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Cyanobacterial toxins in Portugal: effects on aquatic animals and risk for human health

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Abstract

Toxic cyanobacteria are common in Portuguese freshwaters and the most common toxins are microcystins. The occurrence of microcystin-LR (MCYST-LR) has been reported since 1990 and a significant number of water reservoirs that are used for drinking water attain high levels of this toxin. Aquatic animals that live in eutrophic freshwater ecosystems may be killed by microcystins but in many cases the toxicity is sublethal and so the animals can survive long enough to accumulate the toxins and transfer them along the food chain. Among these, edible mollusks, fish and crayfish are especially important because they are harvested and sold for human consumption. Mussels that live in estuarine waters and rivers where toxic blooms occur may accumulate toxins without many significant acute toxic effects. In this study data are presented in order to understand the dynamics of the accumulation and depuration of MCYST-LR in mussels. The toxin is readily accumulated and persists in the shellfish for several days after contact. In the crayfish the toxin is accumulated mainly in the gut but is also cleared very slowly. In carps, although the levels of the toxins found in naturally caught specimens were not very high, some toxin was found in the muscle and not only in the viscera. This raises the problem of the toxin accumulation by fish and possible transfer through the food chain. The data gathered from these experiments and from naturally caught specimens are analyzed in terms of risk for human consumption. The occurrence of microcystins in tap water and the incidence of toxic cyanobacteria in fresh water beaches in Portugal are reported. The Portuguese National Monitoring Program of cyanobacteria is mentioned and its implications are discussed.

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Introduction

Toxic freshwater cyanobacteria are very common worldwide and have been responsible for animal (1-4) and human intoxications (5-7). In Portugal, cyanobacteria have been reported to commonly occur in natural lakes, reservoirs and large slow flowing rivers since the early 30's (8-11). Nevertheless, in all these studies only qualitative and quantitative cyanobacterial data were reported and no toxicity data were available despite the occurrence of fish deaths in summer (12).

In the present study the occurrence of toxic freshwater cyanobacteria in Portuguese

freshwaters is reported. Data from experiments on toxin accumulation by crayfish and naturally caught fish specimens will be presented and analyzed in terms of risk for human consumption. The occurrence of microcystins in tap water and the incidence of toxic cyanobacteria in freshwater beaches are reported. The Portuguese National Monitoring Program of cyanobacteria is mentioned and its implications are discussed.

Occurrence of cyanobacterial toxins in Portuguese freshwaters

A survey of the distribution of toxic freshwater cyanobacteria in lakes, reservoirs and large slow flowing rivers in Portugal was started in 1989. Initially *Microcystis aeruginosa* strains isolated from lakes and reservoirs of North and Central Portugal were analyzed by mouse bioassay, using freeze-dried extracts of cell material injected intraperitoneally into male Charles River mice. The LD_{50} of these samples varied from 15 to 75 mg/kg (dry weight) (13). Two bloom samples also showed hepatotoxicity with LD_{50} of 30 and 175 mg/kg (13). HPLC analysis performed on some of these strains revealed that one to 4 different toxins are present.

A larger study on the occurrence of toxic cyanobacteria was conducted from 1989 to 1992. Thirty lakes, rivers and reservoirs were sampled and a total of 30 bloom samples were analyzed. *Microcystis* strains were also isolated from these samples (14). Eighteen out of 30 water bloom samples were found to be hepatotoxic by mouse bioassay (14). Dominant species were *M. aeruginosa, M. wesenbergii, Anabaena flos-aquae, A. scheremetievi* and *Aphanizomenon flos-aquae*. At this time no neurotoxic blooms were found. In this study, LD_{50} varied from 20 to 700 mg/kg in the blooms and from 15 to 75 mg/kg in *Microcystis* strains (14).

Using high performance liquid chromatography (HPLC) for toxin purification and analysis, several microcystins were identified. The *Microcystis* strains contained up to 4 different microcystins, the most common being microcystin-LR (MCYST-LR), corresponding to 43.9% to 100% of total microcystin content (15). Other less common microcystins such as MCYST-LA, MCYST-AR, MCYST-YR, MCYST-RR and [D-Asp^3]MCYST-LR were also identified in these strains (15). Cyanobacterial blooms were also analyzed and up to 10 different toxins were detected, with 7 being identified (16). Some toxins that were not present in the strain sampled were identified in blooms. These include [Dha^7]MCYST-LR, (L-MeSer7)MCYST-LR and MCYST-HilR (16). MCYST-LR was identified in all samples where microcystins were identified.

Oliveira (17) reported that a bloom collected in Mira Lake (North Portugal) consisting mainly of *Aphanizomenon flos-aquae* (98.8%) was toxic to mice. In fact, an intraperitoneal mouse bioassay revealed that death occurred quite fast (<2 min) with no demonstrable internal organ damage. It is known that *A. flos-aquae* may produce neurotoxins such as PSP or anatoxin-a. Ferreira (18) isolated an *A. flos-aquae* strain from a bloom in Douro River and detected some PSP toxins such as GTX1, GTX3 and GTX4.

Microcystin levels in Portuguese freshwaters

Microcystins have been quantified by using a highly sensitive enzyme-linked immunosorbent assay (ELISA) based on a monoclonal antibody described in a paper by Nagata et al. (19). Samples obtained from 29 lakes and reservoirs in Portugal revealed that 28 of these were positive for MCYST, with concentrations varying from 102 to 37,000 pg/ml (20). Some of these bodies are sources of drinking water (max 37 µg/l) and others are used for recreation (max 34.7 µg/l).

Effects of cyanobacterial toxins on aquatic animals

Aquatic animals that live in eutrophic ecosystems are among those organisms that may be severely affected by cyanobacterial toxins. Although many of the reported animal intoxications involve livestock or waterfowl poisoning (21-24), aquatic animals are also affected. In fact, toxic cyanobacteria are known to cause adverse effects on zooplankton communities. In a previous study (25) we reported that although both toxic and nontoxic *Microcystis aeruginosa* strains have some nutritional value for the cladoceran *Ceriodaphnia pulchella*, they cause death by day 7. A stronger effect was observed for *Daphnia longispina* with total lethality by day 4 (25). Similar conclusions were reached by Lampert (26) and Porter and McDough (27), who considered smaller cladocerans to be less affected during cyanobacterial blooms. In a similar experiment, we (28) reported that the bottom-dwelling cladoceran *Simocephalus vetulus* may feed on a nontoxic strain of *M. aeruginosa* but it can survive only two days after feeding on the toxic strain. The copepod *Acanthocyclops robustus* ingests both toxic and nontoxic strains, surviving up to 26 days (28).

Concerning other invertebrates, some animals such as mollusks seem to be quite tolerant to cyanobacterial toxins. In an experiment with the mussels *M. galloprovincialis* fed on a toxic *M. aeruginosa* strain, we (29) showed that less than 1% mortality occurred during a 16-day exposure time. During this period mussels accumulated up to 10.5 µg MCYST/g dry mussel weight. After this accumulation period animals were fed marine phytoplankton for two weeks and toxins were still detectable by HPLC at day 11 (29). This permits the transfer of the toxins along the food chain. In fact, Lindholm et al. (30) also showed accumulation of a cyanobacterial hepatotoxin by the freshwater mussel *Anodonta*, which was considered to be the most likely reason for a high muskrat mortality. Eriksson et al. (31) also showed the accumulation of high levels of microcystins produced by *Oscillatoria agardii HII* in *Anodonta cygnea*, without any visible damage.

Falconer et al. (32) showed that the edible mussel (*M. edulis*) accumulates nodularins during high densities of *Nodularia spumigena* in the Peel Inlet Estuary. More recently, Amorim and Vasconcelos (33) showed that, although MCYST accumulation in mussels may be quite rapidly cleared during the first two days, the toxins persist up to two weeks and their detectable concentration fluctuates along time. This may indicate a regular release of bound MCYST from PP1 and PP2A complexes. Mollusks seem to be quite resistant to cyanobacterial toxins possibly because MCYST are quite lipophobic and mussels may not have specific carriers that transport these toxins into hepatopancreatic cells.

Crayfish commonly exist in eutrophic freshwater bodies in Portugal (34). Although the autochthonous Iberian species *Austropotamobius pallipes* is decreasing in density and only survives in the very clear northwest Portuguese rivers, *P. clarkii* was introduced in the south of Spain in the 80's (35) and is now spread all over Portugal. Nowadays it is a very important component of many aquatic ecosystems, being part of the diet of birds, fish and mammals. Recently, crayfish started to be harvested for human consumption and for the production of cattle feed.

In a study using *P. clarkii* larvae, Oliveira (17) showed that they are quite resistant to cyanobacterial toxins when the latter were provided at cell densities similar to those occurring during blooms. This high survival enables crayfish to use cyanobacteria and increase their biomass in highly eutrophic ecosystems. Adult crayfish seem to be quite tolerant to cyanobacterial toxins. In fact, they may grow better on a diet of toxic *M. aeruginosa* than on a nontoxic strain (17).

Their productivity, length increase and survival were higher when they were fed the toxic strain (17).

Fish are known to be affected by cyanobacterial blooms and toxins (12,36). Nevertheless, under natural conditions it is sometimes difficult to distinguish between the effects of toxins and the effects of anoxia, ammonia and sulfide production. Laboratory experiments have proved that fish are sensitive to cyanobacterial toxins and that the toxins do not seem to be absorbed via the gills but are assimilated when injected (36).

Fish collected in Portuguese freshwaters do not accumulate very high levels of toxins in their edible parts (Table 1). In fact, as expected, muscle is the part of the fish where the toxin is accumulated less. Nevertheless, values vary widely not only within species but also within specimens of a same species, as shown in Table 1.

Table 1 - Maximum and minimum values of MCYST in edible parts of different fish species collected in Portuguese freshwaters.

Species	MCYST-LR (ng/g)
Carp (*Cyprinus* sp)	50-280
Barbel (*Barbus* sp)	0.7-120
Grey mullet (*Lisa* sp)	8.5-110

Table 2 - Maximum amounts of microcystins in edible parts of aquatic animals.

Species	MCYST-LR (µg/g)
Fish	0.3
Crayfish	2.7
Mussels	16.0

Table 3 - Amount of edible parts of fish, crayfish and mussels contaminated with the levels described in Table 2 that is necessary to ingest to attain WHO limit values for MCYST in drinking water.

Species	Amount of edible part that is necessary to eat to attain the WHO limit for water (g)
Fish	7.0
Crayfish	0.7
Mussels	0.1

Although nothing is known about the dynamics of MCYST in fish muscle, it would not be surprising if we observed a pattern similar to that of crayfish and mussels, i.e., a fluctuation pattern during toxin clearance.

The detectable MCYST in animal tissues is always only a part of the total amount because about 26% of the toxin is bound to protein phosphatases (PP) (36). Nevertheless, during detoxification or even along time PP may be inactivated or destroyed and MCYST may be released, thus becoming detectable. Although the maximum detectable concentration of MCYST in fish is usually low (Table 2) when compared to that of other aquatic animals, it is important to consider that MCYST are potent tumor promoters (37). They may not cause adverse effects in humans, who rarely consume fish contaminated with these levels, but their effect may be increased if water contaminated with MCYST is also ingested. Considering the WHO provisional limit value for the concentration of MCYST in drinking water - 1 µg/l - one may estimate the amount of mussel, crayfish and fish necessary to attain that value. This is shown in Table 3. This indicates that mussels are among the aquatic animals that may represent a higher risk for human health.

In Portugal there is a Monitoring Program for marine toxic phytoplankton species and their toxins in shellfish. Paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) toxins have been found and their values are regularly estimated so as to avoid human health risks. Nevertheless, the animal assay or chemical techniques used in the Program are specific for neurotoxins. Hepatotoxins are not monitored and so in the estuaries of eutrophic rivers such as Minho and Guadiana there is the possibility of the

occurrence of hepatotoxin that may not be detected.

Regarding drinking and recreational waters, no regular monitoring of all the systems is done at present. Nevertheless, those systems that are more problematic in terms of the occurrence of toxic cyanobacteria are currently being analyzed. In many locations, the Portuguese Water Treatment Plants (WTP) are not adequate for the removal of cyanobacteria and their toxins. Oliveira and Monteiro (38,39) reported that in the eutrophic reservoirs of Aguieira and Monte Novo where toxic cyanobacteria commonly occur (40) the WTP efficiency can be as low as 24% for the retention of cells. In these sources of drinking water, values were as high as 7.1×10^2 to 1.2×10^5 cells/ml (38,39). MCYST levels in tap water are usually below the detectable limit of ELISA (<0.1 µg/l). When values higher that 1 µg/l occur, the local health authorities recommend that the population should not use that water for drinking or cooking although other uses may be possible (washing, sanitation, watering plants).

A Monitoring Program has been developed because of the occurrence and significance of toxic cyanobacteria in Portuguese freshwaters. The Ministry of Health coordinates a group of technicians and researchers from the Ministries of Health, Environment, and Agriculture, and from Universities, Municipalities, and Water Companies. This group has met regularly for more than one year and has set up a proposal for a monitoring program that was accepted by the ministers. It was proposed that a network of laboratories should be organized in order to analyze water samples with cyanobacteria and their toxins in a more efficient and rapid manner.

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