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Scientific Editors

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Global Importance of Local Experience

Coimbra • Imprensa da Universidade

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TOXIC CYANOBACTERIA IN THE MONDEGO BASIN RESERVOIRS. AN OVERVIEW

Abstract

Toxic cyanobacteria in Portuguese freshwaters have been studied since 1989. Hepatotoxin producing species have been found to be the dominant contributors to bloom forming populations in a range of Portuguese lakes and reservoirs.

In the Mondego river basin reservoirs few studies have been carried out regarding phytoplankton ecology, and especially cyanobacteria. The eutrophication of this river basin, increases from upstream sites down to Aguieira reservoir but decreases downstream. The Carlson Trophic State Index for chlorophyll was used to assess eutrophication. The most eutrophic sites were Fronhas, Fagilde and Aguieira reservoirs with cyanobacteria blooms recorded during several years.

Microcystins are the only toxins found to date within bloom populations of cyanobacteria with microcystin-LR being the dominant. Other less common microcystins, such as MCYST-HilR and [MeSer^7]MCYST-LR have also been detected. Toxin concentrations within blooms vary with time. However samples collected in 1992 have been found to be the most toxic ones.

The use of the reservoirs of this river basin for drinking water withdraw, irrigation, recreation and for fishing may lead to human health hazard if no proper monitoring is performed.

Introduction

Freshwater cyanobacteria have been studied in Portugal since the early 30's. Initially, these studies focused on the taxonomy and diversity (Sampaio 1933) of potentially toxic species such as *Oscillatoria formosa* and *Lyngbya majuscula*. In 1959, Nauwerck published a paper on the occurrence of phytoplankton species in three locations close to Coimbra, identifying the cyanobacterium *Pseudanabaena catenata* in

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samples collected from Poço do Almegue, in the Mondego river (Nauwerck 1959). Later, Nauwerck (1962), gave an overview of the phytoplankton composition of several Portuguese waterbodies. This study highlighted the occurrence and dominance of cyanobacteria in some of these reservoirs. This author referred to the occurrence of the cyanobacteria *Lyngbya limnetica* and *Anabaena variabilis* in Lagoa Comprida, an oligotrophic lake located in the upper Mondego river basin, during the spring of 1960.

The first data on the toxicity of cyanobacteria in Portugal from the analysis of a *Microcystis aeruginosa* dominated toxic bloom, was recorded in the Douro river (Crestuma reservoir) in 1989 (Vasconcelos et al. 1993).

The Mondego river basin contains several reservoirs (Fig. 1) that are used for irrigation, hydroelectric power generation and also for human consumption and recreational purposes. In these reservoirs, cyanobacteria are now known to reach high concentrations, often forming thick surface scums. Such blooms represent a considerable human health risk. In this paper, an overview of the occurrence and toxicity of cyanobacteria in several reservoirs of the Mondego river basin is presented.

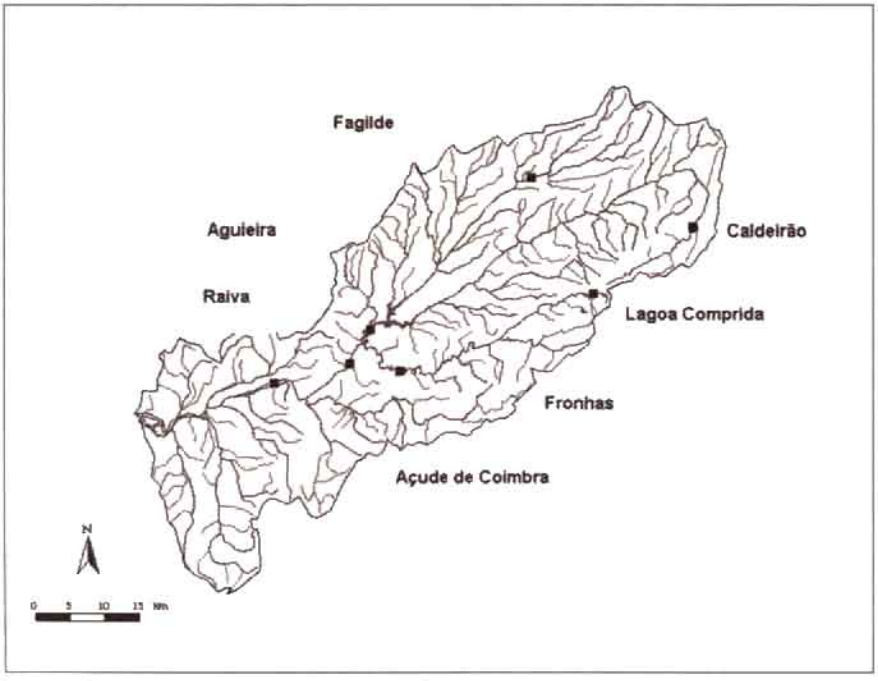

Figure 1. Location of the studied reservoirs of the Mondego river basin.

Eutrophication of Mondego river

Although the Mondego river basin is an important source for the supply of water for human consumption and also for industrial and agricultural purposes, there are few systematic studies concerning the ecology of phytoplankton within its reservoirs.

The eutrophication of two locations of Mondego river (Aguieira and Raiva reservoirs) measured by the Carlson Trophic State index for chlorophyll, is shown in Table 1. Data were obtained from the INAG site (Water Institute- Portuguese Ministry of Environment – www.inag.pt). and calculated according to Carlson (1977). This index uses chlorophyll, phosphorus or secchi disk depth. Higher values indicate more pronounced eutrophy. Taking into account the Carlson's classification of aquatic ecosystems in terms of eutrophy (Carlson 1977), both reservoirs may be classified as meso-eutrophic. In fact, the occurrence of cyanobacteria during summer months is a clear sign of eutrophication. Nevertheless, as it may be seen latter in this work, eutrophication varies considerably along the Mondego River Basin, from highly eutrophic reservoirs such as Aguieira Reservoir (located in an agricultural region) to relatively oligotrophic water bodies close to Coimbra and in the upper reaches of the basin (Lagoa Comprida).

Cyanobacteria and their toxins in Portugal

Freshwater cyanobacteria may produce different kinds of toxins having neurotoxic, hepatotoxic and dermatotoxic effects in humans and other animals (Carmichael 1997). These toxins may adversely affect aquatic animals and also terrestrial animals that drink water contaminated with cyanobacteria. Human populations may also be affected by (i) drinking water not properly treated, (ii) using it for recreation, or (iii) eating aquatic animals contaminated with cyanobacterial toxins (Chorus and Bartram 1999). Recently it was also shown that lethal human intoxication may occur by using contaminated water in dialysis systems (Jochimsen et al. 1998).

Table 1 – Trophic State Index (TSI) of Raiva and Aguieira reservoirs during 1988 to 1998 (x- average, m- minimum, M-maximum and VC- Variation coefficient).

Parameter Reservoir	Average	Minimum	Maximum	Variation Coefficient
Aguieira	49.2	35.2	66.0	15.7
Raiva	48.7	36.4	59.3	12.8

Table 2 – LD50 values (i.p mouse bioassay) of blooms and *Microcystis* strains collected from Portuguese freshwaters - maximum, minimum, average and standard deviation (SD).

	Blooms (n=18)				*Microcystis* strains (n=15)			
	Max.	Min.	Average	SD	Max	Min	Average	SD
LD_{50} SD(mg/kg)	700.0	20.0	136.0	175.0	75.0	15.0	37.5	23.5

A survey of the distribution of toxic cyanobacteria in Portuguese freshwaters began in 1989. Preliminary work surveyed 36 lakes, reservoirs and rivers during the period 1989-1992. At this time only hepatotoxic blooms were found and 60% of the blooms were found to be toxic. The main species present in toxic blooms were *Microcystis aeruginosa* (72%) and *Anabaena flos-aquae* (28%) (Vasconcelos 1994). The toxicity of the bloom samples evaluated by intraperitoneal (i.p.) mouse bioassay – LD_{50} – varied from 20 mg/kg to 700 mg/kg (dry weight of bloom sample to kg mouse body weight). *Microcystis* strains isolated from these blooms were found to be toxic, varying from 15 mg/kg to 75 mg/kg (Vasconcelos 1994) (Table 2). The main hepatotoxins in Portuguese freshwaters are MCYST-LR, MCYST-LA, MCYST-YR and [D-Asp^3]MCYST-LR (Vasconcelos et al. 1995; 1996).

Occurrence and abundance of cyanobacteria in the Mondego river basin

Cyanobacteria blooms are found only in some of the reservoirs in the Mondego river basin. In this work we review the information on cyanobacteria in this river basin from upstream sites (Lagoa Comprida) to the most downstream site (Coimbra reservoir).

Upstream sites, such as Lagoa Comprida are oligotrophic with few cyanobacteria present. Nauwerck (1962) classified this lake as oligotrophic describing *Anabaena variabilis* and *Lyngbya limnetica* as the only cyanobacteria present. Santos and Mesquita (1986) isolated several phytoplankton species from this lake and among them only one cyanobacteria species – *Fortiea crassa*. More recently, Boavida and Gliwicz (in press) analyzed the phytoplankton community during September and October 1993 and did not report any species of cyanobacteria.

Caldeirão reservoir is not well studied from a planktonic point of view but a sample analyzed in April 1997 revealed no cyanobacteria in a total of 7819 cells/ml of total phytoplankton (Vasconcelos et al. 1999).

In Fronhas reservoir, during the autumn of 1998 no cyanobacteria were found in spite of high chlorophyll concentrations. In the Spring 1999, cyanobacteria attained high density, constituting 87.0-98.4% of the phytoplankton biomass. This reservoir was reported as eutrophic with *Oscillatoria limnetica* as the dominant species (Vasconcelos et al. 1999). Total cyanobacteria density reached then 89,141 cells/ml during the spring of 1999.

Fagilde reservoir was found to be dominated by cyanobacteria. In a study performed in 1998 and 1999 cyanobacteria composed 70.9 to 90.6% in autumn and spring samples respectively (Vasconcelos et al. 1999). In the autumn 1998 dominant cyanobacteria species were *Anabaena flos-aquae*, *Microcystis aeruginosa* and *Phormidium mucicola* whereas during spring 1999 *Anabaena spiroides*, *Microcystis aeruginosa* and *Phormidium mucicola* dominated. These species are potentially toxic, being the most common in Portuguese freshwaters (Vasconcelos 1994). A TSI value of 52.3% during autumn confirmed the eutrophy of this reservoir.

In the Aguieira reservoir, bloom samples of *Microcystis aeruginosa* collected in September and October of 1992 showed strong hepatotocity, with a LD_{50} of 31-35 mg/kg (Vasconcelos 1995). This reservoir is the most eutrophic within the Mondego river basin. Oliveira and Monteiro (1992) studied the dynamics of cyanobacteria

Table 3 – Total phytoplankton density and percentage of cyanobacteria in surface samples of Aguieira reservoir – Santa Comba Dão (from Oliveira & Monteiro 1992).

Sampling date	Total phytoplankton (cells/ml)	Cyanobacteria (%)
14.07.92	120,895	80.1
18.08.92	554,744	98.7
20.09.92	164,835	97.8
21.10.92	7,064	58.8

Table 4 – Vertical distribution of total phytoplankton and of cyanobacteria (cells/ml) in Aguieira reservoir– at Santa Comba Dão in October 1992 (from Oliveira & Monteiro 1992).

Depth (m) group	0	1	3	6
Cyanobacteria (cells/ml)	4,154	6,102	18,483	20,181
Total phytoplankton (cells/ml)	7,064	7,286	20,889	22,099

blooms in this reservoir and found out that between July and October 1992, cyanobacteria were dominant in the phytoplankton community (Table 3)

The main cyanobacteria species present were *Raphidiopsis mediterranea, Anabaena cylindrica, Gomphosphaeria lacustris, Microcystis aeruginosa* and *M. flos-aquae* (Oliveira and Monteiro 1992). These authors also stated that cyanobacteria showed a sub-surface peak in abundance in October, with the highest concentrations detected at 3-6 m depths (Table 4). As they did not present temperature data we can not assume that this is due to the thermocline. Nevertheless, in these temperate systems thermocline tend to be deeper (Vasconcelos 1990). This is an important consideration when the reservoir is used for drinking water supply. Most programs monitoring the presence of cyanobacteria are based on surface sampling and in cases where the highest concentrations are found at greater depths, there may be a biased diagnosis of water quality with important consequences in terms of human health.

It was also shown that there is a longitudinal gradient in terms of cyanobacteria density in this reservoir. The density close to the dam wall was four times lower than that of Santa Comba Dão (Oliveira and Monteiro 1992) being a result of the influence of the agriculture runoff and municipal wastewater disposed from Santa Comba. In fact, due to its shape Aguieira reservoir has to be studied and sampled taking this into consideration.

In S. C. Dão, the main cyanobacteria species are *Raphidiopsis mediterranea, Anabaena cylindrica, Gomphosphaena lacustris, Microcystis aeruginosa* and *M. flos-aquae* (Oliveira and Monteiro, 1992). Work done from 1994 to 1999, in collaboration with the health authorities of Santa Comba Dão, Mortágua, Tábua and Carregal do Sal, the

phytoplankton dynamics, with special relevance to cyanobacteria was analyzed (Vasconcelos et al. 1999). The highest concentrations of cyanobacteria were found in the summer and autumn of 1995, with peak concentrations of 1×10^6 cells/ml.

Raiva reservoir is meso-eutrophic reservoir based on its TSI values (Table 1). The occurrence of potentially toxic species such as *Aphanizomenon* sp., *Microcystis aeruginosa* and *Oscillatoria* sp. indicates a clear potential for human health hazards. Nevertheless, maximum concentrations of cyanobacteria measured by Vasconcelos et al. (1999) are not yet very high, attaining only 2,250 cells/ml in the autumn 1998 and 1,244 cells/ml in the spring 1999.

Açude de Coimbra is a reservoir used for the production of drinking water for Coimbra municipality. In a study performed during 1993-1994, Craveiro (1994) analyzed the phytoplankton community of Coimbra reservoir. Chlorophyll attained a maximum concentration of 18.7 mg/m^3 in July 1993 and TSI values calculated with the whole data reached an average of 43.5% with a CV of 20.8%. This clearly indicates a mesotrophic state where cyanobacteria may increase their role. During this study, a total of 11 cyanobacteria species were found with *Aphanothece clathrata* and *Oscillatoria limnetica* as dominant in terms of occurrence. Although no density values measured as cells/ml are available, it was found that, the maximum densities were due to O. limnetica (Craveiro 1994). Data obtained by us (Vasconcelos et al. 1999) revealed that the concentration of cyanobacteria varied from 413 cells/ml in the autumn 1998 to 3,008 cells/ml in the spring 1999, with *Microcystis incerta*, *O. limnetica* and *Coelosphaerium kuetzingianum* as the main species.

Comparing all the sites described in this work in terms of maximum cyanobacteria density (Fig. 2), we may say that eutrophication of this river basin shows a pattern that increases from the upstream sites to Aguieira reservoir and subsequently decreases downstream.

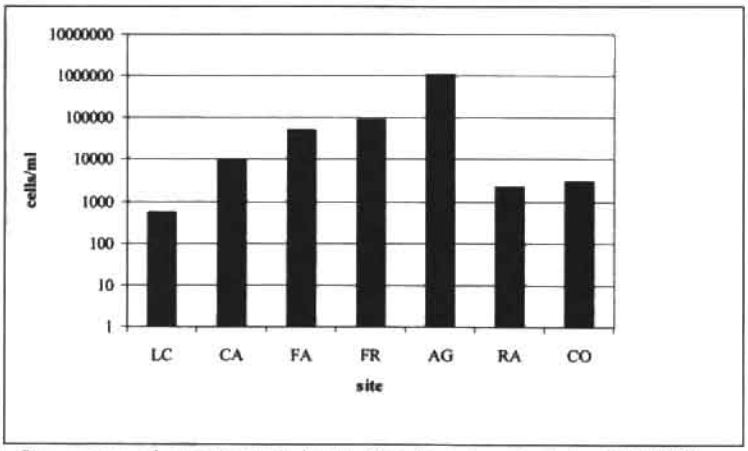

Figure 2. Occurrence of cyanobacteria in the Mondego river basin in 1998-1999 – maximum cyanobacteria values (cells/ml) (LC – Lagoa Comprida, CA – Caldeirão, FA – Fagilde, FR – Fronhas, AG – Aguieira, RA – Raiva, CO – Coimbra reservoirs) (from Vasconcelos et al. 1999)

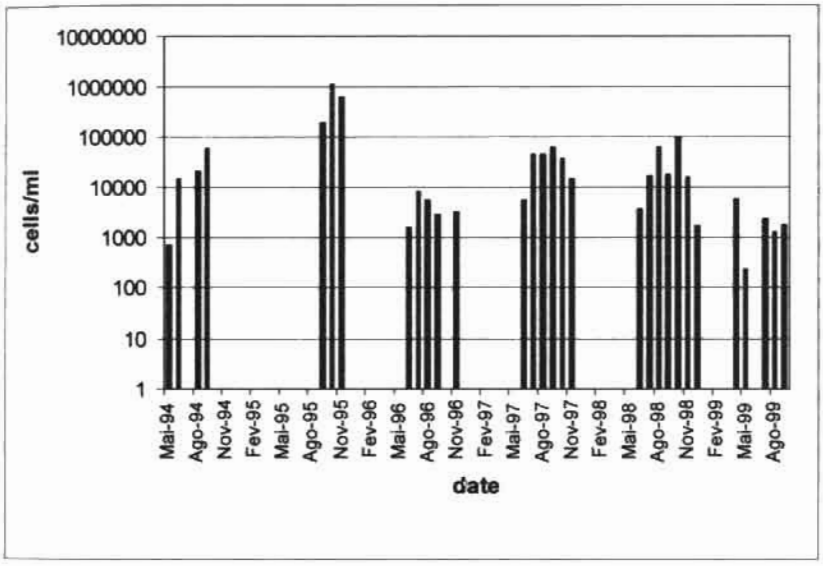

Figure 3. Dynamics of total cyanobacteria density (1994-1999) in the Aguieira reservoir (S.C. Dão) (from Vasconcelos et al. 1999).

Toxins in Mondego river basin

Initially, toxicity was measured by mouse bioassay - LD_{50} (mg/kg animal weight). The results of toxicity (LD_{50}), amount of microcystins and isolated microcystins – MCYST - from blooms collected at Aguieira reservoir in 1992 (Santa Comba Dão) are presented in Table 5, revealing blooms with high hepatotoxicity.

Table 5. Toxicity (LD50), amount of microcystins and isolated microcystins from blooms collected at Aguieira reservoir (Santa Comba Dão) (from Vasconcelos 1995).

Date	Toxicity (LD_{50} mg/kg)	Toxin amount (µg/mg)	Isolated microcystins
10/09/92	31	5,6	MCYST-LR, MCYST-RR, MCYST-HilR [Dha^7]MCYST-LR, [MeSer^7]MCYST-LR, [D-Asp^3]MCYST-LR
04/10/92	35	-	-

The September 1992 sample presented the highest MCYST diversity of the many analyzed from Portuguese cyanobacterial blooms. Seven different MCYST were found and isolated although MCYSTY-LR was the dominant – 64.2% of the total MCYST content. In this sample, it was also found MCYST-HilR (2.3%), a MCYST similar to MCYST-LR but that contains homoisoleucine instead of leucine (Vasconcelos et al. 1996). It was only found before in a *Microcystis* spp. bloom sample in Illinois, USA (Namikoshi et al. 1994). The toxin [L-MeSer^7]MCYST-LR has N-Methylserine instead of dehydroalanine was also present in this sample and it had been reported before only in the same Illinois bloom.

Later, from 1994 to 1997, the evaluation of toxicity revealed negative results in terms of mouse bioassay. This may be due to a decrease on toxicity but also to the fact that mouse bioassays have low sensitivity. Data obtained using a more sensitive method, the immunoassay ELISA (Ann and Carmichael, 1994) are shown in Table 6. Although cyanobacteria density were high during this period (Fig. 1), microcystin concentrations were low. This may be due to a low percentage of toxic strains among the blooms or to unfavorable environmental conditions for toxin production.

Table 6 Microcystin concentration (µg/l) in Aguieira reservoir during 1997 and 1998 measured by ELISA.

Months	Year	1997	1998
August		1,6	0
September		0,3	1,2
October		1,7	1,3
November		0	0,5
December		-	0

Environmental and human health risks

Taking into account the data presented in this paper we may conclude that the occurrence of toxic cyanobacteria in Mondego river basin represent a potential human health hazard.

The variability in patterns of eutrophication along this river basin led us to focus our attention on three sites: Fagilde, Fronhas and Aguieira reservoirs which are most susceptible to blooms of cyanobacteria. Sites downstream of Aguieira should also be monitored regularly, since toxic cyanobacteria are detected in the phytoplankton. Cyanobacteria and their toxins may be transported downstream reaching Raiva and Coimbra reservoirs, so there is a clear need for monitoring microcystin concentrations as well as phytoplankton populations.

The presence of toxic cyanobacteria detected since 1992 in Aguieira reservoir may cause human health problems due to the fact that this reservoir is used for drinking water withdraw and also for recreation and fishing. Aquatic animals, including

fish, may accumulate microcystins (Amorim and Vasconcelos 1999, Vasconcelos 1999) and transfer them along the food chain.

Taking into consideration the WHO provisional guideline values for drinking water – 1 µg/l (Chorus and Bartram 1999), cyanobacteria toxins should be monitored regularly in Mondego river basin reservoirs during the whole bloom season.

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