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Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners

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ABSTRACT

The use of microcystins (MCs) contaminated water to irrigate crop plants represents a human health risk due to their bioaccumulation potential. In addition, MCs cause oxidative stress and negatively influence photosynthetic activities in plants. The present study was aimed at investigating the effect of MCs on photosynthetic parameters and antioxidative response of lettuce. Furthermore, the bioaccumulation factor (BAF) of total MCs, MC-LR and MC-RR in the vegetable after irrigation with contaminated water was determined. Lettuce crops were irrigated for 15 days with water containing cyanobacterial crude extracts (*Microcystis aeruginosa*) with MC-LR (0.0, 0.5, 2.0, 5.0 and 10.0 $\mu g\ L^{-1}$), MC-RR (0.0, 0.15, 0.5, 1.5 and 3.0 $\mu g\ L^{-1}$) and total MCs (0.0, 0.65, 2.5, 6.5 and 13.0 $\mu g\ L^{-1}$). Increased net photosynthetic rate, stomatal conductance, leaf tissue transpiration and intercellular CO_2 concentration were recorded in lettuce exposed to different MCs concentrations. Antioxidant response showed that glutathione S-transferase activity was down-regulated in the presence of MCs. On the other hand, superoxide dismutase, catalase and peroxidase activities were upregulated with increasing MCs concentrations. The bioaccumulation factor (BAF) of total MCs and MC-LR was highest at 6.50 and 5.00 $\mu g\ L^{-1}$, respectively, while for MC-RR, the highest BAF was recorded at 1.50 $\mu g\ L^{-1}$ concentration. The amount of total MCs, MC-LR and MC-RR bioacumulated in lettuce was highest at the highest exposure concentrations. However, at the lowest exposure concentration, there were no detectable levels of MC-LR, MC-RR and total MCs in lettuce. Thus, the bioaccumulation of MCs in lettuce varies according to the exposure concentration. In addition, the extent of physiological response of lettuce to the toxins relies on exposure concentrations.

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1. Introduction

Simultaneous changing global climatic conditions and increasing anthropogenic pressures are altering the composition of aquatic ecosystems and favoring frequent occurrence of cyanobacterial blooms (Houghton et al., 2001; IPCC, 2007; Paerl and Huisman, 2008). Depending on the composition of blooms, the production of bioactive substances such as microcystins, cylindrospermopsin, anatoxins and saxitoxins by cyanobacteria can negatively affect plant and animal life of aquatic ecosystems (Kozdęba et al., 2014). In addition to causing oxidative stress in plants (Cordeiro-Araújo et al., 2015; Pflugmacher et al., 2007; Saqrane et al., 2007), microcystins (MCs) induce changes in development (Máthé et al., 2007; Pflugmacher et al., 2007), photosynthetic rate (Abe et al., 1996; Weiss et al., 2000) and plasma membrane permeability (Cordeiro-Araújo et al., 2015). Although direct natural contact of MCs with land plants is rare, irrigation with contaminated water increases the possibility of this occurring. After exposure to MCs, plants produce higher amounts of reactive oxygen species (ROS) than under normal growth conditions, which can result in oxidative damage to their cells (Peuthert et al., 2007; Pflugmacher et al., 2007). Fortunately, plants are evolutionarily equipped with efficient antioxidant defense systems

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comprising enzymes such as glutathione S-transferase (GST), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) that combat and prevent the damaging effects associated with high ROS production (Babica et al., 2006; Cordeiro-Araújo et al., 2015; Romero-Oliva et al., 2015).

Cyanobacterial blooms pose risks to human and animal health, due to the toxins they produce and release into water (Bittencourt-Oliveira et al., 2014; Carmichael, 1997; Cordeiro-Araújo and Bittencourt-Oliveira, 2013). For example, human deaths have been reported after dialysis, in which patients were exposed to microcystins (MCs) contaminated water (Carmichael et al., 2001; Jochimsen et al., 1998). Cyanotoxins risk assessment and characterization in relation to human health require the identification of common exposure routes, among which consumption of contaminated food has been recognized (Cox et al., 2003; Hereman and Bittencourt-Oliveira, 2012; Murch et al., 2004).

A common agricultural practice involves the use of water from public supply reservoirs, rivers and ponds to irrigate farms/crops. Unfortunately, these surface water sources are sometimes contaminated with cyanobacteria and cyanotoxins, which may be taken up and bioaccumulated in plants tissues (Codd et al., 1999; Crush et al., 2008; Mohamed and Al Shehri, 2009). This makes the consumption of crops and vegetables irrigated with contaminated surface water a potentially dangerous human exposure route to different cyanotoxins including microcystins (Crush et al., 2008; Hereman and Bittencourt-Oliveira, 2012). As a result of the bioaccumulation potential of MCs in crop plants, the World Health Organization (WHO) recommends that total daily intake (TDI) of MC-LR for humans should not exceed 0.04 μg per kilogram of body weight (WHO, 2011). However, recent evidence has demonstrated that values above the recommended TDI limit can be found in crops exposed to MCs at different environmentally relevant concentrations (Hereman and Bittencourt-Oliveira, 2012; Mohamed and Al Shehri, 2009).

To date, more than 80 variants of MCs have been identified, however, the bioaccumulation of these congeners in plants has been poorly investigated (Romero-Oliva et al., 2014). The authors revealed that the bioaccumulation of different MC congeners and total MCs can vary in some aquatic macrophytes. Under natural conditions, water used for irrigation may contain several MCs congeners, in addition to other cyanotoxins (Corbel et al., 2014; El Khalloufi et al., 2012; Pflugmacher et al., 2007). Furthermore, laboratory results suggest that cyanobacterial strains/species can produce more than one MCs congeners (Bittencourt-Oliveira, 2003; Puddick et al., 2014). This implies that crops irrigated with cyanobacterial and cyanotoxins contaminated water may be exposed to more than one MCs congener per time. However, the simultaneous uptake and bioaccumulation of these congeners in vegetables and other crop plants is yet to be extensively studied. This limits our understanding of possible antagonistic, synergistic and/or additive interactions that may occur between cyanobacterial toxins and their congeners in higher plants. Therefore, the objectives of the present study were to determine the effect of cyanobacterial extracts containing different MCs congeners on photosynthetic parameters and antioxidant response of lettuce, as well as their bioaccumulation in this plant after irrigation with contaminated water.

2. Material and methods

2.1. Vegetable

Lactuca sativa L. (Vanda cultivar) seedlings were obtained from IBS MUDAS (Rodovia Piracicaba-Rio Claro, São Paulo, Brazil) and maintained in a growth substrate (*Pinus* bark and vermiculate). At the harvest growth stage, typically from 50 to 70 days after planting (Embrapa, 2010), lettuce plants were used for the experiments. Just before the initiation of the experiments, randomly selected lettuce plants were analyzed to confirm the absence of microcystins contamination.

2.2. Cyanobacterial strain and culture conditions

Microcystins crude extracts were obtained from the cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing, BCCUSP232 strain. The strain produces microcystin-LR and -RR, and belongs to the Brazilian Cyanobacteria Collection of the University of São Paulo (Bittencourt-Oliveira, 2003). Cultivation of the cyanobacterium was done in ASM-1 medium (pH 7.4) (Gorham et al., 1964) under controlled conditions (light intensity, 30 μ mol m^{-2} s^{-1}; photoperiod, 14:10 h light:dark; temperature, 22 ± 1.0 °C). Liquid chromatography/mass spectrometry analysis revealed that the strain produced 3.0 and 9.2 ng mg^{-1} of MC-RR and MC-LR, respectively. The cultures were harvested when the cyanobacterium had 8×10^6 cells mL^{-1} (*ca.* 1 mg mL^{-1}). A detailed description of biomass production and growth rates of *M. aeruginosa* BCCUSP232 can be found in Bittencourt-Oliveira et al. (2015, 2016). At exponential growth phase, the resulting cyanobacterial biomass was centrifuged, frozen in liquid nitrogen, lyophilized and kept frozen at −80 °C until use.

2.3. Microcystins extraction, quantification and lettuce exposure experiment

Prior to use in the experiments, the lyophilized *M. aeruginosa* biomass was sonicated (Microson Ultrasonic Cell Disruptor, Misonix, USA) in an ice bath for 5 min at 15 W and 22.5 kHz to lyse the cells and release of microcystins. The procedure outlined in Hall (2013) was used for MC-LR and MC-RR quantification of the extracted biomass by liquid chromatography, using an Agilent liquid chromatography with mass spectrometers tandem (LC-MS/MS; Agilent Technologies, Santa Clara CA, EUA). The system comprised a triple quadrupole mass spectrometer equipped with an electrospray interface (ESI). A C18 Zorbax Eclipse Pluse chromatographic column (3.0 × 100 mm, 3.5 μm, Agilent – Santa Clara, California, USA) was used for MCs analysis.

Ten days old lettuce seedlings were transplanted to 7 L pots containing approximately 3.5 kg vegetable growth substrate made from the combination of *Pinus* bark and vermiculite (BASAPLANT). The seedlings were manually irrigated by spraying the leaves with 100 mL of deionized water for 30 days. During this period, a nutrient solution (427 mg L^{-1}, $MgSO_4$, 478 mg L^{-1} Ca $(NO_3)_2$ and 134 mg L^{-1} KNO_3) was applied to the plants every 3 days. After 30 days of irrigation with uncontaminated water, the plants were irrigated for another 15 days with water containing *M. aeruginosa* crude extracts with MC-LR (0.0, 0.5, 2.0, 5.0 and 10.0 μg L^{-1}), MC-RR (0.0, 0.15, 0.5, 1.5 and 3.0 μg L^{-1}) and total MCs (0.0, 0.65, 2.5, 6.5 and 13.0 μg L^{-1}). 100 mL of the MCs solution were manually applied on plant leaves with the aid of an Erlenmeyer flask which allowed the flowing of contaminated water excess into the growth substrate.

At the end of the contamination experiment, on day 16, lettuce leaves were randomly collected from the different treatments. Leaf disks of 1 cm in diameter (1 g in total) were cut from lettuce leaves per treatment condition, weighed on a precision scale and stored at −80 °C until MCs analysis.

2.4. Gas exchange parameters measurement

Net photosynthetic rate (*A*), stomatal conductance (*gs*), leaf tissue transpiration (*E*) and intercellular CO_2 concentration (*Ci*) of

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