and CS-1034 *C. ovalisporum* and was absent in the negative one CS-508 *R. raciborskii*.

Table 5
Cyanobacteria species and density (cell/l).

Water bodies	Summer 2016	Autumn 2016	Winter 2016/17	Spring/summer 2017
1	- *Pseudoanabaena* sp	*no ciano*	*no ciano*	*no ciano*
2	- *Limnothrix redekei* - *Borzia* sp. - *Merismopedia* sp - *Pseudoanabaena* sp - *Planktothrix rubescens*	- *Borzia* sp. *(3*10ˆ2)* - *Pesudoanabaena* sp. *(2.0*10ˆ3)* - *Limnothrix redekei (6.0*10ˆ2)*	*picocianobatteri*	*no ciano*
3	- *Planktothrix agardhii*	-	*no ciano*	*no ciano*
4	- *Anabaenopsis* sp - *Pseudoanabaena*	-	*no ciano*	- *Microcystis aeruginosa (6.67*10ˆ8)* - *Cylindrospermopsis raciborskii (1.42*10ˆ7)* - *Pseudoanabena* sp. *(2.15*10ˆ6)*
5	- *Pseudoanabaena* sp. - *Chroococcus* sp.	-	*picocianobatteri*	*picocianobatteri*
6	- *Oscillatoriales* - *Dolichospermum* sp. - *Picocianobatteri*	-	*picocianobatteri*	-
7	- *P. rubescens* - *P. agardhii* - *Oscillatoriales* - *Planktolyngbya* - *picocianobatteri*	*Planktolyngbya* sp. *(4.2*10ˆ3)*	*picocianobatteri*	*picocianobatteri*
8	*Oscillatoria* sp.	-	*no ciano*	*no ciano*
9	*no ciano-*	*no ciano-*	-	-
10	-	-	*P. rubescens (6.6*10ˆ4)*	- *Planktolyngbya* sp. *(1.2*10ˆ3)* - *Limnothrix redekei (6.0*10ˆ2)* - *Oscillatoria* sp. *(1.0*10ˆ3)*
11	-	-	*Pseudoanabaena* sp. *(5.0*10ˆ2) picocianobatteri*	*no ciano*
12	-	-	*picocianobatteri*	*picocianobatteri*
13	*no ciano*	-	*no ciano*	*no ciano*
14	-	-	*no ciano*	*picocianobatteri*
15	-	-	*no ciano*	*-no ciano*

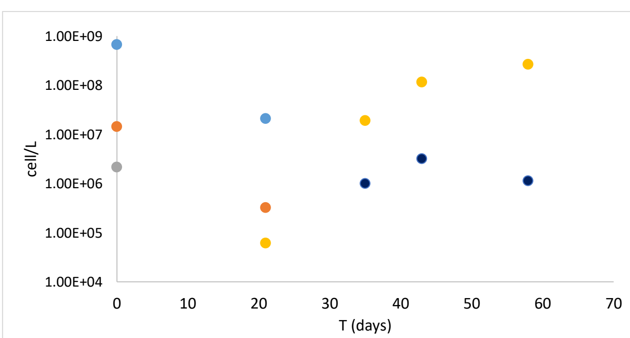

Fig. 2. Temporal variations of CB species density during the bloom in lake #4. Y-axis log scale. Light blue = Microcystis; orange = Cylindrospermopsis; yellow = Anabaenopsis; dark blue = P. rubescens. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. *Statistical analysis*

The two axes of PCA analysis, as shown in the distance biplot (Fig. 3), accounted for a total of 48% of variance of the parameters (PC1 and PC2 were 28% and 20%, respectively). The horizontal axis showed a clear pattern from left to right, with almost all of the parameters localized on the right and lakes divided into two groups: on the left are the lakes that would fit in AII class or that would be unsuitable for drinking only for one parameter; on the right are all the lakes out of class AIII for two and three parameters. The vertical axis further differentiated the lakes in the right sector: from the bottom, where trophic conditions are relevant (TP, TN, NO_3, conductivity vectors are here) towards the top, where turbidity (plus Al and Fe, possibly associated to turbidity), is the main vector. Trophic conditions and turbidity are important drivers for, and as consequence of cyanobacterial blooms: as expected the lakes where CB blooms have been detected in the last decades and in this study (#3, 4, 7, 10, 15) are vertically scattered in the two quadrants on the right.

Table 6
Seasonal mean Microcystins concentration (ng/l) and during the bloom in lake #4.

Water bodies	Summer 2016	Autumn 2016	Winter 2016/17	Spring/ Summer 2017
1	<2.0	4.7	<2.0	3.4
2	669.0 ± 71.4	188.0	35.0 ± 3.0	13.7 ± 6.7
3	37.2 ± 15.0 (87.1 ± 86.3 LR) (54.8 – YR)	n.a.	6.5	52.0 ± 4.7
4	47.8	n.a.	18.7	360.0
5	15.7	n.a.	28.0	21.5
6	3.9 ± 1.2	n.a.	7.2 ± 2.2	n.a.
7	268.8 ± 92.2 (131.5 ± 189.14 LR)	61.4 ± 81.5 (23.1 – LR)	28.2 ± 16.0	25.4 ± 5.0
8	166.3 ± 44.4	n.a.	18.0	554.0
9	2.5	21.6	n.a.	n.a.
10	n.a.	n.a.	728.0	23.6
11	n.a.	n.a.	6.0	61.5
12	n.a.	n.a.	10.2	29.3
13	18.5	n.a.	25.9	30.4
14	n.a.	n.a.	<2.0	11.5
15	n.a.	n.a.	40.5 ± 16.06.4	155.0
	7 Aug 2017	**21 Aug 2017**	**29 Aug 2017**	**12 Sept 2017**
4	393.0	184.0	135.0	65.0

n.a not available since sampling was not performed.

Wilcoxon-Mann-Whitney test highlighted significant differences among the control-case groups for Ammonia (N) (p-value = 0.03996*, Fig. 4), Barium (Ba) (p-value = 0.02797*, Fig. 5), Sodium (Na) (p-value = 0.02797*, Fig. 6), pH (p-value = 0.01958*, Fig. 7), Chloride (Cl) (p-value = 0.01931*, Fig. 8) and, finally, Fluoride (F) (p-value = 0.02897*, Fig. 9).

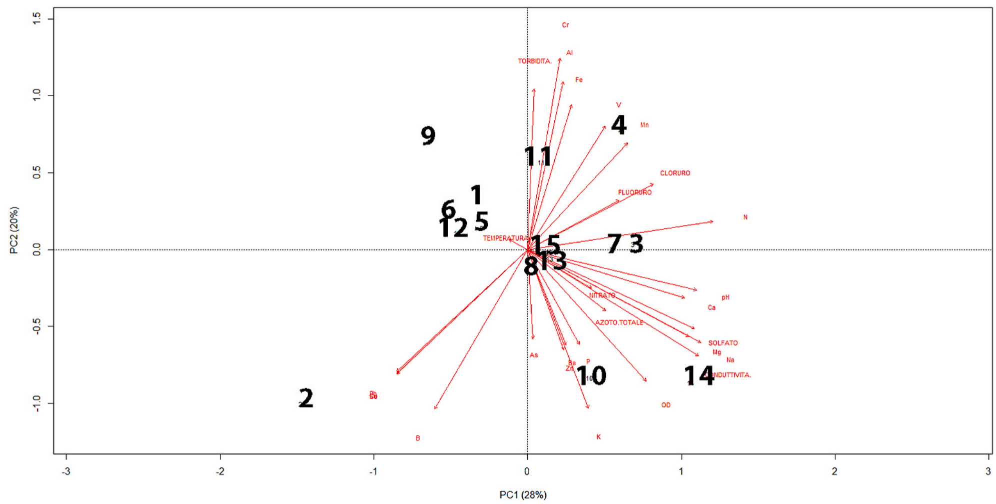

Fig. 3. Distance biplot (PC1 and PC2 were 28% and 20%, respectively) shows spatial disposition of basins and parameters that characterize their water.

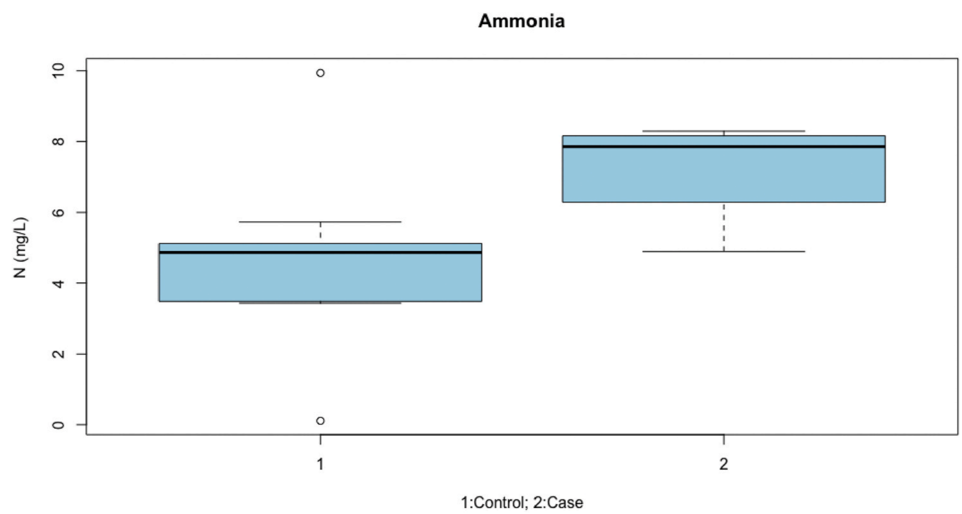

Fig. 4. Wilcoxon-Mann-Whitney test for ammonia among control-case groups (p-value = 0.03996*).

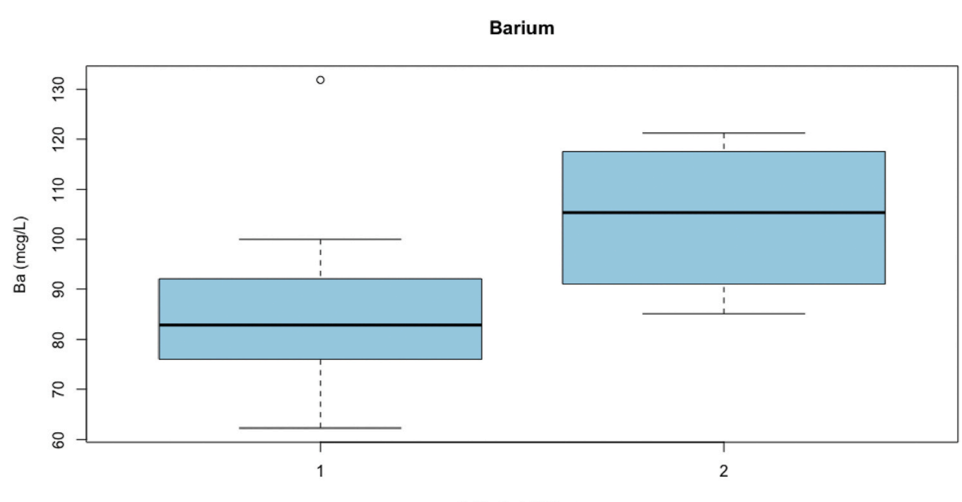

Fig. 5. Wilcoxon-Mann-Whitney test for barium among control-case groups (p-value = 0.02797*).

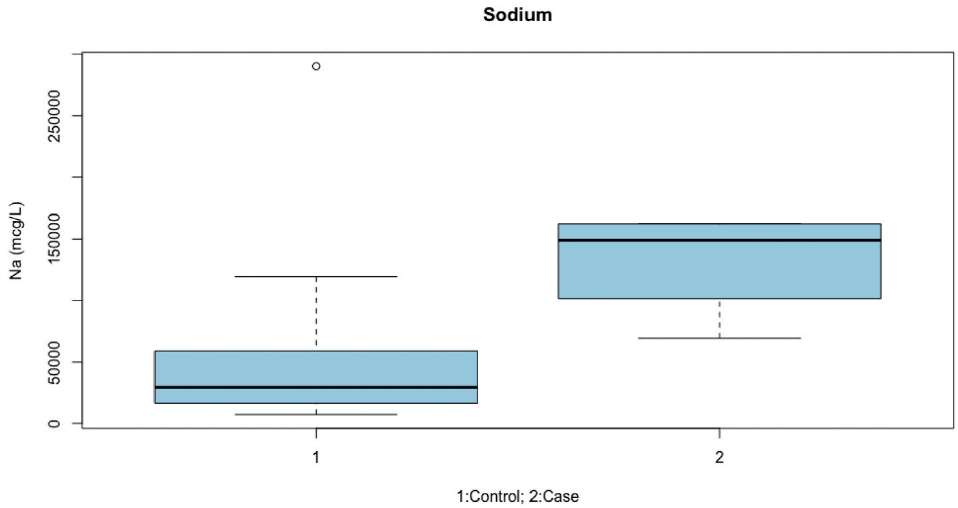

Fig. 6. Wilcoxon-Mann-Whitney test for sodium among control-case groups (p-value = 0.02797*).

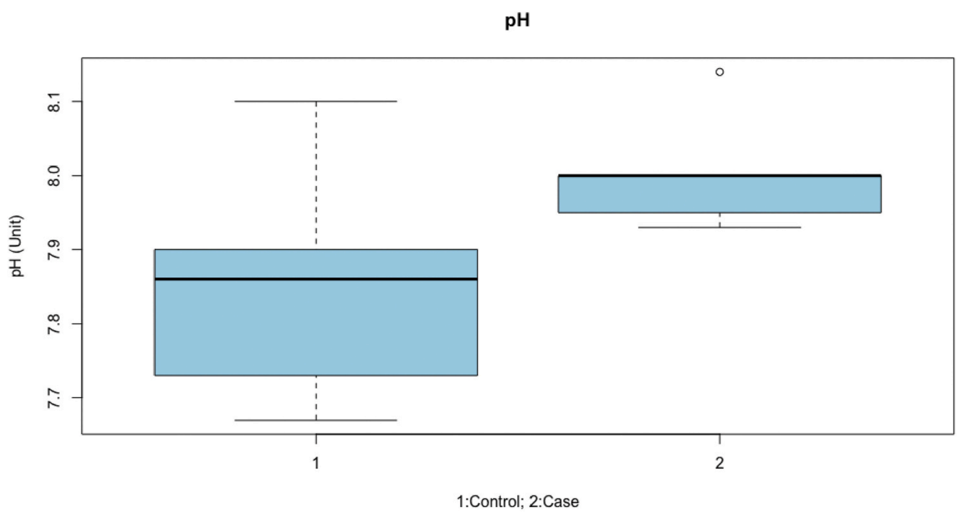

Fig. 7. Wilcoxon-Mann-Whitney test for pH among control-case groups (p-value = 0.01958*).

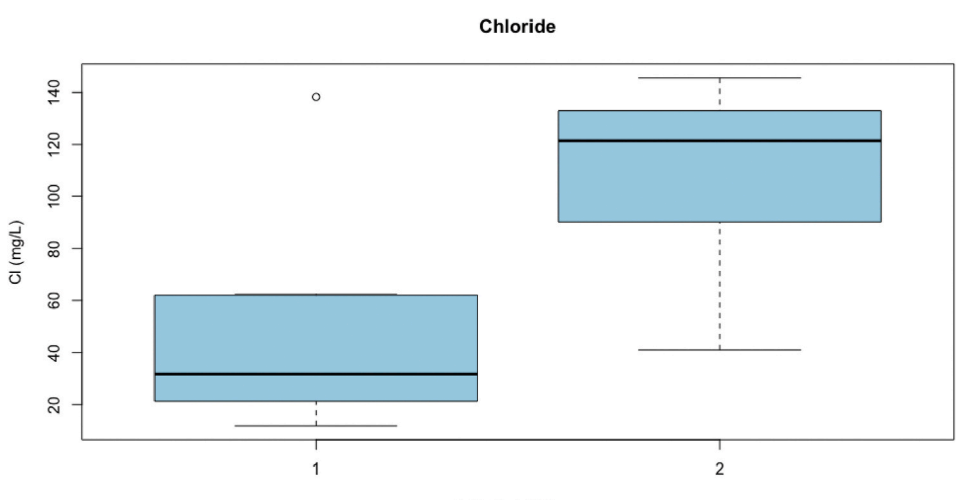

Fig. 8. Wilcoxon-Mann-Whitney test for chloride among control-case groups (p-value = 0.01931*).

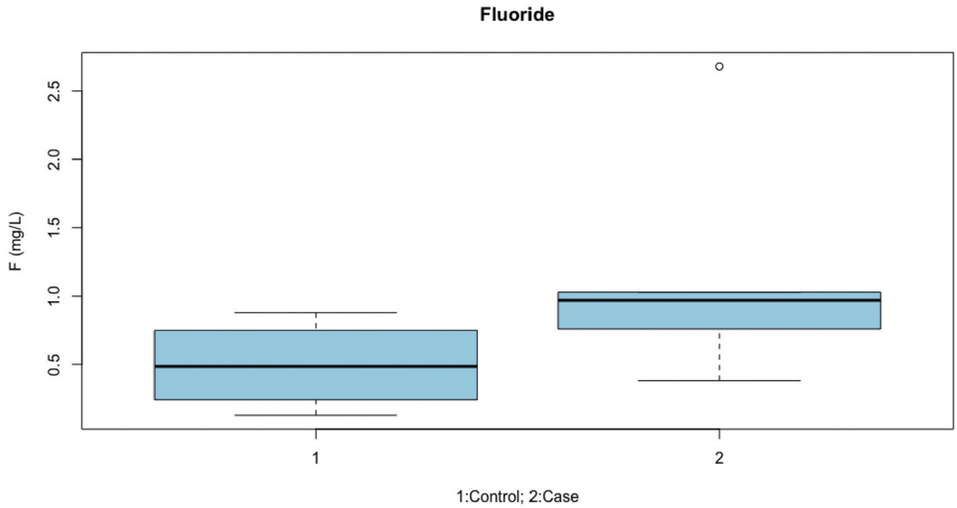

Fig. 9. Wilcoxon-Mann-Whitney test for fluoride among control-case groups (p-value = 0.02897*).

3.6. Needs for monitoring activities

Table 7 shows the EDIs for adults and children, the relative comparison with TDI and the need for monitoring (M) to prevent risky exposure associated to MC occurrence. Overall, only for 3 reservoirs (#2, 4 and 10) monitoring is recommended and other 3 (#3, 7,8) are of low priority on the basis of children's health. The reservoir #4, the one with the bloom detected during the study, was recommended due to results obtained for both children and adult health.

4. Discussion

Our survey on 15 artificial Sicilian water bodies shows that generally their water quality is mainly affected by lakes' geology (excess sulfates) or by organic pollution, as shown by data on BOD5, TP and TN: the high levels of TN (up to 14.5 mg/l) and TP (up to 2.58 mg/l) in line with the high BOD5 indicate the input of partially or completely untreated urban and rural wastewaters in all the reservoirs (Nandakumar et al., 2019; Magwaza et al., 2019) which adds to the sediments internal load. The role of sediments has been thoroughly studied in one of lakes (#15), where peaks of phosphorus and NH_4 are released in summer when, in the absence of precipitation, the lowering of water level allows sediments re-suspension (Naselli-Flores, 2011). The observed nutrient values are in the high range of the ones measured in the past in some of the lakes (Naselli-Flores and Barone, 1994), which are now in the eutrophic or hyper-eutrophic conditions, promoting the excessive algal and microbiological growth (Confesor et al., 2016), as witnessed by the levels of environmental bacteria (Table SM1); expressed as Colonies Forming Units at 22 °C, always >300). Furthermore, at such a high level of nutrients, the effects of physical parameters, like turbidity or the winter filling and summer emptying of the reservoirs, are enhanced and become more important in shaping phytoplankton community, promoting the growth of species able to adapt like *Microcystis aeruginosa* that became dominant in many water bodies (Naselli-Flores, 2011). The role of physical drivers in shaping cyanobacterial community has been observed also in artificial reservoirs of other semi-arid environments, such as in Brazil (Bittencourt-Oliveira Mdo et al., 2012).

Overall, we observed a low density with a rapid succession in the composition of the cyanobacterial community. In 50% of the reservoirs the cyanobacterial communities (including *Limnothrix redekei, Borzia* spp., *Merismopedia, Pseudoanabaena, Anabaenopsis, Oscillatoriales, Dolichospermum, Planktolyngbya*) did not remain stable over time, even during the bloom, characterized by the dominance of a few species in a row. There is no previous information on the ecology of lake #4 but, based on its nutrient concentration, shallowness (especially in summer, when it reached its minimum volume), turbidity and hydrodynamic it is not surprising that in summer 2017 a bloom of *M. aeruginosa* was recorded, with a concomitant presence of *R. raciborskii*. What was unexpected was the presence of *P. rubescens*, a species which is typical of mesotrophic, deep and cold lake, where in summer it is limited at the thermocline depth. Historically, indeed, blooms of *M. aeruginosa* and *P. rubescens* have been reported separately in different Sicilian water bodies (#7, 10 and 15) (Naselli-Flores et al., 2007). However, although the two species are typical of different habitats, their alternation in the same lake has been reported in some Sardinian reservoirs (Stefanelli et al., 2017) and in an Algerian reservoir (Guellati et al., 2017), two areas with a similar climatic condition.

It is also interesting to note that usually both these species have a high toxic phenotypes percentage in the population during the blooms: a maximum cell quota >1pg/cell has been measured in *M. aeruginosa* from Lake Erhai (China), while the range of MC cell quota for *P. rubescens* is between 0.01 and 0.7 pg/cell (Buratti et al., 2017). This feature was previously described also in Sicily: during a *M. aeruginosa* blooms in Lake Arancio in 2001 a cell quota of up to 0.3 pg MC/cell had been measured and, in Lake Pozzillo, during the first bloom of *P. rubescens* recorded in Sicily in winter, a cell quota of ~0.7 pg MC/cell was measured, showing the presence of quite toxic strains (Naselli-Flores et al., 2007).

During our study, very low MC concentration were detected with ELISA test among 0.1–0.3 μg/L; analysis by HPLC-MS/MS on six main congeners of MCs not always confirmed the same concentrations. Therefore, since not even nodularin was be highlighted, it is probable that the presence of MCs showed by ELISA test have to referred to other analogues of MCs having ADDA-group. Moreover, no mcy genes have been detected, both for *Microcystis* and *Planktothrix*. It is plausible, since assuming the presence of strains with the same high toxicity, a percentage <10% of the present populations would have been necessary to produce the amount of MC detected, which may correspond to a too low number of cells to be caught by the traditional molecular method applied. Due to the presence of *Raphydiopsis* and *Anabaenopsis*, we also looked for the CYN and STX genes. However, the potential ability to produce CYN, SXT and MC toxins has been excluded in samples taken during these blooms.

The distance biplot of PCA shows that 9 reservoirs (namely #3, 4, 7, 8, 10, 11, 13, 14, 15) are located on the right side, based on results regarding nutrients, ions, conductivity and turbidity. The lakes #3, 4, 7, 10 and 15 showed the presence of CB. The PCA analysis could give useful indications about the conditions influencing the proliferation of CB: all of the above mentioned water bodies are indeed characterized by different chemical and physical parameters along a vertical gradient

Table 7
Values to determine the need of sampling (M) according to the matrix P/C for adults and for children.

		Adults								Children							
Basins	*MCs Max*	*EDI*	*EDI/TDI*	*MCs*	*CB*	*Probability*	*Consequence*	*M*		EDI	EDI/TDI	MCs	CB	Probability	Consequence	M	
1	4.7	0.2	0.10	1	0	1	1	1	No need	0.5	0.10	1	0	1	1	1	No need
2	669	22.3	0.56	1	1	2	2	4	Low need	66.9	1.67	1	1	2	3	6	Recommended
3	52	1.7	0.10	1	1	2	1	2	No need	5.2	0.13	1	1	2	2	4	Low need
4	393	13.1	0.33	1	2	3	2	6	Recommended	39.3	0.98	1	3	4	2	8	Recommended
5	28	0.9	0.10	1	0	1	1	1	No need	2.8	0.10	1	0	1	1	1	No need
6	7.2	0.2	0.10	1	0	1	1	1	No need	0.7	0.10	1	0	1	1	1	No need
7	268.8	9.0	0.22	1	1	2	2	4	Low need	26.9	0.67	1	1	2	2	4	Low need
8	554	18.5	0.46	1	0	1	2	2	No need	55.4	1.39	1	0	1	3	3	Low need
9	21.6	0.7	0.10	1	0	1	1	1	No need	2.2	0.10	1	0	1	1	1	No need
10	728	24.3	0.61	1	1	2	2	4	Low need	72.8	1.82	1	1	2	3	6	Recommended
11	61.5	2.1	0.10	1	0	1	1	1	No need	6.2	0.15	1	0	1	2	2	No need
12	29.3	1.0	0.10	1	0	1	1	1	No need	2.9	0.10	1	0	1	1	1	No need
13	30.4	1.0	0.10	1	0	1	1	1	No need	3.0	0.10	1	0	1	1	1	No need
14	11.4	0.4	0.10	1	0	1	1	1	No need	1.1	0.10	1	0	1	1	1	No need
15	155	5.2	0.13	1	0	1	2	2	No need	15.5	0.39	1	0	1	2	2	No need

(the second axis of the PCA plot) with nutrients (as a metric for the trophic state) in the bottom quarter, moving up towards other nutrients (ammonia) and micronutrients (Fe, Mn, Cl) and finally turbidity, the latter mainly due to the run-off from the surrounding (Naselli-Flores and Barone, 1994). Water level, external nutrient and matter input, internal nutrient load from sediments, all are related to precipitation. In this contest, Sicily has been divided in three **ecotypes**, characterized by three different levels of humidity and annual average precipitations: humid, dry/semi-humid, and arid-semiarid (identified by green, yellow and red on a color scale) (Sicilia Dip, 2015). The lakes that are in the arid/semiarid zones (red) are more sensitive to the hydrological regime and to the seasonal filling and water outflow, which in turn affects the other parameter. All the lakes on the right (#3,4,7,8,10,11,13,15) are red or yellow; all the others on the left are green, but number 5 that is yellow. This clearly suggests that the lakes on the right are more prone to climatic oscillations and sensitive to environmental conditions and that their phytoplankton community can be dominated by CB. Conflicting opinions have been expressed on the role of phosphorus and nitrogen on cyanobacterial blooms: according to some studies they both act as growth promoters (Davis et al., 2009; Polyak et al., 2013), while other studies showed that the abundance of phosphorus seems to have a more significant impact than nitrogen abundance on the cell growth (Ptacnik et al., 2008; Dolman et al., 2012). The PCA analysis can suggest additional factors associated to the growth of CB: for example, micronutrients like Fe and Mn are important cofactors for some enzymatic activities and may have a positive impact on the growth of *Microcystis* toxic strains (Kaushik et al., 2015). Some authors proposed that MC could act as intracellular chelators of Fe^{2+} and that the synthesis of the toxin would be controlled by the availability of the intracellular free Fe (Ceballos-Laita et al., 2017). An adequate supply of trace metals is essential for optimal grow conditions of CB (Facey et al., 2019). Moreover, high pH could be a positive parameter for cyanobacterial bloom due to their ability in neutral and alkaline environment to use bicarbonate as a form of inorganic carbon, while most of green algae are not able to do it and during blooms, they become CO_2 limited (De Oliveira et al., 2014). Finally, fluoride does not seem to be toxic to CB, which are also used in process of defluorination (Biswas et al., 2018). Moreover, fluoride toxicity is a pH-related phenomenon and it decrease in alkaline environmental (Bhatnagar and Bhatnagar, 2004).

The Wilcoxon-Mann-Whitney test confirmed the influence of ammonia, chloride, pH and fluoride. Furthermore, sodium and barium also appear to play a role in the growth of cyanobacteria. However, these elements could be related to the presence of other anions (Cl, F, sulfate, etc.). Other micronutrients, such as Fe and Mn, lost their statistical significance when evaluated individually. The multifactorial analysis is able to highlight the synergistic or antagonistic activity of two or more elements within the same environment.

Therefore, the PCA analysis seems to give a more representative view, showing the general state of the basins and, rather than the effect of individual parameters, the complexity of the favorable conditions for cyanobacteria growth.

Concerning to the use of surface waters as producer of drinking waters, in our monitoring study no other chemical pollutants (pesticides, polycyclic aromatic hydrocarbons (PAHs) and heavy metals) were detected at significant values, showing that the conditions did not worsen from the last publicly available data from regional authorities, referring to the period 2011–2014, and in few cases they actually improved. In 2011 more than 90% of the artificial water bodies intended for human consumption, including the ones in the present study, fell in the AII class. The analysis in the following years, however, did not always confirm the compliance, for few and annually variable parameters exceeding the regulatory limit (Sicilia Dip, 2016), with sulfate, manganese, BOD5 and total coliform as the most frequent. From our study, of the 7 reservoirs included in the Region's analysis, two lakes (#9, 12) still fell in the AII class, while the others would not comply mainly due to BOD5 and others spotted parameters exceedance.

As for the other lakes, they would not comply with the limits of water bodies intended for human use for organic pollution plus conductivity and sulfate at the same time. All the lakes are characterized by a high ionic content, with Ca^{++} >60 mg/L in all but #9, due to the calcareous nature of the soil, and sulfate rich waters (Naselli-Flores and Barone, 1994).

Worth of note, Al and Fe were sometimes present at relatively high levels. It has been reported that while dissolved Al concentrations in waters with near-neutral pH values are usually low (1–50 μg/l), in waters with low pH or rich in organic matter and with high pH values Al content can rise to 500–1000 μg/l (WHO, 2010): exactly the case of this study's reservoirs, in 5 of which (# 2, 4, 6, 9, 11) spotted values exceeding 1000 μg/l were detected. Moreover, Al and Fe salts can be used as coagulants to eliminate turbidity, promoting the removal by precipitation of the suspended colloidal substances and phytoplankton in the water. Therefore, a possible use of these salts in the lakes could explain the accumulation of the soluble fraction of these metals (Aboubaraka et al., 2017; Deng et al., 2017). Furthermore, most of the highest values are measured during summer, when lakes are subjected to strong water volume reduction due to evaporation, increased outflow of the water for irrigation and/or drinking and consequent, increasing salts concentration (Table 1).

Some debates are underway on the toxicity of Al and Fe in humans. Fe overload is associated with liver disease (cirrhosis, cancer), heart attack or heart failure, diabetes mellitus, arthrosis and osteoporosis, metabolic syndrome, hypothyroidism and hypogonadism (Fiore et al., 2019; Camiolo et al., 2019) such us the progression of neurodegenerative diseases (Alzheimer, early-onset Parkinson's, Huntington's, epilepsy and multiple sclerosis) (Ferrante and Conti, 2017; WHO, 2010, Corkins, 2019). Dietary intake of Al and Fe is the main source of exposure and drinking water is generally a minor source of chronic exposure. The highest Al values found in this study are above the 2010 WHO threshold for chronic daily lifetime exposure (0.9 mg/L for adults) (WHO, 2010; EFSA, 2008). However, considering that the reservoirs are probably not used continuously for drinking purposes but only in case of deficiency and considering the increase in 2011 of the daily intake thresholds from 1 to 2 mg/kg of body weight (WHO, 2011), the found values would not endanger human health. Even for Fe, exceedances of the limit value by national legislation were found in lakes # 4 and # 11 with spotted values, but these levels do not represent any real risk for the population. Indeed, elemental Fe, the amount of which depends on the formulation of the ingested Fe salts, begins to have some toxic effect above 20 mg/kg of body weight (Yuen and Becker, 2020). Furthermore, WHO does not consider providing a guide value (WHO, 2017).

Therefore, since environment risk related to the presence of MCs in waters could represent one of more severe risk of human exposure, we proposed a simple risk-based model combining the presence, current and prospected, of CB and the MC level, providing an useful tool for risk managers to estimate the need of monitoring activities to prevent cyanotoxins exposure through supplying water. Cutpoints have been defined and proposed by the authors of the present study to set the model. Indeed, the present study indicated that these artificial water bodies are characterized by a variable phytoplankton community, whose structure can change over a month, possibly leading to high cyanobacterial biomass. A regular systematic monitoring of all the lakes could be costly and unfeasible but results from PCA and the model suggest the priority for monitoring only for a few of them. The two approaches were in good agreement: 5 out of 6 water bodies for which the model indicated a need for monitoring are located in the right part of the plot, susceptible to CB blooms; the remaining one, (#2) for which monitoring for CB is recommended is the only exception. This outcome is even more relevant, since the reservoir is yet used for drinking purposes and the other results did not flag any concern.

5. Conclusion

Due to climate changes and the growing dramatic water shortage and drought, all water supplies must be consequentially considered for drinking use.

There are a lot of papers that correlates cyanobacteria presence and physico-chemical factors, but no one includes the determination of all water quality parameters, the identification of cyanobacterial species, the quantification of cyanotoxins (MCs), and the identification of cyanotoxins producing cells by PCR. Moreover, the PCA analysis seems to give a more representative view, showing the general state of the basins and, rather than the effect of individual parameters, the complexity of the favorable conditions for cyanobacteria growth.

We proposed, also, a model to be used as warning system for managers to perform further monitoring and prevent cyanotoxins exposure.

Concerning the MCs contamination, although in all analyzed samples the concentration was well below the WHO reference value for drinking waters (1.0 μg/l), due to the high variability of the lakes, to the possible rapid growth and the sudden shift of cyanobacteria species, an implementation of the monitoring network would be necessary. The suggested environmental risk-based model provides an adequate and cost-effective tool for managers, to focus on the few water bodies that really need to be monitored.

Although the present study gives indication about the possible favorable condition for toxic cyanobacteria growth and on the risk evaluation on microcystins exposure associated to the possible use of Sicilian surface freshwater as supplying waters, monitoring activities over time longer than one year for the 6 identified reservoirs is warranted. Our study, through the analysis of a large number of parameters from 15 artificial reservoirs, highlighted the exceedance of only a few of them. Therefore, the chemical status of Sicilian freshwater reservoirs would be easily improved to good status, by adopting a better treatment and management of urban and rural wastewaters. The aim is to reduce the supply of nutrients and, consequently, to improve the microbiological quality of waters and decrease the possibility of cyanobacterial blooms.

Credit author statement

Zuccarello P: Writing - original draft, Writing- Reviewing and Editing, sampling and chemical analysis, statistical data analysis. Manganelli M: Writing - original draft preparation, Cyanobacteria analysis, Data curation. Oliveri Conti G: Conceptualization, Reviewing and Editing, corrisponder author. Copat C: Heavy metals analysis. Grasso A: Microbiological analysis. Cristaldi A: Chemical analysis. De Angelis G: Molecular Analysis. Testai E: Supervision and Conceptualization. Stefanelli M: Cyanobacteria analysis. Vichi S: PCR analysis. Fiore M: Methodology and data curation. Ferrante M: Supervisor and Responsible of research project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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