Supplementary Materials: Occurrence of Microcystins and Toxin-Producing Cyanobacteria in Reservoirs of the Alqueva Water Storage System (Southern Portugal)

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Results:

Table S1. Detection of cyanobacteria 16S rRNA and Phycocyanin gene sequences by PCR, using respectively the pair of primers 27F/209R and PCβF/PCβR. (+) indicate samples where both genes were detected. In contrast, absence of symbol indicate samples where these genes were not detected.

Month/Location	S. Pedro			Magra		Pisão			
	SP1	SP2	SP3	M1	M2	P1	P2	P3	P4
July	+	+	+	+					
August	+	+	+		+			+	
September	+	+	+	+	+		+	+	

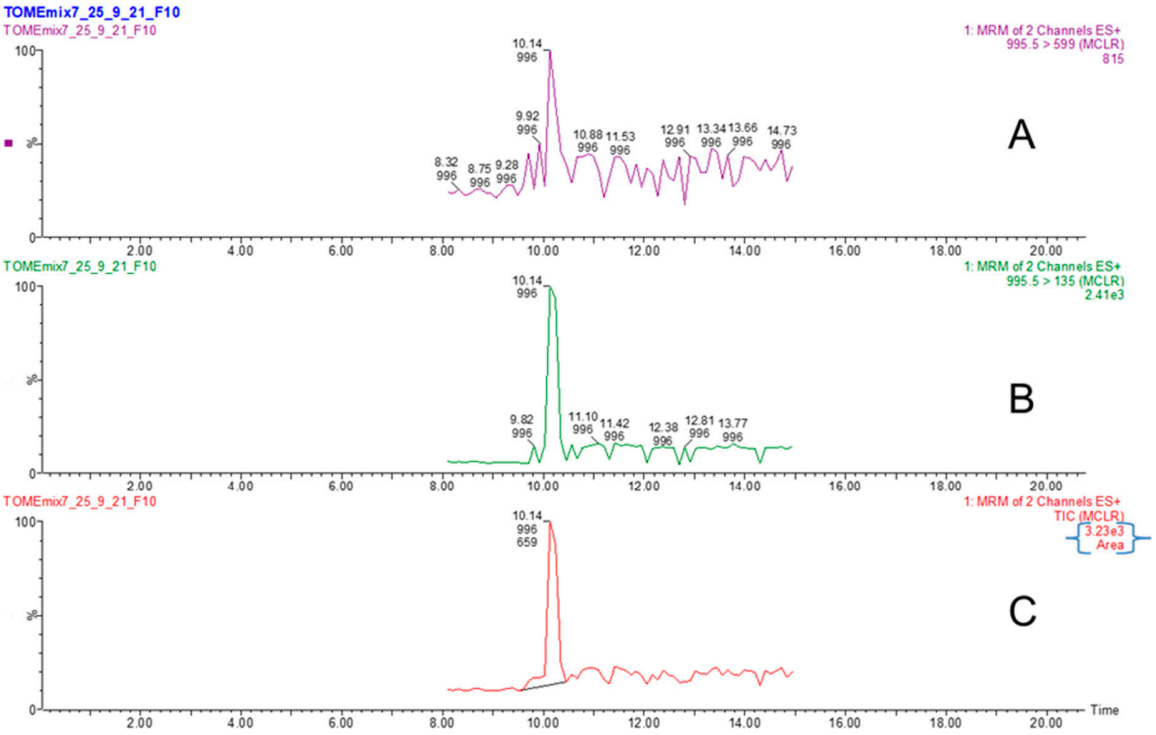


Figure S1. MRM chromatograms and respective transitions of MC-LR in the SP3 sample from July. A) Extracted ion chromatogram (XIC) of the 995.5 > 599 transition; B) Extracted ion chromatogram (XIC) of the 995.5 > 135 transition; C) MRM total ion chromatogram (TIC), with the peak area associated with MC-LR highlighted in blue for quantification.

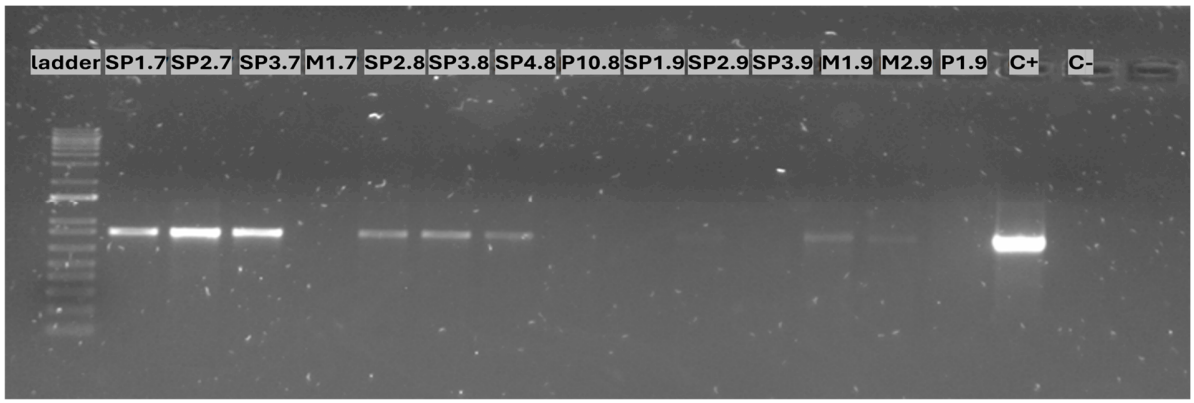
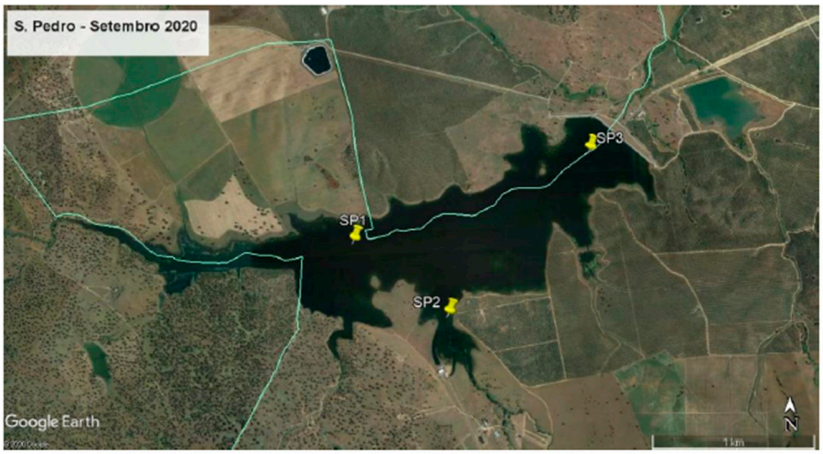


Figure S2. Results of agarose gel electrophoresis of amplified DNA fragments corresponding to mcyD (Microcystis sp.-specific). On the left, the molecular weight marker, followed by PCR amplification products from the environmental samples identified at the top. C+ – positive control; C− – negative control. Samples showing a luminescent band with the same molecular weight as the C+ are considered positive, whereas the remaining samples are negative for the fragment associated with the mcyD gene.

Methods:

a)



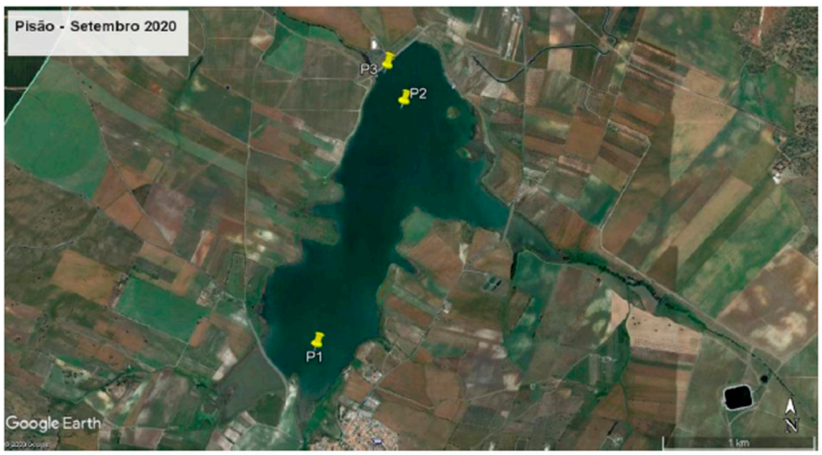
SP1 – 38°05'04.7"N 7°46'08.5"W; SP2 – 38°05'11.4"N 7°45'36.7"W; SP3 - 38°05'23.7"N 7°45'10.1"W

b)



M1 – 38°02'58.0"N 7°46'04.8"W; M2 – 38°02'43.4"N 7°46'00.8"W

c)



P1 - 38°04'43.0''N, 7°58'50.6''W; P2 -38°04'21.7''N, 7°58'29.8''W; P3 - 38°03'48.6''N, 7°59'15.9''W

Figure S3. Sampling points in S. Pedro (a), Magra (b) and Pisão (c) reservoirs and respective GPS coordinates. .

Table S2. Mass-spectrometry operating parameters for detection and quantification of cyanotoxins.

Cyanotoxin	Mass-charge transition (*m/z*)	Colision energy	Limit of detection (µg/L)
Anatoxin-a	166>131 166>149	20 25	5,7 ± 0,3
Cylindrospermopsin	416>336 416>194	20 28	3,8 ± 0,7
Saxitoxin	300>282 300>204	20 25	7,9 ± 1,3
Microcystin-LR	995.5>135 995.5>599	50 50	2,9 ± 0,8
Microcystin-RR	520>70 520>135	50 50	4,1 ± 0,3
Microcystin-YR	1045.5>135 1045.5>175	50 50	3,2 ± 0,8

Cyanotoxin	Retentio Time	Mass-to-charge ratio (*m/z*) transition	Cone (V)	Collision energy (V)
Microcystin-YR	12.90	1045.5>13.2 1045.5>599	20 20	60 55
Microcystin-Lw	16.22	1047.3>579.5	50	20
Microcystin-La	15.49	932.5>135 932.5>599.1	50 50	20 20
Microcystin-LR	13.25 15.00 (gradient 1 and2)	996.5>135.05 995.5>599.2	50 50	30 30
Microcystin-RR	12.30	520.3>105.1 520.3>135.1	20 20	40 30
Microcystin-LF	16.16	1008.6>515.1	50	20
Microcystin-Ly	15054	1024.5>677.2	50	20
Nodularin-R	12.98	825.3>135.5	50	20
Cilindrospermopsin	3.9016	415.9>176	20	80

Table S3. Primer sets used in PCR for the detection of cyanobacteria (16S rRNA and the phycocyanin operon), and of the 16S rRNA gene of *Microcystis*.

Gene	Primer	Primer sequence 5´-3´	Sequence size (base pairs)	Reference
16S rRNA cyanobateria	27F	AGAGTTTGATCCTGGCTCAG	780	[18], [36]
	809R	GCTTCGGCACGGCTCGGGTCGATA		
	740F	GGCYRWAWCTGACACTSAGGGA	754	[18]
	1494R	TACGGTTACCTTGTTACGAC		
Operon phycocyanin	PC?F	GGCTGCTTGTTTACGCGACA	700	[19]
	PC?R	CCAGTACCACCAGCAACTAA		
16S Rrna Microcystis sp.	Mic 184F	GCCGCRAGGTGAAAMCTAA	220	[18]
	Mic 431R	AATCCAAARACCTTCCTCCC		

Table S4. Primer sets used in PCR for the detection of genes responsible for MC synthesis, without taxonomic distinction (general), and in *Microcystis* sp.

Gene	Primer		Primer sequence 5´-3´	Sequence size (base pairs)	Reference
Geral	mcyA	CD1 F	AAAATTAAAAGCCGTATCAAA	297	[37]
		CD1 R	AAAAGTGTTTTATTAGCGGGCTCAT		
	mcyB	FAA	CTATGTTATTTATACATCAGG	758	[38]
		RAA	CTCAGCTTAACTTGATTATC		
Microcystin sp.	mcyA	MSF	ATCACGCAGTTGAGCAAGC	1300	[39]
		MSR	TGCAGATAACTCCGCA		
	mcyB	2156-F	ATCACTTCAATCTAACGACT	955	[21]
		3111-R	AGTTGCTGCTGTAAGAAA		
	mcyC	PSCF1	GCAACATCCCAAGAGCAAAG	674	[20]
		PSCR1	CCGACAACATCACAAAGGC		
	mcyD	PKDF1	GACGCTCAAATGATGAAAC	647	[20]
		PKDR1	GCAACCGATAAAAACTCCC		
	mcyE	PKEF1	CGCAAACCCGATTTACAG	755	[20]
		PKER1	CCCCTACCATCTTCATCTTC		
	mcyG	PKGF1	ACTCTCAAGTTATCCTCCCTC	425	[20]
		PKGR1	AATCGCTAAAACGCCACC		

Table S5. PCR program settings applied for the detection of cyanobacteria, *Microcystis* sp., and microcystin-producing genes.

Gene-alvo		Initial denaturing		35 cycles						Final extension	
				Denaturing		Annealing		Extension			
		Temp.	Time	Temp.	Time	Temp.	Time	Temp.	Time	Temp.	Time
16S rRNA Cyanobacteria		92 °C	2 min	92 °C	20 seg	50 °C	30 seg	72 °C	1 min	72 °C	5 min
Operon phycocyanin		94 °C	3 min	94 °C	30 seg	50 °C	30 seg	72 °C	30 seg	72 °C	5 min
16S rRNA Microcystis sp.		95 °C	2 min	95 °C	90 seg	56 °C	30 seg	72 °C	50 seg	72 °C	7 min
Microcystin SP.	mcy A	95 °C	2 min	95 °C	90 seg	56 °C	30 seg	72 °C	50 seg	72 °C	7 min
	mcy B	94 °C	2 min	93 °C	10 seg	50 °C	20 seg	72 °C	1 min	72 °C	5 min
	mcy A	95 °C	2 min	95 °C	90 seg	60 °C	30 seg	72 °C	50 seg	72 °C	7 min
	mcy B	94 °C	5 min	95 °C	1 min	52 °C	30 seg	72 °C	1 min	72 °C	7 min
	mcy C	94 °C	5 min	95 °C	1 min	52 °C	30 seg	72 °C	1 min	72 °C	7 min
	mcy D	94 °C	5 min	95 °C	1 min	52 °C	30 seg	72 °C	1 min	72 °C	7 min
	mcy E	94 °C	5 min	95 °C	1 min	52 °C	30 seg	72 °C	1 min	72 °C	7 min
	mcy G	94 °C	5 min	95 °C	1 min	52 °C	30 seg	72 °C	1 min	72 °C	7 min