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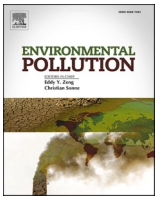

Experimental accumulation and depuration kinetics and natural occurrence of microcystin-LR in basil (*Ocimum basilicum* L.)☆

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ABSTRACT

Microcystin-LR (MC-LR) is a hepatotoxic metabolite that naturally occurs during some cyanobacterial blooms in eutrophic waterbodies, and irrigation of edible plants with MC-LR-contaminated water causes bioaccumulation of the toxin. However, sufficient information about accumulation and depuration mechanics in hydroculture-grown herb plants is still lacking. This work aimed at 1) investigating bioaccumulation and depuration of MC-LR in basil, 2) verifying the possible MC-LR detoxification mechanisms in the plant, and 3) detecting the natural occurrence of MC-LR in basil (n = 50) collected from the Belgian market. Basil plants grown in a hydroculture were exposed to MC-LR (5, 20, and 50 μg L^{-1}) spiked in a Hoagland solution for seven days. MC-LR depuration was also studied by transferring the plants to a non-contaminated Hoagland solution after exposure to MC-LR for another seven days. MC-LR concentrations in Hoagland solution, basil leaves, and roots were quantified using a validated UHPLC–MS/MS method. In addition, ELISA and LC–HRMS (only basil leaves) were used for confirmation. The results showed an increase in the accumulated levels of MC-LR at higher exposure doses, with higher MC-LR levels in roots than in leaves for all the treatment conditions. For MC-LR depuration, significant reductions were observed in all the treatment conditions for roots only. No MC-LR conjugates, potentially related to metabolism, were detected by LC–HRMS. Finally, MC-LR was detected in one store-bought basil sample, representing the first occurrence of cyanotoxins in an edible crop from Belgium.

1. Introduction

Cyanobacterial blooms are common in eutrophic waterbodies worldwide (Svirčev et al., 2019), promoted by, but not limited to, increasing temperatures and other climate change phenomena (O'Neil et al., 2012). During the formation of these blooms, the production and release of a wide array of toxic metabolites into the water can happen (Abdallah et al., 2021; Ibelings et al., 2014). Irrigation of food crops with cyanotoxin-contaminated water from the environment (e.g., basins, lakes, ponds, or canals) may result in bioaccumulation and translocation of the cyanotoxins to edible parts (Xiang et al., 2019; Zhang et al., 2021). Besides their possible negative impacts on the plant by lowering yield and quality (Peuthert et al., 2007), cyanotoxins pose a human health risk (Codd et al., 1999; Tsoumalakou et al., 2021). The most common cyanotoxin is microcystin-LR (MC-LR), a hepatotoxic and possibly hepatocarcinogenic agent (group 2 B) in humans (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010; World Health Organization, 2020). Accumulation of MC-LR was primarily shown in soil-based systems for different edible crops (Bittencourt-Oliveira et al., 2016; Chen et al., 2012; Codd et al., 1999; Corbel et al., 2016; Machado et al., 2017). Moreover, MC accumulation has already been studied in some crop plants. Lettuce is the prime example, as MC accumulation

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(originating from the environment or fortified irrigation water) was observed in roots and leaves (Bittencourt-Oliveira et al., 2016; Codd et al., 1999; Cordeiro-Araújo et al., 2016; Crush et al., 2008; Hereman and Bittencourt-Oliveira, 2012; Levizou et al., 2017; Mohamed and Al Shehri, 2009). Accumulation of cylindrospermopsin, another hepatotoxic cyanobacteria metabolite, was also observed in lettuce (Cordeiro-Araújo et al., 2017; Llana-Ruiz-Cabello et al., 2019). Exposure-dependent accumulation of MCs was also established in rice (*Oryza sativa*) and root vegetables such as carrots, radishes, and rape (Chen et al., 2012, 2004; Crush et al., 2008; Levizou et al., 2020; Machado et al., 2017; Wijewickrama and Manage, 2019). Other laboratory-based studies showed the accumulation of MCs in fruit-bearing plants, including chili, tomato, and strawberries (Corbel et al., 2016; Redouane et al., 2023; Romero-Oliva et al., 2014). Yet, primary accumulation in these products was observed in the roots, stems, and leaves. Most of the observed accumulation of MCs in crops originated from soil-grown crops (Bittencourt-Oliveira et al., 2016; Chen et al., 2004; Codd et al., 1999; Crush et al., 2008; Hereman and Bittencourt-Oliveira, 2012; Levizou et al., 2017; Mohamed and Al Shehri, 2009). However, plants grown in hydroculture systems may be especially vulnerable to MC-LR accumulation due to the direct contact between the contaminated water and plant roots. Accumulation of MCs in hydroculture systems has already been documented for several rice varieties (Wijewickrama and Manage, 2019) and leafy vegetables such as lettuce and spinach (Llana-Ruiz-Cabello et al., 2019; Wijewickrama and Manage, 2019).

In Belgium, MC-LR contamination has been reported in freshwater reservoirs (Van Hassel et al., 2022a; Willame et al., 2005). Recently, cyanobacterial blooms from multiple lakes, ponds, and canals were sampled, and eight microcystins (MCs) were quantified in 86% ($n = 79$) of the samples, with concentrations ranging from 0.1 μg L^{-1} to 2800 μg L^{-1} (Van Hassel et al., 2022a). These water sources can potentially be used for irrigation of crops. Therefore, potential MC-LR accumulation in Belgian crops, including basil (*Ocimum basilicum* L.), is a valid concern that might contribute to a public health risk. Basil is an important crop that is usually grown in hydroculture systems in several countries (Saha et al., 2016). The plant is generally used as a flavoring agent and a main ingredient in Pesto Genovese, a sauce used in many European dishes, especially in Italian cuisines. In addition to foods, basil is used in the cosmetic and pharmaceutical industries since it is a good source of essential oils, valuable antioxidants, and other bioactive compounds (Trajkovska-Broach et al., 2023). In Europe, basil makes up 60–75% of the total consumed herbs, and Belgium is among the top four countries (after Germany, the Netherlands, and France) that import fresh herbs (Centre for the Promotion of Imports from developing countries, 2020). Also, national producers contribute to the total marketed basil in Belgium.

The objectives of this work were to investigate the accumulation and depuration of MC-LR in basil plants grown in a hydroculture, verify the possible mechanisms involved in the depuration, and survey the natural occurrence of MC-LR in basil samples collected from the Belgian market using an in-house validated ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method for the quantification of nine cyanotoxins. The toxins include eight MCs (MC-LR, MC-RR, MC-LA, MC-LF, MC-LY, MC-LW, MC-YR, and MC-WR) and a structurally related cyanotoxin, nodularin-R (NOD-R).

2. Materials and methods

2.1. Experimental setup

Certified basil seeds (Italian large green variety; La Belle Portagere brand, Sebio, Belgium) and potting soil for herbs and aromatic plants (Campo brand, Brico, Belgium) were commercially obtained. After sowing, seeds germinated and grew inside a growth chamber (Mammoth Lite+ 45 type, 450 × 450 × 1200 mm, Mammoth net, Netherlands) where the light (around 200 μmol m^{-2} s^{-1} for 16 h/day), temperature (24.6 ± 1.6 °C), and relative humidity (57.8 ± 12.0) were monitored and kept constant. The plants were watered every other day and after 60 days, the plants were collected and transferred to a Hoagland solution (Hoagland and Arnon, 1950). Each plant was placed in a 100 mL amber glass bottle containing Hoagland solution fortified with MC-LR and sealed with parafilm. Each treatment group ($n = 6$) was exposed to 5, 20, or 50 μg L^{-1} of MC-LR (Enzo Life Sciences, Belgium) for seven days, in addition to an untreated control group. Fortified Hoagland was prepared before the incubation of the plants. A stock solution of 10 μg mL^{-1} of MC-LR in ethanol was used to make a 1:200 dilution in Hoagland solution to obtain the 50 ng mL^{-1} concentration. After that, serial dilutions were used to make 1 L of Hoagland solution at 50, 20, and 5 μg L^{-1} MC-LR. To study the depuration of MC-LR, the same experimental setup was performed, parallel to the accumulation experiment, but the plants were transferred into clean (i.e., non-spiked, MC-LR-free) Hoagland solution for another seven days after exposure to MC-LR as described above. As a preliminary step to investigate any effect of MC-LR on basil, the weight and length of each plant were measured before and after exposure to the toxin. Also, the plant leaves and roots were visually inspected during and after the experiment. To assess the bioaccumulation and depuration of MC-LR, the toxin was quantified in basil leaves, roots, and the Hoagland solution. The basil roots, obtained after the accumulation testing period, were dipped for 2 s into distilled water and dried to ensure that no MC-LR was taken up from the Hoagland solution itself before processing, extraction, and UHPLC–MS/MS analysis. The experiment was repeated twice. Additionally, the accumulation part of the experimental setup was tested before the start of the experiment using an extract of *Microcystis* sp. ULC642 culture obtained from the BCCM/ULC culture collection (Liège, Belgium). The cultures were grown in BG11 at 22 °C and 700 Lux warm-led light intensity on a 10:14 day:night cycle. This extract contained a mixture of MCs, mainly MC-LR and MC-YR (Table S1), and was used to fortify the Hoagland solution to contaminate six basil plants at 50, 20, and 5 μg L^{-1} total MCs.

2.2. Quantification of eight MCs congeners and NOD-R quantification in basil

Samples from Hoagland solution and basil (separated leaves and roots) were collected and kept at −20 °C until analysis. The extraction and UHPLC-MS/MS analysis methods for MC-LR from the different matrices were previously published (Van Hassel et al., 2022b, 2022c). These methods are suitable for analyzing eight MC congeners (MC-LR, MC-RR, MC-LA, MC-LF, MC-LY, MC-LW, MC-YR, MC-WR) and NOD-R in fruit and vegetable matrices and drinking water. The validity of the methods for fruits and vegetables was confirmed for the basil matrix, reassessing repeatability, reproducibility, recovery, specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ), measurement uncertainty (MU), and matrix effect. In brief, this method consisted of extracting the targeted toxins from 0.5 g of homogenized basil material (leaves or roots) using a combination of liquid extraction (methanol-water 80:20), sonication (BRANSON 2510, Analis, Belgium), overhead mixing (Heidolph Reax 2 Mixer, Analis, Belgium), and centrifugation (Sorvall Legend XT, Thermo Scientific, Belgium) at 15303 *g* for 15 min, after which the extracts were partially evaporated and further purified using Agilent C18 cartridges (6 mL, 500 mg) as described by Van Hassel et al., 2022; Van Hassel et al., 2022b, 2022c). Methanol was UPLC/MS grade (Biosolve B.V., Valkenswaard, The Netherlands). Milli-Q water was produced in-house (conductivity ≥18.2 MΩ and TOC ≤4 ppb). After this purification step, the MCs and NOD-R were separated and quantified via UHPLC–MS/MS (Llana-Ruiz-Cabello et al., 2019; Van Hassel et al., 2022c). Except for MC-RR, matrix-matched calibration curves in blank basil matrices were prepared from 0.25 ng L^{-1} to 50 ng L^{-1}. The latter was from 0.75 ng L^{-1} to 50 ng L^{-1} due to matrix interference (Table S2). The blank refers to a sample of the same food matrix free of MCs. The quality control (QC) sample was a

blank matrix spiked with 25 μL of toxin standard solution (100 ng mL^{-1}) at 5 ng g^{-1}, giving a known toxin concentration. Both served to check the quality of the analysis. Diluted toxin standards were prepared in MeOH and Milli-Q water (50:50) with 1% acetic acid. Blank and QC samples were included in the batch to check the sensitivity of the instrument during the analysis. Validation of the method for the basil matrix followed the same methodology described in earlier work for the fruit and vegetables (Van Hassel et al., 2022c). An MC and NOD mixture was spiked in homogenized basil leave matrix at 1, 5, and 25 ng g^{-1} before extraction. However, the limit of quantification (LOQ) was only 1 ng g^{-1} for MC-LR and NOD-R. Therefore, additional concentration levels were spiked at 5 ng g^{-1} for MC-RR and 2.5 ng g^{-1} for MC-YR, MC-LA, MC-LF, MC-WR, MC-LW, and MC-LY. The LOQ was defined as the concentration for which the quantification of the compound adhered to all validation parameters.

2.3. ELISA analysis

Enzyme-linked immunosorbent assay (ELISA) was used to confirm MC-LR accumulation and depuration in basil leaf samples (Samdal et al., 2014). Minor adjustments to concentrations of ELISA assay reagents, such as 0.5 μg mL^{-1} of the plate-coating antigen, 1:4000 of antiserum 80289-5 b, and 1:5500 of the donkey–antisheep IgG (H + L)−horseradish peroxidase conjugate (antisheep−HRP from Agrisera antibodies (Vännäs, Sweden)) were made to optimize the ELISA. The MC-LR standard (NRC CRM-MC-LR (Lot#20070131)) in 50% MeOH (500 ng mL^{-1}) was diluted in phosphate-buffered saline with Tween to give a MeOH concentration of 10% and then in a threefold dilution series in sample buffer, resulting in a standard series of 50, 16.7, 5.56, 0.62, 0.20, 0.069, 0.023, 0.0076, and 0.0025 ng mL^{-1}. Standards and samples were analyzed with serial dilutions and in duplicate on the plate. All incubations were performed at ~20 °C. Absorbances were measured at 450 nm using a SpectraMax i3x plate reader (Molecular Devices, Sunnyvale, CA, USA). Assay standard curves were fitted using a 4-parameter logistic treatment of the data using SoftMax Pro version 6.5.1. (Molecular Devices, Sunnyvale, CA, USA). The assay working range was defined as the linear region at 20–80% of maximum absorbance (A_{max}).

2.4. LC–HRMS analysis

LC-HRMS analysis was performed with a Q Exactive HF Orbitrap mass spectrometer equipped with a heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA) using an Agilent 1200 G1312B binary pump, G1367C autosampler and G1316B column oven (Agilent, Santa Clara, CA, USA) connected to a Symmetry C18 column (3.5 μm, 150 × 2.1 mm; Waters, Milford, MA, USA) held at 40 °C. Analyses were performed with mobile phases A (H_2O) and B (CH_3CN), each of which contained formic acid (0.1% v/v). Gradient elution (0.3 mL/min) was from 15 to 100% B over 21 min, then held at 100% B (6 min), then returned to 15% B over 0.1 min with a hold at 15% B (2.9 min) to equilibrate the column (total run time 30 min), and the injection volume was 2 μL. Full-scan MS data were acquired at 2–27 min, using alternating positive (m/z 500–1400) and negative (m/z 750–1400) ion scan modes, with spray voltages of ±3.7 kV, a capillary temperature of 350 °C, sheath and auxiliary gas flow rates of 25 and 8 units, respectively, a resolution setting of 60,000, an AGC target of 1×10^6, and a max IT of 100 ms. Extracted-ion chromatograms were obtained using exact m/z values with the mass tolerance set to ±5 ppm.

2.5. Basil sample survey

Commercial samples (n = 50) were collected from Belgian food stores between February and October 2022 to screen for the natural occurrence of MCs in basil. The samples were collected from 12 supermarket chains covering 13 brands. More information on sample number, packaging and sample type, sampling date, and origin are available in Table S3.

2.6. Statistical analysis

Data processing and statistical analysis were performed with GraphPad Prism version 10.2.0 (GraphPad Software, Boston, Massachusetts USA). A Two-way ANOVA test was used to investigate the effect of two independent variables (1- different treatments and 2- exposure and depuration) on the detected MC-LR concentrations. Multiple comparisons were performed for all the tested groups and Tukey's test was selected as a recommended post-hoc analysis to identify differences between mean values. Normal (Gaussian) distribution was verified using the Shapiro–Wilk normality test. The threshold for P-value comparison was set at 0.05 for the significant difference. To investigate the effect of different treatments on the length and weight of basil plant, a two-way repeated measures ANOVA test was applied after checking the sphericity and normality. The standard deviation (SD) of the measured values is presented after each value of the calculated mean for these measurements. The SD represents the spread of biological repeats and thus does not constitute a measure of uncertainty. On the other hand, the absolute error for the depuration was calculated using the square root of the error sum of squares and, therefore, constitutes a measure of uncertainty.

3. Results

3.1. Effect of MC-LR on basil growth

As a preliminary step to investigate whether there is an effect of MC-LR on basil growth, the weight and length of each individual plant were measured before and after the MC-LR exposure (seven days post-spiking). Also, the plant leaves and roots were visually inspected before, during and after the experiment. There were no significant differences in the average basil weight and length between the control and the treatment groups (Fig. 1). Furthermore, there no lesions or discoloration were observed on the basil plants. This indicate that the exposure to MC-LR at 5, 20, or 50 μg L^{-1} for one week did not have any noticeable inhibitory effects.

Before the exposure to MC-LR, the average weight of basil plants for the untreated control was 6.0 (SD = 1.1) g, and for the 50 μg L^{-1} group was 7.6 (SD = 1.7) g, while the average length for the untreated control was 52.9 (SD = 5.1) cm and for the 50 μg L^{-1} group was 49.2 (SD = 5.5) cm. After the exposure to MC-LR, the average weights were 6.6 (SD = 1.4) g and 7.8 (SD = 1.6) g, and average lengths were 50.0 (SD = 1.9) and 46.5 (SD = 1.5) cm for the untreated basil plants and 50 μg L^{-1} group, respectively. Averages of the weight and length of each treatment group before and after exposure to MC-LR are detailed in Table S4. Similarly, no significant differences were observed within and among the treatment groups during the depuration part of the experiment (data not shown). Decay of the primary root was observed after seven days for all the plants, independent of the growth condition, after they were transferred from soil to the Hoagland solutions, with multiple secondary roots replacing the primary roots.

3.2. Validation of a UHPLC–MS/MS method for eight MC congeners and NOD-R quantification in basil matrix

Multiple validation parameters were checked for the eight MCs and NOD-R quantification in the basil matrix. The specificity of the method was shown by a lack of MC- and NOD-specific peaks in the blank samples. Moreover, the ion ratios calculated for the points in the calibration curve were consistent with the parameters provided by Directive 96/23/EC of the European Commission (Table S5). The coefficient of determination (R^2) of the linear calibration curve for all the MCs and NOD-R showed the appropriateness ($R^2 > 0.99$) of the linear regression during quantification (Table S6). Additionally, comparisons of the slopes of calibration curves in the blank matrix and solvent (50:50 MeOH–H_2O,

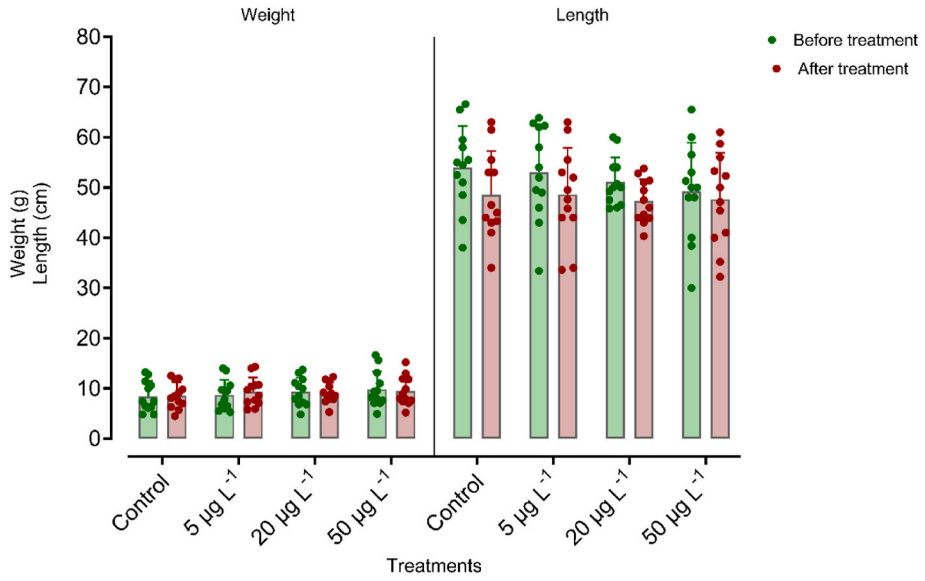

Fig. 1. Effect MC-LR on basil growth by measuring the weight and length of the plant before and after treatment (repeated measure). No significant differences were detected across the tested groups.

containing 1% acidic acid), using t-tests, showed matrix effects for all MCs during quantification (Table S6). Recovery, repeatability, reproducibility, and MU were calculated at three concentration levels: 1, 5, and 25 ng g^{-1} for MC-LR and NOD-R; 5, 10, and 25 ng g^{-1} for MC-RR; and 2.5, 10, and 25 ng g^{-1} for MC-YR, MC-LA, MC-LF, MC-WR, MC-LW, MC-LY. A complete overview of the validation data (recovery, repeatability, reproducibility, and MU) can be found in Table S7. Averages for recovery, repeatability, reproducibility, and MU are reported in Table 1. Repeatability and reproducibility remained below their calculated Horwitz ratios, 14.7% and 22.0%, respectively.

The LOQ value was considered to be the lowest validated concentration: 1 ng g^{-1} for MC-LR and NOD-R; 5 ng g^{-1} for MC-RR, and 2.5 ng g^{-1} for MC-YR, MC-LA, MC-LF, MC-WR, MC-LW, MC-LY. The signal-to-noise (S/N) for the quantifier and qualifier analyte peak was always >10. To determine the LODs, the quantifier and qualifier peak should have S/N > 3. The lowest point in the calibration curve for each MC was selected as the LOD, as these were the lowest concentrations at which S/N was determined.

3.3. Accumulation and depuration of MC-LR in basil

Exposure of basil plant to higher doses of MC-LR resulted in more accumulation in basil roots and leaves (*i.e.,* higher accumulation occurred with a higher concentration of MC-LR in the Hoagland solution). Moreover, MC-LR was detected in higher concentrations in roots than in leaves for all three treatment groups (Fig. 2). The mean values of the MC-LR concentration in basil leaves were 1.6 (SD = 0.8), 5.7 (SD = 2.0), and 24 (SD = 15) ng g^{-1} fresh weight (fw) for the 5, 20, and 50 µg L^{-1} treatment groups, respectively. In roots, the MC-LR concentrations were 8.5 (SD = 3.1), 17.3 (SD = 3.1), and 49 (SD = 17) ng g^{-1} fw for the 5 µg L^{-1}, 20 µg L^{-1}, and 50 µg L^{-1} treatment groups, respectively. During the preliminary experiment with the culture extract, both MC-LR and MC-YR accumulated in the roots and leaves, similar to their ratio in the original extract (Table S1). This preliminary test shows that MCs other than MC-LR could also accumulate in plants, which is essential because multiple MCs are often present in natural cyanobacterial blooms.

To investigate the depuration potential of basil plants, the depuration percentage was calculated using a simple formula:

$$Depuration\ Percentage = 100 - \frac{MC-LR\ concentration\ after\ depuration\ period \times 100}{MC-LR\ concentration\ after\ accumulation\ period}$$

The percentage of MC-LR depuration from the leaves at lower MC-LR concentrations was higher than the percentage depurated at higher concentrations (5 µg L^{-1} > 20 µg L^{-1} > 50 µg L^{-1}). For the 5 µg L^{-1} treatment group, no MC-LR was detectable (LOD = 0.6 ng g^{-1}) after seven days in leave samples, the depuration was ≥62 ± 11%. The depuration percentages were 34 ± 8% (mean MC-LR was 4 (SD = 2) ng g^{-1} fw) and 32 ± 14% (mean MC-LR 16 (SD = 14) ng g^{-1} fw) for the 20 and 50 µg L^{-1} treatment groups, respectively (Table S8). MC-LR concentrations in leaves after the depuration testing period were lower, but not significantly different, than MC-LR concentrations after the accumulation period for the three treatment groups (Fig. 2).

On the other hand, the average MC-LR concentrations in roots were significantly lower after depuration than in the corresponding treatment group after the accumulation period (Fig. 2). The 20 µg L^{-1} treatment group showed the lowest depuration percentage (53 ± 19%), with a mean of 8.0 (SD = 4.5) ng g^{-1} fw MC-LR after depuration, followed by a depuration percentage of 57 ± 13% for the 5 µg L^{-1} treatment group,

Table 1
Averages for recovery, repeatability, reproducibility, measurement uncertainty (MU), LODs, and LOQs for MCs and NOD-R in basil leaf matrix.

Validation parameter	Cyanobacterial toxins								
	MC-RR	MC-LA	MC-LF	MC-LR	MC-LY	MC-LW	MC-YR	MC-WR	NOD-R
Repeatability (%)	6.2	5.0	6.0	4.4	4.9	4.2	6.4	7.6	5.5
Reproducibility (%)	9.7	21.2	20.4	17.1	20.7	20.5	9.6	14.0	12.1
MU (%)	19.4	42.4	40.8	34.1	41.4	41.0	19.7	28.0	24.2
Recovery (%)	78.7	89.7	81.3	90.0	89.3	83.3	83.0	82.0	82.7
LOD (µg kg^{-1})	4.5	1.5	1.5	0.6	1.5	1.5	1.5	1.5	0.6
LOQ (µg kg^{-1})	5.0	2.5	2.5	1.0	2.5	2.5	2.5	2.5	1.0

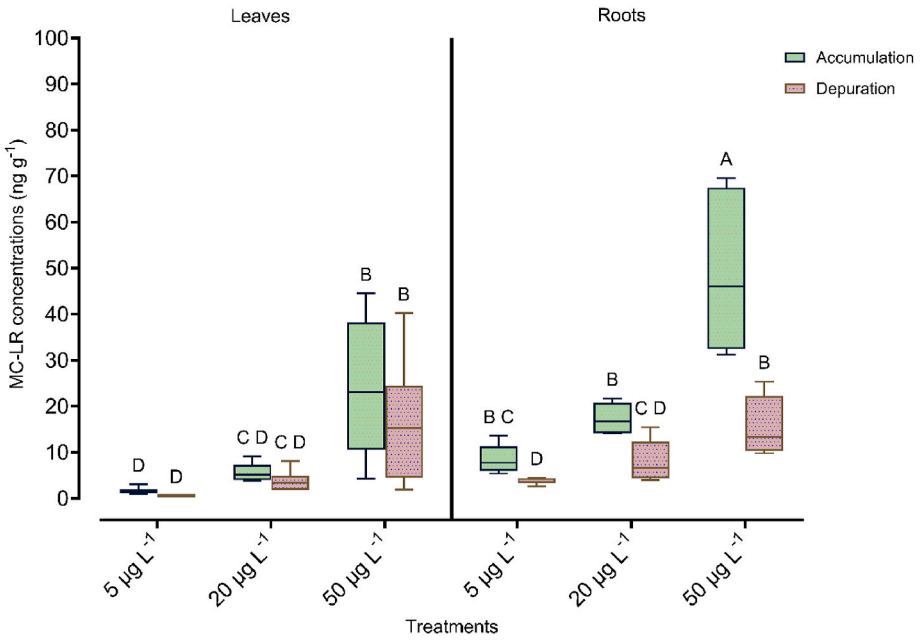

Fig. 2. MC-LR Concentrations in basil (leaves and roots) after the accumulation and depuration periods. Different letters above the boxes indicate statistically significant differences according to a two-way ANOVA test followed by Tukey's multiple-comparison test as a post-hoc analysis.

with a mean value of MC-LR at 3.7 (SD = 0.6) ng g^{-1} fw (Table S8). The depuration period for the 50 µg L^{-1} treatment group resulted in 68 ± 29% depuration and a mean concentration of MC-LR of 15.5 (SD = 6.2) ng g^{-1} fw.

The MC-LR in the Hoagland solution was also quantified after the accumulation and depuration periods. The mean concentrations of MC-LR after accumulation were 2.1 (SD = 1.2), 7.6 (SD = 3.9), and 25.0 (SD = 9.1) µg L^{-1} for the 5 µg L^{-1}, 20 µg L^{-1}, and 50 µg L^{-1} treatment groups, respectively (Fig. S1). Interestingly, MC-LR levels were below the LOD value (0.1 µg L^{-1}) in the Hoagland solutions used for the depuration experiment (Van Hassel et al., 2022b).

To further confirm the results of the accumulation and depuration of MC-LR in basil leaves, four samples (two samples from each experiment from the 50 µg L^{-1} treatment group) were analyzed by two alternative analytical approaches, ELISA and LC–HRMS, at two different laboratories. Table 2 shows the results of the analyzed samples and the analysis of the same samples using UHPLC–MS/MS. For the basil samples from the accumulation period, the MC-LR concentrations by ELISA were higher than those detected by UHPLC–MS/MS. However, due to matrix effects, the MC-LR concentrations determined by ELISA in the two basil samples from the depuration period were lower than the LOQ value (0.2 ng g^{-1}). It is important to mention that the ELISA determines the total concentration of MCs and could include other metabolites and conjugates than the concentration of MC-LR alone, resulting in reporting a total MCs.

The analysis of the same samples using LC–HRMS showed that the MC-LR was depurated to give a significantly lower peak area compared to the semi-quantified MC-LR in the basil samples analyzed after the accumulation period (Fig. 3). No conjugates of MC-LR with glutathione (GSH) or cysteine (Cys), potentially originating from metabolism, were

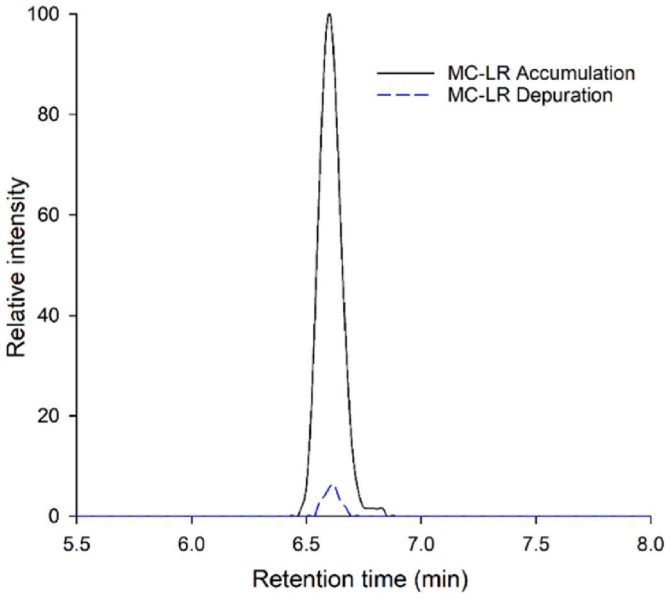

Fig. 3. Extracted-ion (*m/z* 995.5560) chromatograms of MC-LR by LC–HRMS in positive ionization mode in extracts of basil leaves after accumulation and depuration.

Table 2
Mean values of total MCs measured by ELISA and MC-LR measured by UHPLC–MS/MS in two samples of basil leaves in the 50 µg L^{-1} treatment group after the accumulation and depuration periods.

Sample	Sample number (*n*)	Detection method (ng g^{-1} fw)		
		Mean MCs by ELISA	**Mean MC-LR by UHPLC–MS/MS**	**Detection of MC-LR by LC– HRMS**
Basil leaves (accumulation)	2	62.3	47.4	Detected
Basil leaves (depuration)	2	<LOQ (0.2)	8.0	Detected

detected during the LC–HRMS screening of the samples. The identity of MC-LR in basil was further confirmed by its isotopic pattern (Fig. S2).

3.4. Occurrence of MC-LR in basil collected from Belgian markets

The UHPLC–MS/MS analysis of the basil samples ($n = 50$) collected from the Belgian market showed the contamination of one sample (sample ID: S22FD02911) with MC-LR at a level higher than LOD but lower than the LOQ value (*i.e.,* between 0.6 and 1.0 ng g^{-1}). Other MC congeners and NOD-R were under the LOD levels of the method in all the analyzed samples. Fig. 4 shows an extracted-ion chromatogram for MC-LR in the contaminated samples and the matrix-matched calibration curve. It is worth noting that this market sample was collected in September 2022 (**Supplementary data,** Table S3), the end of the cyanobacterial bloom season in Belgium. The results further indicate that the basil was irrigated with water contaminated with MC-LR of 'environmental' origin during its growth.

4. Discussion

4.1. Effect of MC-LR on basil growth and its accumulation and depuration potentials

Several plant species readily take up MCs, accumulating them in leaves, stems, and roots (Abdallah et al., 2021; Bittencourt-Oliveira et al., 2016; Peuthert et al., 2007; Xiang et al., 2019). This accumulation depends on various factors, including the concentration of the toxin in water or soil, the plant species, and the stage of plant growth (Xiang et al., 2019). Plants are generally more susceptible to MC uptake during their vegetative growth stage when they actively take up water and nutrients from the soil. The results obtained from the preliminary assessment of the plant length and weight before and after MC-LR treatment showed that MC-LR had no detectable inhibitory effects on basil growth. Therefore, no further work or assessment was done to investigate the impact of the toxin on the plant. However, the primary basil roots did decay after the transfer to the Hoagland solution, while smaller secondary roots developed. This change in root structure probably resulted from the manipulation, as it was also observed in the untreated control group. Previous work reported different potential negative or positive effects of MCs (including MC-LR on plant root growth depending on the toxin concentration and the plant species (Chen et al., 2012; Freitas et al., 2015; McElhiney et al., 2001; Pflugmacher et al., 2001). The effects of MC exposure on plant growth have been observed in tomatoes (Corbel et al., 2015), potato shoots and mustard seedlings (McElhiney et al., 2001), wheat, carrots, cucumber, beans, and spinach (Bittencourt-Oliveira et al., 2016; Llana-Ruiz-Cabello et al., 2019; Machado et al., 2017; Mohamed et al., 2022; Peuthert et al., 2007).

The results obtained during this study clearly show that basil can accumulate MC-LR when the toxin is available at relevant

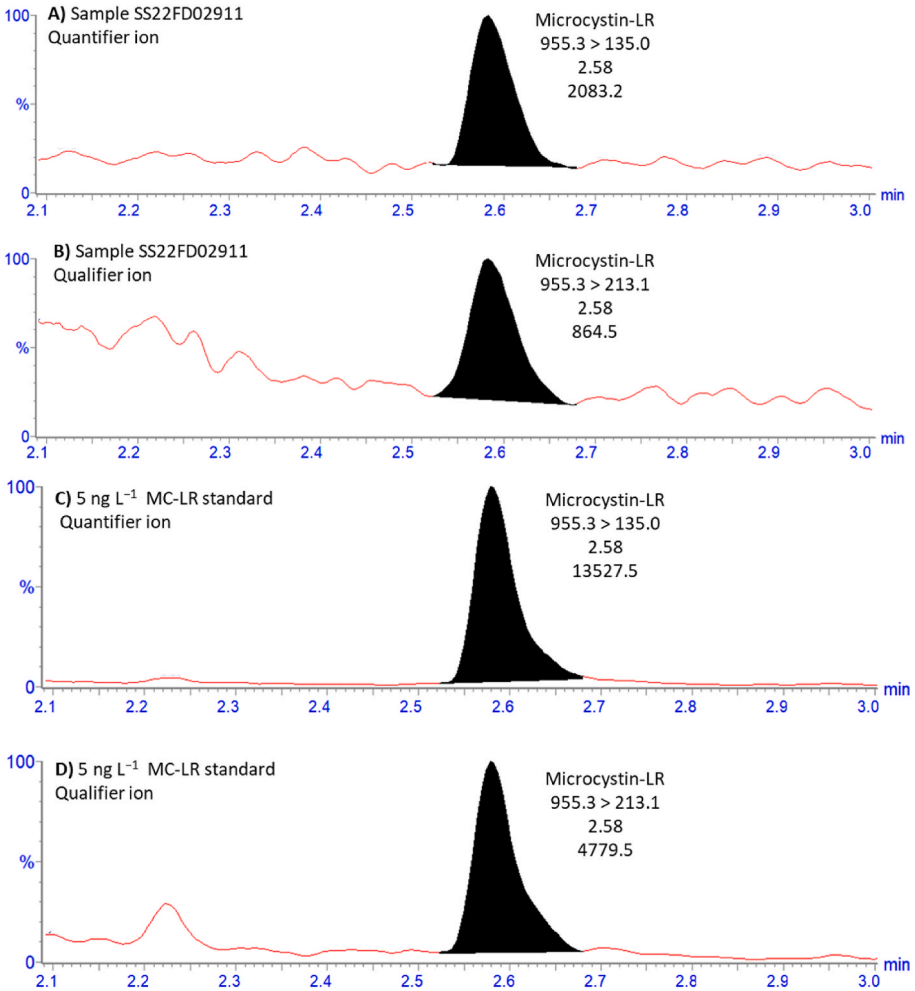

Fig. 4. Extracted-ion chromatograms of an MC-LR standard in a QC sample and the commercial sample contaminated with MC-LR. A) MC-LR quantifier ion; B) MC-LR qualifier ion; C) MC-LR quantifier ion in the 5 ng L^{-1} MC-LR standard in a QC sample, and D) MC-LR qualifier ion in the 5 ng L^{-1} MC-LR. For each chromatogram, the toxin name, precursor- and product-ion, retention time, and area under the peak are described from top to bottom.

concentrations during naturally occurring cyanobacterial blooms. Accumulation of MCs, including MC-LR, through water irrigation was reported in a range of edible crops (Abdallah et al., 2021; Hereman and Bittencourt-Oliveira, 2012; Machado et al., 2017). In general, this matches the results obtained in the current work. Peuthert et al. investigated MC accumulation both in the shoots and roots of several agricultural plants (e.g., soybean, lentil, alfalfa, pea, and wheat) using ELISA (Peuthert et al., 2007). The roots of these plants accumulated MC-LR at higher concentrations than leaves. The MCs concentration in the roots and leaves, detected with ELISA, ranged from 13 to 127 μg kg^{-1} fw and 3.1–31.1 μg kg^{-1} fw, respectively. Moreover, foliar bioaccumulation of MCs in lettuce (8.3–178 μg kg^{-1} fw) was shown to be linearly proportional to the treatment concentration (0.6–12.5 μg L^{-1}) (Hereman and Bittencourt-Oliveira, 2012), which is inconsistent with our findings in basil roots and leaves.

Accumulation of MC-LR in crops could also depend on the irrigation method used (Machado et al., 2017). Earlier ELISA research established that spray irrigation with water contaminated with MC-LR results in accumulation in leafy crops (Bittencourt-Oliveira et al., 2016; Codd et al., 1999; Crush et al., 2008). Our study showed that MC-LR can also be taken up through the roots and transported to the leaves. In previous research, lettuce and spinach were exposed to a mixture of MCs and cylindrospermopsin, each at 5 and 25 μg L^{-1}, using hydroculture, resulting in 0.2 ± 0.1 to 1.3 ± 0.1 μg kg^{-1} fw of MCs in roots and no detected MCs (LOD = 0.06 μg kg^{-1} fw) in leaves while using UHPLC-MS/MS (Llana-Ruiz-Cabello et al., 2019). Similarly, MC-LR was quantified with UHPLC-photodiode-array (PDA) in edible parts of rice and water spinach (*Ipomoea aquatica*), 429.8 ± 4.4 to 567.5 ± 4.9 μg kg^{-1} fw and 350.8 ± 2.9 μg kg^{-1} fw respectively, after exposure to cyanobacteria-contaminated water through hydroculture (Wijewickrama and Manage, 2019). Since plants are constantly in contact with the enriched solution, the bioavailability of MCs in hydroculture cultures might be higher than in soil-based systems because, in soil, the toxins can be adsorbed to clay minerals and organic matter, flow to deeper layers, or be degraded by microorganisms (Zhang et al., 2021).

The depuration of natural toxins by plants is a well-known phenomenon. A few studies have documented this for MCs, including MC-LR in edible crops. One study showed that 25% of the accumulated MC-LR in lettuce leaves could be depurated after seven days of exposure (10 μg L^{-1} MC-LR), followed by seven days of irrigation with clean water, while full detoxification of the toxin was estimated to occur after 37 days (Cordeiro-Araújo et al., 2016). The study also reported that the depuration was less efficient at higher levels or concentrations of exposure. In a second study, MC-LR depuration rates of 9.5 and 8.1 μg kg^{-1} dw day^{-1} were reported over 12 days for lettuce and spinach leaves, respectively (Cao et al., 2019). Those depuration rates were obtained after a 12-day bioaccumulation period of irrigation with water containing MC-LR at 10 μg L^{-1} preceded the depuration period of equal length (Cao et al., 2019). These two studies are consistent with our findings, with the observed trends in the accumulation and the depuration of MC-LR in basil plants depending on the MC concentration in the Hoagland solution. However, further investigation of the MC-LR depuration in edible crops, including basil, should be considered under other lab and field conditions.

Different metabolic pathways may be relevant for the derivatization of MCs in plants (Cao et al., 2019; Chen et al., 2012). However, in our study, no known conjugates of MC-LR were detected during LC–HRMS screening of the basil leaf samples. An explanation could be that conjugates might be present at ultra-low concentrations in the basil samples and, therefore, not possible to detect with our current methods. Metabolic pathways should be examined in the future with other approaches. For instance, radio-labeled MCs could be used to explore the mechanics and kinetics of accumulation and depuration, as was previously applied to study MC-LR accumulation in tomatoes (Corbel et al., 2016). Crop type should also be taken into account when studying metabolic mechanisms. Finally, understanding the depuration dynamics for different crops might allow the development of approaches to salvage crop harvests exposed to irrigation water contaminated with MCs.

4.2. Natural occurrence of MC-LR in basil samples collected from the Belgian market

The detection of MC-LR in basil leaves during this (limited) survey further showed that human exposure to MC-LR through food crops is a growing concern, even with the presence of MC-LR in only one basil sample. The concentration of MC-LR in the sample can be estimated between 0.6 (LOD) and 1 ng g^{-1} (LOQ), which would probably not constitute a health risk. However, it does confirm that water containing naturally occurring toxic cyanobacterial blooms is being used to irrigate crops in Belgium. Until now, two studies found higher MC accumulation in multiple field samples in Asia irrigated with contaminated water (Wijewickrama and Manage, 2019; Xiang et al., 2019). These field samples consisted of various crops (e.g., rice, beans, fruits, leafy and root vegetables). Rice and water spinach contained 21 and 133 μg kg^{-1} fw MC-LR, respectively, as quantified with UHPLC- PDA (Wijewickrama and Manage, 2019). Leafy vegetables, including lettuce, celery, cabbage, and spinach, contained the highest concentration of MCs (mean values from 9.2 to 118 μg kg^{-1} fw) compared to fruit (mean values from 1.4 to 47 μg kg^{-1} fw) and root vegetables (mean values from 4.9 to 17 μg kg^{-1} fw) (Xiang et al., 2019). For some samples, the content of MCs exceeded the tolerable daily intake (i.e., maximum allowed daily exposure to a compound before it becomes harmful) (World Health Organization (WHO), 2020; Xiang et al., 2019). The current work shows that MC-LR accumulation in basil is concentration-dependent. Similar results were observed as vegetables cultivated around two Chinese lakes with higher concentrations of MCs (mean values from 66 to 276 μg L^{-1} fw) accumulated more MCs compared to vegetables associated with a third lake with lower a lower concentration of MCs (mean values from mean 7.7–53 μg L^{-1}) (Xiang et al., 2019). Consequently, higher concentrations of MC-LR in the irrigation water will probably lead to a significantly higher level of bioaccumulated MC-LR in the edible parts of the plant. This scenario is also likely in Belgium due to prevalent cyanobacterial blooms throughout Belgium and the dwindling of available water sources during climate-change-driven hot summers. Ideally, the occurrence of cyanotoxins in crops on the Belgian market should be regularly monitored in the future. Additionally, processed foods based on basil leaves, where their water content is reduced (e.g., pesto sauce and herbs), could lead to a concentration of the toxin, resulting in potential health risks. The effect of storage, cooling, and heating on MC-LR stability during the preparation of basil-based foods and herbs should be considered for future studies.

5. Conclusions

Accumulation of MCs in food crops is becoming more relevant since it poses an environmental and public health risk. The current study showed that basil can bioaccumulate MC-LR in roots and leaves when grown in a hydroculture system with MC-LR-contaminated water. More MC-LR concentrations were detected in basil roots and leaves at higher MC-LR exposure doses. The bioaccumulations were significantly higher in roots than in leaves for all the treatment conditions. Partial depuration was achieved when basil plants were placed in a clean Hoagland solution. The detected MC-LR levels after the depuration were significantly lower than the accumulated MC-LR levels in roots but not in leaves. Both accumulation and depuration of MC-LR were analyzed using UHPLC–MS/MS, ELISA, and LC–HRMS. Basil plants did not show any obvious signs of phytotoxicity since no significant differences in the weight and length were found between the different basil groups before and after the treatment. As the LC-HRMS analysis did not show any MC-LR conjugates formed by the plant, more research is required to unravel the depuration mechanism of MC-LR in basil. Although the collection of basil samples from the market showed a contamination of only one

sample with MC-LR, this confirms that contamination via cyanotoxin-contaminated irrigation water is a fact in Belgium. Given that the effects of climate change are getting more serious, the occurrence of cyanotoxins in crops might increase. This study describes the first detection of MC-LR in basil and food crops from the Belgian market. Therefore, more research on MC accumulation in basil is needed, and yearly monitoring of human food crops for MCs is recommended.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wannes Hugo R. Van Hassel reports financial support was provided by Federal Public Service Health Food Chain Safety and Environment. Mohamed F. Abdallah reports financial support was provided by Ghent university special research fund. Andreja Rajkovic reports financial support was provided by EU Framework Programme for Research and Innovation Euratom. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.123715.

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