ECOLOGICAL IMPLICATIONS OF HEPATOTOXIC *MICROCYSTIS AERUGINOSA* IN THE JACAREPAGUÁ LAGOON, BRAZIL

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ABSTRACT

Increasing eutrophication in Brazilian water bodies has led cyanobacteria to become dominant in several lagoons and reservoirs. The Laboratory for Culture and Physiology of Microalgae (NPPN-UFRJ) has confirmed toxic *Microcystis aeruginosa* blooms producing microcystins (hepatotoxic heptapeptides) in the Jacarepaguá Lagoon, located in the west zone of the city of Rio de Janeiro. This Lagoon communicates with the sea and is used for recreation and fishing. Since August 1996, a project was started to analyze the causes and ecological consequences of toxic cyanobacterial dominance in this Lagoon. The present study has evaluated microcystin bioaccumulation in fish and the presence of this hepatotoxin in bottom sediments harvested fortnightly. Purification and detection of microcystins were carried out by reverse phase HPLC with diode-array detector. By the first week of November a new toxic *Microcystis* bloom started to be observed. Analysis confirmed the presence of microcystins in plankton samples. Hepatotoxins were detected in fish liver and visceral organs since the end of the same month. However, these toxins have not so far been detected in sediment samples. These results suggest that the hepatotoxin intoxication can be fast and that human health concerns should consider possible food chain bioaccumulation of cyanobacterial microcystins in the aquatic food chain.

INTRODUCTION

The presence of hepatotoxic cyanobacteria has been documented in several areas of the world. Exposure to microcystins, the most commonly detected cyanobacterial hepatotoxic peptides, is a health risk and it has been related to several incidents of animal poisoning [1, 2]. Furthermore, human intoxication and death by microcystins were confirmed for haemodialysis patients from the city of Caruaru (Pernambuco State - Brazil) in 1996 [3].

The stability of cyanobacterial hepatotoxins in water is variable. A recent study [4] showed that part of the loss of these toxins in the water column is due to adsorption on sediments.

Increasing eutrophication in Brazilian waterbodies has led cyanobacteria to become dominant in several lagoons and reservoirs[5].

Jacarepaguá Lagoon is a brackish waterbody located in the west zone of Rio de Janeiro city (Fig. 1). It has an oblique communication with the sea and presents ideal conditions for cyanobacterial bloom formation.

The water in this Lagoon has a pH between 6 and 9 and an accelerated artificial eutrophication with high levels of nutrients, mainly ammoniacal nitrogen and total phosphorus. The elevated nutrient conditions are due to industrial and domestic effluents and earth embankment.

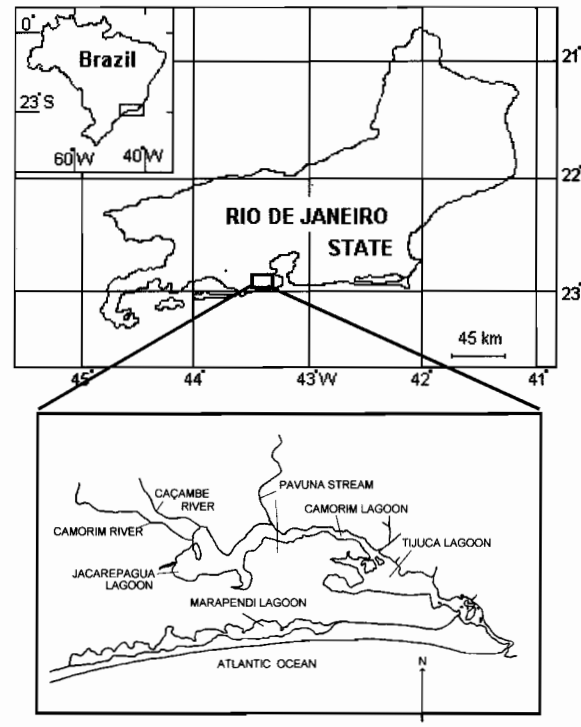

Fig. 1. Location of Jacarepaguá Lagoon.

These characteristics have caused physical, chemical and biological alterations and limit the water circulation [6].

Previous studies confirmed toxic *Microcystis* blooms producing microcystins (hepatotoxic heptapeptide) in this lagoon [unpublished data].

A project was started in August 1996 to analyse the causes and ecological consequences of toxic cyanobacterial dominance in this lagoon. This project aims to verify the occurrence of phytoplanktonic species and their toxicity, presence of microcystins in the water and in zooplankton, fishes and sediments samples of the Jacarepaguá Lagoon.

Since the lagoon is used for recreation and fishing and there is a population of fisherman which consumes and markets the fish, the aim of this paper is to verify the bioaccumulation of microcystin in the fish of the lagoon and to analyze the possible adsorption of microcystins onto sediments to evaluate the potential health hazards for the local population.

MATERIALS AND METHODS

In order to analyze microcystin concentrations, fish (5 specimens) and sediment samples were collected fortnightly. Five grams of dry sediments were extracted with 100% v/v methanol three times, the extracts were dried, redissolved in deionised water and passed through C-18

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cartridges. The cartridges were first washed with deionized water and then eluted in sequence with 20 ml of 20% and 100% methanol.

Whole fresh fish liver, visceral organs and mature female gonads and 5 g of muscle were treated twice with a suitable volume to cover the samples with 100% v/v methanol. The methanol extracts were then extracted with hexane three times, using the same methanol volume. Hexane layers were discarded and the methanol extracts were dried and redissolved with deionized water using the same volume. These extracts were loaded onto a Bond Elut C-18 cartridge and then washed and eluted with 30 ml of methanol 20% and 50 ml of 100% v/v methanol.

The 100% methanol fractions of sediments and fish samples were dried and redissolved in 1 ml of methanol:water (1:1, v/v) which was used to verify the microcystin presence by reverse phase HPLC with diode array detector (Shimadzu SPD-M10A).

The HPLC system used a Lichrospher 100 RP-18 reverse phase column (5µm - Merck), the separation was carried out under isocratic conditions with a mobile phase of 20 mM ammonium acetate, pH 5,0 and acetonitrile (7:3), for 10 minutes. The volume injected was 20µl with UV detection at 238 nm and a flow rate of 10 $mL.min^{-1}$.

RESULTS

Microcystins were not detected in fish samples until the beginning of the *Microcystis aeruginosa* bloom. By the first week of November an atypical surface bloom started to be observed.

The maximum phytoplankton density reached was 170 $x10^6$ cells L^{-1}. The percentage of Cyanophyceae increased 10 times from August to November and microscopic analysis showed the dominance of *Microcystis aeruginosa,* representing 13% of the total phytoplankton community.

At that time, laboratory analysis confirmed microcystin presence in the plankton samples, reaching 12 $\mu g.l^{-1}$. Furthermore, HPLC analysis identified four different toxic peaks in the chromatograms with the same UV spectra as microcystin.

Water samples from this time showed no microcystin. This observation is compatible with the bloom condition, that was growing actively so that toxin release to the water was minimized.

Microcystins were not detected in sediment samples until the beginning of December.

Hepatotoxins started to be detected in fish liver and visceral organs at the end of November, just after the beginning of the bloom. Four different toxic peaks in the chromatograms of visceral organ samples from January, as well as in water samples, were also registered.

From December to March there were some fish samples with mature female gonads and in January some of these showed typical microcystins peaks. All muscle sample analysis showed no evidence for microcystins yet.

In all seston samples and in almost all fish samples, toxin continued to be detected until March.

Table 1 shows the mean microcystin-LR equivalent concentrations in different fish samples, realized by the comparison between standard microcystin-LR peak area and the sample toxic peak area.

Table 1: Microcystin-LR equivalent levels in fish samples in $\mu g.g^{-1}$ (wet weight).

	Liver	visceral organs	female mature gonads	muscles
10/15/96	-	-	x	-
11/12/96	-	-	x	-
11/15/96	-	-	x	-
11/26/96	+	+	x	-
12/11/96	-	++	x	-
12/23/96	++	+++	-	-
01/08/97	-	+++	+	-
01/22/97	-	-	-	-
02/06/97	+	+	-	-
02/22/97	-	+++	-	-
03/20/97	-	-	-	-

(-) below detection limit of $1\mu g.g^{-1}$; (+) 1-6 $\mu g.g^{-1}$; (++) 7-30$\mu g.g^{-1}$; (+++) > 30 $\mu g.g^{-1}$; (X) no sample.

DISCUSSION

It is already known that microorganisms are able to decompose cyanobacterial hepatotoxins but this degradation depends on the history of the lake. The degradation is faster in sediments from lakes with previous blooms than from lakes in which no blooms have been observed [4].

In Jacarepaguá Lagoon microcystins were not detected before or during the bloom in sediment samples. If *Microcystis aeruginosa* blooms had occurred in Jacarepaguá Lagoon in the past, the absence of this hepatotoxin in sediments can be related to the occurrence of populations of microorganisms capable of degrading microcystins.

In a laboratory experiment with rainbow trout, microcystin-LR was quickly taken up by the liver 3 hours after exposure [7]. In our field study we consider that fish from Jacarepaguá Lagoon showed a rapid uptake of microcystins once the toxin had been detected in visceral organs just after the beginning of the bloom. This observation is in agreement with experiments with mussels [8].

The route of exposure to microcystin is through oral ingestion and it is absorbed primarily by a single transport mechanism. Some reports [9, 10, 11] have shown that mouse liver accumulates roughly 50 to 70% of the total microcystin injected, followed by the intestine and kidneys, but other authors have shown a greater accumulation of microcystin-LR in the digestive tract of mussels [8].

The concentration of free microcystins in visceral organs and fish liver from Jacarepaguá Lagoon varies from 1 to 150 $\mu g.g^{-1}$ and 3 to 15 $\mu g.g^{-1}$ respectively.

These results indicate that these fish consumed cyanobacteria and part of the toxin can be accumulated by the liver, bound to protein phosphatases or metabolized, and the other part was excreted by feces.

The concentration of 150 $\mu g.g^{-1}$ probably is enough to cause severe damage to fish; however, microcystin can be excreted quickly. In rats, for example, 75% of the total amount is excreted within 12 hours in feces and urine [9]. Mussels

can accumulate high levels of this toxin and do not suffer visible damage [8, 12].

Our results showed a rapid transfer of microcystins to fish. This transfer is primarily due to the ingestion of *Microcystis aeruginosa* colonies. The toxin is taken up by bile-acid transporter in intestinal and liver cells and accumulated mainly in liver.

It has been verified that only 24% of the total microcystins-LR was extractable with methanol [13] and in spite of many barriers to the digestion and absorption of *Microcystis aeruginosa* cells in fish, hepatic lesions were observed in more than 50% of the analyzed fish [14]. These facts suggest that the microcystin concentrations found in Jacarepaguá Lagoon fish are underestimated and thus the risk associated with their consumption is very high.

These findings show that it is necessary to monitor and to control the occurrence of toxic cyanobacteria and their toxins in Brazilian brackish lagoons as well as verify the possible bioaccumulation of these toxins in the food chain. This will alert authorities to possible public health hazards from these potent hepatotoxic tumor promoters. Furthermore the ecological implications of these toxins need to be more investigated.

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