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HYALELLA AZTECA USED FOR THE EVALUATION OF CYANOTOXINS BEFORE AND AFTER IRRADIATION WITH ELECTRON BEAM: PRELIMINARY RESULTS

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ABSTRACT

The occurrence of cyanobacteria blooms in water bodies used for human consumption represents risks to the population due to the ability of these microorganisms to produce metabolites, known as cyanotoxins. They can be classified as hepatotoxins, dermatotoxins and neurotoxins. Cyanotoxins are water soluble and are not removed by the conventional water treatment system for public supply. Several studies have been carried out to minimize this type of contamination. Electron beam irradiation has been applied in aqueous solution containing the cyanobacteria of the genus Microcystis, in 40% of growth medium content and applying 2.5 kGy. The amphipod *Hyalella azteca* was the aquatic organism selected for the evaluation of the potential of cyanotoxins risks to the biota of the reservoirs where the cyanobacteria blooms occurs, being demonstrated high toxicity.

1. INTRODUCTION

The cyanobacteria or cyanophytes are aerobic photoautotrophic microorganisms, therefore their vital processes require only water, carbon dioxide, inorganic substances and light. The eutrophication of aquatic environments allow the bloom of cyanophytes as a result of anthropic activities, causing artificial enrichment of ecosystems. The main sources of this enrichment have been identified as domestic and industry sewage discharges from urban centers and agricultural regions [1]. The occurrence of cyanobacteria blooms development in water bodies used for human consumption represents risks to the population due to the ability of these microorganisms to produce metabolites, known as cyanotoxins.

Due to the need to implement other technologies for the treatment of cyanotoxins from water for human consumption, the present study analyzed the ecotoxicological effects of microcystin toxin samples produced by *Microcystis aeruginosa* cyanophytes in laboratory before and after treatment by ionizing radiation (electron beam). This technology for cianobacteria control has been developed also in China [2, 3]. Toxins from algae may also be kept into sediments of rivers and be a start-source of a new bloom.

Few authors worked on the assessment of toxicity of cianotoxins in sediments. Nonetheless there is a consensus that sediments concentrate many pollutants and it can be a source of cyanophytes cells able to start a new bloom in a favorable time, reflecting in the amount of toxins into the reservoirs. The amphipoda *Gammarus zaddachi* (sea gammarids) was exposed to algal toxins [4]. The distribution and annual variations of MCs in sediments of Lake Taihu, China were reported by Chen et al [5].

The use of ionizing radiation through electron beams is one of many technologies to be used for the abatement of pollution from different sources. The ionizing capacity of radiation is based on the water radiolysis and can be effective in reducing whole toxicity with relatively low doses of radiation [2, 3].

The objective of this paper was the determination of acute effects of cianobacteria to the *Hyalella azteca* amphipod before and after irradiation at electron beam accelerator (EBA).

2. MATERIALS AND METHODS

This study refers to the use of an amphipod, *Hyalella azteca*, exposed to samples containing a culture medium suitable for *Microcystis aeruginosa* cianobacteria and the following step was using ionizing radiation trough electron beam to observe the resulting effects. The use of *H. azteca* was related to the potential of sediments as a source of cianotoxins blooms and *H. azteca* and it is very dependent on sediments as biological characteristics of its group. All the experiments were carried out at the Laboratory for Biological and Environment Assays, at Centro de Tecnologia das Radiações (CETER), Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP).

2.1. Cyanobacterial cultures

The present study analyzed samples of microcystin toxins produced by laboratory-grown *Microcystis aeruginosa* cyanophytes.

The ASM-1 was the culture medium used as reference for growing *Microcystis aeruginosa* cyanophyte [6]. Four stock solutions were prepared and named as solutions A, B, C and D. The aforementioned solutions and their respective volumes were prepared. To prepare each liter of culture medium in a volumetric flask, 300mL of distilled water was added and the following solutions: 20mL of solution A, 2mL of solution B, 0.1mL of solution C and 0.4mL of solution D. This solution was homogenized and and the volumetric flask was completed to one liter with distilled water. The pH was adjusted to 8.0 using 1M NaOH (sodium hydroxide) or 1M HCl (hydrochloric acid). The culture medium was autoclaved before use it.

2.2. Toxicity tests with *Hyalella Azteca* (Amphipod)

The experiments with the freshwater amphipod of the genus *Hyalella* were carried out in controlled laboratory conditions. The assays method followed the recommendation of Brazilian Standard Method, ABNT NBR 15470 (2007) [7]. *Hyalella azteca* is a suitable organism for studies concerning pollution into sediments in rivers and water reservoirs, Figure 1.

The samples analyzed undergone treatment with ionizing radiation (electron beam), in order to reduce the toxicity of cyanotoxins present in the samples. In order to enable ecotoxicological analysis, bioassays were performed with the organism-test (organisms used to perform the toxicity test) described below.

Figure 1 – *Hyalella azteca* from LEBA culture (4X)

Test vessels were prepared prior to the start of the experiment. Each vessel contained an artificial substrate made with a small nylon mesh with an opening of one hundred and fifty to six hundred micrometers (μm). Four concentrations and one negative control group were prepared for each sample. Ten replicates were performed for each studied concentration. In the first experiment, the organisms were exposed to the control group (20mL of culture water in each vessel), and more four concentrations: the first concentration corresponds to 16mL of culture water and 4mL (20%) of cyanobacteria (*Microcystis aeruginosa*) in each vessel; the second concentration in which the organisms were exposed corresponds to 8mL of culture water and 12mL (40%) of cyanobacteria in each vessel; the third concentration corresponds to 8mL of culture water and 12mL (60%) of cyanobacteria in each vessel and the fourth concentration in which the organisms were exposed, corresponds to 4mL of culture water and 16mL (80%) of cyanobacteria in each vessel.

In the second test, the samples used during the period of exposure of the organisms were submitted to treatment by 2.5kGy ionizing radiation (electron beam). To irradiate the samples, 492mL of cyanobacteria (*Microcystis aeruginosa*) were distributed in pyrex borosilicate vessels and covered with plastic film in order to avoid contamination and loss of contents. In each pyrex 246mL of the sample was placed, ensuring that the thickness of the sample in 4mm, in order to obtain more homogeneous processing during the irradiation.

For the experiment with the treated samples, the organisms were exposed to the control group (20mL of culture water in each vessel), and more four concentrations: the first concentration corresponds to 16mL of culture water and 4mL (20%) of irradiated cyanobacteria in each vessel; the second concentration in which the organisms were exposed corresponds to 8mL of culture water and 12mL (40%) of irradiated cyanobacteria in each vessel; the third concentration corresponds to 8mL of culture water and 12mL (60%) of irradiated cyanobacteria in each vessel and the fourth concentration in which the organisms were exposed, corresponds to 4mL of culture water and 16mL (80%) of irradiated cyanobacteria in each vessel.

2.3. Treatment by ionizing radiation (electron beam)

A Dynamitron Electron Beam Accelerator was applied for the irradiations EBA. The machine energy was fixed at 1.4 MeV with variable electric current according to the needed dose. In this first step we have applied 2.5kGy to ASM-1 solution. After irradiation this ASM-1 solution was exposed to young *H. azteca* (7 days) in four different concentrations: 20%, 40%, 60% and 80%.

3. RESULTS AND DISCUSSION

The previous results obtained through the exposition of *Hyalella Azteca* to cianotoxins before and after irradiaion were presented as Figure 2.

To validate the biological assays the sensitivity of the test organism was determined each month, using potassium chloride (KCl) as a reference substance. The Average Lethal Concentration (LC50) to *Hyalella Azteca* varied from 183.35 ppm up to 223.47 ppm for 96 hours of exposition.

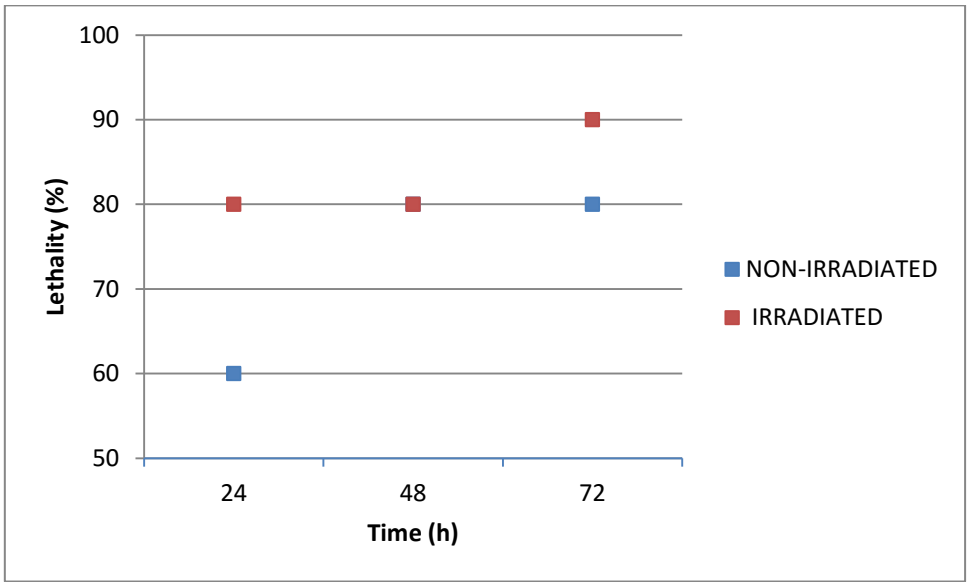

Figure 2 – Toxicity of cyanotoxins to *Hyalella azteca* when exposed to 40% content of ASM-1 media

According to Fig. 2, it was noted 60% up to 90% lethality of exposed organisms for 24 hours up to 72 hours. There was an enhancement of effects when samples were irradiated at 2.5 kGy and in the presence of ASM-1 media.

Ionizing radiation application and the occurrence of cyanotoxins in China has also been reported. Zheng et al [2] and Liu et al [3] are studying ionizing radiation for the control of cyanotoxins, using gamma source and electron beam accelerator, respectively.

Remaining cyanobacteria produce toxins that can kill wildlife and domestic animals and cause death in humans through exposure to contaminated freshwater or by the consumption of contaminated drinking water, fish or shellfish.

Regarding the Microcystins in sediments, there is a possibility that this compartment is a deposit of Microcystis cells and in favorable conditions, they grow and expand cells to the aquatic system. Because of the difficulty in extracting toxins from sediments, analysis of MCs in aquatic environment has been limited to determining the MC content in water. The distribution and annual variations of MCs in sediments of Lake Taihu, China were reported by Chen et al [5].

3. CONCLUSION

For now the irradiation conditions and applied dose did not improved toxicity and whole toxicity of miycrocystins determined for amphipods in the present study showed *Hyalella azteca* as a very sensitive organism. There is a need for the confirmation of radiation benefits for cyanotoxins removal in raw water.

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