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Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security

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ABSTRACT

Microcystin (MC), a hepatotoxin that can adversely affect human health, has become more prevalent in freshwater ecosystems worldwide, owing to an increase in toxic cyanobacteria blooms. While consumption of water and fish are well-documented exposure pathways of MCs to humans, less is known about the potential transfer to humans through consumption of vegetables that have been irrigated with MC-contaminated water. Likewise, the impact of MC on the performance of food crops is understudied. To help fill these information gaps, we conducted a controlled laboratory experiment in which we exposed lettuce, carrots, and green beans to environmentally relevant concentrations of MC-LR (0, 1, 5, and 10 μg/L) via two irrigation methods (drip and spray). We used ELISA and LC-MS/MS to quantify MC-LR concentrations and in different parts of the plant (edible vs. inedible fractions), measured plant performance (e.g., size, mass, edible leaves, color), and calculated human exposure risk based on accumulation patterns. MC-LR accumulation was positively dose-dependent, with it being greater in the plants (2.2–209.2 μg/kg) than in soil (0–19.4 μg/kg). MC-LR accumulation varied among vegetable types, between plant parts, and between irrigation methods. MC-LR accumulation led to reduced crop growth and quality, with MC-LR persisting in the soil after harvest. Observed toxin accumulation patterns in edible fractions of plants also led to estimates of daily MC-LR intake that exceeded both the chronic reference dose (0.003 μg/kg of body weight) and total daily intake guidelines (0.04 μg/kg of body weight). Because the use of MC-contaminated water is common in many parts of the world, our collective findings highlight the need for guidelines concerning the use of MC-contaminated water in irrigation, as well as consumption of these crops.

1. Introduction

Toxic cyanobacterial blooms have become commonplace in freshwater ecosystems around the world, owing to human-induced eutrophication (Lu, Tian, Pei, Hu, & Xie, 2013). A general consensus also exists that continued climate change will increase their prominence by promoting growing conditions for harmful cyanobacteria (O'neil et al., 2012; Paerl et al., 2016; Xia, Li, Deng, & Hu, 2013). This increase is problematic because cyanobacteria can negatively affect aquatic ecosystems by 1) altering physicochemical characteristics of the water (e.g., transparency, dissolved oxygen availability), 2) modifying interactions among aquatic organisms (e.g., fish, shellfish, macrophytes, and zooplankton), and 3) producing cyanotoxins that can harm the health and development of aquatic organisms (Cheung, Liang, & Lee, 2013; Eisenhut et al., 2008; Li, Zhang, Zhu, Xiao, & Chen, 2013; Paerl & Huisman, 2009; Whitton & Potts, 2012). Additionally, the hepatotoxins, neurotoxins, dermatotoxins, and cytotoxins produced by various cyanobacterial bloom genus (e.g. *Anabaena*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, and *Plaktothrix*) can threaten human health by tainting recreational water, drinking water, and seafood supplies (Cheung et al., 2013; Lee, Lee, & Jiang, 2017). Therefore, harmful cyanobacterial bloom formation has become a public health concern worldwide (Cheung et al., 2013; Larsen et al., 2004). Additionally, cyanobacteria blooms can cause economic hardship through lost tourism (~$1 billion per year in the USA), increased drinking water treatment costs (~$13 million in Ohio in 2011–2012), and reduced

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fishing revenues ($10 million per year) (US EPA, 2015; US EPA, 2016).

Microcystins (MCs), which are a family of cyclic heptapeptides produced by species from *Microcystis*, *Anabaena*, and several other cyanobacterial genera, are the most common cyanotoxins found in freshwater ecosystems worldwide, including the United States, China, Japan, and Europe (Carmichael, 2013). Microcystins are produced inside the bacterial cells but are released into the environment when the cells rupture (Younos, 2016). Ingestion of MCs can cause widespread and serious animal and human health problems, including fever, vomiting, weakness, liver-, kidney-, heart-, brain-, and skin-damage, neurological impairment, and even death (Rastogi, Sinha, & Incharoensakdi, 2014). In one mouse study, MCs induced DNA mutations (genotoxicity), damage to mitochondria and membranes, cytoskeletal damage, and loss of cell morphology (Li, Zhao, Zhou, Xu, & Wang, 2015). Recent studies also have shown that MC-LR, which is the most common congener and has high toxicity, can be transmitted from adults to offspring in rats and fish (Li et al., 2015; Liu, Qiao, Chen, Wu, & Zhang, 2014; Zhao, Li, & Chen, 2015).

Ingestion of contaminated water, inhalation, and dermal contact are the major routes for exposure to MCs. However, past studies also have shown that cyanotoxins can accumulate in both consumable animal and plant tissues, which may increase the risk of MC exposure to humans (Lee et al., 2017). While a rich body of literature has shown that MCs can accumulate in the organs and muscles of seafood that are living in eutrophic environments (e.g., Bittencourt-Oliveira et al., 2016; Ibelings & Chorus, 2007; Magalhaes et al., 2000; Ni, Zhang, & Luo, 2015; Poste, Hecky, & Guildford, 2011; Schmidt et al., 2013), fewer studies have explored MC accumulation in edible crops and soils. Those that have, however, provided clear evidence to indicate that MCs associated with the irrigation water can accumulate in edible plant tissues (Saqrane & Oudra, 2009). For example, MC accumulation was documented in Saudi Arabian crops (e.g., radish, lettuce, dill parsley, arugula, and cabbage) that were cultivated with MC-contaminated water (Mohamed and Al Shehri, 2009), as well as in tomatoes grown in a hydroponic solution contaminated with MC-LR (Corbel, Mougin, Nélieu, Delarue, & Bouaïcha, 2016). Despite these findings, our understanding of the factors that influence accumulation of MCs in plants, such as irrigation method (e.g., drip versus spray) and MC concentration in the irrigation water, is limited. In addition, knowledge gaps exist with respect to how MC uptake varies among different crop types and their associated soils, how MC accumulation differs among plant parts (e.g., roots vs. shoots), how MCs influence plant growth and quality, and how persistent MCs are in the soils after harvest. All of these unanswered questions limit the ability of regulatory agencies and policymakers to understand the negative impact on food safety, food quality, and crop yield of irrigating crops with MC-contaminated water. These are critically important questions as irrigating crops with MC-laden water appears to be a common practice in many parts of the world (Mohamed and Al Shehri, 2009; USGS, 2010).

Towards improving our understanding of how irrigating crops with MC-contaminated water influences MC accumulation, crop growth, and crop quality, such that appropriate consumption guidelines and irrigation practices can be established, we conducted multiple controlled laboratory experiments to determine 1) the fate of MCs in crops and soil when irrigated with contaminated water; and 2) the effects of MCs on crop quality and productivity. More specifically, we examined the systemic transfer of MC-LR in crop cultivation systems, including different parts (i.e., shoots, roots) of three types of vegetables (lettuce, carrot, green bean) that were watered with 1 of 4 environmentally relevant MC-LR concentrations (0, 1, 5, or 10 μg/L), using either a drip or spray irrigation method. We also measured the yield of edible portions of each produce type (in terms of mass and length), as well as the quality (e.g., color, number of edible leaves) of each food type. In addition, we quantified the accumulation and persistence of MC-LR in plant soils before, during, and after plant harvest. Finally, we calculated the risk to humans from ingesting the edible fractions of the vegetables grown in our experiments.

2. Materials and methods

2.1. Fresh produce growing conditions

We cultivated romaine lettuce (*Lactuca sativa* L.), carrots (*Daucus carota*), and green beans (*Phaseolus vulgaris* L.) in an Ohio State University (Columbus, OH, USA) greenhouse and laboratory. Plants were grown from seed in a greenhouse in individual pots that were filled with 300 g of dry soil (Professional Growing Mix, Sung Gro Horticulture, Agawam, MA, USA). The temperature and humidity ranged 19–21 °C and 40–50%, respectively. Six weeks after germination, which is when plants were stable enough to be moved, we transferred them to the laboratory and where they were put into chambers to protect them against other potential contaminants. The temperature (20–22 °C) and humidity (~42%) were similar to the greenhouse, with the light cycle being controlled using a T5 fluorescent grow light (AgroBrite Petaluma, CA, USA) in a way that simulated a natural environment (16:8 light: dark hours).

To explore MC-LR accumulation rates and the impact of MC-LR on plant performance, we irrigated each pot with either 100 mL of uncontaminated water (negative control) or a 100 mL solution that contained MC-LR (Beagle Bioproducts Inc., Columbus, OH, USA) at 1 of 3 concentrations (1 μg/L, 5 μg/L, or 10 μg/L). To test the simultaneous effect of irrigation type, water was applied to each pot using either drip irrigation (around the root area) or spray irrigation (on the surface of the leaves). All pots were irrigated three times per week for four weeks (12 total applications). Each treatment group (4 MC-LR levels × 2 irrigation types) was replicated 15 times for each plant type, with each replicate consisting of one plant in an individual pot. All experimental trials were repeated three times (n = 360 pots per produce type; n = 1080 total pots).

2.2. Measurements of MC-LR in fresh produce and soil

When the plants were ready for harvest, both fresh produce and soil samples were collected. To remove extra soil and debris on the produce and tissue surfaces, the samples were gently washed with deionized water and then dried. Our preliminary tests showed that this washing step did not alter the accumulated MC-LR concentrations between the washed and unwashed plants (t-test, $p > 0.05$).

We first used enzyme-linked immunosorbent assay (ELISA; Microcystins/Nodularins (ADDA) kit, Abraxis, Warminster, PA, USA) to measure MC-LR levels in all plants. ELISA offers a cost-effective way to quantify MC levels at low concentration and has been widely used in MC studies, including for both biological (Drobac et al., 2016; Kohoutek et al., 2010; Trifirò et al., 2016) and non-biological (Hu, Rea, Yu, & Lee, 2016; Graham, Loftin, Meyer, & Ziegler, 2010; Grützmacher et al., 2010) samples. We followed established protocols from previous studies that used ELISA to quantify MC in fresh produce samples (Mohamed and Al Shehri, 2009; Chen, Han, Wang, Zhang, & Shi, 2012; Gutiérrez-Praena et al., 2014; Liang & Wang, 2015; Trifirò et al., 2016).

To prepare plant tissues for MC analysis with ELISA, we divided each sample into roots and shoots with a sterile knife, with the beans also being collected separately for green beans. All of the fresh produce samples were handled using sterile aluminum foil or dark glass vials and contact with plastics was avoided. About 10 to 20 g of tissue samples (roots, shoots, or beans) were homogenized with dry ice using a stainless steel container (Waring commercial, Torrington, CT, USA) to make a fine powder. The powder sample was mixed with 40 or 80 mL 75% methanol (1:4 ratio) and the slurry then centrifuged at 10,000 xg at 4 °C for 10 min. The supernatants were purified by Sep-park C18 cartridges. The purified supernatants of each sample were evaporated to dryness. All the samples were extracted in triplicate. The remaining residue was re-suspended in 5% methanol for analysis of MC-LR with

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