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Lettuce facing microcystins-rich irrigation water at different developmental stages: Effects on plant performance and microcystins bioaccumulation

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ABSTRACT

This study investigated the microcystins (MCs)-rich irrigation water effect on lettuce of different developmental stages, i.e. during a two months period, covering the whole period from seed germination to harvest at marketable size of the plant. We followed four lettuce plant groups receiving MCs-rich water (1.81 μg l^{-1} of dissolved MCs), originating from the Karla Reservoir, central Greece: 1) from seeds, 2) the cotyledon, 3) two true leaves and 4) four true leaves stages, all of which were compared to control plants that received tap water. Lettuce growth, photosynthetic performance, biochemical and mineral characteristics, as well as MCs accumulation in leaves, roots and soil were measured. The overall performance of lettuce at various developmental stages pointed to increased tolerance since growth showed minor alterations and non-enzymatic antioxidants remained unaffected. Plants receiving MCs-rich water from the seed stage exhibited higher photosynthetic capacity, chlorophylls and leaf nitrogen content. Nevertheless, considerable MCs accumulation in various plant tissues occurred. The earlier in their development lettuce plants started receiving MCs-rich water, the more MCs they accumulated: roots and leaves of plants exposed to MCs-rich water from seeds and cotyledons stage exhibited doubled MCs concentrations compared to respective tissues of the 4 Leaves group. Furthermore, roots accumulated significantly higher MCs amounts than leaves of the same plant group. Concerning human health risk, the Estimated Daily Intake values (EDI) of Seed and Cotyledon groups leaves exceeded Tolerable Daily Intake (TDI) by a factor of 6, while 2 Leaves and 4 Leaves groups exceeded TDI by a factor of 4.4 and 2.4 respectively. Our results indicate that irrigation of lettuce with MCs-rich water may constitute a serious public health risk, especially when contaminated water is received from the very early developmental stages (seed and cotyledon). Finally, results obtained for the tolerant lettuce indicate that MCs bioaccumulation in edible tissues is not necessarily coupled with phytotoxic effects.

1. Introduction

Cyanobacteria produce numerous toxic substances, i.e. cyanotoxins and other compounds, which are released to their aquatic environment, exhibiting allelopathic effects against planktonic microalgae, macroalgae, macrophytes, other cyanobacteria species as well as higher aquatic plants (Pflugmacher, 2002). Among various cyanotoxins, microcystins (MCs) are one of the predominant types, commonly found in 40–75% of the cyanobacterial blooms worldwide (Corbel et al., 2014). The MCs can extend their toxic action to terrestrial plants, when contaminated water is used for irrigation purposes. MCs are known to affect a number of processes in plant tissues, having considerable and well documented impacts on the growth and development of some crops (Machado et al., 2017 and references there-in). Some of the major MCs impacts on plants are the inhibition of seed germination, impaired root development, adverse effects on plant growth and crop yield, promotion of oxidative stress and lipid peroxidation (Saqrane et al., 2008; Prieto et al., 2011) as well as decreased photosynthetic performance (El Khalloufi et al., 2011, 2012; Saqrane et al., 2009). Moreover, MCs have been associated with plant tissue subcellular alterations, such as in chromatin and cytoskeletal organization, indicating an interference of toxins with plant cell cycle regulation (Beyer et al., 2012). Furthermore, cyanobacterial crude extracts containing MCs induced changes in mineral assimilation and content in tomato (El Khalloufi et al., 2012) and faba bean (Lahrouni et al., 2013).

Irrigation water from sources containing cyanobacterial blooms and

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cyanotoxins, such as water reservoirs constructed for agricultural purposes, is not subject to any treatment to control MCs. Thus, in addition to the adverse effects on plant growth per se, bioaccumulation of MCs in edible plant tissues may induces a food chain contamination with considerable human health risks (Carmichael and Boyer, 2016). The problem of increased cyanotoxins abundance is expected to further intensify due to mainly two on-going processes, i.e. climate change and anthropogenic eutrophication of aquatic environments (O'Neil et al., 2012). In face of the intensification of this problem of public health, crop and environmental concern, it is important to advance the assessment of MCs effects on plants as an affirmative step to risk management. For this purpose, it is important to know if MCs can be transferred and accumulated in edible tissues of various plant species under realistic experimental conditions. Recent reviews and articles highlight the key-factors ensuring ecologically relevant experimental designs: long time of exposure, realistic MCs concentrations, mature plants - not restricted to early developmental stages and soil as growth medium (Corbel et al., 2014; Freitas et al., 2015a). It is clear that all the above-mentioned parameters mediate plant sensitivity and response to MCs contaminated irrigation water.

There are several studies demonstrating the adverse effects of MCs on seedlings and early developmental stages of crop plants (Peuthert et al., 2007; Pichardo and Pflugmacher, 2011; Saqrane et al., 2008) or mature plants (Cordeiro-Araújo et al., 2015; Gutiérrez-Praena et al., 2014). Since, to the best of our knowledge, there are no studies on the long-term effects of MCs when supplied in different developmental stages of the crop plant, the purpose of this work was to fill this gap conducting an ecologically relevant experiment with realistic conditions in terms of time, concentrations and substrate. Therefore, we tested the hypothesis of whether the supply of MCs-containing irrigation water at different plant developmental stages results in differential effect on plant growth and MCs accumulation. To achieve this, we experimentally irrigated lettuce with MCs-containing water from an artificial reservoir at four different developmental stages. The impact of MCs was assessed by following growth, physiological responses and tissue contained MCs concentrations for the whole growth period until the plants reached the mature marketable size.

2. Materials and methods

2.1. Karla reservoir

The reconstruction of Lake Karla (Greece) is referred to as the most significant European environmental project in terms of environmental benefits and also financial cost. Lake Karla was an important Greek lake that was dried in 1960s and re-constructed in 2010. The re-constructed reservoir is located in the region of Thessaly, Central Greece, which is one of the most productive agricultural regions of Greece. According to the water allocation and management plan, an irrigation network that will supply farmers with water from the Karla reservoir is in the immediate plans. However, Karla Reservoir has already been adversely affected by both agricultural and industrial pollution from the surrounding area. Given the poor quality and ecological status of Penios river water that flows into Karla, worrisome harmful algal blooms have already been documented (Oikonomou et al., 2012). Also, MCs have already detected in Karla, ranging from 1.5 $\mu g\, l^{-1}$ to 33 $\mu g\, l^{-1}$ (Papadimitriou et al., 2013). HPLC analyses confirmed the presence of MC-LR and MC-RR in water of Karla Reservoir, as well as the presence of many other organic compounds. Among them, the co-existence of different MCs variants is very possible (unpublished data).

In September 2014, a total volume of 150 l was collected from the Karla Reservoir in order to be used for experimental plants irrigation. Since this study is focused on the effect of dissolved (extracellular) MCs, we removed all living organisms (larger than bacteria) and non-living (detritus) particulate matter from the water prior to irrigation by sequential filtering through 180 μm and 20 μm nylon filters and eventually from 0.2 μm polycarbonate filters. After this procedure water was stored at 4 °C in the dark. MCs concentration in the water at the beginning of the experiment was 1.81 $\mu g\, l^{-1}$ of dissolved MCs. In order to check the stability of MCs in the water, during the experimental period, MCs were measured not only at the beginning of the experiment, but also in the middle (4th week) and in the end of the experiment (8th week). According to these measurements, there were no significant differences between measurement times, concerning the concentration of MCs in water ($p > 0.05$, $F = 13.5$).

2.2. Growth conditions

Lettuce (*Lactuca sativa* L., var. Parris Island Cos) seeds were put in seed trays containing peat and then transplanted at the stage of cotyledons in 2 L pots containing peat (Klassman-Deilmann KTS2) and perlite in a ratio of 2:1 (v/v). Plants were grown in a greenhouse for two months, from October to December.

The experimental design included 5 treatments:

a. *Control*, i.e. plants receiving tap water from seed to final harvest.
b. *Seed*, i.e. plants receiving dissolved MCs-containing water (MC-w) from Karla reservoir from seed to final harvest.
c. *Cotyledon*, i.e. plants receiving tap water from seed to cotyledons appearance and then receiving MC-w until final harvest.
d. *2 Leaves*, i.e. plants receiving tap water from seed to the first two true leaves appearance and then receiving MC-w until final harvest.
e. *4 Leaves*, i.e. plants receiving tap water from seed to the four true leaves stage and then receiving MC-w until final harvest.

The plants were watered three times a week (a weekly total of 300 ml/plant). The experiment was laid out in a completely randomized design with each pot (which contained one plant) comprising one replicate, i.e. a total of 15 replicates per treatment.

2.3. Measurements during growth period

During the growth period various plant physiological and morphological characteristics were measured. Gas-exchange measurements were performed with a portable photosynthesis system (LCpro+, ADC BioScientific Ltd, Hoddesdon, UK) in natural sunlight and in ambient concentrations of CO_2 and H_2O. Photosynthetic light-response curves, i.e. net photosynthetic rate vs. photosynthetically active radiation (An/PAR) were determined on mature leaves at the same time for all treatments (12 to 8 days before final harvest). This measurement requires > 25 min per leaf, so with the adequate replications lasted 5 consecutive days. From these curves the photosynthetic efficiency, i.e. quantum yield of photosynthesis was assessed. This refers to the maximum quantum yield which is measured when photosynthesis is light-limited, a situation diagnosed by the initial, linear relationship between photosynthesis and PAR.

Chlorophyll content was measured with a portable chlorophyll meter (Minolta, SPAD-502). The chlorophyll meter values were transformed into actual chlorophyll concentrations by using a reference curve. For this purpose, leaves from plants not used in the experiment and having various values according to the chlorophyll meter, were extracted with 80% acetone and chlorophylls were estimated using the equations of Lichtenthaler and Wellburn (1983).

Concerning morphological parameters, total leaf area and leaf number at plant level were recorded throughout the experimental period. Due to the specific plant architecture with many erect leaves, leaf area of attached leaves was measured as length*max width and then transformed into actual area by using a reference curve. The latter was created by measuring detached leaves as length*max width and their subsequent measurement with LI-3000C Portable Area Meter (LI-COR Inc. Lincoln, Nebraska, USA). During the same date range leaf thickness measurements were performed using a leaf micrometer