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NOTE

BIOACCUMULATION OF MICROCYSTINS IN LETTUCE[1]

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The contamination of lettuce (*Lactuca sativa* L.) by water-borne crude extracts of the cyanobacterium microcystin-producing *Microcystis aeruginosa* (Kützing) Kützing was investigated. The aim of the study was to determine whether bioaccumulation of microcystins occurs in lettuce foliar tissue when sprayed with solutions containing microcystins at concentrations observed in aquatic systems (0.62 to 12.5 μg · L^{-1}). Microcystins were found in lettuce foliar tissues (8.31 to 177.8 μg per Kg of fresh weight) at all concentrations of crude extracts. Spraying with water containing microcystins and cyanobacteria may contaminate lettuce at levels higher than the daily intake of microcystins recommended by the World Health Organization (WHO), underscoring the need to monitor such food exposure pathways by public authorities.

Key index words: **alimentary security; cyanobacteria; food contamination; irrigation; *Microcystis aeruginosa*; vegetables**

***Abbreviations*: MC, microcystin; WHO, World Health Organization**

Cyanobacterial blooms have been recorded with increasing frequency due to global warming and increased eutrophication in water bodies (Paerl and Huisman 2009), with some cyanobacteria producing toxins that may affect water quality and represent a public health risk (Carmichael 1992). Microcystin (MC) is the most common cyanotoxin in water bodies and is produced mostly by species of the genus *Microcystis* Kützing ex Lemmerman. These toxins inhibit protein phosphatases 1 and 2A, causing loss of cytoskeleton integrity and considerable harm to mammals (Yoshida et al. 1997, Jochimsen et al. 1998). Humans can also be exposed to MC by consumption of contaminated fish and mollusks (Magalhães et al. 2001, Mohamed et al. 2003, Xie et al. 2007) or vegetables irrigated with water containing toxic cyanobacteria. Codd et al. (1999) found MCs, as well as individual cells and colonies of *M. aeruginosa* (Kützing) Kützing, in the foliar tissue of commercial lettuce that was spray irrigated with water containing the microcystin-producing cyanobacteria; the total concentration of MCs and exposure time were not established.

Accumulation of MCs in vegetables entering the human diet has been reported in lettuce (Codd et al. 1999, Crush et al. 2008), rice and rape seedlings (Chen et al. 2004), broccoli and mustard roots (Järvenpää et al. 2007), and wheat and corn (Saqrane et al. 2009), however, many studies utilized concentrations of MCs greater than normally found in the environment (Chen et al. 2004, Crush et al. 2008, Saqrane et al. 2009). The estimated ingestion of lettuce by the Brazilian population ranges from 60 to 106 mg · d^{-1} (Arabbi et al. 2004). Conventional lettuce cultivation demands irrigation using water from reservoirs, which are mostly eutrophied and may contain toxic cyanobacteria (Bouvy et al. 2000, Bittencourt-Oliveira et al. 2011, 2012, Moura et al. 2011). The aim of this study was to determine whether the bioaccumulation of MCs occurs in the foliar tissue of lettuce (*Lactuca sativa* L.) when sprayed with solutions containing MCs at concentrations generally found in water bodies (~10 μg · L^{-1}).

MATERIALS AND METHODS

Culture of the toxic MC-LR and MC-RR-producing strain of *M. aeruginosa* (BCCUSP232) was centrifuged and freeze dried for the acquisition of a crude extract, which was quantified for MC-LR, MC-RR, and total MCs using HPLC/MS, following the method described by Bittencourt-Oliveira et al. (2005). Solutions with four different concentrations of total MCs (0.62, 2.5, 6.23, and 12.5 μg · L^{-1}) were prepared using the extract, which was resuspended in distilled water and lysed by sonication (5 min, 15 W and 22.5 kHz) in an ice bath. Cell lyses of the solutions were confirmed by examination of the lysate under an optical microscope.

Early-growth lettuces (30-d-old; Vanda cultivar, Sakata Seed, Brazil) were transplanted to pots with a vermiculite substrate (Basaplant, Base Substratos, Artur Nogueira, Brazil) and maintained in a greenhouse. After reaching full growth, the plants were sprayed with distilled water and a nutrient solution over 10 d, prior to being exposed to the four different total MC solutions (Table 1). Each plant received 100 mL of solution sprayed over the leaves for 15 consec-

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TABLE 1. Concentration of total microcystins (MCs) ($g \cdot L^{-1}$), MC-LR ($\mu g \cdot L^{-1}$), MC-RR ($\mu g \cdot L^{-1}$) in the crude extract of *Microcystis aeruginosa* BCCUSP232 strain, spread over the lettuce leaves for 15 consecutive days and, last column, the MCs in the foliar tissues ($\mu g \cdot Kg^{-1}$ fresh weight). Average ± standard deviation, $n = 3$.

Treatment	Total MCs	MC-LR	MC-RR	MCs in foliar tissue
1	0.62	0.50	0.12	8.31 ± 0.2
2	2.50	2.00	0.50	19.8 ± 4.1
3	6.23	5.00	1.23	16.8 ± 6.3
4	12.50	10.00	2.50	177.8 ± 3.4
Control	0.0	0.0	0.0	0.0

utive days; plants sprayed only with distilled water were used as a control. Three plants were used for each treatment and for the control ($n = 3$). Following this period, whole medium-sized leaves from each plant in each treatment were collected and washed in distilled water.

The extraction of total MCs of the foliar tissue was performed by maceration of 1 g of wet mass from each plant using liquid nitrogen. The foliar tissue cells were broken down by sonication (cell lysis was confirmed by optical microscopy) and used directly for the analysis of total MCs. Toxin quantification was carried out with a commercial ELISA kit (Beacon Analytical Systems Inc., Portland, ME, USA), following the manufacturer's protocols. The detection limit for MC by ELISA is 2.0 ppb. Negative and positive controls for the ELISA analysis were included in the commercial kit and all analyses were performed in triplicate.

RESULTS AND DISCUSSION

Microcystins were found in the foliar tissue of all plants exposed to different concentrations of the toxin (Table 1). No morphological alterations were found in the plants exposed to the toxin, such as leaf necrosis, lesser plant development, deformations, etc.

The bioaccumulation of MCs corresponding to a concentration of 12.50 $\mu g \cdot L^{-1}$ was 20 times higher than for 0.62 $\mu g \cdot L^{-1}$, the lowest concentration in this experiment. Since 177.8 $\mu g \cdot Kg^{-1}$ was also 20 times higher than 8.31 $\mu g \cdot Kg^{-1}$, we conclude that bioaccumulation was linearly proportional to the concentrations from the lowest dose (0.62 $\mu g \cdot L^{-1}$) to the highest dose (12.50 $\mu g \cdot L^{-1}$). However, a discontinuity was observed between doses of 2.50 and 6.23 $\mu g \cdot L^{-1}$ (Fig. 1).

The MC values in the foliar tissues (8.31 to 177.8 $\mu g \cdot Kg^{-1}$ of fresh weight) were similar to those reported in previous studies (Codd et al. 1999, Crush et al. 2008), considering that 1 g of dry foliar tissue is equivalent to 10 g of fresh weight. Crush et al. (2008) found MC bioaccumulation of 84 $\mu g \cdot Kg^{-1}$ of fresh weight when using a concentration of 1700 $\mu g \cdot L^{-1}$ for 6 d. Although the highest MC concentration in this study was roughly 100 times lower (12.5 $\mu g \cdot L^{-1}$), the bioaccumulation of MCs (177.8 $\mu g \cdot Kg^{-1}$ of fresh weight) was more than twice the value obtained by Crush et al.

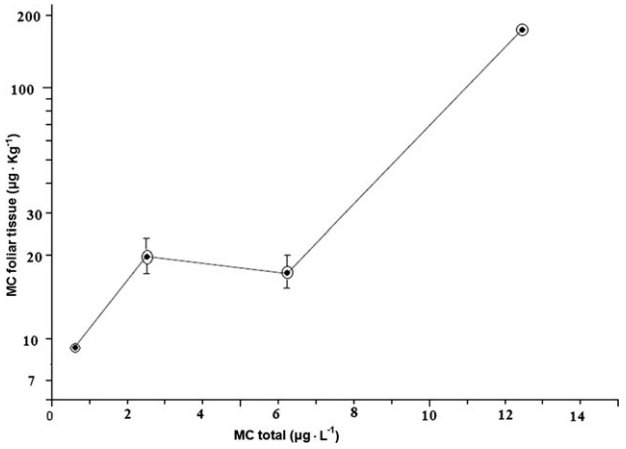

FIG. 1. Log-linear plot of microcystins (MCs) in foliar tissue as a function of total MC amounts. The (a), (b), and (c) labels refer to the three bioaccumulation stages described in the text, and corresponds to the following bioaccumulation rates: $r_a = 6.1$ ($\mu g \cdot Kg^{-1}$) ($\mu g \cdot L^{-1}$)$^{-1}$, $r_b \approx 0$ and $r_c = 16.1$ ($\mu g \cdot Kg^{-1}$) ($\mu g \cdot L^{-1}$)$^{-1}$.

(2008). This may have occurred because of the longer exposure time of the plants in this study, which corroborates previous findings that a longer exposure time results in a greater bioaccumulation of MCs in different organisms (Ibelings and Chorus 2007).

Despite the low number of points, it is evident that the accumulation process of total MC in the foliar tissues presents three stages with the following bioaccumulation rates:

(a) $r_a = (19.8–8.31)/(2.50–0.62) = 6.1$ ($\mu g \cdot Kg^{-1}$) ($\mu g \cdot L^{-1}$)$^{-1}$;
(b) $r_b \approx 0$; and
(c) $r_c = (117.8–16.8)/(12.50–6.23)^{-1} = 16.1$ ($\mu g \cdot Kg^{-1}$) ($\mu g \cdot L^{-1}$)$^{-1}$.

Interestingly, in the low dose range (stage-a) the rate is approximately one-third smaller than in the higher dose range (stage-c). The observation of a plateau between 2.50 and 6.23 $\mu g \cdot L^{-1}$ is intriguing and we found no explanation for this behavior, indicating that further studies will be necessary. The following issues deserve future investigation: (1) Why, if reproducible, is there a zero accumulation between 2.5 and 6.23 $\mu g \cdot L^{-1}$? (2) Is the accumulation pattern, as in Figure 1, also true for other vegetables? and (3) If 1 and 2 are confirmed, what kind of biokinetics is involved in MCs uptake?

Besides possible economic consequences (Chen et al. 2004), exposure to vegetables grown in water contaminated with cyanotoxins represents an increased risk of food contamination. If an individual weighing 60 Kg consumes 40 g of lettuce (approximately four leaves) that had been exposed to MC concentrations of 0.62–12.5 $\mu g \cdot L^{-1}$, they could ingest 0.33–7.11 μg of MC per meal (0.005–0.118 $\mu g \cdot Kg^{-1}$ of body weight), therefore, exceed-

ing the tolerable daily intake of 0.04 $\mu g \cdot Kg^{-1}$ of body weight recommended by the WHO (Chorus and Bartram 1999). Hence, even low concentrations of MCs in the irrigation water can put humans at risk, thus underscoring the need for monitoring of this food exposure pathway by public authorities.

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