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MICROCYSTIN BIOACCUMULATION IN CULTURES IRRIGATED WITH EUTROPHIZED WATERS IN BRAZILIAN SEMIARID

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ABSTRACT

The present investigation was aimed to investigate the bioaccumulation of microcystin and cylindrospermopsin in *Capsicum annuum* (bell pepper) and *Coriandrum sativum* (coriander) irrigated with waters from a eutrophic reservoir and persistent cyanobacterial blooms. Samples of bell pepper and coriander were collected in ten areas distributed along Epitacio Pessoa reservoir. To bell pepper, samples (leaves and fruits) were collected in plants in three developmental stages (sprout, green fruit and ripe fruit). To coriander plants, leaves and stems are collected in different time of development, 30 days old and 60 days old. Cyanotoxins were determined by immunoassay ELISA kits. Phytoplankton samples were beneath the water surface next the irrigated areas. Cyanobacteria is dominant in phytoplankton community. Microcystin and cylindrospermopsin were detected in water reservoir. Only microcystin were detected in plants. For bell pepper, the highest concentrations of microcystin occurred in the leaves, however, no differences were observed between leaves, fruits and maturation time. Significant differences were observed between time of development to microcystin concentrations in leaves and steam of coriander. Microcystin can uptake by bell pepper and coriander cultures irrigated with contaminated water and the bioaccumulation varied as a function of exposure time and species dependent. This is a potential risk to consumers, since microcystin can biomagnified in the trophic chain.

KEYWORDS: *Coriandrum sativum*, *Capsicum annuum*, Cyanotoxin, Eutrophication, Health risk.

INTRODUCTION

Cyanobacterial blooms are not a new problem. Cyanobacteria are a group of gram-negative, aerobic and photoautotropic bacteria. Morphologically, these organisms did not change significantly throughout evolution (about 2.700 million years ago), yet their adaptability and tolerance to environmental conditions allowed colonization across the planet. Although found in many different environments, cyanobacteria are present mainly in aquatic ecosystems as part of phytoplankton and periphyton, playing an important role as primary producer.[1,2]

In environments where cyanobacterial blooms occur, cyanotoxins released from cyanobacteria have been demonstrated to have deleterious effects on human, animal and environmental health.[3,2,4]

Cyanotoxin can be accumulated into aquatic products via contaminated feeds, direct contact with contaminated water (living environment) and biomagnification through the food web.[4,5] Plants are not usually killed by the environmentally relevant concentration, but their growth and crop yields are affected.[6,7,8] In addition, the soil may retain toxins when extra water flows through, and it can bioaccumulate toxins during non-bloom seasons [9], [10]. Humans are exposed to cyanotoxin by drink water, inhalation, dermal contact and foods.[2,3,11]

Microcystin and cylindrospermopsin can cause toxic effects on terrestrial plants,[12] thus the significance of the use of surface water contaminated with cyanotoxins for agricultural purposes is a field of increasing interest. Furthermore, to the potential effects on plant growth and development, this issue may pose concerns for food safety if the possible absorption of toxins by plants can lead to its bioaccumulation in edible tissues.

Most of studies about bioaccumulation of cyanotoxin in plants are experimental. A lack of information occurs in relation of environmental conditions and the bioaccumulation of cyanotoxin in cultures exposed at ecologically relevant concentrations. Moreover, this study aimed to investigate the bioaccumulation of microcystin and cylindrospermopsin in *Capsicum annuum* (bell pepper) and *Coriandrum sativum* (coriander) natural cultures, irrigated with waters from a eutrophic reservoir and persistent cyanobacterial blooms.

MATERIAL AND METHODS

Study Area and Sampling

The study was conducted in Epitacio Pessoa reservoir (7°29'20.08"S, 36°8'26.59"W) located in the Paraiba River Basin in a semiarid region of Brazil. This reservoir is used to supply water to approximately one million people, irrigation, animal desedentation and recreation. Throughout reservoir, 10 sampling areas with irrigated agriculture activity were selecting (Figure 1), and during February of 2019, samples of leaves and fruits of *C. annuum* (bell pepper) plants and leaves and stems of *C. sativum* (coriander) plants, in different maturation stages are collect. Water samples were collected in reservoir, next water catchment sites for irrigation.

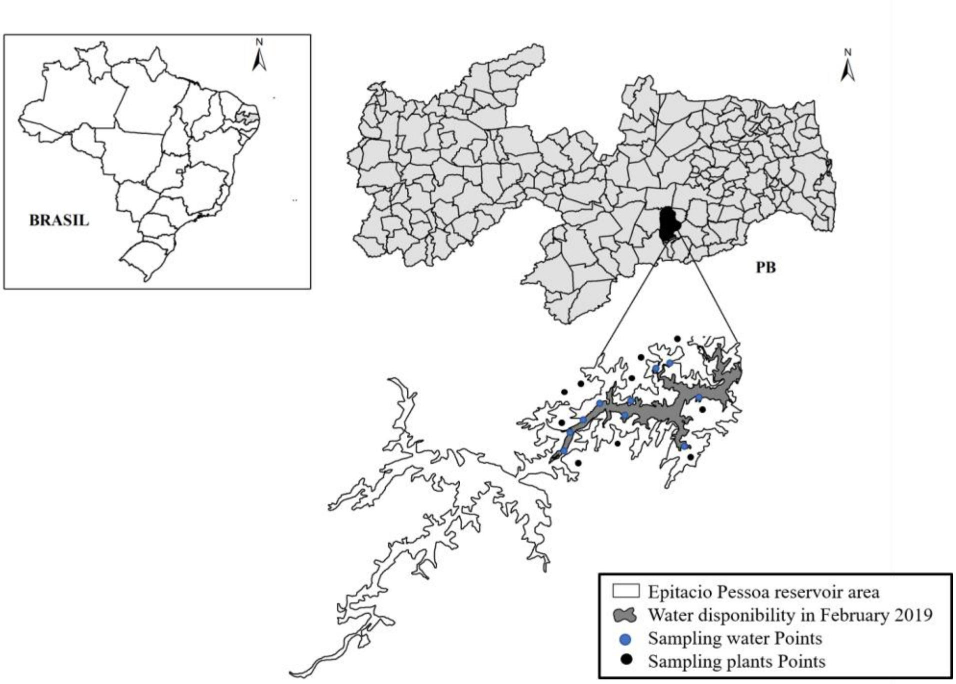

Figure 1: Location of sampling areas throughout Epitacio Pessoa reservoir.

Analysis of Water

Water samples were collected in PVC bottles previously cleaned with distilled water to evaluated cyanotoxins using immunoassay Elisa kits to microcystin, and cylindorpermopsin produced by Abraxis®. Samples with 300mL were fixed with 2% lugol solution to quantitative phytoplankton analyzes using inverted microscope (Zeiss Axiovert), as described by Uthermol.[13] Cyanobacterial genera and species were identified via microscopic observation by distinguishing morphological characteristics cited in the literature.[14]

Cyanotoxin Analysis in Plants

Leaves and fruits of bell pepper were collected from plants with different maturation stages (sprout, green fruit and ripe fruit), in triplicate for the ten sampling areas. To coriander plants, leaves and stems were collected in different time of development, 30 days old and 60 days old, but only in six sampling areas, according disponibilities in areas. Portions of stems, leaves and fruits of all sampling plants were removed, washed with deionized water and dried. 1g of each portion was weighed and freeze at -80ºC until analysis. Extraction of cyanotoxins were done following the procedure described by Hereman and Bittencout-Oliveira.[15] Concentration of microcystin and cylindropermopsin were evaluated using immunoassay Elisa kits to microcystin, and cylindorpermopsin produced by Abraxis®.

Statistical Analysis

Differences in cyanotoxins concentration among maturation stages of plants were compared using oneway ANOVA ($P < 0.05$) using R software.

RESULTS AND DISCUSSION

Phytoplankton and Cyanotoxin in Water

The phytoplankton community in Epitacio Pessoa reservoir was represented by 37 taxa from the phylo/divisions, Cyanobacteria, Chlorophyta, Bacillariophyta, Charophyta, Miozoa, Euglenozoa (Table 1). Cyanobacteria was the dominant group representing 88,4% of total phytoplankton density. *Pseudoanabaena galeata* Bocher 1949 and *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno 2018 were the most abundant species in all samples. These species were reported like potential producers of microcystin, saxitoxin.[16] and cylindrospermopsin.[17]

Table 1: List of phytoplankton species observed in Epitacio Pessoa Reservoir in February 2019.

Cyanobacteria	**Bacillariophyta**
Chroococcus dispersus	*Aulacoseira granulata*
Amphiheterocytum lacustre	*Cyclotella striata*
Aphanocapsa elachista	*Eunotia sp*
Coelomorum tropicalis	*Navicula sp.*
Cuspidothrix tropicalis	*Nitzschia closterium*
Limnothrix planctonica	*Pinnularia sp.*
Microcystis aeruginosa	*Stauroneis sp*
Microcystis protocystis	*Synedra ulna*
Phormidium breve	**Charophyta**
Planktothrix agardhii	*Closterium lanceolatum*
Planktothrix isothrix	*Mougeotia sp.*
Pseudanabaena galeata	*Staurastrum leptocladum*
Raphidiopsis raciborskii	**Miozoa**
Chlorophyta	*Ceratium furcoides*
Ankistrodesmus spiralis	*Peridinium sp.*
Chlorella vulgaris	**Euglenozoa**
Crucigeniella crucifera	*Lepocinclis acus*
Dictyosphaerium pulchellum	*Phacus curvicauda*
Kirchneriella lunaris	*Trachelomonas armata*
Monoraphidium contortum	*Trachelomonas volvocina*
Oocystis lacustres	
Scenedesmus acuminatus	

Microcystin and cylindrosperrmopsin were detected in reservoir water, in all samples, with higher concentration of cylindrospermopsin. No significant differences were observed to cyanotoxin concentration among sample sites (Table 2). In Brazilian semiarid, usually faces periods of drought, besides reducing the reservoirs to critical volumes, favors toxic cyanobacterial blooms.[18]

Table 2: Cyanotoxins concentrations (Mean and standard deviation (SD)) in Epitacio Pessoa reservoir in February 2019.

Cyanotoxins	**Mean**	**± SD**	**p**
Microcystin	0,73	0,35	0,07
Cylindrospermopsin	1,73	0,95	0,06

Cyanotoxins in Plants

The bioaccumulation of cyanotoxins in plants varied as function of exposure concentration, time.[10] and dependent on the plant species.[19] All vegetable plants collected presented bioaccumulation of microcystin in leaves and fruits to bell pepper, leaves and stem to coriander. Cilyndrospermopsin were not detected. Appear microcystin presented lower concentration in water, it is very stable and many persistent in aquatic systems for weeks after being released for cells.[20] In addition, the soil may retain toxins when extra water flows through, and it can be a via to bioaccumulate toxins during non-bloom periods.[9]

In Epitacio Pessoa reservoir, toxic bloom of cyanobacteria is frequent, persistent and toxic.[21] and can explain the accumulation of microcystin in plants. In other hand, studies regarding bioaccumulation of cyanotoxin in the environment are scarce,[12] so more field studies are necessary to explain the bioaccumulation of cyanotoxins with low toxins in water.

For bell pepper, the highest concentrations of microcystin occurred in the leaves, however, no differences were observed between leaves and fruits ($F_{2;60}$=2,5; p=0,5). The mechanisms of microcystin bioaccumulation in plants are relatively unexplored. Some authors suggest that the toxin is absorbed by root system and translocated, by specific transporters, dependent of plant metabolism, to shoots.[22,23]

Our study corroborates with this, because with the drip irrigation mechanism, the font of microcystin was the complex soil-water. According Romero-Oliva et al.,[24] in *Capisicum annunt*, microcystin-LR translocation goes further into fruits and even into new plants by their seed, indicating a new rote of contamination.

The green fruit plants presented higher concentrations of microcystin, with lower concentrations to ripe fruit plants (Figure 2A). Appear no significant differences were observed to microcystin concentration among maturation fruit stages to leaves ($F_{1;30}$=6,2; p=0,3) and fruit ($F_{2;30}$= 1,3; p=0,3), we suggest a potential depuration of microcystin in bell pepper, during maturation time, but studies of bioaccumulation and depuration kinetics to this species are necessary. Some plants were reported with capability to depurate microcystin, like lettuce (*Lactuca sativa*), however this process cannot be efficient.[12,25]

Instead bell pepper, significant differences were observed between time of development to microcystin concentrations in leaves ($F_{1;18}$=46,0; p=0,003) and stem ($F_{1;18}$=40,1; p=0,002) of coriander. For this no depuration mechanisms are suggested. Higher concentrations of microcystin were observed 60 days old, to leaves and stem (Figure 2B), so we suggest that mechanisms of accumulation differ between plants in study. No significant difference was observed between leaves and stem microcystin concentration ($F_{1;36}$=5,3; p=0,4).

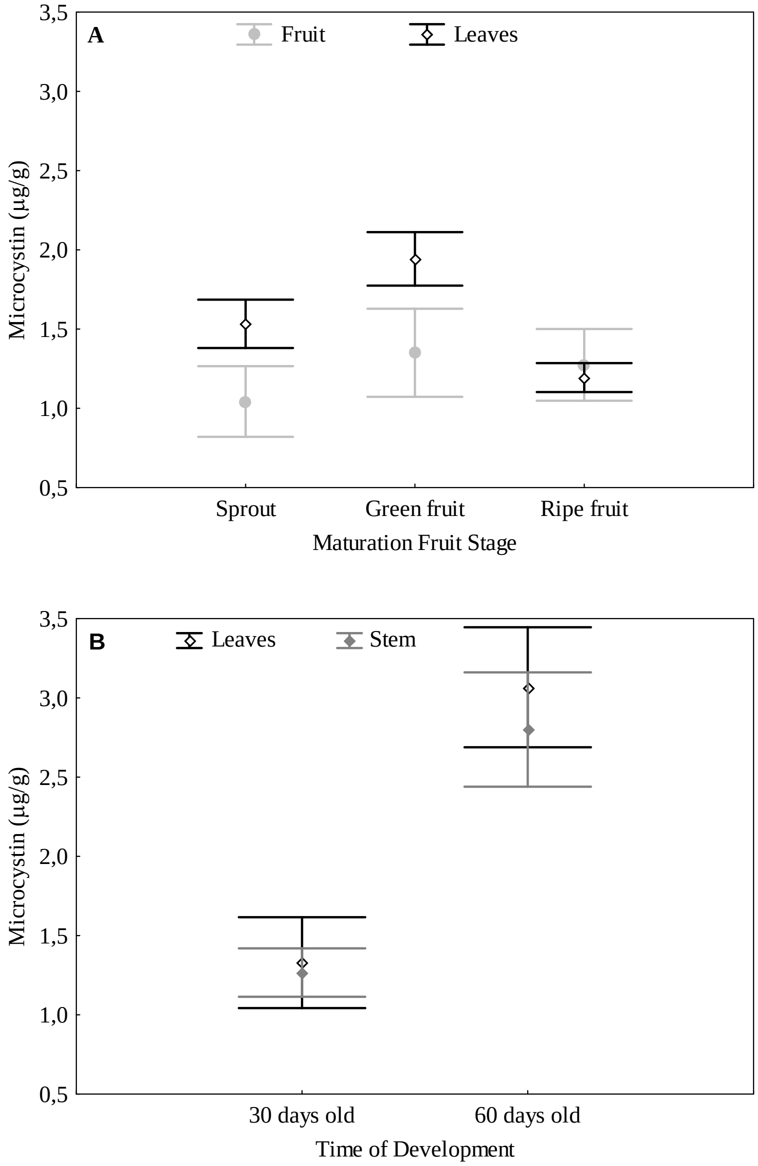

Figure 2: Microcystin concentrations in bell pepper (A) and coriander (B) from cultures throughout Epitacio Pessoa reservoir in February 2019.

Independent of mechanisms of bioaccumulation, from the human health perspective, the control of toxic cyanobacterial bloom in waters of multiple uses need management measure to avoid risks. Agricultural contaminated products are an emergent rout of human exposition to cyanotoxin and even in low concentrations, potential risks to health, depending on exposure time.[26] There are significant lack of studies correlating the effects of chronic natural low concentration of cyanotoxin and carcinogenic effects, for example. In China the development of primary liver cancer was linked to long-term chronic exposure to cyanotoxins.[27]

To agricultural production, some effects of cyanotoxin were observed, inducing morphological and physiological alterations, and consequently cause putative loss of productivity due to the inhibition of germination, growth and development.[28]

CONCLUSION

Microcystin can uptake by bell pepper and coriander cultures irrigated with contaminated water and the bioaccumulation varied as a function of exposure time and species dependent. The real impact of cyanotoxins on agricultural plant food safety are not fully understood, so more research ins need to assess the effects of environmental concentrations of cyanotoxin in different cultures and consequences to human health.

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