



Phytotoxicity, bioaccumulation and potential risks of plant irrigations using cyanobloom-loading freshwater

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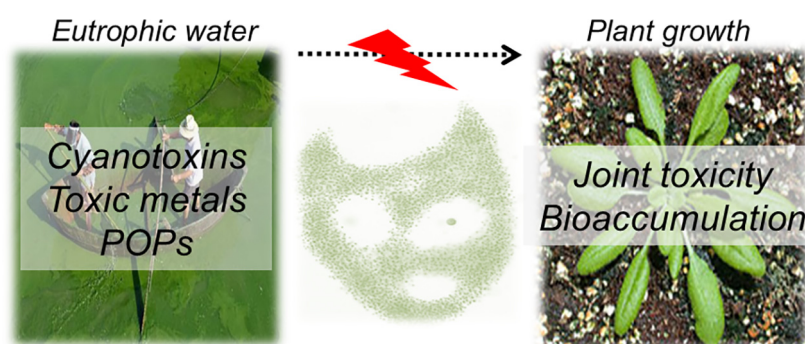
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HIGHLIGHTS

- MC-LR and PCB-28 exhibits synergistic impacts on seeds germination;
- MC-LR and Cd exerts antagonistic effects on seeds germination;
- Strongest growth inhibition were observed under ternary mixture;
- Irrigation with cyanobloom broth led to inhibition on plant growth;
- Direct irrigation by eutrophic water led to multiple contaminations of crops.

GRAPHICAL ABSTRACT



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ABSTRACT

The toxicity of cyanotoxins on plant has been reported. However, in eutrophic waters harmful cyanobacteria are associated with other environmental pollutants, such as persistent organic pollutants (POPs) and metals. Information on the phytotoxicity and bioaccumulation of coexisted cyanotoxins and these environmental pollutants is still lacking. In this study, the combined phytotoxicities of three types of cyanobacteria-associated pollutants, i.e., microcystin-LR (MC-LR), cadmium (Cd), 2, 4, 4'-Trichlorobiphenyl (PCB-28) were systematically investigated. After 7-days exposure, strong synergistic effects can be detected when *Arabidopsis thaliana* seeds and seedlings exposed to binary mixtures of MC-LR + PCB-28 and PCB-28 + Cd. The strongest inhibition occurred when *A. thaliana* exposed to their ternary mixture under both glasshouse and semi-field conditions. Moreover, bioaccumulation of MC-LR, Cd and PCB-28 was enhanced when seedlings exposed to their binary/ternary mixtures, especially when seedlings were treated with higher concentrations of toxicants (MC-LR, 1 mg L⁻¹; Cd, 10 mg L⁻¹; PCB-28, 1 µg L⁻¹). Additionally, pronounced toxic effects could be determined under 7-days after seedlings were irrigated with raw cyanobloom-containing water (collected from Lake Taihu in China) and its dilutions. Seeds production decreased significantly after the continuous irrigation with cyanoblooms-containing water. Collectively, this work will be an informative implication for risks of cyanoblooms and adequate utilization of freshwater containing cyanoblooms for crop irrigation.

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1. Introduction

The occurrence of harmful cyanobacterial blooms in eutrophic freshwaters has received increasing attention worldwide as their harmful metabolites, such as cyanotoxins and taste and odor compounds, could lead to serious environmental and health threats (Codd et al., 2005). Due to rapid economic development and the intensive use of water resources in China, many freshwater lakes are becoming more seriously polluted by cyanobacterial blooms (Song et al., 2007). Lake Taihu, the third largest freshwater lake in China, has a total water surface area of about 2338 km², and a mean water volume of approximately 4.43×10^{12} L. The lake serves as an important resource for drinking water, irrigation, aquaculture and industrial use, in addition to being a popular recreational and tourist attraction. In 2007, a serious drinking water crisis caused by a cyanobacterial bloom left approximately two million people without drinking water for over a week in the city of Wuxi (Yang et al., 2008).

To avoid and reduce the potential risks associated with the occurrence of harmful cyanobacterial blooms, many strategies, including the use of chemical algaecides, physical adsorption, biological control, and mechanical collection of algal biomass have been implemented. Amongst these approaches, mechanical collection was the most effective and widely used as an emergency measure in Lake Taihu and other freshwater lakes during the bloom seasons. From May to August in 2008, over 1000 tons of fresh cyanobacterial blooms were harvested daily from Lake Taihu. As mechanically collected biomass usually contained over 90% of moisture, the dewatering, deposit, transportation and further utilization of toxic blooms were neither cost-effective nor energy-efficient. In this sense, some of the collected blooms were discharged directly into croplands, irrigation canals, and forest land near the lakeshore as plant fertilizer for crop irrigations, without any treatment (Chen et al., 2012a).

However, this practice may lead to potential risks associated with the bioaccumulation and transfer of contaminants in crops and soils. In addition to the toxic secondary metabolites, cyanobacterial blooms can also phytoextract and accumulate other environmental pollutants (e.g., metals, persistent organic pollutants (POPs)) from water-columns as cyanobacterial cells are natural thickeners and have specific extracellular feature and metabolic nature (De Philippis et al., 2011). In view of this, mechanical collection of bloom biomass and the subsequent treatments with soil-based technologies, particularly the crop irrigations/fertilizations with toxic cyanobacterial blooms would significantly transfer high amounts of contamination into the soil interface. *Microcystis* are the dominant bloom-forming cyanobacteria in Lake Taihu. Microcystin-LR (MC-LR) is a well-studied cyanotoxin in their toxicological effects on both animals and plants. In higher aquatic plants, it's known to affect many processes, such as germination, root elongation, photosynthesis as well as the formation of reactive oxygen species (ROS) (Pflugmacher et al., 2007). Metals are also considered very harmful to plants due to their non-biodegradable nature. Exposure to even very low concentrations of some metals, such as cadmium (Cd), will unavoidably lead to the uptake of Cd by roots and the further transport of Cd to vegetative and reproductive organs, which have a detrimental effect on plant growth, reproducibility and development (Clemens, 2006). Polychlorinated biphenyls (PCBs) are ubiquitous global pollutants belonging to the group of POPs, which can be found in almost every compartment of terrestrial and aquatic ecosystems (Zhang et al., 2008). Because of the similarity to dioxins in shape and size, PCBs were thought to exhibit dioxin-like toxicity (Bright et al., 1995).

Due to the limited information on combined phytotoxicity of cyanotoxins and other cyanobloom-associated environmental pollutants, in the current study, firstly, joint toxicity of three types of cyanobloom-associated toxicants was examined on plant. Meanwhile, further risks associated with toxicant bioaccumulations in crop plants and food contamination, caused by crop irrigations/fertilizations with toxic cyanoblooms, should also be characterized and evaluated. For

these purposes, a model plant—*Arabidopsis thaliana* was employed to investigate the acute and long-term toxicity due to exposure to single and/or mixtures of cyanobloom-derived pollutants as well as raw cyanoblooms-containing water, to imitate the crop irrigation process with toxic cyanoblooms. Seed germination, sprouting, seedling growth, development, seed production and accumulated hydrophilic toxicants (MC-LR and Cd) and hydrophobic toxicants (PCBs) as well as biomass of harvested cyanobloom in *Arabidopsis* seedlings and seeds were also systematically investigated. Results from this study provide valuable information to characterize joint toxicity of three types of cyanobloom-derived toxicants on plant, and further avoid/reduce potential risks of using cyanobloom-loading eutrophic waters for crop irrigations.

2. Materials and methods

2.1. Chemicals

Microcystin-LR (MC-LR) used in this study was purified from a laboratory culture of *Microcystis aeruginosa* 905 (FACHB 905) according to the procedure described by Chen et al. (2004a) The purity was 95% as determined by HPLC (Shimadzu LC-10A, Japan). MCs standards for HPLC analysis were purchased from Wako (Japan). CdCl₂·2.5H₂O was purchased from Sigma-Aldrich (USA), and 2, 4, 4'-Trichlorobiphenyl (PCB-28) was obtained from Dr. Ehrenstorfer (Germany).

2.2. Seed germination acute toxicity

Wild-type *A. thaliana* (Columbia) seeds were obtained from the Arabidopsis Biological Resource Center (ABRC) (Columbus, OH). For the germination assay, seeds were surface-sterilized in 70% ethanol for 2 min, soaked in 50% bleach for 15 min, and then rinsed four times with sterile water (Xiang and Oliver, 1998). The surface sterilized seeds were sown in glass dishes on seed germination test papers and all seeds were placed an equal distance from each other. Germination test filter papers were soaked in different toxicants. After one week, the number of germinated seeds (the emergence of 1 mm or more of the radical from the seed coat) was recorded for each plant. For each treatment, triplicates were conducted. Seven concentrations were set at 0, 0.1, 0.5, 1, 5, 10, and 25 mg L⁻¹ for MC-LR, 0, 1, 5, 10, 15, 30, and 60 mg L⁻¹ for Cd, and 0, 0.05, 0.25, 1, 5, 20, and 50 µg L⁻¹ for PCBs, respectively. In order to generate the EC₅₀ values, high concentrations of toxicants are used in this study. Although the high concentrations of these toxicants are much higher than their environmental-relevant levels, the set-up of the low concentrations was according to the monitoring of cyanobloom samples from Lake Taihu (see table S1). And co-exposure treatments (MC-LR + Cd, MC-LR + PCB-28, Cd + PCB-28, and MC-LR + Cd + PCB-28 mixtures) were designed according to these individual toxicant treatments.

2.3. Seedling growth acute toxicity

For acute toxicity experiments, the seeds were sown in square dishes on half-strength Murashige and Skoog (MS) media. At 10 days, seedlings were transferred into artificial soil system and placed in a growth chamber with the temperature of 22 ± 2 °C, 16 h–8 h light-dark cycle (light intensity approximately 100 µmol photos m⁻² s⁻¹) and the relative humidity of 55%. These seedlings were sprayed twice a week with Hoagland nutrient solution until the adult stage (e.g., rosettes with around nine well developed leaves). From then the nutrient solution was replaced by distilled water containing MC-LR, Cd, PCB-28, and their binary/ternary mixtures. The treatments nutrient solutions were spiked with different toxicants under the concentrations of 0, 0.1, 0.5, 1, 5, and 10 mg L⁻¹ for MC-LR, 0, 1, 5, 10, 15, and 30 mg L⁻¹ for Cd²⁺ and 0, 0.05, 0.25, 1, 5, and 20 µg L⁻¹ for PCBs, respectively. The binary and ternary mixtures of MC-LR, Cd²⁺ and PCB-28 were in corresponding to each of the above six concentrations of the three toxicants.

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