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Microcystins adsorption on sediment particles

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Abstract

Previous studies confirmed toxic *Microcystis aeruginosa* blooms producing microcystins and the accumulation of this toxin in fish from Jacarepaguá Lagoon (Rio de Janeiro – Brazil). Furthermore microcystins had also detected in sediment sample. Thus, sediment and water samples were collected in Jacarepaguá Lagoon with the aim of investigating microcystins kinetics within sediment and water column. Fifty grams of coarse (> 250 mesh) and fine (< 250 mesh) sediment previously sieved and sterilized were put in a vial with 100 mL of local filtered water and incubated with a lysed toxic *Microcystis aeruginosa* cells. In each sampling time, microcystins concentration in sediment and water samples were performed by HPLC and samples with microcystins concentration below the HPLC detection limit were analysed by ELISA (ENVIROLOGIX INC.®). Twelve hours after the incubation, only 18% of inoculated microcystins were observed in water and its concentration in fine and coarse sediment increased 15 and 2 times respectively. Fine sediment adsorbed, on the average the double of microcystins than coarse sediment. On the average, 82% of added microcystins were not recovered. Probably the technique used was not enough for the extraction of the adsorbed microcystins to the sediment; thus it is probable that microcystins are strong adsorbed to these particles confirming that this compartment represents an important microcystin deposit. This strong adsorption would difficult the re-enter of these toxins in the water column. This fact decreases the risk of the food chain contamination, however the risk can be related to the food chain via particle feeding.

Introduction

The growth of toxin producing cyanobacteria in water bodies can decrease water quality and increase the risk of toxicity to animal and human health. One of the most studied groups of cyanotoxins is the cyclic heptapeptide hepatotoxin called microcystins (MCYSTs). Exposure to MCYSTs represents a health risk to animals (Azevedo and Carmouze, 1994; Carmichael, 1994 and 1997 and Falconer, 1998) and humans (Jochimsen *et al.*, 1998).

Exposure to cyanotoxins can occur by different routes including dermal, inhalation, oral and intravenous (medical dialysis treatment). Two oral routes can lead to cyanotoxin

exposure: direct ingestion of water containing cyanobacteria cells and toxin and the consumption of animals, which have ingested cyanobacteria and accumulated their toxins.

The bioaccumulation of cyanotoxins by aquatic animals including fish, mollusks and zooplankton has been reported by Amorim and Vasconcelos (1999), Tencala *et al.*, (1994), Thostrup and Christoffersen (1999) and Williams *et al.*, (1997). Thus means that oral consumption by animal tissues containing cyanotoxins is possible and could lead to human toxicity.

The maximum allowable concentration for MCYSTs in drinking water was established as 1 $\mu g\ L^{-1}\ day^{-1}$ (Falconer *et al.*, 1994). A Tolerable Daily Intake (TDI) value of 0.04 $\mu g\ Kg^{-1}$ of body weight day^{-1} was proposed as a provisional guideline value by WHO (Chorus and Bartram, 1999).

In Brazil, problems associated with cyanobacteria are increasing, especially in areas experiencing population growth without adequate water treatment. Jacarepaguá Lagoon, a coastal water body located in the western part of Rio de Janeiro City is an example of an aquatic ecosystem suffering environmental impacts due to anthropogenic influences.

As part of our ongoing studies to analyse the causes and ecological consequences of toxic cyanobacteria in Jacarepaguá Lagoon, we have confirmed the occurrence of toxic *Microcystis* sp. blooms that produce MCYSTs in this lagoon and the bioaccumulation and persistence on MCYST in fish muscle tissue therefore a potential risk for human health (Magalhães *et al.*, 2001).

It is already known that MCYST degradation is higher when using sediment from a place with previous occurrence of cyanobacterial blooms than from a place with no bloom occurrence (Rapala *et al.*, 1994). Part of the loss of this toxin is due to adsorption on sediment and part is degrading by bacteria. The persistence of dissolved MCYST-LR (30 d) is longer than the persistence in particulate material (15 d) (Lahti *et al.*, 1997).

The major part of MCYST-LR can be removed from water by clay material. This fact offers a method of stripping this toxin from drinking water supplies. Nevertheless the adsorbed toxin is probably protected by degradation and can be transported at long distance thereafter can re-enter the food chain (Morris *et al.*, 2000).

With the confirmation of cyanobacteria toxicity in Jacarepaguá Lagoon, the growing eutrophication resulting the ideal condition for cyanobacteria water bloom formation and

the confirmation of MCYST bioaccumulation in fish it will be necessary to investigate a compartment still not study: the sediment.

Therefore, studies about the behavior of this toxin in this compartment, will evaluate its possible transfer for the water column and the re-enter in the food chain, as well as it will esteem the potential of this sediment in degrading MCYTs. Thus, in this paper we report the microcystin adsorption on sediment particles.

Material and Methods

One sample of sediment was harvested with a VanVeen grab in Jacarepaguá Lagoon (RJ, Brazil). At the laboratory, sediment were sieved (250 mesh). The fine (<250 mesh) and coarse (>250 mesh) sediment were dry at 80^0C and sterilised in an autoclave to avoid bacteria activity that are able to degradate MCYSTs. MCYST concentration in fine and coarse sediment was analysed previously.

Fifty grams of coarse (> 250 mesh) and fine (< 250 mesh) sediment were put in a vial with 100 mL of local filtered and sterilised water and incubated with a lysed toxic *Microcystis aeruginosa* cells (NPLJ-4). This strain of *M. aeruginosa* was previously isolated from the Jacarepaguá Lagoon. Water samples (100 mL) and fine and coarse sediment samples (50 g) were collected just after the incubation; 0.5; 1; 2; 4; 7; 14; 21; 28 and 35 days after the incubation. The experiment was performed in triplicate.

Water samples were filtered under vacuum onto fiberglass filters. The filtrate was passed through a C18 reverse phase (Bound-Elut) cartridge which was washed with 20ml distilled water, followed by 20ml of 20% methanol and finally 20ml of 100% methanol. This last fraction was again purified on silica cartridges and eluted with 30mL of 100% methanol and 20mL of a solution of H2O:TFA:metanol (10:0,1:89,9 - v/v) (Tsuji *et al.*, 1994). This last fraction was dried and stored at -20^0C for subsequent MCYST analyses.

The sediment samples were extracted using methanol:buthanol:water (20:5:75 - v/v). Three extractions were performed at room temperature and the pooled fractions were centrifuged at 510 g. The extract was evaporated to 1/3 of its initial volume and then passed through another C18 cartridge. This cartridge was washed and eluted with distilled water, with methanol 20% and then with methanol 100%. This last fraction was dried and stored at -20°C for subsequent MCYST analyses (Krishnamurthy *et al.*, 1986).

Analyses for MCYSTs were performed by PDA-HPLC (Shimadzu SPD-M10A) and a Lichrospher 100 RP-18 reverse phase column (5µm - Merck). Chromatography 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. Volume injected was 20µl with a flow rate of 1 mL.min-1. UV detection was done at 238 nm and the absorption spectrum of each peak was analyzed over the range of 190-300 nm.

Samples with MCYSTs concentration below the HPLC detection limit were analysed by immunoassay using ELISA microcystin Plate kit (ENVIROLOGIX INC.®).

Results

Water samples were analysed by HPLC and sediment samples were analysed by immunoassay using ELISA microcystin Plate kit (ENVIROLOGIX INC.®) because the concentrations were below the HPLC detection limit therefore these samples.

Twelve hours after the incubation, only 18.9% of inoculated MCYSTs were observed in water (Figure 1). On the 2nd day after, this value increases to 44.8% in the experiment with fine sediment. In spite of this increase the percentage of MCYSTs in water, corresponded to 9.8% of the initial concentration (average value).

MCYSTs concentration in the water of the experiment with coarse sediment, showed the same tendency of decrease as observed for the experiment with fine sediment (Figure 1). Only 17% of incubated MCYSTs were observed in water 12 hours after the incubation and this percentage were in average 8.8% until the end of the experiment (35 days).

MCYSTs concentration in fine and coarse sediment increases 15 and 2 times respectively 12 hours after the incubation (Figure 2).

MCYSTs concentration in fine sediment increase gradually until the 21st day when reached the highest concentration (91 times above the initial concentration of the sediment). Then, this value decreased even almost the half and it stayed like this until the end of the experiment (Figure 2).

The kinetics of MCYST adsorption on coarse sediment was slower comparing with fine sediment, reaching the highest concentration on the 28th day. This maximum concentration was only 8.6 times higher than the initial value. Then, this value decreases until almost the half (Figure 2).

In sediment it was detected 8.3% and 4.7% of the initial MCYSTs concentration in fine and coarse experiment respectively. Comparing MCYSTs concentration between fine and coarse sediment during the whole experiment, it was noticed that fine sediment adsorbed, on the average, 13.2 times more MCYST than the coarse one (Figure 2). Therefore, fine sediment adsorbed, on the average, the double of the detected MCYST than coarse sediment.

However, comparing MCYSTs concentration in sediment and water with the inocula concentration, it was observed that 82% of added MCYSTs were not recovered (78% and 86% in fine and coarse sediment respectively) (Figures 1 and 2).

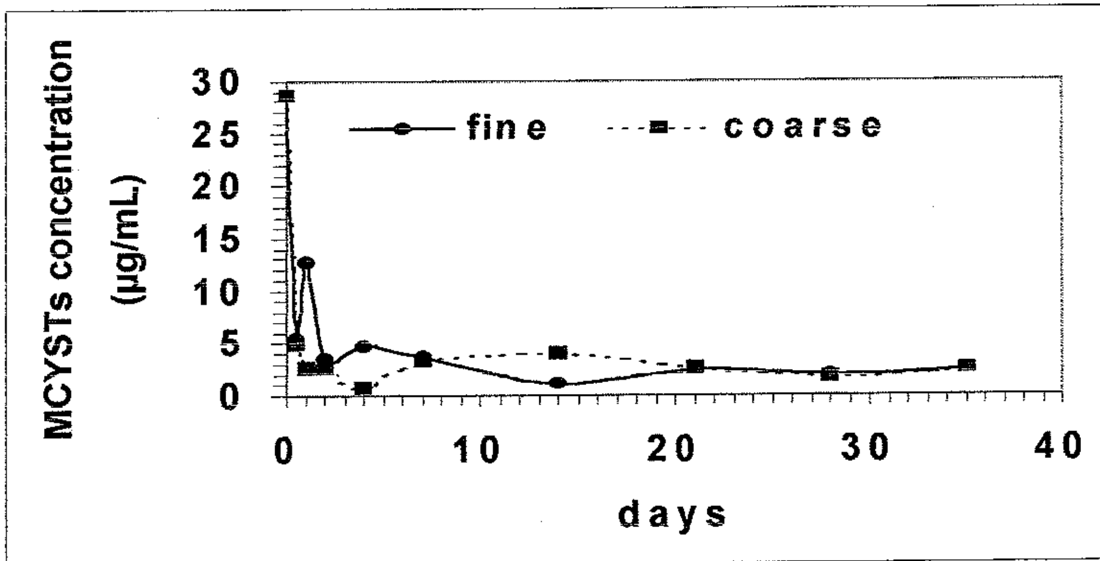

Figure 1: MCYSTs concentration in water (µg/L).

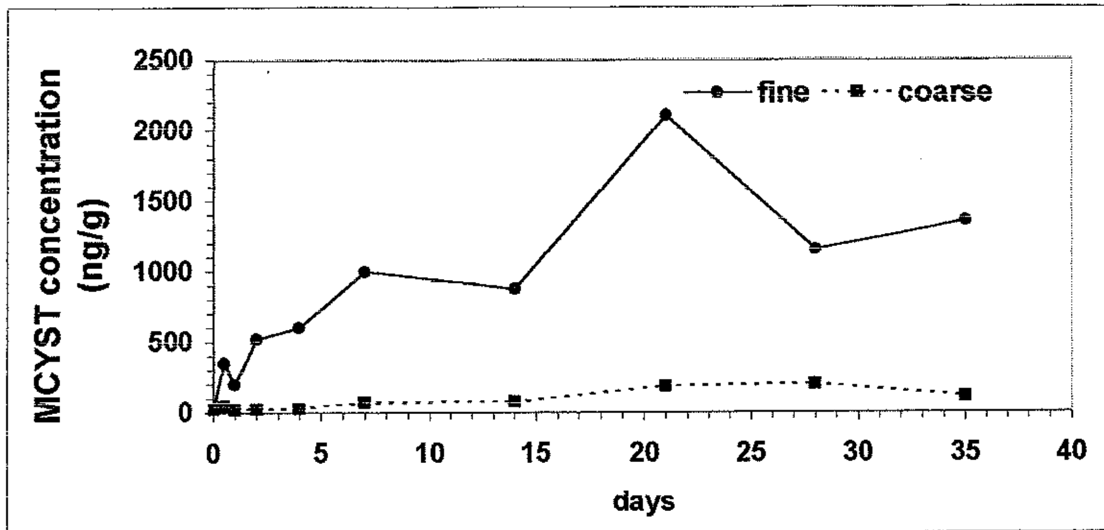

Figure 2: MCYSTs concentration in sediment (ng/g).

Discussion

Our results demonstrated that there was a recover of only 18.9% in the water from the fine sediment experiment and 17% from the coarse sediment experiment in the first 12 hours. Therefore, 81.1% or 83% of MCYSTs added would be bound to sediment particles. However, sediment analyses showed a little amount of MCYST corresponding to 3% and 1.7% of the total MCYST added in fine and coarse sediment experiment respectively at the same time.

According to Rapala *et al.,* (1994), the degradation of MCYSTs started faster with sediment samples from lakes with previous occurrence of cyanobacterial blooms due to the presence of bacteria. The sediment tested in this paper was coming from an euthrophic lagoon with constant cyanobacterial blooms, but the MCYSTs degradation by bacteria was discarded because all material were sterilised before the beginning of the experiment, including the sediment.

Dissolved MCYSTs can be submitted to different degradation process; even so they are more persistent (the decimal reduction time for dissolved MCYST-LR was 30 days). But the particulate MCYSTs, that can be bound to the sediment particles can possibly become protected from degradation in spite of they be less persistent (15 days) (Lahti *et al.,* 1997

and Morris *et al.*, 2000). Thus, after 35 days of experiment, it was still possible to detect dissolved MCYSTs.

Although the experiments have been accomplished in amber vials, Lahti (1997) stated that is also possible the MCYST biodegradation in the dark. This could be the reason of the loss of MCYSTs. Meanwhile it was already known that MCYST, mainly the hydrophilic ones were adsorbed strongly on the sediment particles and thus they were difficult to recover (Morris *et al.*, 2000).

More recently, Tsuji *et al.*, (2001) tested different techniques of MCYSTs extraction from sediment and concluded that the usual process using solvent system, like used in this experiment, is not efficient due to the interaction of the MCYST with the sediment particles.

So, the technique used was probably not enough for the extraction of the adsorbed MCYSTs to the sediment because they were strongly adsorbed to these particles confirming that this compartment represents an important MCYSTs deposit. This strong adsorption would difficult the re-enter of these toxins in the water column. This fact decreases the risk of the food chain contamination, however the risk can be related to the food chain via particle feeding.

As observed, fine sediment adsorbed great amount of MCYST than the coarse one. This observation linked to the fact that MCYSTs is strongly bound to the sediment particles, suggest that besides being difficult the entrance of these toxins in the water column is possible that they are transported at long distances, contaminating other areas.

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