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ELSEVIER

Toxicon xx (2005) 1–10

TOXICON

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Intracellular and dissolved microcystin in reservoirs of the river Segura basin, Murcia, SE Spain

Marina Aboal[a,*], Maria-Ángeles Puig[b]

[a]*Laboratorio de Algología, Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia,*
Campus de Espinardo, E-30100 Murcia, España,
[b]*Departamento de Biogeoquímica Aquatica, Centro de Estudios Avanzados de Blanes (CEAB-CSIC).*
Carrer d'accès a la Cala Sant Francesc, 14, 17300 Blanes, Girona, España

Received 24 June 2004; revised 9 December 2004; accepted 10 December 2004

Abstract

The seasonal sampling of irrigation or drinking water reservoirs in the province of Murcia (SE Spain) in the hydrological year 2000–2001 revealed the presence of intracellular microcystins in the benthic cyanophyte communities throughout the year. The total microcystin levels, as measured by HPLC, were relatively high but lower than others published for planktonic communities of the European continent or certain African countries. The diversity of forms was also very high and comparable to those found for other European countries. The concentrations of microcystins dissolved in the water were always below limits recommended by the WHO for drinking water and, during most of the year, undetectable by immunological methods. We discuss the need for setting up a control network for detecting benthic cyanobacteria growth to prevent long-term undesirable effects in the human population in small towns (through drinking water or the consumption of vegetables) and in wild animals. In the particular case of the Iberian Peninsula, a joint strategy between Portugal and Spain is recommendable.

Keywords: Cyanotoxins; Microcystins; Benthic cyanobacteria; Reservoirs; SE Spain

1. Introduction

The proliferation of planktonic cyanobacteria in lakes and reservoirs is a worldwide problem (Jones, 1994; Carmichael, 1997; Chorus and Bartram, 1999) and cases of toxicity have been registered from the Antarctic to the tropics (Brittain et al., 2000; Hitzfeld et al., 2000; Park et al., 2001; Freitas de Magalhaes et al., 2001; Li et al., 2001).

Recent years have seen an increased frequency in this type of episode, partly due to the increased degree of eutrophy of lacustrine systems. Associated to this, a greater production of toxins has been detected, which, in some parts of the world, has reached very high levels: Scandinavia (Sivonen et al., 1990), Denmark (Henriksen and Moestrup, 1997), Portugal (Vasconcelos, 1994), Germany (Fastner et al., 1999) and France (Vezie et al., 1997).

Besides high nutrient concentrations, high temperatures also favour microalgal proliferation. Many cases describing the massive development of cyanobacteria have been recorded in the literature and there is generalised concern in this respect because of the likely effects of global warming, especially as regards those species that produce toxins.

The principal toxins detected in cyanobacteria are microcystins (peptides) and neurotoxins (alkaloids), both of which may be lethal even in low concentrations. The WHO has published protocols concerning their detection and recommendations for maximum permitted concentrations in water destined for human consumption and recreational use (Chorus and Bartram, 1999).

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* Corresponding author. Tel.: +96 836 4990; fax: +96 836 3963.
E-mail address: maboal@um.es (M. Aboal).

0041-0101/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.toxicon.2004.12.012

Even sub-lethal doses are cause for concern as they may induce certain types of cancer. These substances must be carefully controlled since they are extremely refractory and accumulate in the food chain. There is also evidence that these toxins may be retained in plants irrigated with water containing toxic cyanobacteria (Codd et al., 1999a,b). All this, then, explains the preoccupation of several countries, which, after observing the numerous cases of massive growth of these cyanobacteria in lakes, rivers and reservoirs, have implemented networks for their control (Chorus and Bartram, 1999).

However, there are few data about the proliferation of planktonic cyanobacteria and the production of cyanotoxins in the Mediterranean area, although some reports have been published for Greece (Lanaras et al., 1989), Egypt (Yanni and Carmichael, 1997; Brittain et al., 2000), Israel (Banker et al., 1997), Italy (Bruno et al., 1992, 1994; Prati et al., 2002) and Morocco (Oudra et al., 1998, 2001). In addition, very few references can be found about the toxic potential of benthic cyanobacteria (Carmichael et al., 1997, Codd et al., 1999a,b; Marsalek et al., 1997; Hitzfeld et al., 2000).

In Spain, apart from some isolated data for a reservoir serving Madrid (Padilla et al., 1999) and the coastal lagoon of Albufera in Valencia (Bradt and Villena, 2001), little information is available on toxicity in reservoirs and lakes. Probably the most thorough investigation has been carried out in calcareous rivers and streams (Ríos et al., 2000; Aboal et al., 2000, 2002).

The massive development of planktonic and benthic species occurs on a seasonal basis in some reservoirs destined for human supply or crop irrigation in the River Segura basin (Aboal et al., 1996). Consequently, it would seem advisable to verify and quantify any toxins that may be present with the aim of setting up measures for their control.

This led us to make an extensive sampling in the reservoirs of the River Segura basin (both in Murcia and neighbouring provinces, whose waters are destined for Murcia) to confirm the production of microcystins by benthic communities and the presence of dissolved microcystins in the water.

2. Material and methods

2.1. Study area

The River Segura rises in the Sierra del Segura, Jaén, and crosses the provinces of Albacete, Murcia and Alicante before entering the Mediterranean Sea near Guardamar in

Fig. 1. Map with the location of reservoirs.

Table 1
Main characteristics and use of the reservoirs of Murcia region studied

Reservoir	River	Altitude (m.b.s.l)	Drainage area (km^2)	Capacity (Hm^3)	Regulated volume (Hm^3)	Use
Alfonso XIII	Quipar	256	834	22	12	Irrigation, flooding control
Argos	Argos	276	505	10	55	Irrigation
Cenajo	Segura	350	2593	437	105	Irrigation, flooding control
Cierva	Mula	335	156	7	2	Irrigation, flooding control
Ojos	Segura	–	–	1	–	Irrigation, human supply
Pedrera	Alcoriza	–	–	246	–	Irrigation, flooding control
Puentes	Guadalentín	402	1389	48	10	Irrigation, flooding control, hydroelectric power
Valdeinfierno	Guadalentín	638	311	13	6	Irrigation, flooding control

the last of these provinces (Fig. 1). The flow regime is very irregular and the river frequently rises considerably and floods the surrounding countryside.

Within the Segura basin, 29 structures exist to regulate the flow, prevent flooding, produce electricity or provide drinking and irrigation water. After an initial inspection, we chose eight reservoirs (Cierva, Ojos, Alfonso XIII, Puentes, Valdeinfierno, Argos and Cenajo in Murcia and Pedrera in Alicante), all belonging to the middle or lower basin and whose waters are principally destined for irrigation, with a small proportion for human consumption (Table 1).

2.2. Sampling

Seasonal samplings were carried out in the eight reservoirs; temperature, pH, conductivity and oxygen content were recorded in situ at the same time.

The water samples collected for chemical analysis were kept refrigerated for transport to the laboratory, while samples for detecting toxins were frozen (in triplicate) by liquid nitrogen in the field.

Benthic material was collected in littoral areas by scraping an area of 12 cm^2 of benthic communities with a scalpel (two replicates, one of which was fixed in

Table 2
Minima, maxima and mean of physico-chemical variables of water from river Segura basin reservoirs

Reservoirs	Conductivity (μS/cm^2)	pH	Temperature (°C)	Si (μM/l)	Ortophosphate (μM/l)	Nitrite (μM/l)	Nitrate (μM/l)
ALFONSO XIII							
Minimum–maximum	4900–9360	8.1–8.6	13.9–28	45.3–105.9	0.07–0.7	0.6–1.8	4.1–29.2
Mean	7497	8.3	19.3	73.9	0.4	1.1	13.5
ARGOS							
Minimum–maximum	1670–2090	8.2–8.7	12.9–30	1.5–100.8	0.4–2.2	0.5–8.3	1.8–152.5
Mean	1847	8.5	20.9	57.4	0.9	2.7	50.7
CENAJO							
Minimum–maximum	428–4730	8.5–8.9	11.8–22.5	46.2–86	0–0.05	0.4–1.2	34.1–70.5
Mean	1574	8.6	16.5	66.4	0.01	0.8	49.3
CIERVA							
Minimum–maximum	854–1090	8.4–8.7	13.7–25.7	47.4–110.4	0–0.2	0.9–2.3	39.1–89.2
Mean	1019	8.7	17.4	25.2	0.1	1.0	34.3
OJOS							
Minimum–maximum	1020–1160	8.1–8.3	13.7–25.7	47.4–110.4	0–0.3	0.9–2.3	39.1–89.2
Mean	1056	8.2	18.2	84.4	0.1	1.6	66.8
PEDRERA							
Minimum–maximum	1020–1200	8.2–9.2	14.3–26.8	7.6–81.5	0.02–0.1	0.6–5.6	14.8–160.9
Mean	1131	8.6	18.7	43.5	0.05	2.2	69.3
PUENTES							
Minimum–maximum	2870–3960	7.7–8.3	11.3–26.4	166.2–242.9	0.3–0.5	0.3–1	26.0–96.6
Mean	3555	8.2	18.3	218.9	0.36	0.7	57.9
VALDEINFIERNO							
Minimum–maximum	2040–8000	8–8.2	12.4–25.1	10.6–115	0.2–1.9	0–1	5.6–39
Mean	4150	8.1	18.7	64.6	0.6	0.5	19.1

glutaraldehide for taxonomic study and the other frozen with liquid nitrogen for toxicity tests).

Samples (250 ml) of water were collected and refrigerated to analyse the phytoplanktonic community in the laboratory.

2.3. Laboratory analysis

The levels of the nutrients were determined according to Standard Methods (APHA, 1998).

Prior to the immunological analysis, the water samples were filtered through Whatman G/C filters, while the microcystins dissolved in the water were quantified with commercial ELISA kit (Platekit 75400, Strategic Diagnostics Inc. Envirogard®).

The samples were extracted with methanol according to Gjolme and Utkilen (1996) and the toxin content was measured using, high performance liquid chromatography (HPLC-PAD) with an internal surface reverse-phase column (Meriluoto et al., 1990; Gjolme and Utkilen, 1996). The mobile phase was in a gradient from 70% TFA (0.5 ml/l) to 0 and 30% TFA in acetonitrile (=ml per l) to 100% at 45 min and back to 70% TFA in water and 30% TFA in acetonitrile at 46 until 55 min. The flow rate was 1 ml min^{-1}. A photodiode array set between at 200–300 nm was used as detector. The toxin content was quatified by calibrating against purified toxin standards (MC-RR, MC-LR and MC-YR) from Calbiochem. Other unidentified micocystin varieties were detected by their UV-spectra and quantified as equivalent MC-LR.

2.4. Statistical analysis

The correlations between variables were calculated using the STATISTICA program, version 5.5.

3. Results

The physical–chemical variables were within the range expected for reservoirs on a calcareous substrate (Table 2). The high conductivity (1019–7497.50 µS/cm^2) and sulphate concentration (32.18–8743.5 ppm) were due to

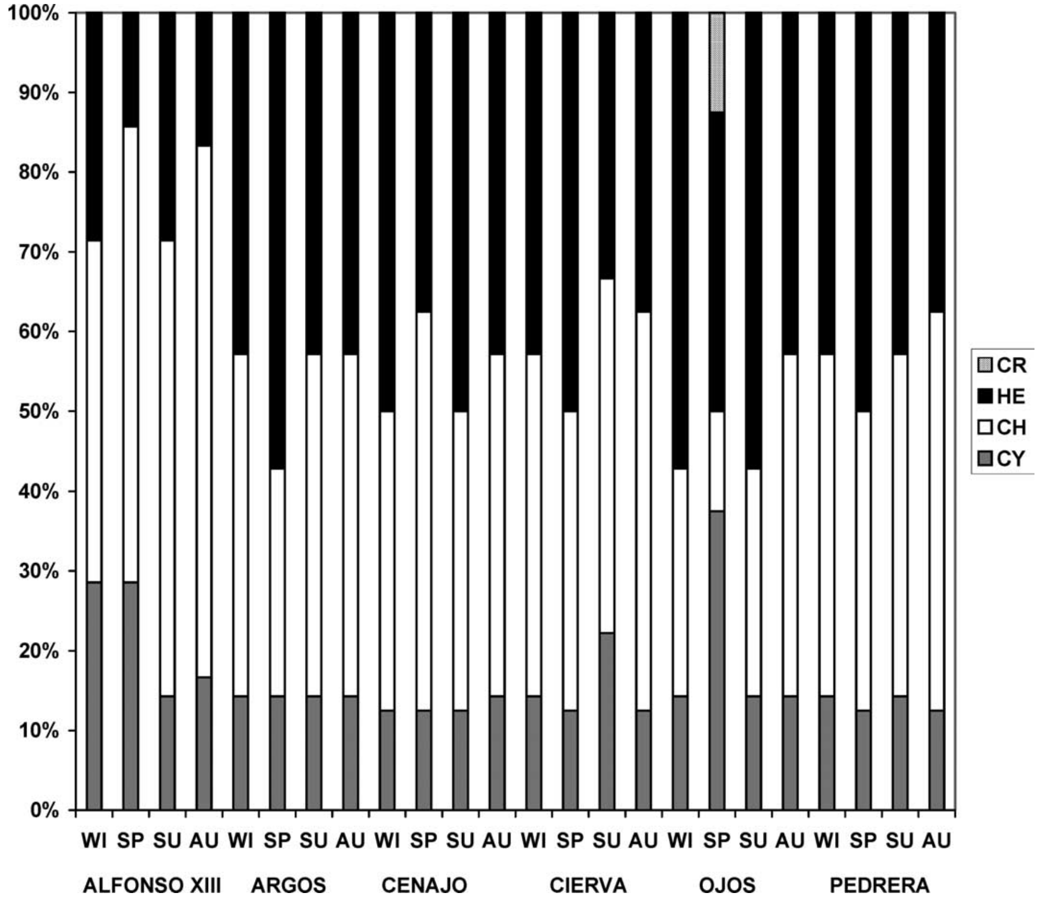

Fig. 2. Seasonal variation of phytoplankton communities in reservoirs, where microcystins were detected (CR, Cryptophyta; HE, Heterokontophyta; CH, Chlorophyta; CY, Cyanophyta; WI, winter; SP, spring; SU, summer; AU, autumn).

Table 3
Cyanophyta developping macroscopic assemblages in planktonic or benthic habitats in Murcia reservoirs, where microcystins were detected.

	ALFONSO XIII				CENAJO				CIERVA				OJOS				PEDRERA				PUENTES			
	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI
PLANKTON																								
Aphanizomenon flos-aquae												■					■		■	■				
BENTHOS																								
Rivularia biasolettiana					■	■	■																	
Tolypothrix distorta					■	■	■	■																
Phormidium sp.		■			■	■	■	■									■	■						■
Calothrix parietina											■													
Oscillatoria sp.		■					■						■	■	■	■								
Lyngbya sp.		■												■		■	■	■					■	
Microcoleus sp.																							■	

the lithology, since patches of gypsum-rich loams exist throughout the study area.

The phosphorus and the nitrogen levels varied greatly: from <0.01 μg/l of orthophosphate in Cenajo and Cierva to 208.9 in Argos and from 0.11 mg/l of nitrate in Argos to 9.98 in Pedrera. Four of the reservoirs can be considered oligotrophic and four eutrophic. The high N/P molar ratio in excess of 16 pointed to a phosphorus deficiency throughout the year. Ammonium was not usually detected in any of the reservoirs but reached the highest concentration in Ojos in winter.

Although the presence of blooms of planktonic cyanobacteria, such as *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, has been detected in Pedrera and other reservoirs in other hydrological years, no such phenomenon was detected during the year studied.

In general, planktonic communities were poor (Fig. 2) and the presence of cyanobacteria scarce, although more or less permanent benthic communities may develop as a result of the use to which the waters are put. The predominant genera in these benthic communities were *Scytonema*, *Calothrix*, *Phormidium*, *Rivularia*, *Lyngbya* and *Tolypothrix*, which form very conspicuous carpets (Table 3).

The occurrence of microcystins in water differed in the reservoirs studied. Dissolved microcystins were detected in Cenajo throughout the year with values of between 0.032 and 0.078 ppb. In Ojos they were detected only in summer (0.170 ppb) and were not detected in the other reservoirs in any season. The evolution of the concentrations of both intracellular and dissolved microcystins seems to follow an opposite pattern (Fig. 3), although no statistical correlation could be detected. The concentrations were always lower than those recommended by the WHO for drinking water.

As many as five intracellular microcystin varieties were identified and quantified. Generally all the varieties were present in the same sample but MC-LR was the dominant variety in most (Table 4). The proportion of MC-LR ranged from 2.6 to 74%, of MC-RR from 3 to 75%, and of MC-YR from 1 to 53%. Only in spring and autumn in Ojos, in spring in Cenajo and in autumn and winter in Puentes was MC-RR the predominan microcystin, which was probably associated with the dominance of oscilatoriales (*Oscillatoria*, *Lyngbya* and *Phormidium*). In the last reservoirs, intracellular microcystins were detected throughout the year, while in Pedrera, Cierva and Alfonso XIII they were detected only in summer–autumn (Table 4). Only in two of the reservoirs were dissolved intracellular microcystins never detected: Valdeinfierno and Argos.

The absolute values of total microcystins per dry weight varied from 0.098 μg/g in Alfonso XIII and 8.611 μg/g in Cenajo in summer. The highest absolute values of concentration per dry weight were detected for all the varieties in summer in Cenajo, except in the case of MC-1 which was at its highest concentration in autumn, also in Cenajo (Table 4).

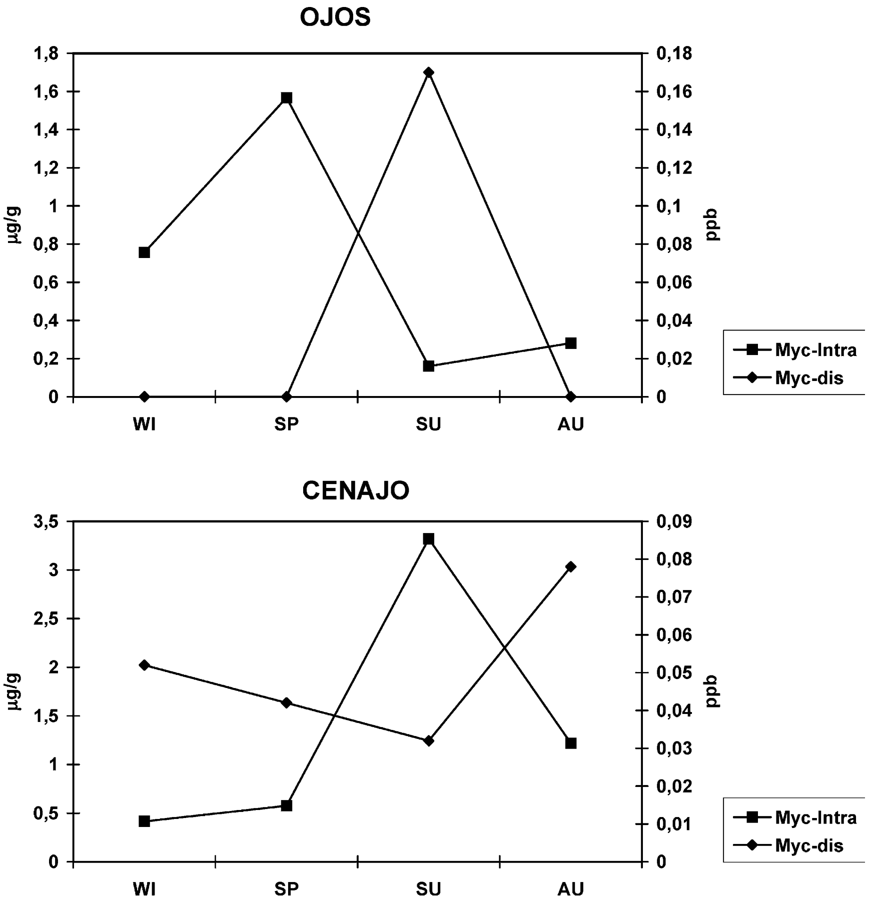

Fig. 3. Seasonal variation of intracellular and dissolved microcystins in the reservoirs of Ojos and Cenajo (WI, winter; SP, spring; SU, summer; AU, autumn).

All the microcystin varieties were correlated with silicates, except MC-RR. MC-LR was correlated negatively with conductivity and MC-2 with chloride, although the correlation was slight in both cases (Table 5). No correlation with nitrogen or phosphorus was observed.

4. Discussion

The present study clearly demonstrates the extensive occurrence of microcystin-producing cyanobacteria in the benthos of the reservoirs of Murcia (South East Spain) and neighbouring provinces. The percentage of sites containing microcystins (75%) is similar to the 60% found in Portugal (Vasconcelos, 1994) and the 70% in France (Vezie et al., 1997). Only two of the reservoirs included in the study were free of microcystins throughout the year (Valdeinfierno and Argos). However, the data concerning the percentage of water bodies with microcystins must be treated with caution because of the difficulties involved in comparing results obtained with different sampling methods and because the published data refer to planktonic toxins.

We detected toxins in the benthic cyanophyte communities growing in the reservoirs throughout the year, although the pattern of growth varied. It seems that microcystin production is more seasonally dependent in some places than in others (Mez et al., 1997) as a response to ecological conditions (Hitzfeld et al., 2000).

A correlation was found between silicates and the increased production of toxins in Alfonso XIII, Cenajo, Cierva, Ojos and Pedrera. This high correlation seems to indicate a relationship between the presence of microcystins and diatom decline. The predominant varieties in most samples were MC-LR and MC-RR, which reached their highest concentrations in summer in Cenajo. However, in most of the reservoirs studied, the production of microcystins did not seem to be linked to nutrient levels. No statistical correlation was observed and the presence of microcystins was more frequent in oligotrophic water. Microcystins were never detected in the most eutrophic reservoirs (Valdeinfierno and Argos).

Table 4
Concentrations of the varieties of intracellular microcystins identified

	MC-1 (μg/g)	MC-RR (μg/g)	MC-YR (μg/g)	MC-LR (μg/g)	MC-2 (μg/g)	Tot-MC (μg/g)	Dis-MC (ppb)
Alfonso XIII winter	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alfonso XIII spring	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alfonso XIII summer	n.d.	n.d.	0.003	0.037	0.027	0.067	n.d.
Alfonso XIII autumn	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cenajo winter	0.229	0.051	0.029	0.079	0.028	0.415	0.052
Cenajo spring	n.d.	0.265	0.311	n.d.	n.d.	0.576	0.042
Cenajo summer	0.747	0.023	0.308	0.473	1.770	3.320	0.032
Cenajo autumn	0.096	0.111	0.025	0.174	1.178	1.219	0.078
Cierva winter	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cierva spring	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cierva summer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cierva autumn	n.d.	n.d.	0.040	0.185	0.093	0.318	n.d.
Ojos winter	0.300	0.169	0.020	0.266	n.d.	0.756	n.d.
Ojos spring	0.212	0.546	0.092	0.360	0.386	1.568	n.d.
Ojos summer	n.d.	0.040	n.d.	0.120	n.d.	0.160	0.170
Ojos autumn	0.082	0.069	0.007	0.099	0.026	0.281	n.d.
Pedrera winter	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pedrera spring	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pedrera summer	n.d.	0.107	0.006	0.143	0.053	0.308	n.d.
Pedrera autumn	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Puentes winter	n.d.	0.026	0.023	0.073	0.114	0.235	n.d.
Puentes spring	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Puentes summer	n.d.	n.d.	0.013	0.045	0.040	0.098	n.d.
Puentes autumn	n.d.	0.041	0.056	0.157	0.089	0.341	n.d.

The level of detection was 0.2 μg/ml for intracellular microcystins and 0.01 ppb for dissolved microcystins (n.d., not detected).

The diversity of the microcystins found in the benthic samples from Murcia is similar to that found in planktonic samples in Portugal (Vasconcelos et al., 1996), even though the Portuguese samples covered a much wider area and included a greater variety of water systems. However, in other geographical zones, e. g. Australia, the data suggest that benthic species (of the genera *Nostoc*, *Lyngbya* and *Gloeotrichia*) are not toxin producers (Baker and Humpage, 1994). This confirms that the capacity to synthesize microcystins is not a taxonomic character, since even different strains of the same species may behave differently in this respect.

The values for total microcystins reported in Europe vary from 1.276 μg/mg DW in Denmark (Henriksen, 1996) to 7.1 μg/mg DW in Portugal (Vasconcelos et al., 1996). The values reported here are lower and it should be borne in mind that all the samples were benthic samples, which usually contain a high proportion of precipitates.

In the hydrological year studied, cyanophyte blooms were not detected and cyanobacteria were never the predominant element in the phytoplanktonic communities; furthermore, the N/P ratio was always higher than 29 (Smith, 1983). However, cyanobacteria were the principal elements in the benthic communities and, even though it cannot be ruled out that microcystins might come from upstream reservoirs, a portion of the intracellular microcystins produced by benthic species are most probably released into water during their senescent stages. In this particular case, there were no reported cases of cyanobacteria proliferation in any upstream reservoir of the basin to explain the presence of dissolved microcystins.

The composition and abundance of the benthic communities normally varied during the year. The release of large amounts of water for irrigation (for example) may bring about the complete disappearance of the benthic communities, although the intervening periods between such releases seems to be sufficient for these communities to be completely renewed because of the favourable temperature conditions prevailing in the study area. In addition, the stress caused by the changes in water level may induce the release of the intracellular toxins into the water. It is necessary to study the potential contribution of benthic communities to total microcystin production in these systems, especially in shallow waters with high retention times.

The massive development of algae is common in warm countries and in eutrophic reservoirs. Within the Mediterranean Basin, in Egypt (Mohamed and Carmichael, 2000) and Morocco (Oudra et al., 2001), microcystins have been detected in reservoirs during the warmest months, although never above the maximum limits recommended by the WHO for drinking water. However, the presence and production of microcystins in the study area is more constant and it is not linked with eutrophy.

Table 5
Correlation matrix between physico-chemical variables and microcystins ($P<0.05$)

	Sil	PO_4	NO_3	NO_2	Cond.	PH	Temp.	Cl	MC- 1	MC- RR	MC- YR	MC- LR	MC- 2	Tot- MC
Sil.	1.00	−0.06	0.04	−0.10	−0.06	−0.08	0.16	0.22	**0.56**	−0.02	**0.61**	**0.63**	**0.75**	**0.71**
PO_4	−0.06	1.00	−0.21	−0.07	0.31	−0.07	0.34	−0.20	−0.21	−0.15	−0.16	−0.21	−0.14	−0.19
NO_3	0.04	−0.21	1.00	**0.80**	−0.31	0.08	**−0.39**	−0.08	0.07	0.24	0.10	0.09	−0.02	0.07
NO_2	−0.10	−0.07	**0.80**	1.00	−0.18	0.16	−0.29	−0.13	−0.08	0.02	−0.13	−0.10	−0.12	−0.12
Cond.	−0.06	0.31	−0.31	−0.18	1.00	−0.31	0.19	−0.27	−0.33	−0.17	−0.07	**−0.39**	−0.27	−0.31
PH	−0.08	−0.07	0.08	0.16	−0.31	1.00	−0.14	0.35	0.10	−0.13	0.04	−0.16	0.07	0.00
Temp.	0.16	0.34	−0.39	−0.29	0.19	−0.14	1.00	−0.02	−0.05	−0.14	−0.13	−0.05	0.01	−0.05
Cl	0.22	−0.20	−0.08	−0.13	−0.27	**0.35**	−0.02	1.00	0.12	−0.08	0.07	0.22	**0.41**	0.30
MC − 1	**0.56**	−0.21	0.07	−0.08	−0.33	0.10	−0.05	0.12	1.00	0.32	**0.47**	**0.77**	**0.62**	**0.75**
MC − RR	−0.02	−0.15	0.24	0.02	−0.17	−0.13	−0.14	−0.08	0.32	1.00	**0.42**	**0.54**	0.18	**0.48**
MC − YR	**0.61**	−0.16	0.10	−0.13	−0.07	0.04	−0.13	0.07	**0.47**	**0.42**	1.00	**0.53**	**0.59**	**0.72**
MC − LR	**0.63**	−0.21	0.09	−0.10	**−0.39**	−0.16	−0.05	0.22	**0.77**	**0.54**	**0.53**	1.00	**0.73**	**0.89**
MC − 2	**0.75**	−0.14	−0.02	−0.12	−0.27	0.07	0.01	**0.41**	**0.62**	0.18	**0.59**	**0.73**	1.00	**0.93**
Tot − MC	**0.71**	−0.19	0.07	−0.12	−0.31	0.00	−0.05	0.30	**0.75**	**0.48**	**0.72**	**0.89**	**0.93**	1.00

Significant correlations in bold.

Although it is common to associate the massive development of cyanobacteria with increases in nutrients, it has not always been possible to establish a clear relationship between nutrient levels and toxicity, some authors even claiming that the production of toxins is not necessarily connected with eutrophication (Willén and Mattsson, 1997). It has been proposed that reactive phosphorus, rather than a low N:P ratio, is the key regulatory factor for establishing the dominance of the non-nitrogen-fixing cyanobacteria, at least in highly eutrophic systems (Xie et al., 2003). The massive development of benthic cyanobacteria, which frequently causes fatalities in cattle and domestic animals, usually occurs in oligo- or meso-trophic lakes (Owen, 1994; Mez et al., 1997). In Swedish lakes, there seems to be some correlation with the intensity of light, although in most cases reported there seems to be no relation with environmental variables (Willén and Mattsson, 1997).

The microcystins present in water destined for irrigation may not only affect crop growth and development, but may also accumulate in plant tissues (McElhiney et al., 2001). This is of special concern taking into consideration that most of the water stored in arid and semiarid areas is destined for the irrigation of horticultural crops, which are frequently consumed fresh. The long-term effect of low concentrations of microcystins is unclear, although there is some evidence that they may increase the frequency of gastrointestinal and hepatic cancers (Falconer, 1999), since MC-LR is the strongest liver carcinogen known (Dow and Swoboda, 2000).

It has been shown that lettuce leaves may contain both cyanobacteria and microcystins after irrigation with contaminated water (Codd et al., 1999a,b). Although the most usual route of human intoxication is drinking contaminated water, the accumulation of toxins in vegetables may increase the number of intoxications and heighten the long-term effects, including the risk of cancer.

Cattle and wildlife, including protected species, may also suffer from the massive development and the presence of microcystin-producing cyanobacteria (Frazier et al., 1998). Such would be the case with many species of birds that drink from the reservoirs. Cases of mortality in wild birds as a result of drinking contaminated water have been recorded in Japan (Matsunaga et al., 1999) and Denmark (Onodera et al., 1997). This is of very special concern in the Iberian Peninsula, which is located in the middle of main migratory routes.

Microcystins can accumulate in fish, especially cyprinids, since cyanobacteria may form an important part of their diet (Northcott et al., 1991; Freitas de Magalhaes et al., 2001). Some authors have suggested that these fish may avoid grazing toxic species (Beveridge et al., 1993), although in some reservoirs in Murcia, such as Ojos, where fishing is commonly practised, toxins have been detected in the benthic species throughout the year. Cyanobacteria mats not only form a fringe around the perimeter but also cover most of the bottom in Ojos, so it is therefore, extremely probable that they form an important (if not the only) part of the diet of the fish found there.

The consequences of the production and potential liberation into the water of benthic intracellular microcystins must be taken into consideration and benthic cyanobacteria must be included in control networks in an attempt to elucidate their influence in the final concentration of dissolved microcystins and to ascertain their potential effects on fauna.

The fact that the two countries that make up the Iberian Peninsula share the principal hydrographic basins points to the need to establish integral control systems applicable to the peninsula as a whole. Lending weight to this assertion is the fact that toxic blooms have been identified in the rivers Miño and Guadiana as they flow through Portugal (Vasconcelos et al., 1996) and that cases of fish mortality and human intoxication have been associated with the latter river (Oliveira, 1991).

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