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Genetic toxicity of water contaminated by microcystins collected during a cyanobacteria bloom

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ABSTRACT

Microcystin-LR (MCLR) is a toxin mainly produced by *Microcystis aeruginosa*, cyanobacteria most commonly found in eutrophic environments. Cyanobacteria blooms have affected Salto Grande reservoir (Americana, State of São Paulo/Brazil) for several decades, often observed during periods of drought. In this study, the genotoxic effects of MCLR (95% purity) and water samples contaminated by this toxin were evaluated during cyanobacteria bloom using assays with the test organism *Allium cepa*. The results showed genotoxic action for pure microcystin and cytotoxic, genotoxic and mutagenic action for water samples collected during flowering. Chromosomal aberration assays have shown that MCLR induces chromosomal breaks that persist in the daughter cells as MN. Therefore, it is possible to infer a clastogenic action for this toxin. The MCLR present in the environmental samples was shown to be more cytogenotoxic for the cells than the different concentrations tested in this study with the pure substance. This amplified toxic action can be related to a synergistic effect between the MCLR and other compounds present in the environmental samples. The genotoxicity studies with MCLR show inconsistent and inconclusive results, so this toxin needs to be better investigated in order to obtain further information about the action mode of it is on the biological system.

1. Introduction

The state of São Paulo is considered to be the most developed state of Brazil in terms of urbanization and industrialization (CETESB, 2008), it is also stands out in the field of agriculture, as it is the largest producer of sugarcane of Brazil (Corbi et al., 2006; INPE, 2018). However, this greater progress ends up generating a lot of waste that places environmental quality at risk. As the aquatic environment suffers most from environmental contamination, since it is the final destination of all pollutants, rivers and reservoirs end up receiving many compounds that deteriorate the quality of their waters (Manzano et al., 2015), resulting in a risk to both the aquatic life and health.

This deterioration of water bodies can induce cyanobacteria bloom, which results in contamination by cyanotoxins.

Cyanotoxins are toxic substances produced by various species of cyanobacteria. Among this toxin group, the Microcystin-LR (MCLR), which is mainly produced by *Microcystis aeruginosa*, is the most commonly found in the environment (Downing et al., 2005; EPA, 2017; Jang et al., 2006; Zhang et al., 2017). The influx of nitrogen and nutrients in aquatic systems causes eutrophication and not the occurrence of MCRL (Rietzler et al., 2016; Sinha et al., 2012; Trout-Haney et al., 2016). In general, these cyanotoxins present high hepatotoxic, dermatotoxic and neurotoxic potential (Drobac et al., 2013; Hercog et al., 2017; Müller, 2017).

Since these molecules have a high molecular stability, regarding temperature, pH, and hydrolysis, and because they present high toxicity and persistence in the environment. Then is a great concern about their presence in water resources due to the risk they may present to the aquatic health and human life (Lone et al., 2015).

The major water resources affected by cyanotoxin contamination are lakes and reservoirs. In Brazil, the Salto Grande reservoir, located in Americana city, State of São Paulo, stands out as a reservoir with recurrent cyanobacteria blooms since 1969, mainly observed in drought periods associated with high temperatures (Espíndola et al., 2004; Tucci et al., 2004). This reservoir, which is located near large urban centres of São Paulo State, has great economic and social value for the region (Deberdt, 2002). Besides being used by the population as public supply and leisure, which arouses great concern with its contamination by cyanobacteria and their toxins.

Cyanotoxins are known for the capable of inducing damage to DNA molecules, in the case of microcystins, the damage is linked to the inhibition of phosphatases 1 and 2A (PP1 and PP2A) which leads to the phosphorylation of cytoskeletal proteins, causing disorganization (Chen and Xie, 2016).

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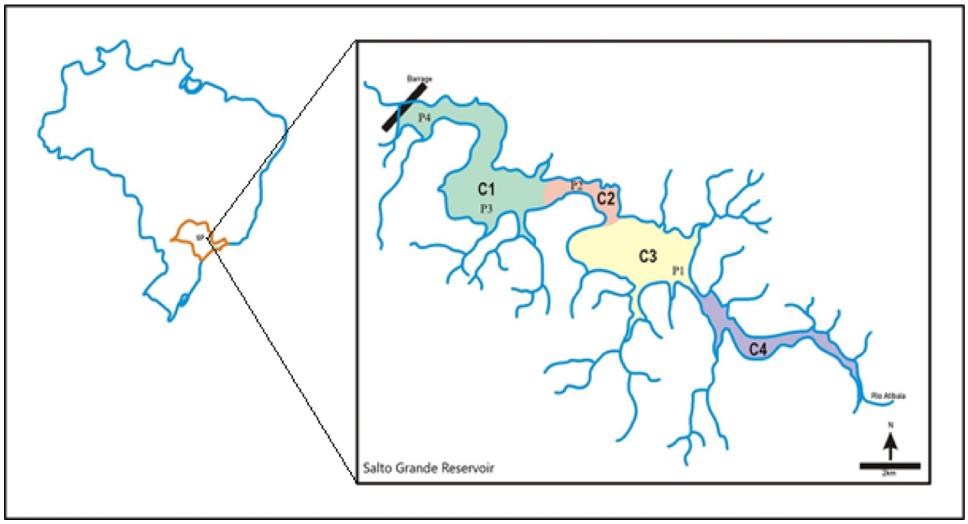

Fig. 1. Scheme of the Salto Grande reservoir Americana – SP, with the collection sites P1- Site located in compartment 3(C3), shortly after the narrowing of compartment 4 (C4); P2- Site located where a narrowing occurs between compartment 3 (C3) and 2(C2); P3- Site located in compartment 2 (C2); P4- Site located in compartment 1 (C1), near the reservoir dam.

The observation of these damages in the DNA of superior plants has shown a good correlation with human cells (Hara and Marin-Morales, 2017; Palmieri, 2016). Due to the high human exposure to these toxins, there is a real and urgent demand to better understand the effects of these toxins on DNA, as well as to monitor water resources that have history of seasonal contamination by cyanobacteria blooms.

Cytogenetic tests are widely used to monitor the extent of pollution and also to assess the effects that different substances can cause in the biological environment (Bafana et al., 2018; Caritá and Marin-Morales, 2008; Leme and Marin-Morales, 2009). Many short-term cytogenetic assays are applied to study the mutagenic and genotoxic potential of environmental contaminants (Bafana et al., 2018; Leme and Marin-Morales, 2009).

The tests developed with higher plants provide accurate information on the toxic potential of a substance and are easier to carry out than tests with animal cells or embryos (Fiskesjö, 1985; Grant, 1999, 1998, 1994; Leme and Marin-Morales, 2009; Rank and Nielsen, 1994). For this reason, these tests are often used to monitor contaminated environments (Leme and Marin-Morales, 2009; Mazzeo et al., 2011; Oudalova et al., 2017). *Allium cepa* test organism is considered an efficient bioindicator because it readily responds to genotoxicity and provides important information about the mechanism of action of pollutants (Oudalova et al., 2017; Rank and Nielsen, 1994; Roberto et al., 2016).

Some studies indicate that there is a relationship between cyanotoxins with chromosomal abnormalities and chromatidic breaks (Ding et al., 1999; Gaudin et al., 2008; Zegura, 2016; Zegura et al., 2004) such as the induction of free radical production, the formation of DNA adducts and cytoskeleton disorders and repair machinery. The aim of this study was to evaluate both the effects of the commercially acquired cyanotoxin, used as a parameter from the e isolated substance effect, and of those present in water extracts collected during the cyanobacteria bloom in an important reservoir in Americana city (São Paulo State-Brazil), using the test organism *A. cepa*

2. Materials and methods

Two tests were carried out separately: the first one with the commercially acquired pure substance (CAS No. 101043-37-2- Cayman) and the second with samples extracts obtained by solid-phase extraction (C18 cartridge) from water collected in Salto Grande reservoir (Americana city, São Paulo State, Brazil) during cyanobacteria bloom. During the collection it was observed great flowering of cyanobacteria in all points, but with certain different characteristics: in P1, we observed the greatest drought of all and the flowering was brown in color; in P2, we observed a larger water column and the flowering presented a more vivid and dense green coloration; in P3, we observed a flowering with characteristics similar to those of P2 and in P4 a less dense flowering was observed.

2.1. Microcystin LR

Three concentrations of pure MCLR (CAS No. 101043-37-2-Cayman) were tested in this study: 1 µg/L (concentration considered as level 1 alert by WHO); 1.5 µg/L, simulating a beginning of cyanobacteria bloom; 2 µg/L (simulating an advanced cyanobacteria bloom).

2.2. Collection water samples

We also evaluated the water collected in four distinct points of the Salto Grande reservoir (Americana - SP). The collections, carried out in a school boat, used to transport students who visit the dam, followed the standards NBR 9.898, established for preservation and sampling techniques of effluent and receiving bodies, proposed by ABNT (Brazilian Association of Standards and Techniques). Four liters of water were collected from each point (P) and stored in dark glass bottles for later extraction of toxins. The location of the sample points are shown in Fig. 1. P1- Site located in compartment 3, shortly after the narrowing of compartment 4; P2- Site located where a narrowing occurs between compartment 3 and 2; P3- Site located in compartment 2; P4- Site located in compartment 1, near the reservoir dam (Fig. 1).

2.3. Extraction of cyanotoxins from environmental samples

After collecting the water samples from the reservoir with cyanobacteria bloom, extracts (Solid Phase Extraction - SPE) of the organic contaminants present in the 4 collection sites were obtained, using C18 cartridges, considered the most suitable for extraction of cyanotoxins. Cartridges were eluted in methanol. Then, the methanol was evaporated using $N_{2\ (G)}$ in Manifold apparatus. The material obtained from the elution was used for the dosage of the microcystin present in the samples, by the kit specific for this cyanotoxin (Abraxis, No. 522015/ Microsystins-DM),

Then, the preliminary tests of germination of *A. cepa* seeds were performed on Petri dishes lined with filter paper, according to Grant (1999), they were maintained at constant temperature (24–26 °C) and humidity (5%), with 12/12 photoperiod. As the seeds did not germinate when exposed to the crude extracts, different dilutions of these extracts were tested to estimate the concentration that allowed a viability of germination higher than 80%. This concentration was of 10% of the crude extract (1 of crude extract for 10 of Mili-Q water). The Negative Control (NC) was performed in mineral water and the Positive Control