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Potential human health risk assessment of cylindrospermopsin accumulation and depuration in lettuce and arugula

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ABSTRACT

The cyanobacterial toxin cylindrospermopsin (CYN) has become a globally important secondary metabolite due to the negative effect it has on human and animal health. As a means of evaluating the risk of human exposure to CYN, the bioaccumulation and depuration of the toxin in lettuce (*Lactuca sativa* L.) and arugula (*Eruca sativa* Mill.) were investigated, after irrigation with contaminated water. The vegetables were irrigated for 7 days with CYN (3, 5 and 10 μg/L) contaminated water (bioaccumulation phase), and subsequently, irrigated for 7 days with uncontaminated distilled water (depuration phase). In general, the bioaccumulation of CYN in both vegetables decreased with increasing exposure concentration. Bioconcentration factor (BCF) of CYN increased with the progression of the experiment at 3.0 μg/L CYN, while the reverse occurred at 5 and 10 μg/L CYN. In arugula, BCF increased at all CYN exposure concentrations throughout the study. The depuration of CYN decreased with increasing exposure concentration but was highest in the plants of both species with the highest bioaccumulation of CYN. Specifically, in plants previously irrigated with water contaminated with 3, 5 and 10 μg/L CYN, the depuration of the toxin was 60.68, 27.67 and 18.52% for lettuce, and 47, 46.21 and 27.67% for arugula, respectively. Human health risks assessment revealed that the consumption of approximately 10 to 40 g of vegetables per meal will expose children and adults to 1.00-6.00 ng CYN/kg body mass for lettuce and 2.22-7.70 ng CYN/kg body mass for arugula. The irrigation of lettuce and arugula with contaminated water containing low CYN concentrations constitutes a potential human exposure route.

1. Introduction

The occurrence of cyanobacterial blooms increases the concentration of bioactive secondary metabolites in aquatic ecosystems during cell lyses or death (Bittencourt-Oliveira et al., 2014). The bioactive secondary metabolites produced by cyanobacteria have been demonstrated to have deleterious effects on human, animal and environmental health (Codd et al., 1999a; Metcalf et al., 2004).

The cyanotoxin cylindrospermopsin (CYN) is a polycyclic uracil derivative that contains guanidine and sulphate groups (Welker, 2008), and is produced by several genera of cyanobacteria such as *Cylindrospermopsis* (Ohtani et al., 1992), *Umezakia* (Harada et al., 1994), *Aphanizomenon* (Banker et al., 1997), *Lyngbya* (Seifert et al., 2007) and *Anabaena* (Spoof et al., 2006). The cyanotoxin is genotoxic and carcinogenic (Falconer and Humpage, 2006), inhibits glutathione and general protein synthesis (Runnegar et al., 1994, 1995), and animals injected or orally given CYN containing extracts have pathological symptoms in organs such as kidney, spleen, thymus, and heart (Sivonen and Jones, 1999). A number of studies have investigated the physiological effects of CYN on different plants (Beyer et al., 2009; Prieto et al., 2011; Kittler et al., 2012; Mathé et al., 2013; Freitas et al., 2015; Garda et al., 2015). For example, Máthé et al. (2013) showed that CYN can stimulate and inhibit lateral root formation, inhibit xylem differentiation in roots and leaves, and alter cell division; and Freitas et al. (2015) implicated the cyanotoxin in oxidative stress induction and mineral content alteration of plants.

Abbreviations: CYN, cylindrospermopsin; LC–MS/MS, liquid chromatography tandem–mass spectrometry; BCF, bioconcentration factor; ToDI, total daily intake.

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In the past, human exposure to CYN was mainly attributed to direct contact with the toxin, for example, during swimming and aquatic sport activities, and the use of contaminated potable water (Kittler et al., 2012). Recently, several cyanotoxins have been detected in the human diet, due to their bioaccumulation in the tissues of aquatic organisms (Saker et al., 2004; Mohamed and Hussein, 2006; Ibelings and Chorus, 2007) and irrigated agricultural crops (Codd et al., 1999b; Crush et al., 2008; Mohamed and Al-Shehri, 2009; Prieto et al., 2011; Kittler et al., 2012; Hereman and Bittencourt-Oliveira, 2012; Gutiérrez-Praena et al., 2013, 2014; Bittencourt-Oliveira et al., 2016; Corbel et al., 2014; Cordeiro-Araújo et al., 2016; Drobac et al., 2017).

Despite the human health risk associated with CYN (Sivonen and Jones, 1999; Kinnear, 2010), very little attention has been given to its bioaccumulation in agricultural crops (Kittler et al., 2012). Compared to the high number of studies available on the bioaccumulation of microcystins in plant tissues, a few studies have shown the lack of bioaccumulation of CYN in plant tissues (White et al., 2005; Kinnear et al., 2007; Silva and Vasconcelos, 2010; Kittler et al., 2012). Although it is important to study the bioaccumulation of cyanotoxins in plant tissues as a means of evaluating human health risks, the ability of plants to depurate these bioactive metabolites is of equal importance. Currently, most studies on the time required to totally depurate cyanotoxins have been conducted on aquatic animals (Ozawa et al., 2003; Soares et al., 2004; Mohamed and Hussein, 2006; Smith and Haney, 2006; Deblois et al., 2008; Garcia et al., 2010) and aquatic macrophytes (Mohamed, 2017), which are in natural contact and bioaccumulate the secondary metabolites in their tissues.

There are no studies that have simultaneously considered the bioaccumulation and depuration of CYN in irrigated agricultural crops. This knowledge gap is of human health importance because it provides valuable information on public health risks associated with bioaccumulation and depuration of CYN in plant tissues. Therefore, the objectives of the present study were to (1) investigate the bioaccumulation and depuration of CYN in leaf tissues of lettuce (*Lactuca sativa* L.) and arugula (*Eruca sativa* Mill.); and (2) estimate the amount of CYN consumed daily by adults and children per Kg body mass. The leaves of both vegetables were selected because they are consumed worldwide (McMichael, 1994).

2. Material and methods

2.1. Vegetables

Lettuce (*Lactuca sativa* L.; Vanda cultivar) and arugula (*Eruca sativa* Mill.; Folha cultivar) were obtained from IBS MUDAS (Piracicaba/Rio Claro-SP, Brazil) and used for the experiments. The experiments were carried out when the vegetables had attained their respective harvest growth stages, which were 50 to 70 days for lettuce and 40 to 60 days for arugula. Prior to the start of the experiments, six seedlings of each vegetable were randomly selected and analyzed for CYN contamination using liquid chromatography tandem-mass spectrometry (LC–MS/MS, see Section 2.3). This was done to confirm the absence of the toxin in lettuce and arugula.

2.2. Accumulation and depuration of CYN in lettuce and arugula

Ten days old lettuce and arugula seedlings were transplanted into 7 L pots containing 3.5 kg vegetable growth substrate made of a mixture of *Pinus* bark and vermiculite. The plants were maintained at 25 ± 2 °C temperature, 50 μmol.m^{-2}.s^{-1} irradiance and 10:14 h (light:dark cycle) photoperiod in a greenhouse.

Lettuce and arugula plants were irrigated daily with 100 mL of distilled water for 30 and 20 days, respectively. During the growth and maintenance of both vegetables, a nutrient solution containing calcium nitrate, potassium nitrate, magnesium sulphate, boric acid, monoamonium phosphate, iron, and a micronutrient cocktail (Cordeiro-Araújo et al., 2015), was applied to the plants every 3 days. Thirty (30) and 20 days after transplantation of lettuce and arugula seedlings, respectively, the bioaccumulation phase of the study commenced. During the bioaccumulation phase, the plants were irrigated with CYN (3, 5 and 10 μg/L) contaminated water daily for 7 days. Purified CYN (> 95% purity) was obtained from Abraxis (Abraxis, LCC, USA). Toxin application was done manually on the leaves of both vegetables with 100 mL of CYN contaminated water, which allowed the excess contaminated water to spill into the growth substrate. At the end of the bioaccumulation phase, lettuce or arugula plants received 2.1, 3.5 and 7.0 μg of CYN, corresponding to daily irrigation with contaminated water having 3, 5 and 10 μg/L of the toxin, respectively. Immediately after the bioaccumulation experiment, the previously CYN contaminated plants were irrigated with uncontaminated distilled water for 7 days. This represented the depuration phase of the experiment. The control plants were irrigated with uncontaminated distilled water throughout the study. The CYN concentrations (3, 5 or 10 μg/L) were selected based on the levels of the toxin recorded in Brazilian reservoirs used for public water supply and irrigation of agricultural crops (Bittencourt-Oliveira et al., 2014).

Leaf samples were collected during the bioaccumulation experiment on days 1, 4 and 7 and during the depuration experiment on day 7. Immediately after collection, the leaves of the vegetables were washed with distilled water to remove CYN residues on their surfaces. All bioaccumulation and depuration experiments were carried out in triplicates ($n = 3$). For each replicate, several 1 cm diameter leaf discs were cut using a calibrated cutter to give 1 g fresh weight (FW). The samples were frozen at −80 °C until CYN extraction. The total cultivation times, including the bioaccumulation and depuration experimental phases, were 55 days for lettuce and 45 days for arugula.

2.3. Analysis of lettuce and arugula leaves for CYN bioaccumulation and depuration

Bioaccumulation and depuration of CYN in lettuce and arugula leaves were investigated by LC–MS/MS and enzyme linked immunosorbent assay (ELISA) methods. Extraction of CYN was done following the procedure described by Hereman and Bittencourt-Oliveira (2012). Briefly, 1 g of lettuce and arugula leaf samples were ground to powder in liquid nitrogen, resuspended in deionized water and sonicated (15 W and 22.5 KHz, Microson Ultrasonic Cell Disruptor, Misonix, New York, USA) on ice in a water bath for 5 min. Afterwards, the samples were centrifuged for 5 min, and the supernatant filtered through 0.2 μm pore size PTFE DISMIC-13 filters (Advantec, Shibuya-ku, Tokyo, Japan). The filtrates were used for CYN quantification.

For LC–MS/MS analysis of CYN in lettuce and arugula leaf tissues, an Agilent 1200 series model 6420 liquid chromatography tandem-mass spectrometers system (Agilent Technologies, Santa Clara, California) was used. The LC–MS/MS system comprised a triple quadrupole mass spectrometer equipped with an electrospray interface (ESI), and a C18 Zorbax Eclipse Plus (2.1 × 150 mm, 3.5 μm, Agilent – USA) column. Chromatographic and MS conditions were based on those described in Guzmán-Guillén et al. (2012) and Hindle and Noot (2014) with modifications. Chromatographic conditions were: column temperature = 30 °C; injection volume = 20 μL; flow rate = 0.2 mL/min; runtime = 5 min; mobile phase: A = 1% methanol in water + 5 mM ammonium acetate, and B = 60% methanol in water + 5 mM ammonium acetate.