



Short communication

Saxitoxins accumulation by freshwater tilapia (*Oreochromis niloticus*) for human consumption

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ABSTRACT

The accumulation of saxitoxins (STXs) in fish from freshwater aquaculture was investigated for the first time in the present study. Cyanotoxins have been monitored in liver and muscle samples of *Oreochromis niloticus* by chromatographic methods, both before and after the depuration process. The results show that tilapia can accumulate STXs. Our findings suggest that depuration with clean water is an alternative process to eliminate STXs from fish and, therefore, improve the safety of tilapia for consumers.

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Toxic cyanobacterial blooms in lakes and reservoirs have been reported all over the world (Ibelings and Chorus, 2007), and they are often dominated by hepatotoxins, i.e. microcystins (MCs), nodularins (NOD) and cylindrospermopsin (CYN), and sometimes by neurotoxins, i.e. anatoxin-a (ANA) and saxitoxins (STXs) (Chorus and Bartram, 1999).

The STXs are produced by bloom-forming microalgae (mainly marine dinoflagellates). These compounds are neurotoxins (more specifically, potent voltage-gated

sodium channel antagonists) that can cause numbness, paralysis and death in mammals via respiratory arrest (Humpage, 2008). STXs are also produced by freshwater cyanobacteria; therefore, there is a potential for these toxins to be transferred through the freshwater food web to pose a risk to human consumers of freshwater products contaminated by STXs (Pereira et al., 2004; Samsur et al., 2006). In Brazil, STXs and ANA are produced in freshwater systems by cyanobacterial genera *Anabaena* (Monserrat et al., 2001), *Aphanizomenon*, *Planktothrix*, *Lynbya* (Yunes et al., 2003) and, in contrast to cases reported in Europe and the USA, *Cylindrospermopsis* (Lagos et al., 1999; Molica et al., 2002).

The accumulation of STXs is well known in marine organisms, mostly in shellfish (Negri and Jones, 1995; Wiegand and Plugmacher, 2005; Sephton et al., 2007;

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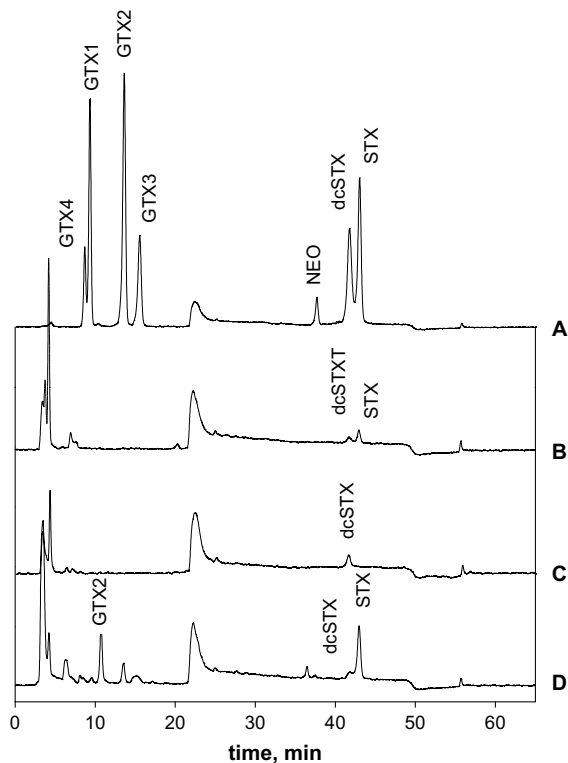


Fig. 1. Chromatograms showing peaks of saxitoxin and related compounds (STXs): (A) STXs standard mixture, (B) fish liver, (C) fish muscle and (D) water by HPLC-FD.

Deeds et al., 2008). High concentrations of cyanotoxins in freshwater primarily result from surface scum formation, which to date have been the focus of most risk assessments. However, cyanotoxins may accumulate in fish via direct feeding on phytoplankton, through uptake of dissolved toxins after lysis of blooms via epithelial absorption, or from exposure through the food web. Accumulation of MCs in freshwater “seafood” has been reported. In particular, MC-LR has been found in the muscle of flounder, Nile tilapia, redbreast tilapia, silver carp and roach fish, and MC-RR has been detected in silverside fish (Magalhães et al., 2001; Ibelings and Chorus, 2007).

Herein, Nile tilapia (*Oreochromis niloticus*) cultivated in an artificial lake in Brazil was collected both before and after the depuration process in order to investigate the accumulation of cyanotoxins in the presence of cyanobacterial blooms. Water and fish (liver and muscle) from the freshwater aquaculture were analysed by LC-ESI-MS for microcystins (MC-LR, -RR, -LA, -LF, -LW and -YR), NOD, ANA and CYN. HPLC-FD was used to detect STXs (STX, neosaxitoxin (NEO), gonyautoxins (GTX1, GTX2, GTX3, GTX4), and decarbamoyl STX (dcSTX)). The efficiency of depuration was also studied to evaluate reduction of cyanotoxin content in Nile tilapia.

Nile tilapia were collected in July 2005 in a fish farm from Garça city, situated in the Northeast of São Paulo state in Brazil (22°12′38″S 49°39′22″W). The tilapia was cultivated in cages from freshwater aquaculture. Twenty-four adult tilapia, each approximately 1 kg in size, were

collected with a net. Twelve tilapia were examined without depuration, and the other twelve underwent depuration in a depuration tank (1.40 × 2.40 m) with good quality running water and without food for five days. After this period, the living fish were put directly into coolers in layers intercalated with ice prepared from drinking water, where they suffered thermal shock. The hyperthermic fish were transported to the Food Processing Sector of the Agroindustry, Food and Nutrition Department of ESALQ-USP where the samples were processed. At the same time, water samples (three 1 liter bottles) were collected by vertical hauls for determination of cyanotoxin concentrations. Cyanobacteria samples were obtained using a phytoplankton net (25 µm mesh, $\theta = 30$ cm) for microscopic analyses, with one part transported fresh and another preserved with Transeau solution for phytoplankton identification. All the samples were lyophilized and stored at -80°C in glass bottles for the determination of MC-LR, -RR, -LA, -LF, -LW and -YR, NOD, ANA, CYN, STX, NEO, GTX1, GTX2, GTX3, GTX4, and dcSTX by chromatographic analyses.

For the extraction of cyanotoxins from fish and water, samples were treated according to AOAC (1990). The MCs, NOD, ANA and CYN were isolated and identified following the modified methods described by Dahmann et al. (2003) and Bittencourt-Oliveira et al. (2005). Briefly, the cyanotoxins were extracted twice with water-methanol mixture (50:50, v/v) by 10 min sonication in an ultrasonic bath and then treated for 2 min in an ultrasonic homogenizer (Sonopuls GM 70, Bandelin®, Berlin, Germany). The extracts were centrifuged (14,000 rpm), and the supernatant was filtered using 0.22 µm PTFE syringe filters (Roth®, Karlsruhe, Germany). The extracts (20 µL) were analysed via liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS). Quantitative MCs, NOD, ANA and CYN standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The STXs were isolated following the modified method from Anjos et al. (2006). Briefly, 50 mg lyophilised samples were extracted by adding 1 mL acetic acid (0.03 N), sonicated at 35 kHz for 60 s in an ice bath, and centrifuged at 14,000 rpm for 5 min. The supernatant was then filtered with a single-use syringe filter (0.45 µm). Finally, 10 µL of the extract was analysed via HPLC with fluorescence detection (FD) (Diener et al., 2006). An ion-pair buffer gradient composed of a solution of octanesulfonic acid and ammonia phosphate at pH 6.9 and acetonitrile was used to separate STXs. After post-column oxidation with alkaline periodic acid in a heated derivatisation unit at 50°C , the resulting products were detected using a fluorescence detector with λ_{EX} 330 nm and λ_{EM} 395 nm. STXs were identified by comparing chromatograms obtained from sample extracts with those remaining after injection of standard solutions. Quantitative STX, NEO, GTX1, GTX2, GTX3, GTX4, and dcSTX standards were purchased from the National Research Council Canada, Halifax, NS, Canada. Quantification of cyanotoxin content was performed using the factor response (peak area/toxin concentration) obtained from the injection of certified quantities of toxin standards.

The chromatograms (Fig. 1) show that only STXs were detected in the samples. The microscopic analyses revealed

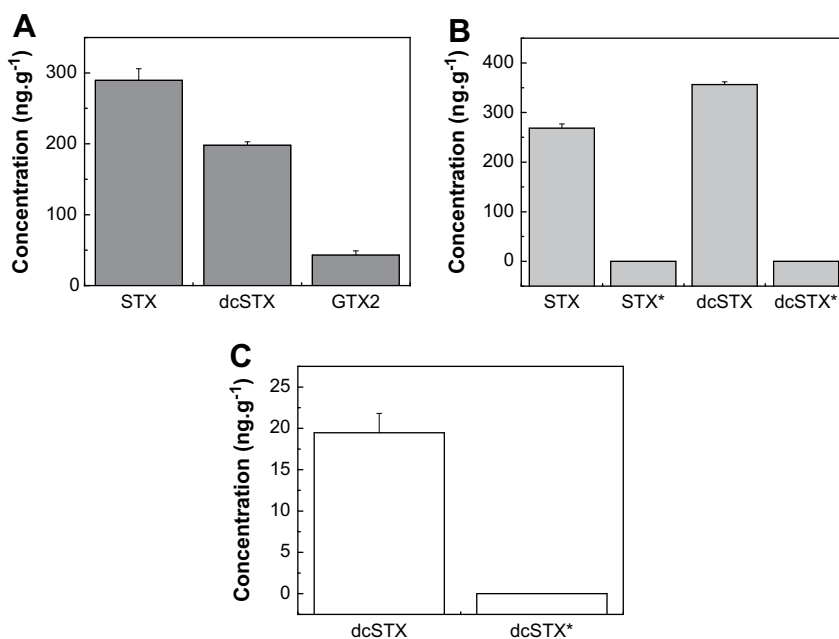


Fig. 2. Saxitoxin and related compound concentrations in the (A) water of the tank, (B) liver fish and (C) muscle fish before and (*) after depuration process. Results were obtained by HPLC-FD. Values are presented as the mean \pm SE of three water samples and twelve fish per group (before or after depuration process).

the occurrence of *Anabaena spiroides* Klebahn in the lake, which can produce the STXs found in the water samples (Fig. 2A) as well as in the liver (Fig. 2B) and muscle (Fig. 2C) tissue collected from fish. In Fig. 2B and 2C it can be also seen that Nile tilapia tissues were free of STXs after a depuration process. MCs, NOD, ANA, and CYN were not detected by LC-ESI-MS, and NEO, GTX1, GTX3 and GTX4 were not detected by HPLC-FD (limit of detection on column (ng): 0.5 for NOD, 0.7 for MC, 1.0 for ANA, 1.5 for CYN, 0.3 for NEO, 0.8 for GTX₁, 0.015 for GTX₃ and 0.96 for GTX₄).

The release of nutrients from aquaculture production using cage farms in protected areas is thought to be responsible for eutrophication and pollution, which may have effects on the environment, ecosystems and fishery resources (Prieto et al., 2006). Because of the high input of nutrients due to feeding activity, toxic cyanobacterial blooms have frequently occurred in aquatic ecosystems used for aquaculture and fishery (Soares et al., 2004). Freshwater culture of tilapia is common in Brazil, Africa and China (Canónico et al., 2005). The depuration routine can be used for fish cultivated in freshwater in order to decrease the number of microorganisms, to improve flavour and to eliminate/decrease the presence of phytoplankton metabolites. In this process, fish are transferred from culture tanks or artificial lakes to ponds with clean water, and are kept there for several days without feeding (Stickney, 2005).

The current study analysed, for the first time, the accumulation of STXs in fish cultivated in freshwater. Our results indicated that Nile tilapia may accumulate STXs, including STX and dcSTX, in freshwater aquaculture, during toxic cyanobacteria bloom episodes.

The toxicological database for STXs is limited and is mostly composed of studies on their acute toxicity

following intra-peritoneal (i.p.) administration in mice. No data on the chronic effects of STXs in animals or humans are available, so the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) could not establish a tolerable daily intake. From the available reports on exposure in humans, a lowest-observed-adverse-effect-level (LOAEL) in the region of 1.5 μ g STXs/kg b.w. could be set, and an estimated no-observed-adverse-effect-level (NOAEL) of 0.5 μ g STXs/kg b.w. was established. Thus, the CONTAM Panel has defined an acute reference dose (ARfD) of 0.5 μ g STXs/kg b.w. (EFSA, 2009). In accordance with the FAO and WHO, the total STX-group toxin content must not exceed 80 μ g STXs/100 g of mollusc flesh (FAO/WHO Codex, 2008). However, the CONTAM Panel observed that consumption of a 400 g portion of shellfish meat containing STXs at the current limit of STXs would result in an intake of 320 μ g of toxin (equivalent to 5.3 μ g/kg b.w. in a 60 kg adult). This level of intake is considerably higher than the ARfD guideline (equivalent to 30 μ g of STXs per portion for a 60 kg adult), and is a health concern (EFSA, 2009).

Although the levels found in tilapia muscle in our study were lower than the ARfD limit, this may signal that an imminent danger exists since a longer exposure time may result in greater bioaccumulation of toxins, as occurs with the MCs (Ibelings and Chorus, 2007).

Therefore, freshwater fish monitoring of STXs should be considered an important issue in quality control. The occurrence of toxic cyanobacteria blooms producing STXs in aquaculture can represent a risk to the quality of the fish and, consequently, this exposure route should be considered an important concern for public health authorities (Dittmann and Wiegand, 2006). Our results suggest that depuration is a simple process that can be readily adopted by producers as a way to eliminate STXs.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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