Aperito Online Publishing
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Aperito Journal of Aquatic and Marine Ecosystems

Received: Jan 14, 2015
Accepted: Mar 11, 2015
Published: Mar 13, 2015

http://dx.doi.org/10.14437/AJAME-1-101 Research Pflugmacher S, Aperito J Aquat and Mar Ecos 2015, 1:1

# Transfer of Cyanobacterial Toxins into Edible Plants via Irrigation with Lake Water - A Chinese Case Study

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## Abstract

The rising pollution of water bodies goes along with an increase of cyanobacteria in the microbiotic community. The ability of cyanobacteria to produce toxins is well known and many cases of poisoning of animals and humans have been described worldwide. Thereby the exposure routes are mainly *via* drinking water. Another possible risk is the irrigation of edible plants with cyanotoxin contaminated water, since plants are able to take up these toxins. This scenario is observed at the lake Chao (China). In this study the cyanotoxin content in vegetables (spring onion, pak choi and courgette) grown on the shore of lake Chao was investigated. This lake regularly exhibited massive cyanobacterial blooms (mainly *Microcystis* and *Anabaena*) for years. Most of the blooms exhibit two different cyanobacterial microcystins, microcystin-LR and –RR. The continuous irrigation of the plants with lake water led to high accumulations of microcystins in all vegetables. The microcystin concentrations in the vegetables were above the tolerable daily intake recommended by the World Health Organization.

**Keywords**: Cyanotoxin, human food plants, toxin transfer, irrigation

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**Abbreviations**: HPLC: High Pressure Liquid Chromatography; LC-MSMS: Liquid Chromatography Mass Spectrometry; MC: Microcystin; MeOH: Methanol; MRM: Multiple Reaction Monitoring; N: Nitrogen; P: phosphor; TDI: Tolerable daily intake; TFA: Trifluoracetic Acid; UHPLC: Ultra High Pressure Liquid Chromatography; WHO: World Health Organization; v/w: volume/weight

## Introduction

Eutrophication in combination with rising temperatures due to climate change processes are the main driving force for massive proliferation of primary producers such as cyanobacteria [1,2]. Many of the bloom-forming cyanobacteria produce different types of toxic secondary metabolites, which cause a variety of ecological and even human health effects [3]. The most frequent, highly toxic and best studied cyanobacterial toxins are the group of microcystins (MCs) with over 65 congeners detected [4,5]. Since MCs are known to be acutely liver toxic, and chronically act as tumour promoters (liver and colon), they pose certain serious risk to human health when contaminated surface waters are used as a drinking water source, for recreational purposes or for spray irrigation of plants used in agriculture [6, 7, 8, 9, 10].

Lakes in the PR China are heavily eutrophicated, with the consequence of yearly cyanobacterial bloom formation. Monitoring for toxins in chinese lakes by [11] resulted in the detection of mainly microcystin-LR and –RR (MC-LR, MC-RR) in most of the studied lakes.

Lake Chao, located in the Anhui Province, a subtropical region in the south-east of China, is the fifth largest freshwater lake in China. Due to the construction of two lock-gates (Chao- and Yuxi-gate) the water level of the lake is artificially controlled. The lake is located 10 km south-east of the Anhui Province capital Hefei, which is planned to grow to a city with a population of 5 millions inhabitants. Analyses of N- and P-contents revealed in the sediment 400-1000 µg $g^{-1}$ total N and 200-640 µg $g^{-1}$ total P, respectively, and in the water phase 0.61-2.5 mg $l^{-1}$ total N and 0.038-0.242 mg $l^{-1}$ total P, respectively [12]. This nutrient level in combination with the low lake depth and the relatively high ambient temperatures provides the best conditions for cyanobacterial mass proliferation.

Consequently, this lake regularly exhibited cyanobacterial blooms (mainly *Microcystis* and *Anabaena*) in the past decades [12]. The presence and seasonal variation of microcystin-LR and –RR at different sample stations in the lake were shown by [12]. A risk for humans is given, since the lake is used for fishery and drinking water supply [13]. Furthermore, the untreated lake water is used by people living around the lake to irrigate their vegetables for human consumption (Fig. 1).

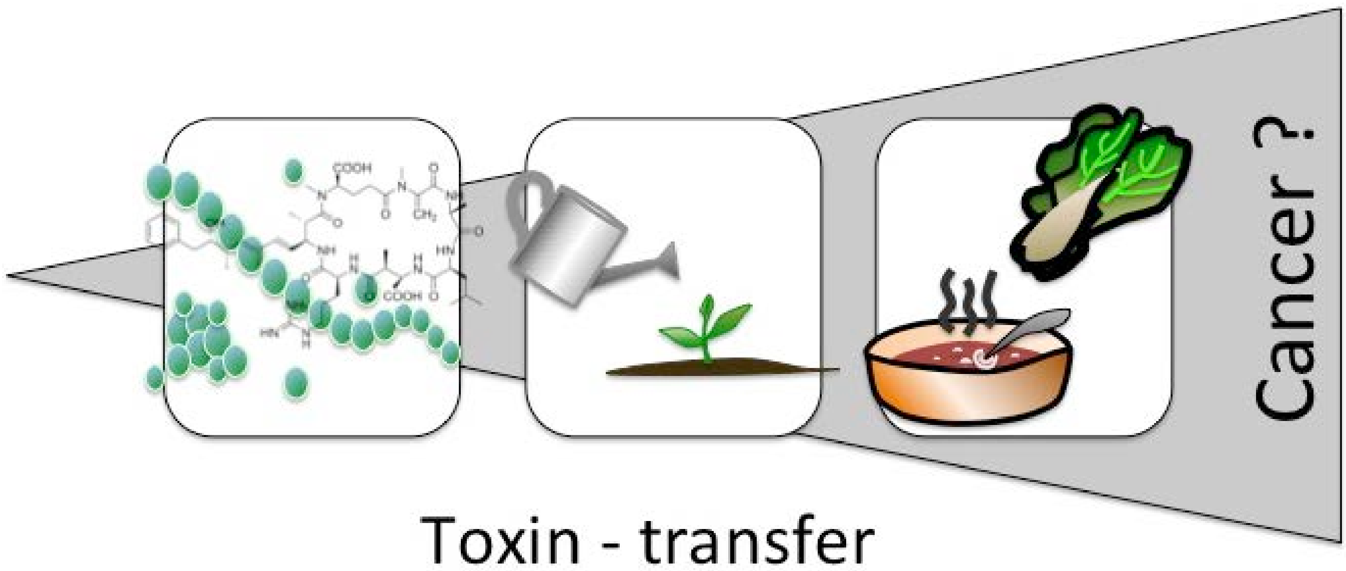


**Figure 1:** Possible transfer of cyanobacterial toxins into human food web by simple spray irrigation of crop plants. The use of lake surface water for irrigation of crop plants is the usual way in the Chao area.

Aim of this study was to investigate if there is a transfer of cyanotoxins from lake water into vegetables and to calculate daily intake levels to assess associated human health risks.

## Material and Methods

### Cyanobacterial bloom material

The shallow subtropical Lake Chao (North: 31° 25’- 31° 43’; East: 117° 17’ – 117° 52’) is located in the Anhui province (PR China) with a total surface area of ca. 780 $km^2$ and a mean depth of 3.06 m. Close to the sampling site of the vegetables, in the north-western area of the lake, a bloom sample (volume: 1.5 L) was taken. The bloom sample was filtered through cellulose filters and the filters were dried for 8 hrs at 70°C.

### Plant Material

The plant material was collected in 2008 from micro-agricultural fields located directly on the shore of Lake Chao. Five different samples from three agricultural fields were taken for each plant species. Sampled plant species were spring onion (*Allium fistulosum*), pak choi (*Brassica rapa chinensis*) and courgette (*Cucurbita pepo*). Plant material was transferred to the laboratory and washed intensively with water to remove soil residues. Then plants were divided into the different cormus parts and dried overnight at 70°C.

### Microcystin reference material

Three microcystin congeners MC-LR, MC-YR and MC-RR (Axxora, Grünberg Germany) were used as reference materials. They were diluted with HPLC grade methanol (Karl Roth, Karlsruhe Germany) to concentrations fitting the calibration curves on LC-MS/MS.

### Toxin Extraction and Analysis

### Cyanobacterial bloom material and plants

The cellulose filters (containing cyanobacterial bloom material) were shredded into small pieces and the plant material was ground to a fine powder using liquid nitrogen. Extractions of cyanotoxins started by adding 25 % (v/w) of methanol (100 % MeOH, Karl Roth, Germany) in 50 ml falcon tubes (Sarstedt, Germany) and shaking for 1 h. Then the methanolic slurry was ultra-sonicated for 1 min followed by turnover shaking for 24 h. After centrifugation at 12.500 x g for 10 min at 4°C, the supernatant was collected and the pellet subjected to a second methanolic extraction step similar to the first extraction procedure. The resulting supernatant was pooled with the first one and evaporated to dryness at 35°C under a constant nitrogen flow and redissolved in 1 ml MeOH for quantification of cyanotoxins by LC-MS/MS.

### Toxin measurement via LC-MS/MS

Chromatographic separation of MC-LR, MC-RR and MC-YR was performed with the Sunfire C18 column (3.5µm, 4.6 x 75 mm) using an Alliance 2695 UHPLC combined with a Micromass Quattro micro$^{TM}$ (Waters, Germany). The column oven temperature was set to 40°C and the injection volume was 10 µL at a flow rate of 0.25 mL min$^{-1}$ using a solvent gradient with 0.1% TFA in $H_2O$ (MS grade, mobile phase A) and 0.1% TFA in acetonitrile (MS grade, mobile phase B): mobile phase B increases from 0 to 35% within 2 min followed by an increase to 65% for 0.5 min, an abidance at this condition until 2.5 min, a decrease to 0% until 0.2 min and an abidance at this condition until 2.8 min (total running time of 8 min including a post-run). The retention times of MC-LR, MC-RR and MC-YR were 5.40, 4.97 and 5.18 min, respectively. For the subsequent MS–MS detection the MRM mode (positive polarity) was used with a mass transfer of 995.5 (Q1) and 107.3, 135.1, 213.2 and 357.2 (Q3) for MC-LR, 519.9 (Q1) and 107.3, 135.3, 213.3 and 329.3 (Q3) for MC-RR and 1045.5 (Q1) and 107.3, 135.0, 213.1 and 375.2 (Q3) for MC-YR. Calibrations were linear ($R^2$ = 0.999) between 5 and 500 µgL$^{-1}$. Lowest detection limit was 1 µgL$^{-1}$.

## Results and Discussion

Surface water encounters very often eutrophication and therefore may contain an excess of potentially toxic cyanobacteria. In many countries, this surface water is used for spray irrigation of agricultural fields. *Via* irrigation, crop plants could come into contact with cyanobacterial toxins. The transfer of cyanotoxins into plants used in agriculture and effects due to cyanotoxin exposure have been shown [10, 14]. The transfer of cyanotoxins into food web structures can pose a human health risk.

Some terrestrial plants species, such as the white mustard (*Sinapis alba*), were used as a biomarker test system for the presence of cyanobacterial toxins [15]. For other terrestrial plant species, the knowledge concerning uptake of cyanotoxins is very limited [14, 16, 17].

http://dx.doi.org/10.14437/AJAME-1-101 

Bloom material from Lake Chao in China analysed for cyanotoxins revealed the congeners MC-LR (58.99 µg $l^{-1}$), MC-YR (1.72 µg $l^{-1}$) and MC-RR (42.64 µg $l^{-1}$). This is in agreement with analyses done by [12] showing also the presence of MC-LR and MC-RR. Different vegetables were collected from agricultural fields on the shore of Lake Chao, to screen for cyanotoxins: pak choi, spring onion and courgette. In this monitoring study at Lake Chao in China, in all plant material the three different microcystins: MC-LR, MC-RR and MC-YR, had been detected, in roots and other plant parts such as leaves, flowers and fruits as well.

High MC-LR values were detected in the edible parts, e.g. in courgette fruits (0.98µg $g^{-1}$ FW) or pak choi leaves (0.56 µg $g^{-1}$ FW). Other toxin congeners (MC-YR and MC-RR) were detected in lower concentrations (Table 1), however these congeners might contribute to a high total toxicity. Highest MC-LR concentrations were detected in spring onions roots (2.39 µg $g^{-1}$ FW) and bulbs (1.69 µg $g^{-1}$ FW). It should be considered thatthere might be a high contamination of the soil due to thecontinual irrigation with toxin-containing water. This hypothesis is supported by the detection of toxins in the roots of all plant samples from the shore of Lake Chao

| Plant species | Plant part | Microcystins [µg $g^{-1}$ FW] | | | | $MC_{total}$ in a 100 g meal [µg $MC_{total}$ per 100 g FW] | $MC_{total}$ intake per 100 g FW [$MC_{total}$ per kg body weight per 100 g meal] | |
|---|---|---|---|---|---|---|---|---|
| | | MC-LR | MC-RR | MC-YR | $MC_{total}$ | | for a 60-kg person | for a 35-kg person |
| *Brassica rapa chinensis* (pak choi) | Roots | 0.51 | 0.03 | 0.06 | 0.60 | | | |
| | Leaves | 0.56 | 0.10 | 0 | 0.66 | 66 | 1.10 | 1.88 |
| *Allium fistulosum* (spring onion) | Roots | 2.39 | 0.68 | 1.48 | 4.55 | | | |
| | Bulbs | 1.69 | 0.64 | 0 | 2.33 | 233 | 3.88 | 6.65 |
| | Leaves | 0.53 | 0.49 | 0 | 1.02 | 102 | 1.70 | 2.91 |
| *Cucurbita pepo* (courgette) | Leaves | 1.32 | 0.29 | 0 | 1.61 | | | |
| | Flower | 0.40 | 0 | 0 | 0.40 | 40 | 0.66 | 1.14 |
| | Fruits | 0.98 | 0.25 | 0.12 | 1.35 | 135 | 2.25 | 3.85 |

**Table 1:** Microcystin concentration (MC-LR, -RR, -YR) in vegetables from Lake Chao [µg $g^{-1}$ FW]. Values are means from five different plants in three different agricultural used areas. Standard derivations within the samples were between 5- 25%. Calculations of an oral toxin intake are related to the total microcystin content ($MC_{total}$) in a meal of 100 g fresh weight of eatable plant parts per kilogram body weight. Intake per 100 g meal was calculated for a 60-kg person and 35-kg child. WHO recommended a TDI of 0.04 µg per kg body weight per day for MC-LR.

The uptake of cyanotoxins in salad lettuce (*L. sativa*) after spray irrigation with water containing *Microcystis* was shown by [14]. This process resulted in colonies and single cells being lodged on the leavesten days after the last irrigation [14]. In this study, MC-LR was present in the central leaves (2.5 mg $kg^{-1}$ DW), in the distal zone of mature leaves (0.83 mg $kg^{-1}$ DW), and in the basal zone of mature leaves (0.094 mg $kg^{-1}$ DW).

In the study by [18], the cuticle properties of plants were discussed as a factor influencing the cyanotoxin uptake. The authors used lake water containing MC congeners to irrigate *L. perenne*, *T. repens*, *B. napa*, and *L. sativa*. Plants received three or six applications of toxin contaminated water on the soil or on the shoots. MCs could be detected in roots of all plants after both application methods. Using *B. napa* and *L. perenne*, no measurable absorption of MCs by the shoots occured, because the water applied on the shoots ran off the leaves, with little wetting on the foliage. In contrast, *L. sativa* and *T. repens* leaves were visibly wetted and retained therefore a higher concentration of MCs. The authors suggested that leaf cuticle properties are a very important factor in controlling absorption of MC´s applied to plant shoots. The correlation between cuticle properties and absorption of substances is well established for organic agrochemical compounds [19]. Furthermore the stomata also may play an important role in the uptake of toxins and contribute to the overall toxin uptake [14].

The tolerable daily intake (TDI) recommended by the World Health Organization (WHO) is 0.04 µg $kg^{-1}$ of body weight per day [20]. A 60-kg person could orally take in 2.40 µg MC-LR per day all life without any toxicological effects, equivalent to 1.40 µg MC-LR for a 35-kg child.

In the courgette fruits harvested on the shore of Lake Chao 0.98 µg MC-LR $g^{-1}$ FW was detected. A meal made out of 100 g fresh weight (FW) of these courgette fruits would contain 98 µg MC-LR, resulting for a 60-kg person in an intakeof 1.63 µg $kg^{-1}$ body weight and therefore already exceeding the TDI by WHO.

Taking into account, that not only MC-LR is present in the fruits, but also MC-RR and –YR, the total microcystin content ($MC_{total}$) in 100 g courgette is 135 µg, corresponding to a calculated intake for a 60-kg person of 2.25 µg $MC_{total}$ $kg^{-1}$ body weight per meal.

A calculation for a child with 35 kg results in an intake of 3.85 µg $MC_{total}$ $kg^{-1}$ person. Comparing the WHO TDI value of 0.04 µg $kg^{-1}$ of body weight per day, which was only calculated for the MC-LR congener, all intake values are well above this recommendation, for the 35-kg child even by factor of 100. The vegetables from the Lake Chao therefore pose a human health risk factor.

All calculations in this study were related to a 100 g portion of vegetables to refer to a realistic meal portion. A meal would of course contain several vegetables with a total weight of more than 100 g. In contrast, the TDI is a recommendation for a daily intake. Taking into account that microcystins are known to be unaffected by cooking, as shown by [21], and furthermore cooking might also increase the bioavailibility of microcystins [22], the values measured and calculated here, are far more problematic concerning human health. Even more so considering the consumption of toxin contaminated vegetables on a daily basis in the lake Chao-Hu region.

## Conclusion

Microcystins are known to be tumor promotors. The high incidence of hepatic tumors in a chinese population has been associated with consumption of raw waters containing MCs [11]. Now a second exposure pathway via irrigated vegetables seems to contribute to these incidences.

## Acknowledgement

This work was supported by the German Ministry for Education and Research (BMBF; grant number 02WT0529). For this German-Chinese cooperation, the authors would like to thank especially the Anhui Environmental Protection Bureau (AEPB), Zhang Fu Hai, as well as the farmers giving permission to sample vegetables from their fields.The authors would also like to thank Sandra Kühn for technical assisstence on LC-MSMS in the laboratory.

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