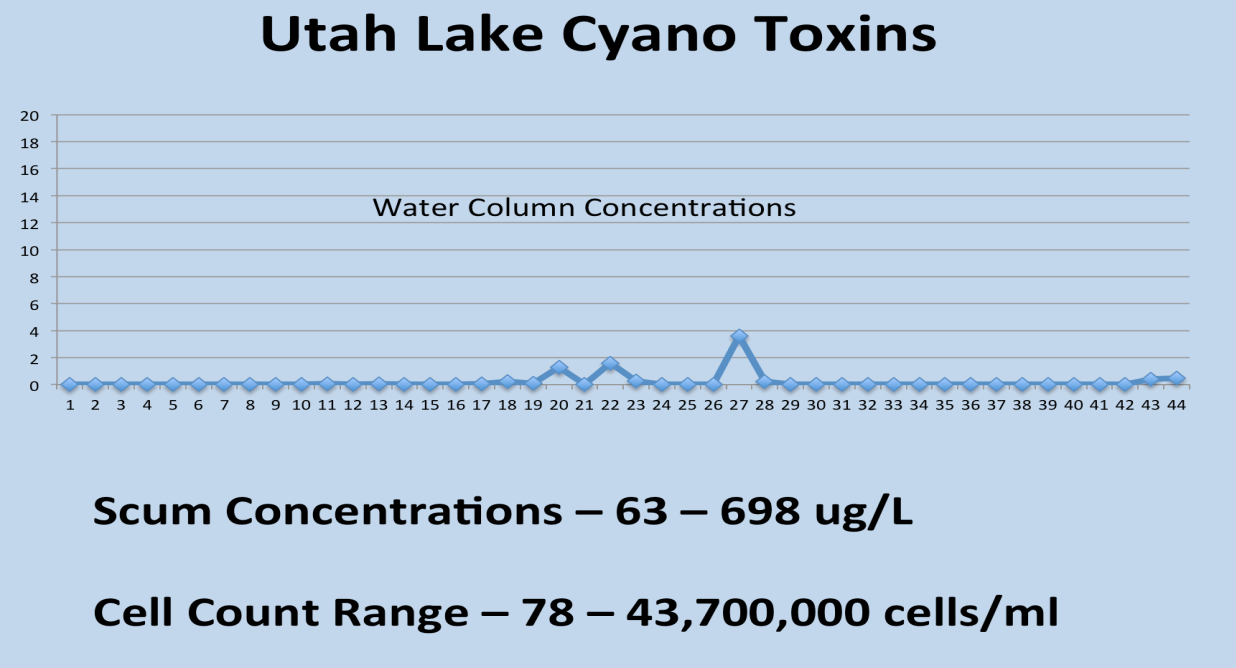
# Agriculture and Cyanotoxins

(Summary by Leland Myers)

Attached are two review articles on cyanobacteria and their toxins and their impacts on agriculture. Both reviews were very cautious to insure that readers assume that cyanotoxins may impact plants. Both reviews also stressed that there is not a lot of research to draw certain conclusions. From many of the sources cited in the reviews, uptake from water containing cyanotoxins was identified when toxin concentrations were high, generally above 25-100 µg/L. As a reference, Utah Lake whole water samples never exceeded 4 µg/L in 2016. Below is a graph of Utah Lake samples.




In addition, the cited literature generally used hydroponic conditions to grow the plants tested. As such, they did not take into account the attenuation of toxics by the soil. In general, if you are a "sky is falling" type of person, irrigation with any cyanotoxin containing water is a risk. However, if you were more pragmatic worry would only begin after reaching 50-100 µg/L.

## Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: A review of their relevance for agricultural plant quality and public health

J. Machado, et.al.

Below are relevant quotes from this review:

> 1. Interestingly, it has been suggested that when a more realistic experimental design is established (i.e., environmentally relevant concentrations, longer exposure period and comparable soil growth

conditions), the effects on plant growth are less pronounced. Corbel et al. (2015a) studied the effects of MC-LR in tomatoes following irrigation with water containing 5–100 $\mu$ g/L for 90 days and demonstrated that the toxin did not disturb the global growth of the tomatoes.

2. Freitas et al. (2015a) also suggested that lettuce plants are able to cope with low concentrations (1 and 10 $\mu$ g/L) of MC-LR, CYN and an MCLR/CYN mixture by ensuring the maintenance of and even increasing their fresh weight.

3. Azevedo et al. (2014) in rice plants exposed to 13 μg MC-LR/L. In this case, however, the authors did not find significant differences in photosynthetic efficiency.

4. Finally, it is important to notice that most of these studies have been performed in hydroponic conditions, which to an extent maximizes the availability of the toxin to the root system. Therefore, the accumulation rate can be overestimated because the soil and other plant substrates can retain the toxins, reducing their bioavailability for the plants' uptake. In the only published study where a more realistic exposure scenario was created, i.e., the plants were grown in soil/vermiculite conditions, MC accumulation did not occur (Järvenpää et al., 2007).

5. More recently, a study performed by Kanzo et al. (2013) demonstrated that in hydroponic conditions, MCs were able to accumulate in the roots, stems and leaves of Brassica rapa after exposure to 100 and 1000 μg MC-LR/L. Interestingly, in soil cultivated B. rapa, no accumulation was detected after exposure to the same MCLR concentrations.

6. The current literature shows that both cyanobacterial toxins, MCLR and CYN, can cause toxic effects in agricultural plants, especially at the biochemical level. Furthermore, MC-LR can accumulate in a wide range of agricultural plants, and the predicted human exposure would be higher than the TDI proposed by WHO. However, because most of these studies have been performed using cyanotoxin concentrations that are higher than those usually found in the environment and in hydroponic conditions, these effects can be overestimated in some studies because the accumulation of MC-LR and CYN seems to be dependent on the exposure concentration, and their uptake by plants can be reduced due to the adsorptive effects of soil particles and the potential biodegradation, photolysis and hydrolysis.

7. Irrigation water monitoring programs should be initiated, and when the concentrations of MC-LR and CYN are higher than 100 μg/L, dilutions should be made to avoid major risks.

## Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops

Sylvain Corbel. et.al.

Below are relevant quotes from this review:

1. Humans are exposed to cyanobacteria toxins through many routes, including drinking water, recreational contact, and health food products made from cyanobacteria, and food chain. In recent years, several cyanobacterial toxins were investigated in regard to their ability to enter the food chain via freshwater seafood (Ibelings and Chorus, 2007; Ettoumi et al., 2011), however, their ability to enter the food chain via agricultural crops has not been thoroughly investigated to date. Although no case of poisoning by these products has been reported in the literature, this eventuality must not be ignored.

2. Once they enter in aquatic and soil ecosystems, cyanotoxins can be removed according to various processes such as photochemical degradation by UV, adsorption in particles in suspension or onto sediments, and biodegradation.

3. Therefore, biodegradation would appear to be the main fate for most cyanotoxins in aquatic systems and the relative performance of this process would be very site specific and dependent upon local sediment characteristics and microbial activity.

4. It was recently reported that the data generated in laboratory and field studies strongly indicate that, in shallow lakes, low persistence and natural eliminations of MCs are due to biodegradation; suggesting that sediments play a crucial role in biodegradation by continuously supplying toxin-degrading bacteria to the water column (Chen et al., 2008, 2010; Mazur-Marzec et al., 2009).

5. They found that the soils with the high clay and/or organic carbon contents had the higher toxins adsorption coefficients. In similar experiments, Miller and Fallowfield (2001) found that the soils with the highest organic carbon content (2.9%) and the highest clay content (16.1%) were the most effective at removing these toxins in batch experiments. However, the sandy soil (98.5% sand) was incapable of the removal of cyanotoxins.

6. However, slow sand filters can be expected to remove 99% of dissolved cyanotoxins (Keijola et al., 1988; Grützmacher et al., 2002). This can be explained by the formation of a biofilm on top of the filter that it allows for some biodegradation of cyanotoxins in slow sand filtration.

7. However, Saqrane et al. (2007) reported that L. gibba could take up and biotransform microcystins. The chronic exposure of plant led to dose-dependent MCs accumulation which reached 2.24 μgm/g dry weight after being exposed to 0.3 μg/mL[300 μg/L] of MCs (Saqrane et al., 2007).

8. Several studies have been shown that cyanotoxins could be absorbed by roots, transported to shoots, and then be translocated to grains and/or fruits. Nevertheless, the concentration of MC-LR detected, for example, in rice grains was very low and thus may not pose a threat to human health currently.

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Review article

# Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: A review of their relevance for agricultural plant quality and public health

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ABSTRACT

Toxic cyanobacterial blooms are recognized as an emerging environmental threat worldwide. Although microcystin-LR is the most frequently documented cyanotoxin, studies on cylindrospermopsin have been increasing due to the invasive nature of cylindrospermopsin-producing cyanobacteria. The number of studies regarding the effects of cyanotoxins on agricultural plants has increased in recent years, and it has been suggested that the presence of microcystin-LR and cylindrospermopsin in irrigation water may cause toxic effects in edible plants. The uptake of these cyanotoxins by agricultural plants has been shown to induce morphological and physiological changes that lead to a potential loss of productivity. There is also evidence that edible terrestrial plants can bioaccumulate cyanotoxins in their tissues in a concentration dependent-manner. Moreover, the number of consecutive cycles of watering and planting in addition to the potential persistence of microcystin-LR and cylindrospermopsin in the environment are likely to result in groundwater contamination. The use of cyanotoxin-contaminated water for agricultural purposes may therefore represent a threat to both food security and food safety. However, the deleterious effects of cyanotoxins on agricultural plants and public health seem to be dependent on the concentrations studied, which in most cases are non-environmentally relevant. Interestingly, at ecologically relevant concentrations, the productivity and nutritional quality of some agricultural plants seem not to be impaired and may even be enhanced. However, studies assessing if the potential tolerance of agricultural plants to these concentrations can result in cyanotoxin and allergen accumulation in the edible tissues are lacking. This review combines the most current information available regarding this topic with a realistic assessment of the impact of cyanobacterial toxins on agricultural plants, groundwater quality and public health.

## 1. Introduction

The eutrophication is recognized as an important problem worldwide, being an unequivocal consequence of the intensification of agricultural and industrial activities. In the last decades, its environmental significance has also been enhanced by the global climate change (O'Neil et al., 2012). In eutrophic systems, this process promotes a rapid proliferation of phytoplankton, resulting in the phenomena acknowledged as blooms (Smith et al., 1999; Codd, 2000; Vasconcelos, 2006).

Cyanobacteria, commonly designated as "blue-green algae", are a group of unicellular and multicellular photosynthetic prokaryotes with ubiquitous distribution (Sivonen and Jones, 1999). Currently, there are about 150 cyanobacterial genera identified, which comprise nearly 2000 species (Mur et al., 1999; Hitzfeld et al., 2000). Cyanobacterial blooms can be potentiated by a combination of several environmental factors besides nutrient availability, such as water temperature, light intensity, salinity and water stagnation (Vasconcelos, 2006; Merel

*Abbreviations:* Adda, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; APX, ascorbate peroxidase; CAT, catalase; CYN, cylindrospermopsin; Fv/Fm, chlorophyll fluorescence; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-S-transferase; IARC, International Agency for Research on Cancer; MC(s), Microcystin(s); MC-LR, Microcystin-LR; Mdha, N-methyldehydroalanine; OATP, organic-anion transporting polypeptides; OECD, Organization for Economic Co-operation and Development; POD, peroxidase; PP, protein phosphatases; PP1, protein phosphatases 1; PP2A, protein phosphatases 2A; ROS, reactive oxygen species; SOD, superoxide dismutase; TDI, tolerable daily intake; TNFα, tumor necrosis factor α; WHO, World Health Organization

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et al., 2013). These blooms are documented as a threat to human and environmental health because some species produce secondary metabolites (cyanotoxins) with a demonstrated toxic activity to humans and other mammals, birds, fish, crustaceans, mollusks, and zooplankton (Sivonen and Jones, 1999). Due to the production of these metabolites, the Organization for Economic Co-operation and Development (OECD) classified the cyanobacteria as emerging pathogens, even though they do not have the ability to colonize or invade hosts (OECD, 2005). Among the cyanobacterial toxins, microcystins (MCs) are the most widespread group, being microcystin-LR (MC-LR) the main variant in eutrophic freshwaters (WHO, 2011). Nevertheless, concerns are also focused in the increasing occurrence of cylindrospermopsin-producing cyanobacteria, including temperate areas (Kinnear, 2010; Poniedziałek et al., 2012).

Recent studies have suggested that MC-LR and cylindrospermopsin (CYN) cause toxic effects on terrestrial plants (Corbel et al., 2014a). Indeed, the significance of the use of surface water contaminated with cyanotoxins for agricultural purposes is a field of increasing interest. In addition to the potential effects on plant growth and development, this issue may pose concerns for food safety if the possible absorption of toxins by plants can lead to its bioaccumulation in edible tissues. Furthermore, the impact of cyanotoxins on agricultural plants and the ability of cyanotoxins to enter the food chain by this pathway is not fully understood, especially at ecologically relevant concentrations. The concentration of MCs in surface waters used as irrigation source range from 4 to 50 μg/L up to 6500 μg/L, however, the higher concentrations would be found in blooms and scums and comprise intracellular and dissolved MCs (Corbel et al., 2014a). Although the studies reporting the concentrations of CYN in the environment are scarce, the concentration of total extracellular CYN in water seem to vary from undetectable values up to 126 μg/L (Corbel et al., 2014a) (see Table 1 of Corbel et al., 2014a). In addition, due to the chemical stability of MC-LR and CYN in irrigation water, these cyanotoxins may leach into the soil, which can compromise groundwater quality and lead to negative public health consequences (Corbel et al., 2014a; Eynard et al., 2000). The aim of this review was to provide the most current information regarding the effects of MC-LR and CYN on plant-soil systems due to the use of contaminated water for irrigation, to better understand the true impact of ecologically relevant concentrations of these cyanotoxins in agricultural plants and the potential implications for groundwater quality and public health.

### 1.1. Microcystin-LR

The most widespread and studied cyanotoxins are the cyclic heptapeptide hepatotoxins, MCs (MW 900–1200) (Dawson, 1998; Sivonen and Jones, 1999). The *Microcystis* genus is recognized as the most common bloom forming and the main producer of MCs (Sivonen and Jones, 1999). However, other genera such as *Anabaena, Oscillatoria, Planktothrix, Nostoc* and *Anabaenopsis* can also produce MCs (Sivonen and Jones, 1999; Hitzfeld et al., 2000; Apeldoorn et al., 2007). The general structure of MCs is: cyclo-(D-alanine-*X*-DMeAsp-*Z*-Adda-D-glutamate-Mdha), in which *X* and *Z* are variable L-amino acids, D-MeAsp is D-erythro-*b*-methylaspartic acid, Mdha is N-methyldehydroalanine and Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Dawson, 1998). Although more than 100 structural variants have already been reported (Puddick et al., 2014; Qi et al., 2015), MC-LR, mainly produced by *Microcystis aeruginosa*, is the most studied due to its toxicity and dominance in cyanobacterial blooms (Kuiper-Goodman et al., 1999).

The primary mechanism of toxicity of MC-LR in both animals and higher plants is well recognized and consists in the irreversible inhibition of serine/threonine protein phosphatases 1 and 2A (PP; PP1 and PP2A) by covalent binding (MacKintosh et al., 1990; Dawson, 1998). The induction of oxidative stress by the production of reactive oxygen species (ROS) seems also to be an important biochemical mechanism of MC-LR toxicity in both mammal and plant cells (Pflugmacher, 2004; Pflugmacher et al., 2006, 2007a, b; Pichardo and Pflugmacher, 2011; Zegura et al., 2011; Zhou et al., 2015). Although the target molecules appear to be the same in both animals and higher plants, one relatively unexplored question regarding MCs concerns the mechanism of uptake by plants. In fact, specific transporters of these toxins have not been yet described for vegetable organisms. Nevertheless, several types of membrane transporters with affinity to different peptides and amino acids have been identified (Tegeder and Rentsch, 2010). Since MC-LR is a peptide, it is plausible to put forth the hypothesis that peptide transporters might potentially be involved in the transport of MC-LR in plants. In mammals, once MC-LR has been ingested it concentrates mainly in liver but cannot move across cell membranes easily. It becomes able to enter in hepatocyte cell membranes through active uptake by nonspecific organic-anion transporting polypeptides (OATP) for bile salts (Fischer et al., 2005). Inside the hepatocytes, the inhibition of PP1 and PP2A occurs according to the following two-step mechanism: (1) a non-covalent binding between the ADDA residue of the toxin and the active center of PP1 and PP2A, which seems to be the responsible for the main inhibitory effects of the toxin; (2) a covalent binding between the Mdha residue of the toxin and the cysteine-273 of the catalytic subunit of PP1 or the cysteine-266 of the catalytic subunit of PP2A (Craig et al., 1996; MacKintosh et al., 1990, 1995). Moreover, although the liver is the primary target organ of MC-LR, due to OATP membrane transport system, MC-LR can also affect other organs, such as brain (Kist et al., 2012), heart (Milutinovic et al., 2006), intestine (Zegura et al., 2008), kidney (Qin et al., 2010) and reproductive organs (Wu et al., 2014). MC is able to modulate the expression of oncogenes, proto-oncogenes, cytokines and tumor necrosis factor α (TNFα), affecting cell division, cell survival and apoptosis (IARC, 2010). Epidemiological studies have associated chronic oral exposure to MC-LR with the increasing incidence of liver (Yu, 1995; Ueno et al., 1996; Hitzfeld et al., 2000) and colorectal cancer (Zhou et al., 2002). Moreover, the *International Agency for Research on Cancer* (IARC) classified MC-LR as a possible carcinogenic to humans (IARC, 2010).

### 1.2. Cylindrospermopsin

CYN is a low molecular weight (MW 415) tricyclic alkaloid known for its cytotoxicity in both animal and plant cells. It is considered an emerging threat worldwide due to the progressive distribution of its main producer, *Cylindrospermopsis raciborskii* (Kinnear, 2010; Poniedzialek et al., 2012). Nevertheless, other cyanobacterial species such as *Anabaena bergeii, Aphanizomenon ovalisporum, Raphiodiopsis curvata, Umezakia natans, Aphanizomenon flos-aquae, Anabaena lapponica* and *Lygnbya wollei* have been described as CYN producers (Ohtani et al., 1992; Harada et al., 1994; Banker et al., 1997; Li et al., 2001; Schembri et al., 2001; Preussel et al., 2006; Spoof et al., 2006; Seifert et al., 2007). The mechanism of CYN toxicity is still under investigation. So far, it is known that the uptake of this toxin is relatively fast and the complete and irreversible block of protein synthesis occurs after 1 h of exposure in *in vitro* assays (Froscio et al., 2003). It is also recognized that the inhibition of protein synthesis occurs at the ribosome during the peptide chain elongation step, with the uracil moiety and the hydroxyl at the C7 position, being crucial for toxicity (Banker et al., 2001; Froscio et al., 2003). In mammals, CYN can cause liver, kidney, thymus and heart damage and it is considered hepatotoxic, cytotoxic, and neurotoxic; and also a potential carcinogen (Falconer and Humpage, 2006). Additionally, CYN appears to be capable to inhibit glutathione synthesis (Runnegar et al., 1995) and the activation of CYN by cytochrome P450 seems to enhance even more the toxicity (Norris et al., 2002; Humpage et al., 2005). CYN has also two structural variants, 7-epi-CYN and deoxy-CYN (Li et al., 2001; Norris et al., 1999) whose origin is still unclear, although it has been hypothesized that they could be

precursors, variants or degradation products (Banker et al., 2000; Seifert et al., 2007). Nevertheless, both have been proven to be less toxic than CYN (Sukenik et al., 2001).

## 2. Effects of MC-LR and CYN in edible plants

Surface water originating from sources that contain toxic cyanobacteria is often used in agriculture for irrigation. MC-LR can be released from toxic cyanobacterial cells into water during the senescence phase (Apeldoorn et al., 2007) or, in the case of CYN, due to their natural metabolism (Chiswell et al., 1999; Rücker et al., 2007). MCs are very stable and may persist in aquatic systems for weeks after being released from cells (Apeldoorn et al., 2007). Additionally, CYN can persist in water for long periods because it has a very low photodegradation rate under natural conditions (Wörmer et al., 2010).

Because PPs regulate important molecular and cellular processes (Sheen, 1993; Takeda et al., 1994) in vascular plants, the exposure to MC-LR can lead to various perturbations in their physiology and growth (Saqrane et al., 2008). It is well known that the inhibition of PPs in plants affects: (1) tissue development; (2) activation of enzymes involved in $CO_2$ fixation; (3) starch storage; (4) gene expression; (5) regulation of ionic channels; (6) carbon and nitrogen metabolism and (7) the photosynthetic process (Siegl et al., 1990; Sheen, 1993; Smith and Walker, 1996; Luan, 1998; Toroser and Huber, 2000). Furthermore, Garbers et al. (1996) demonstrated that PPs play an important role in auxin transport; therefore, the inhibition of these proteins (PPs) by MC-LR may affect hormone transport, as it was observed in rice (Chen et al., 2013), and disrupt plant growth. MCs can also exert dual effects on plant cells by either stimulating or inhibiting mitosis, depending on the exposure dose (Máthé et al., 2013a).

Studies regarding the effects of CYN on plants are relatively scarce, although it is recognized that this toxin inhibits protein synthesis in eukaryotic cells with similar intensity in both plant and mammalian cell extracts (Terao et al., 1994; Runnegar et al., 2002). The few published studies regarding the effects of CYN on plants indicate: (1) the induction of oxidative stress (Prieto et al., 2011); (2) a reduction in germination rate (Metcalf et al., 2004) and (3) the inhibition of growth (Vasas et al., 2002; Beyer et al., 2009).

In this section, the studies that have reported that MC-LR and CYN produce effects on the physiology and metabolism of agricultural plants will be reviewed and critically discussed in light of potential risks.

### 2.1. Effects of MC-LR and CYN on plant growth and development

An effect of MCs that has been investigated in several agricultural plants is the inhibition of seed germination (Table 1). This effect attracted the interest of some plant breeding researchers when the results of most studies suggested that the exposure of plants to MC-LR-contaminated water may represent a threat to the quality and productivity of crops, which can then lead to economic losses. In addition, several other studies have demonstrated that MC-LR may have a negative impact on the growth and development of exposed plants (Table 1). Overall, the inhibition effect seems to be dependent on the: (1) plant species; (2) stage of development (seedlings are generally more susceptible than adult plants); (3) time of exposure (prolonged exposures are associated with increased inhibition); (4) range of toxin concentrations applied (positive relation of toxin concentration and inhibition effects); and (5) the nature of the toxin used (e.g., purified toxin or crude extracts). According to some authors, the exposure of plants to MC-LR, either purified or contained in a crude extract, may induce histological, cytological and morphological modifications (McElhiney et al., 2001; Saqrane et al., 2008; Chen et al., 2013; Máthé et al., 2013b), which seem to be related to the negative impacts on the growth and development of the plants.

The effects of CYN on seed germination are still unknown, and to the best of our knowledge, the only study that intended to investigate them demonstrated that these effects are also dependent on the plant species (Table 1). The authors exposed four plant species (*Lactuca sativa, Phaseolus vulgaris, Pisum sativum* and *Solanum lycopersicum*) to the same concentration range of CYN (0.57–57 μg/L), but the inhibition of germination occurred only in *S. lycopersicum* (Silva and Vasconcelos, 2010).

Interestingly, it has been suggested that when a more realistic experimental design is established (i.e., environmentally relevant concentrations, longer exposure period and comparable soil growth conditions), the effects on plant growth are less pronounced. Corbel et al. (2015a) studied the effects of MC-LR in tomatoes following irrigation with water containing 5–100 μg/L for 90 days and demonstrated that the toxin did not disturb the global growth of the tomatoes. These results are contradictory to those submitted by El Khalloufi et al. (2012), probably because the concentrations used were 20-fold higher. Freitas et al. (2015a) also suggested that lettuce plants are able to cope with low concentrations (1 and 10 μg/L) of MC-LR, CYN and an MC-LR/CYN mixture by ensuring the maintenance of and even increasing their fresh weight. The growth increase promoted by low concentrations of cyanotoxins can be explained by the hormesis concept, which is characterized by an inverted U-shaped dose response (Bibo et al., 2008).

### 2.2. Biochemical effects of MC-LR and CYN on plants

The inhibitory effect of MC-LR on photosynthesis has been described in several plant species (Table 2), although the mechanism behind this process remains unknown. A direct effect on the photosynthetic apparatus is hypothesized, which presupposes that the toxin would be assimilated particularly at the root level and then translocated to the leaves by crossing cell barriers. Nevertheless, although this hypothesis cannot be excluded, it is thought that the inhibition occurs through an indirect action of the toxin by the induction of oxidative stress in plants (Peuthert et al., 2007; El Khalloufi et al., 2011). Along with the specific inhibition of PP1 and PP2A (Dawson, 1998), the increase in antioxidant defenses induced by MC-LR suggests that oxidative stress is a major mechanism contributing to the phytotoxicity of this toxin (Pflugmacher et al., 2006, 2007a, b; Pichardo and Pflugmacher, 2011). However, although the inhibition of photosynthetic processes due to increased concentrations of ROS has been documented (Noctor and Foyer, 1998), recently Garda et al. (2016) have shown that under long-term exposure PP inhibition was the primary cause of MC-LR induced mitotic spindle disorders in *Vicia faba* and not ROS induction. Nevertheless, in a study performed by Gutiérrez-Praena et al. (2014) in which tomato plants were exposed to MC-LR, changes were detected in the function of various proteins related to ATP synthesis, carbon fixation, photosynthesis and carbohydrate metabolism that appear to be linked with the observed decrease in photosynthetic efficiency. A decrease in the expression of some proteins involved in photosynthesis was also observed by Azevedo et al. (2014) in rice plants exposed to 13 μg MC-LR/L. In this case, however, the authors did not find significant differences in photosynthetic efficiency. Recently, a study conducted by Corbel et al. (2015a) also demonstrated that, with regard to the photosynthetic process, low concentrations of MC-LR did not alter the concentrations of chlorophyll *a* and *b* or the chlorophyll fluorescence (Fv/Fm) of *L. esculentum*, emphasizing the possibility that environmentally relevant concentrations might not adversely affect exposed plants. We hypothesize that in the exposure of plants to low concentrations of MC-LR, the effects are manifested primarily or solely at the subcellular level, which highlights the importance of choosing suitable biomarkers for this research. It is important to emphasize that we are not assuming that these effects on plants are deleterious; by ensuring plant tolerance, the potential changes may even be beneficial.

As a result of the photosynthetic process, ROS production is a natural phenomenon in plants. However, the excessive formation of

**Table 1**
Effects of MC-LR and CYN on seed germination, growth and development of several agricultural plant species.

| Plant species | Endpoint | Effect | Range of exposure concentrations (µg/L) | Reference |
|---|---|---|---|---|
| | | **MC - LR** | | |
| *Brassica napus* | Germination Rate | ↓ | 600 – 3000 b | Chen et al., 2004 |
| | Height of seedlings | ↓ | 120 – 3000 b | |
| *Brassica rapa* | Shoot length | ↓ | 400 – 6400 b | Chen et al., 2012b |
| *Lactuca sativa* | Root growth | ↓ | 5.9 – 56.4 b | Pereira et al., 2009 |
| | Root fresh weight | ↑ | 1–100 b | Freitas et al., 2015a |
| | Fresh weight of leaves | ↑ | 1–50 a | |
| | | ↓ | 100 a | |
| *Lens esculenta* | Germination Rate | ↓ | 8700 – 11,600 b | Saqrane et al., 2008 |
| | Epicotyl length; primary root length; lateral root number | ↓ | 11,600 b | |
| | Height (30th day) | ↓ | 1050 – 42,000 b | |
| | Leaf number; (30th day) | ↓ | 4200 b | |
| | Fresh weight | ↓ | 500 – 4200 b | |
| | Dry weight | ↓ | 1050 – 4200 b | |
| *Lepidium sativum* | Fresh weight; (6th day) | ↓ | 10 a and b | Gehringer et al., 2003 |
| | Root and leaf length | ↓ | 1 a and b | |
| *Lycopersicon esculentum* | Germination Rate | ↓ | 16,680 – 22,240 b | El Khalloufi et al., 2012 |
| | Fresh biomass, stem length | ↓ | 2220 – 22,240 b | |
| *Malus pumila* | Growth | ↓ | 300 – 3000 b | Chen et al., 2010 |
| *Medicago sativa* | Germination Rate | ↓ | 5 a and b | Pflugmacher et al., 2006 |
| | Primary root length | ↓ | | |
| | Germination Rate | ↓ | 2220 – 22,240 b | El Khalloufi et al., 2011 |
| | Plants length; nodules number; biomass (30th day) | ↓ | 2222 – 22,240 b | |
| | Root length | ↓ | 11,120 – 22,240 b | |
| | Dry weight | ↓ | 10 – 20 b | El Khalloufi et al., 2013 |
| *Oryza sativa* | Fresh weigh and length of roots | ↓ | 120 – 3000 b | Chen et al., 2004 |
| | Dry weight of roots | ↓ | 24 – 600 b | |
| | Height of seedlings | ↓ | 600 – 3000 b | |
| | Fresh weight of root; length and number of crown root | ↓ | 2000 – 4000 b | Chen et al., 2013 |
| | Number of lateral root on seminal root | ↓ | 1000 – 4000 b | |
| *Pisum sativum* | Germination Rate | ↓ | 1600 – 11,600 b | Saqrane et al., 2008 |
| | Epicotyl length; primary root length; lateral root number | ↓ | 1600 b | |
| | Height (30th day) | ↓ | 500 – 4200 b | Saqrane et al., 2009 |
| | Leaf number (30th day) | ↓ | 1050 – 4200 b | |
| | Fresh and dry weight | ↓ | 500 – 4200 b | |
| *Sinapis alba* | Growth | ↓ | 2000 a | Kurki-Helasmo and Meriluoto, 1998 |
| | Fresh mass, length (total, hypocotyl, cotyledon, root), primary root growth, lateral root number | ↓ | 3500 – 30,000 b | M-Hamvas et al., 2003 |
| | Growth | ↓ | 500 – 5000 b | McElhiney et al., 2001 |
| | Growth | ↓ | 7800 b<br>18,200 a | Vasas et al., 2002 |
| *Solanum tuberosum* | Fresh weight; shoot length | ↓ | 500 – 5000 b | McElhiney et al., 2001 |
| | Number of roots | ↓ | 10 – 500 b | |
| *Spinacia oleracea* (var. Balta. Saran, Gamma, Merlin) | Growth | ↓ | 0.5 b | Pflugmacher et al., 2007a |
| *Spinacia oleracea* (var. Parys, Matador) | Number of leaves; leaf size | ↓ | | |
| *Triticum aestivum* | Germination Rate | ↓ | 0.5 a and b | Pflugmacher et al., 2007b |
| | Shoot and root length | ↓ | | |
| *Triticum durum* | Germination Rate | ↓ | 2900 – 11,600 b | Saqrane et al., 2008 |
| | Epicotyl length; primary root length; lateral root number | ↓ | 1600 b | |
| | Height (30th day), fresh and dry weight | ↓ | 500 – 4200 b | Saqrane et al., 2009 |
| | Leaf number (30th day) | ↓ | 4200 b | |
| *Vicia faba* | Germination Rate | ↓ | 50 – 100 b | Lahrouni et al., 2012 |
| | Shoot dry weight, root length, root and nodule dry weight, total number of nodules | ↓ | | |
| *Zea mays* | Germination Rate | ↓ | 5 a and b | Pflugmacher, 2007 |
| | Shoot and root length | ↓ | | |
| | Germination Rate | ↓ | 2900 – 11,600 b | Saqrane et al., 2008 |
| | Epicotyl length; primary root length; lateral root number | ↓ | 11,600 b | |
| | Height (30th day); fresh weight | ↓ | 500 – 4200 b | Saqrane et al., 2009 |
| | Leaf number (30th day) | ↓ | 1050 – 4200 | |

*(continued on next page)*

**Table 1** *(continued)*

| Plant species | Endpoint | Effect | Range of exposure concentrations (µg/L) | Reference |
|---|---|---|---|---|
| | | **CYN** | | |
| *Lactuca sativa* | Root length | ↑ | 0.57 – 5.7 b | Silva and Vasconcelos, 2010 |
| | | ↓ | 57 b | |
| | Stem length | ↑ | 0.57 – 57 b | |
| | Fresh weigh of roots | ↑ | 1-100 a | Freitas et al., 2015a |
| | Fresh weigh of leaves | ↓ | 100 a | |
| *Nicotiana tabacum* | Germination Rate | ↓ | 5000 – 10,000,000 a | Metcalf et al., 2004 |
| *Phaseolus vulgaris* | Root length | ↑ | 0.57 – 57 b | Silva and Vasconcelos, 2010 |
| *Pisum sativum* | Root length | ↓ | 0.57 – 57 b | Silva and Vasconcelos, 2010 |
| | Stem length | ↑ | | |
| *Oryza sativa* | Root fresh weight | ↑ | 2.5 b | Prieto et al., 2011 |
| *Sinapis alba* | Lateral root emergence | ↑ | 10 | Máthé et al., 2013b |
| | | ↓ | 5000 – 20,000 a | |
| *Lycopersicon esculentum* | Germination rate | ↓ | 0.57 – 57 b | Silva and Vasconcelos, 2010 |
| | Root length | ↓ | | |
| | Stem length | ↓ | | |
| *Vicia faba* | Epicotyl and main root elongation | ↑ | 100 a | Garda et al., 2015 |
| | | ↓ | 5000 – 20,000 a | |
| | Number of lateral root | ↑ | 2500 a | |
| | | ↓ | 10,000 – 20,000 a | |

↑ Increased in comparison to control group; ↓ Decreased in comparison to control group; a, pure toxin; b, crude extract.

these molecules is often triggered by external factors, such as various xenobiotics and their biotransformation, which may lead to damage of the DNA, proteins, carbohydrates and lipids. Plants have a well-developed antioxidant defense system that works to relieve the negative effects caused by ROS. It consists of a network of enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione-S-transferase (GST), glutathione reductase (GR) and also a non-enzymatic complex comprised of reduced glutathione (GSH) and vitamins (e.g., ascorbic acid as well as phenolic, tocopherol and carotenoid compounds) (Cadenas, 1995; Apel and Hirt, 2004). The evaluations of changes in the enzymatic and non-enzymatic components of plants have also demonstrated the promotion of oxidative stress by MC-LR (Table 2). For instance, Peuthert et al. (2007) detected cellular damage (lipid peroxidation) in both the roots and shoots of several agricultural plants (*Pisum sativum*, *Cicer arietinum*, *Vigna radiata*, *Phaseolus vulgaris*, *Glycine max*, *Medicago sativa*, *Lens culinaris*, *Triticum aestivum* and *Zea mays)* that were exposed to MC-LR, either purified or in crude extract. These observations highlighted the impact of cyanotoxins on the nutritional value of plants because the content of antioxidants can be changed as a physiological response mechanism. Furthermore, some studies have demonstrated that MC-LR can be responsible for changes in the mineral content of plants; however, the existing information on this topic is still scarce. In general, the macro mineral content of the roots is increased after exposing the plants to MC-LR (Saqrane et al., 2009; El Khalloufi et al., 2012; Lahrouni et al., 2013) in a concentration-dependent manner (Saqrane et al., 2009; El Khalloufi et al., 2012). Lahrouni et al. (2013) suggest that these changes may be derived from a disruption of the membrane permeability caused by MC-LR. However, it is important to note that almost all of the studies that have been performed used crude extract containing MC-LR (Saqrane et al., 2009; El Khalloufi et al., 2012; Lahrouni et al., 2013) instead of pure toxin, which can be relevant from an ecological point of view. Nevertheless, doubt remains regarding whether the effect is the outcome of the negative impact of the toxin in plants or a consequence of the increase in minerals provided by the extract because it is known that these extracts are rich in minerals (Rajeshwari and Rajashekhar, 2011). The exposure of *Lactuca sativa* (Freitas et al., 2015a) and *Vicia faba* (Lahrouni et al., 2013) to purified MC-LR and MC-LR contained in crude extract, respectively, generally produced a decrease in the mineral content of the leaves. These contradictory results may be associated with differences in the concentrations of the exposures, which were much lower in the latter experiments. After being assimilated by the roots, minerals are translocated to various parts of the plant where they are used in numerous biological functions (Taiz and Zeiger, 2002), such as ensuring the growth and development of the plant (Grusak, 2001). The oxidative stress and cellular damage potentiated in the roots by MC-LR may considerably affect the translocation of nutrients and water to the aerial tissues of the plants, and may therefore explain the decrease in mineral content and even the impairment in the growth and development of leaves (Tables 1, 2).

As for the effects on plant growth, the induction of biochemical effects due to CYN exposure is poorly documented. This cyanotoxin seems to generate stress/defense responses such as the lignification of cell walls and the formation of a callus-like tissue in *in vitro* cultures, and this may play a role in the inhibition of toxin uptake (Beyer et al., 2009; Máthé et al., 2013a). Moreover, a study conducted by Prieto et al. (2011) demonstrated that CYN is able to induce oxidative stress after 48 h of exposure to 2.5 µg CYN/L. This concentration of CYN appears to be sufficient to cause an increase in GST and GPx activities in the roots, contrary to what occurred in the leaves, where a non-significant effect was observed. A study developed by Freitas et al. (2015a) also showed an increase in the GST activity in the roots of lettuce plants exposed to CYN, which seemed to be time- and concentration-dependent. However, the GPx activity was significantly decreased in both the roots and the leaves of lettuce plants exposed to 100 µg CYN/L for 5 days.

Although Freitas et al. (2015b) reported a significantly increased abundance of proteins involved in photosynthesis in lettuce plants exposed to CYN, the effects of CYN in this process have not yet been investigated; the effects on the mineral content, however, have already been reported. Freitas et al. (2015a) found that the exposure of lettuce to purified CYN, in contrast to MC-LR, produced an enhancement in leaf micro (Fe, Mn, Cu, Zn, Mo) and macro (Ca, Mg, P, K, Na) mineral content. Because crop plants generally only need small amounts of micronutrients, the excessive increase in concentrations of these elements in the leaves of the lettuce plants exposed to CYN could provoke impairment in their metabolism and physiology.

**Table 2**
Effects of MC-LR and CYN on different biochemical processes (photosynthesis, enzymatic and non-enzymatic components and induction of cellular damage) of several agricultural plant species.

| Plant species | Endpoint | Effect | Range of exposure concentrations (µg/L) | Reference |
|---|---|---|---|---|
| | **MC – LR** | | | |
| *Brassica napus* | SOD | ↓ | 24 – 3000 b | Chen et al., 2004 |
| | POD | ↑ | 120 – 3000 b | |
| *Brassica rapa* | Cu/Zn-SOD; APX and CAT | ↑ | 4230 b | Chen et al., 2012b |
| *Lactuca sativa* | Shoot Mineral Content (Ca, Mg, K, P, Mn, Fe, Zn, Cu, Mo) | ↓ | 10 – 100 a | Freitas et al., 2015a |
| | GST (Roots) | ↑ | | |
| | GPx (Roots) | ↓ | 100 a | |
| | Net photosynthetic rate | ↑ | 0.65 – 2.5 b | Bittencourt-Oliveira et al., 2016 |
| | Transpiration | ↑ | | |
| | Intercellular $CO_2$ concentration | ↑ | | |
| | Stomatal conductance | ↑ | | |
| | GST | ↓ | 0.65 – 13 b | |
| | SOD | ↑ | 2.5 – 13 b | |
| | CAT | ↑ | 13 b | |
| *Lens esculenta* | Total chlorophyll content (a+b) | ↓ | 2100 – 4200 b | Saqrane et al., 2009 |
| | Root Mineral Content (Na; N, K, P and Ca) | ↑ | 500 – 4200 b | |
| *Lepidium sativum* | GST; GPx | ↑ | 1 – 10 a and b | Gehringer et al., 2003 |
| | Lipid peroxidation | ↑ | 0.5 a and b | Stüven and Pflugmacher, 2007 |
| | α- and β-tocopherol, GST; GPx; GR | ↑ | | |
| | ϒ- and δ-tocopherol | ↓ | | |
| *Lycopersicon esculentum/ Solanum tuberosum* | Fv/Fm fluorescence | ↓ | 2220 – 22,240 b | El Khalloufi et al., 2012 |
| | POD, Phenols content, Protein Content | ↑ | | |
| | Root Mineral Content (Na; K and Ca) | ↑ | | |
| | Fv/Fm fluorescence | ↓ | 100 b | Gutiérrez-Praena et al., 2014 |
| | Total chlorophyll content (*a+b*) | ↓ | 50 – 5000 a | McElhiney, et al., 2001 |
| *Malus pumila* | POD, SOD | ↑ | 300 – 3000 b | Chen et al., 2010 |
| *Medicago sativa* | SOD, CAT, POD, GST, GR, Lipid peroxidation, Protein content | ↑ | 5 a and b | Pflugmacher et al., 2006 |
| | Fv/Fm fluorescence | ↓ | 11,120 – 22,240 b | El Khalloufi et al., 2011 |
| | POD, Phenols content | ↑ | | |
| | Protein Content | ↑ | 2220 – 22,240 b | |
| | α- and β-tocopherol | ↑ | 0.5 – 5 a and b | Peuthert and Pflugmacher, 2010 |
| | Total chlorophyll content (*a+b*) | ↓ | 5 – 20 b | El Khalloufi et al., 2013 |
| | POD, CAT, PPO | ↑ | 10 – 20 b | |
| *Oryza sativa* | Phenols content | ↑ | 24 – 120 b | Chen et al., 2004 |
| | GST | ↑ | 50 b | Prieto et al., 2011 |
| *Pisum sativum* | Fv/Fm fluorescence | ↑ | 500 – 4200 b | Saqrane et al., 2009 |
| | Root Mineral Content (Na; N, K, P and Ca) | ↑ | 500 – 4200 b | |
| *Sinapis alba* | Anthocyanin content | ↓ | 3500 – 30,000 b | M-Hamvas et al., 2003 |
| | PP1 and 2A activity | ↓ | 10 – 10,000 a | Máthé et al., 2013b |
| *Solanum tuberosum* | Total chlorophyll content (*a+b*) | ↓ | 50 – 5000 a | McElhiney, et al., 2001 |
| *Spinacea oleracea* | Photosynthetic oxygen production | ↓ | 0.5 b | Pflugmacher et al., 2007a |
| | CAT; SOD; POD; GST (microsomal and cytosolic); GR, Ascorbate; dehydroascorbate; α- and – ϒ-tocopherol | ↑ | | |
| *Triticum aestivum* | Total chlorophyll content (*a+b*); photosynthetic oxygen production | ↓ | 0.5 a and b | Pflugmacher et al., 2007b |
| | GST; GPx; GR | ↑ | | |
| *Triticum durum* | Fv/Fm fluorescence | ↓ | 500 – 4200 b | Saqrane et al., 2009 |
| | Root Mineral Content (Na; N, K, P and Ca) | ↑ | | |
| *Vicia faba* | Total chlorophyll content (*a+b*) | ↓ | 100 b | Lahrouni et al., 2013 |
| | Fv/Fm fluorescence | ↓ | 50 – 100 b | |
| | POD; CAT, PAL; PPO; phenolic compounds | ↑ | | |
| | Shoot Mineral Content (Ca, N and K) | ↓ | | |
| | Shoot Mineral Content (Na) | ↑ | | |
| | Root Mineral Content (N and P) | ↓ | | |
| | Root Mineral Content (Na) | ↑ | | |
| | | ↓ | 1000 – 20,000 a | Garda et al., 2016 |
| *Zea mays* | POD | ↑ | 5 a and b | Pflugmacher, 2007 |
| | Total chlorophyll content (*a+b*) | ↓ | 4200 b | Saqrane et al., 2009 |
| | Fv/Fm fluorescence | ↓ | 500 – 4200 b | |
| | Root Mineral Content (Na; N, K, P and Ca) | ↑ | | |
| | **CYN** | | | |
| *Lactuca sativa* | Shoot Mineral Content – (Na, P, Mn, Fe, Zn, Cu, Mo) | ↑ | 1 – 100 a | Freitas et al., 2015a |
| | GST (Roots) | ↑ | | |
| *Nicotiana tabacum* | Protein synthesis | ↓ | 138,000 a | Metcalf et al., 2004 |
| *Oryza sativa* | GST and GPx (Roots) | ↑ | 2.5 b | Prieto et al., 2011 |
| *Sinapis alba* | *de novo* protein synthesis in roots | ↓* | 18,000 a | Garda et al., 2015 |
| | PP1 and 2A activity | ↓ | 10 – 10,000 a | Máthé et al., 2013b |

↑ Increased in comparison to control group; ↓ Decreased in comparison to control group; ↓* Delayed in comparison to control group; a, pure toxin; b, crude extract.

**Table 3**
Accumulation of MC-LR in several edible plant species and the daily consumption calculated based on the concentration reported in plant tissues.

| Plant species | Concentration of exposure (μg/L) | Exposure time (days) | Analyzed organ | Concentration reported in plant tissues (ng/g F.W) | Daily consumption (μg/kg BW)[a] | Reference |
|---|---|---|---|---|---|---|
| *Brassica napus* | 24 | 10 | Extract of plant (excluding roots) | 2.61 | 0.01 | Chen et al., 2004 |
| | 120 | | | 8.32 | 0.02 | |
| | 600 | | | 123.57 | **0.31** | |
| | 3000 | | | 651 | **1.63** | |
| *Cicer arietinum* | 5 | 1 | Shoots | ≈10 | ≈0.03 | Peuthert et al., 2007 |
| *Glycine max* | 5 | 1 | Shoots | ≈17 | **≈0.04** | Peuthert et al., 2007 |
| *Lactuca sativa* | 2 | 15 | Leaf | ≈ 33 | 0.02–**0.09**[b] | Bittencourt-Oliveira et al., 2016 |
| | 5 | | | ≈ 103 | | |
| | 10 | | | ≈ 143 | | |
| *Lactuca sativa* | 5 | 1 | Leaf | ≈ 1.30[c] | 0.02[b] | Cordeiro-Araújo et al., 2016 |
| | | 4 | | ≈ 1.59[c] | 0.03[b] | |
| | | 7 | | ≈ 2.05[c] | 0.03[b] | |
| | 10 | 1 | | ≈ 2.94[c] | **0.05**[b] | |
| | | 4 | | ≈ 3.83[c] | **0.06**[b] | |
| | | 7 | | ≈ 4.04[c] | **0.07**[b] | |
| *Lens culinarian* | 5 | 1 | Shoots | ≈20 | **≈0.05** | Peuthert et al., 2007 |
| *Lycopersicon esculentum* | 100 | 7 | Green Fruits | ≈5 | ≈0.01 | Gutiérrez-Praena et al., 2014 |
| | | | Mature Fruits | ≈10 | ≈0.03 | |
| | 5 | 90 | Leaves | n.d | – | Corbel et al., 2016 |
| | | | Roots | ≈ 4.5 | ≈0.01 | |
| | 20 | | Leaves | ≈ 0.29 | ≈0.00 | |
| | | | Roots | ≈ 4.8 | ≈0.01 | |
| | 50 | | Leaves | ≈ 0.33 | ≈0.00 | |
| | | | Roots | ≈ 5.7 | ≈0.01 | |
| | 100 | | Leaves | ≈0.55 | ≈0.00 | |
| | | | Roots | ≈8.1 | ≈0.02 | |
| *Malus pumila* | 30 | 14 | Shoots | 14.76 | **0.04** | Chen et al., 2010 |
| | 300 | | | 43.94 | **0.11** | |
| | 3000 | | | 510.23 | **1.28** | |
| *Medicago sativa* | 5 | 1 | Shoots | ≈27 | **≈0.07** | Peuthert et al., 2007 |
| *Oryza sativa* | 120 | 10 | Extract of plant (excluding roots) | 2.94 | 0.01 | Chen et al., 2004 |
| | 600 | | | 5.12 | 0.01 | |
| | 3000 | | | 5.40 | 0.01 | |
| *Pisum sativum* | 5 | 1 | Shoots | ≈ 18 | **≈ 0.05** | Peuthert et al., 2007 |
| *Phaseolus vulgaris* | 5 | 1 | Shoots | ≈ 38 | **≈ 0.10** | Peuthert et al., 2007 |
| *Vigna radiata* green | 5 | 1 | Shoots | ≈ 18 | **≈ 0.05** | Peuthert et al., 2007 |
| *Vigna radiata* red | 5 | 1 | Shoots | ≈ 4 | ≈ 0.01 | Peuthert et al., 2007 |
| *Triticum aestivum* | 5 | 1 | Shoots | ≈ 28 | **≈ 0.07** | Peuthert et al., 2007 |
| *Zea mays* | 5 | 1 | Shoots | ≈ 40 | **≈ 0.10** | Peuthert et al., 2007 |

Daily consumption values highlighted in bold indicate TDI values higher than those recommended by WHO. n.d., non-detectable.
[a] The daily consumption of MC was calculated assuming that a person of 60 kg consumes 150 g of the vegetable species per day.
[b] The daily consumption of MC was calculated by the authors assuming that a person of 60 kg consumes 40 g of lettuce leaves per day.
[c] Value expressed in μg per 40 g of lettuce leaves.

## 3. Accumulation of MC-LR and CYN in edible tissues of plants

The ability of MC-LR and CYN to accumulate in the tissues of several agricultural plants has been described previously, and it was recently reviewed by Corbel et al. (2014a). Nonetheless, the mechanism of MC-LR and CYN uptake by plants is relatively unexplored. The accumulation of MC-LR in plants appears to occur in a time- and concentration-dependent manner (for relatively short periods of exposure, ≤15 days), and a higher uptake in the roots has frequently been observed (Table 3). Indeed, it has been suggested by several authors that, in general, the toxin is absorbed via the root system, and it is then translocated to the shoots (Peuthert et al., 2007; Crush et al., 2008; Saqrane et al., 2009). As above mentioned, specific transporters of MC-LR have not yet been described, although several types of plant membrane transporters with affinities for different peptides and amino acids have been identified (Tegeder and Rentsch, 2010). Because MC-LR is a peptide, the hypothesis that peptide transporters might potentially be involved in toxin uptake by plants is plausible. In addition, a study developed by Romero-Oliva et al. (2014) suggests that MC-LR translocation goes further into fruits and even into new plants via their seeds as they observed in *Capsicum annuum*. Gutiérrez-Praena et al. (2014) have already described the accumulation of MC-LR in *L. esculentum* fruits (tomato). However, recently, Corbel et al. (2016) have shown that for the same range of exposure concentrations (100 μg eq. MC-LR/L) the accumulation of MC-LR in the *S. lycopersicum* cv. MicroTom, cultivated in a soil–plant system, occurred only in leaves and roots but not in tomato fruits. These contradictory results may be explained by the duration of exposure experiment, which was of 2 weeks in the study of Gutiérrez-Praena

et al. (2014) and 90 days in the study of Corbel et al. (2016). Indeed, Gutiérrez-Praena et al. (2014) have detected MC-LR in tomato fruits only in the first week of the exposure experiment, since in the second week the MC-LR concentration in fruits decreased to below limit of detection. This is potentially associated to the chemical modification of MC-LR over time as a result of its binding to intrinsic biomolecules (e.g., PPs) or its detoxification by conjugation with the GSH, catalyzed via GST (Pflugmacher et al., 2001); or simply, MC-LR was more diluted in the 2-week fruits (by increase in volume and water accumulation related to the fruit growth and maturation) leading to the inability of its detection (Gutiérrez-Praena et al., 2014). In a recent study of bioaccumulation and depuration kinetics of MC-LR in leaf tissues of lettuce, Cordeiro-Araújo et al. (2016) have shown that it is possible to decontaminate this vegetable, once lettuce gradually eliminated the accumulated MC-LR over time. However, although lettuce was capable to depurate MC-LR, when it was exposed to 5 and 10 μg/L for 7 days, it required approximately 29 and 37 days, respectively, to eliminate the toxin, which indicates that time is needed to recover the contaminated vegetable and higher exposure concentrations tend to turn depuration less efficient (Cordeiro-Araújo et al., 2016).

Nevertheless, Pflugmacher et al. (2001) reported that the exposure of *Phragmites autralis* to 0.5 μg of $^{14}$C-labeled MC-LR/L for 3 days resulted in a rapid uptake (from 0.5 h) of the toxin. The main uptake route appeared to be in the rhizome and stem, from which the toxin seemed to be transported into the higher parts of the plant. However, the authors hypothesized that uptake directly through the leaves may also occur (Pflugmacher et al., 2001). In a study performed by Crush et al. (2008), water containing toxic cyanobacteria was applied to the shoots of four different crops, and one of them (*Lactuca sativa*) was able to retain the toxin (0.68 mg MC-LR/kg dw), possibly by transdermal absorption. Furthermore, MC-producing *M. aeruginosa* cells can accumulate in the leaves of spray-irrigated lettuce, and these cyanobacterial cells and MCs are not completely removed after washing (Codd et al., 1999). Another unexplored but interesting issue is related to groundwater, which can also contribute to the potential accumulation of MCs by agricultural plants. In a study conducted *in situ* by Mohamed and Al Shehri (2009), the MC accumulation in the leaves and roots of six vegetable plants (*Raphanus sativus, Eruca sativa, Lactuca sativa, Anethum graveolens, Petroselinum hortense* and *Brassica oleracea*) were recorded. These plants were collected from farms that used MC-contaminated groundwater (0.3–1.8 μg/L) for irrigation. The levels of MC in plant tissues ranged from 0.07 to 1.2 μg/g fresh weight, and the roots were found to accumulate significantly higher concentrations of MC than the leaves; these findings were positively correlated with the concentration of MCs in the groundwater wells.

The uptake mechanism of CYN by terrestrial plants has been minimally studied. In a study performed by White et al. (2005), the authors hypothesized that in the aquatic macrophyte *Hydrilla verticillata,* CYN is not taken up by cells, but instead is adsorbed in the plant cell walls. However, Prieto et al. (2011) detected CYN in the leaves of *O. sativa* plants, which suggests that in addition to MC, the toxin seems to be transported through the vascular system (with an uptake at the root level via plasma membrane and symplastic transport). Because CYN is more often found in the environment in dissolved form than within cyanobacterial cells, the transdermal absorption of this toxin may be a relevant route of plant uptake (Chiswell et al., 1999; Rücker et al., 2007). Concerning the accumulation of CYN in agricultural plants, it seems to follow a similar pattern of MC-LR (Table 3). The concentration-dependent accumulation of CYN was reported in the roots and leaves of *Brassica* vegetables after a treatment using a cyanobacterial extract containing the toxin (Kittler et al., 2012). Prieto et al. (2011) have found the accumulation of CYN in the roots and leaves of *O. sativa* plants exposed to an extract containing 2.5 μg CYN/L, with a significantly higher concentration measured in the roots than in the leaves. Additionally, in a study developed by Silva and Vasconcelos (2010), the roots of *L. sativa*, *P. vulgaris* and *P. sativum* had higher concentrations of CYN in comparison to the stems.

From the human health perspective, it is important to emphasize that most of these studies were carried out using vegetables in which the leaves are the edible parts, and in many cases the concentration of MCs detected would exceed the tolerable daily intake (TDI) of 0.04 μg/kg of body weight/day recommended by the World Health Organization (WHO), assuming that a person weighing 60 kg consumes 150 g or 40 g (Bittencourt-Oliveira et al., 2016; Cordeiro-Araújo et al., 2016) of each vegetable (Table 3). To the best of our knowledge, the only edible root vegetables that were studied with regard to MC accumulation were *R. sativus* and *Eleocharis dulcis*, and MC was detected in both (Mohamed and Al Shehri, 2009; Xiao et al., 2009). Because in most of the existing studies (Chen et al., 2004; Peuthert et al., 2007; Crush et al., 2008; Saqrane et al., 2009) the roots were found to accumulate higher toxin concentrations than the leaves, edible root vegetables will require increased attention with respect to food safety. Likewise, the MC-LR- or CYN- conjugates formed due to plant metabolism must be considered. The *in vitro* study performed by Pflugmacher et al. (2001) with the macrophyte *Phragmites australis* suggested that cysteine-MC and glutathione-MC conjugates may be produced during MC detoxification in plants. Although these metabolites are less toxic than MC-LR, their toxicological properties have already been described in animals (Ito et al., 2002), and they should therefore also be considered within the evaluation of the total toxicity of the MCs. To date, these potential CYN derivatives have neither been investigated nor are any analytical data available. Furthermore, the majority of the studies published are based on assessments carried out with individual cyanotoxins. However, in an aquatic or terrestrial environment, plants are exposed to several chemical contaminants simultaneously, and the interactions of each component of the mixture may result in different effects than each component applied alone, including changes in the bioaccumulation rate. A study conducted by Wang et al. (2011) showed that the uptake of MC-LR by lettuce plants was significantly higher in the presence of the anionic surfactant linear alkylbenzene sulfonate than when plants were exposed to the same concentration of MC-LR only. This finding highlights the potential for the enhancement of MC-LR and CYN accumulation in plants due to their co-occurrence with other chemical contaminants, and it underlines the importance of further research regarding the joint effects of cyanotoxin mixtures.

Finally, it is important to notice that most of these studies have been performed in hydroponic conditions, which to an extent maximizes the availability of the toxin to the root system. Therefore, the accumulation rate can be overestimated because the soil and other plant substrates can retain the toxins, reducing their bioavailability for the plants' uptake. In the only published study where a more realistic exposure scenario was created, i.e., the plants were grown in soil/vermiculite conditions, MC accumulation did not occur (Järvenpää et al., 2007). More recently, a study performed by Kanzo et al. (2013) demonstrated that in hydroponic conditions, MCs were able to accumulate in the roots, stems and leaves of *Brassica rapa* after exposure to 100 and 1000 μg MC-LR/L. Interestingly, in soil cultivated *B. rapa*, no accumulation was detected after exposure to the same MC-LR concentrations. Likewise, all of the CYN studies were carried out in a soil-free cultivation system, and therefore adsorptive effects of environmental soil particles were not considered. Future studies must be designed using more realistic experimental conditions to contribute to the development of management policies regarding the use of cyanotoxin-contaminated water for irrigation and on the acceptability of these possibly contaminated plants for human consumption.

## 4. MC-LR and CYN bioavailability in soil and potential implications to the groundwater quality

In addition to the potential toxic effects for crops, the use of water

from eutrophic systems for irrigation raises questions about the persistence of cyanotoxins in the soil, their bioavailability to plants and the groundwater contamination due to infiltration into the soil. Despite the scarcity of information available regarding the MC adsorption in cropland soils, it is suggested that the adsorption is generally low, which can potentially result in higher bioavailability for plants. However, it is known that soils with high clay and/or organic carbon contents have high adsorption coefficients of toxins. Chen et al. (2006) highlighted MC-LR as a pollutant of high mobility in soil, and mobility was mainly related to the clay content in the soil. Miller and Fallowfield (2001) found that the soils with the highest organic carbon content and the highest clay content were the most effective at removing these toxins in batch experiments. Additionally, a study developed by Järvenpää et al. (2007) demonstrated that MC elimination from the water phase by soil and vermiculite alters the concentration of the toxin available to the plants, and the success of the toxin elimination is dependent on the soil characteristics. Sandy soil (98.5% sand) was incapable of removing cyanotoxins. This finding was supported by Morris et al. (2000), who reported that the clay content and its quality may be more important for adsorption than other soil characteristics. Chen et al. (2006) also proposed that the adsorption mechanism of MCs in soil is not only due to sorption, but also chemical binding with the metal ions on the surface of the soil particles. Due to the nitrogen and oxygen atoms in the toxin structures, MCs can chelate with the metal ions in soil clay. Moreover, the persistence of MC-LR in agricultural soils is dependent on the degradation efficiency (e.g., photolysis, hydrolysis or microbial degradation). It seems that the major dissipation process for cyanotoxins in soil ecosystems is mainly via microbial degradation (Miller and Fallowfield, 2001; Chen et al., 2006). Indeed, several soil bacteria, such as *Arthrobacter* sp., *Brevibacterium* sp. and *Rhodococcus* sp., appear to be able to break down MC (Manage et al., 2009). Bourne et al. (2001) also reported that *Sphingomonas* sp. possesses a gene cluster that is involved in MC-LR degradation. However, a recent study based on the use of radiolabeled MC-LR showed that when this cyanotoxin was introduced to a silty sand soil, it underwent a weak microbial mineralization under aerobic conditions, and therefore large amounts of the toxin remained in soil aqueous extracts (Corbel et al., 2014b). Similarly, during plant irrigation practices, a portion of the MCs may be degraded rapidly by sunlight or by some of these soil bacteria, but another portion can persist and become available in the ecosystem. Chen et al. (2006) reported that the half-life of MC-LR is between six and eighteen days. However, Corbel et al. (2015c) detected MC in soil in concentrations ranging from 1.3 and 3.9 ng MC-LR/g (dry soils) after 90 days of irrigation, which corroborates with the half-life of $^{14}$C-MC-LR described by Corbel et al. (2014b), which exceeded 60 days in the same agricultural soil. No studies have yet examined the persistence of CYN in agricultural soil.

The persistence of cyanotoxins is of particular interest because it may lead to significant accumulations in soils after consecutive cycles of watering and planting. Therefore, in addition to the exposure from irrigation water containing cyanotoxins, plants may also be exposed to the toxins already present in the soil at the time of planting. Nevertheless, while soil may be able to reduce the availability of cyanotoxins to the crops, these can migrate from the surface to deeper layers of the soil following precipitation, leading to possible groundwater contamination (Chen et al., 2006). Indeed, Eynard et al. (2000) reported that the soil was not able to protect groundwater from the toxins originating from blooms occurring in the rivers and lakes of Riga, which led to the contamination of this resource. Recently, Corbel et al. (2014b) also suggested a high risk of cyanotoxins leaching from the soil toward groundwater. However, the investigation of groundwater contamination due to the use of cyanotoxin-contaminated water for crop irrigation is still in a very early stage. Further studies should be developed to investigate the real risks, especially depending on the soil characteristics and seasons. The hydrophilic characteristics of MC-LR and CYN make them very prone to leach into groundwater, and we therefore hypothesize that the use of contaminated water for irrigation or the use of harvested cyanobacterial blooms for plant fertilization are likely to cause unsafe groundwater contamination, especially in rainy seasons.

## 5. Balance of risks for agriculture and public health

Water is essential for growing agricultural plants, and its availability is becoming scarcer and of lower quality (FAO, 2012). The control of toxic cyanobacterial blooms in surface water would be the best management measure to avoid risks for agriculture and human health. However, the proliferation of these blooms has been forecasted to increase, and the use of surface water contaminated with MC-LR in agriculture has already been reported in several countries, such as Finland (Spoof et al., 2003), Spain (Aboal and Puig, 2005), Tunisia (El Herry et al., 2008), Turkey (Gurbuz et al., 2009), Morocco (Oudra et al., 2001), Saudi Arabia (Mohamed and Al Shehri, 2009), India (Prakash et al., 2009), China (Liu et al., 2011; Chen et al., 2012a), New Zealand (Wood et al., 2006), Guatemala (Romero-Oliva et al., 2014) and Algeria (Nasri et al., 2008). MC content of irrigation water examined in these studies ranged between 4 and 50 μg/L up to 760 μg/L. In Algeria, however, concentrations up to 29,000 μg MC-LR/L were reported (Nasri et al., 2008). CYN, which was also detected in water intended for agricultural irrigation in Australia (McGregor and Fabbro, 2000; Everson et al., 2011; Saker and Griffiths, 2001), is usually found in concentrations ranging from 2.0 to 18.9 μg/L.

As discussed previously, the absorption of these cyanotoxins by plants is thought to induce morphological and physiological alterations, and consequently cause a putative loss of productivity due to the inhibition of germination, growth and development. However, it has recently been suggested that MC-LR and CYN are not always associated with toxic effects, and when tested in environmentally relevant concentrations ( < 100 μg/L), they may not be as harmful as initially thought, and may even accelerate plant development (Corbel et al., 2015a; Freitas et al., 2015a). Indeed, in a plant-soil system, Corbel et al. (2015b) noticed that environmental concentrations of MC-LR (0–100 μg/L) had no deleterious effects on the dry weight of tomatoes (var. MicroTom), leading therefore to a significant increase in the dry weight of the aerial parts of the plant. The biological processes underlying the tolerance of plants to relevant concentrations of MC-LR and CYN could be related to the increased actions of antioxidant components of the defense system (enzymatic and/or non-enzymatic) in response to oxidative stress. Therefore, the physiological stress promoted by cyanotoxins may alter the chemical composition of plants and consequently may change their nutritional quality. It has been reported that the content of minerals and non-enzymatic antioxidants, such as phenolic compounds and α- and β-tocopherol, were enhanced in plants after their exposure to CYN and MCs, respectively (Freitas et al., 2015a; El Khalloufi et al., 2012; Lahrouni et al., 2013; Stüven and Pflugmacher, 2007; Saqrane et al., 2009). The ability of crop plants to cope with the stress promoted by environmentally relevant concentrations of cyanotoxins also maximizes their productivity and nutritional quality, a finding that is of major relevance for agriculture and human nutrition. However, there is a significant lack of studies correlating the effects of low concentrations of cyanotoxins with the productivity and nutritional quality of agricultural plants. Nevertheless, in spite of the apparent benefits, there are attendant risks and unexpected consequences threatening food safety. Some proteins with defensive or protective functions against stress that are secreted by plants are recognized to also have allergenic potential (Abreu et al., 2013). Freitas et al. (2015b) reported an increase in the abundance of pathogenesis-related proteins that have allergenic proprieties in leaf-lettuce plants exposed to CYN and an MC-LR/CYN mixture. Furthermore, the potential tolerance of agricultural plants to low concentrations of MC-LR and CYN raises the possibility of its

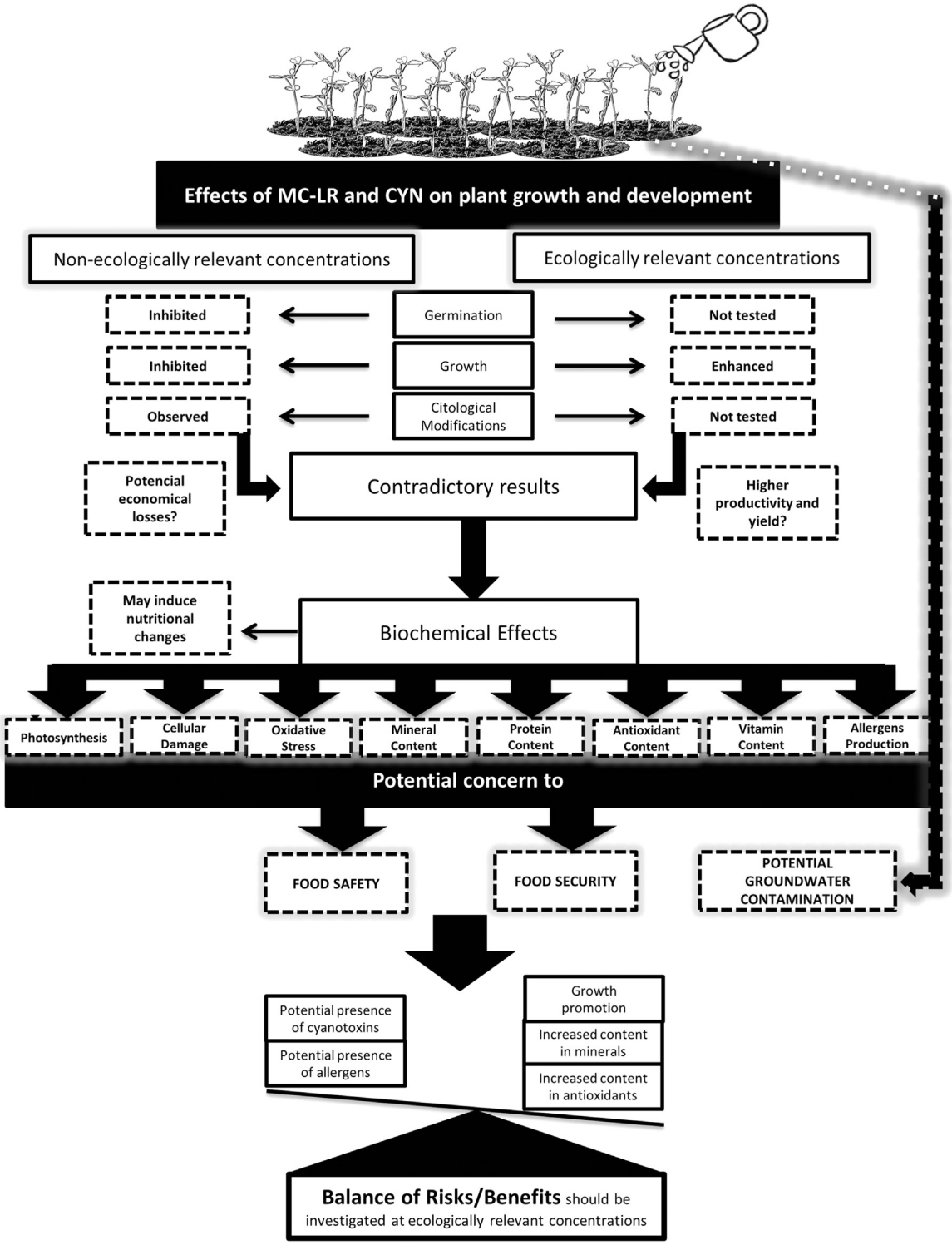


**Fig. 1.** Balance of the effects of MC-LR and CYN on plant growth, development and biochemical processes as well as human health implications considering ecologically relevant concentrations.

accumulation in edible tissues. Provisional TDI amounts of 0.04 and 0.03 μg/kg (body weight) were established for the presence of MC-LR and CYN in food, respectively (Sivonen and Jones, 1999; Humpage and Falconer, 2003). Although levels of CYN exceeding the TDI have not been verified, the concentration detected in the tissues of several vegetables exceeds the TDI proposed by WHO for MC-LR (assuming a consumption of 150 g of vegetables) (Table 3). Again, however, the accumulation studies were performed using non-ecologically relevant concentrations and with plants in the early stages of development. In light of the available information, the real impacts of cyanotoxins on agricultural plant food safety (dangerous levels of cyanotoxins and allergenic potential) are not fully understood, and more research is needed to assess the effects of realistic concentrations of cyanotoxins during a long-term exposure. In addition, another factor of uncertainty in assessing human exposure derives from the fact that it is not clear whether the levels of MC-LR and CYN measured in raw edible matrices correspond to the bioavailable amount. Freitas et al. (2014, 2016) have shown that factors such as food storage, processing and human

digestion can change the MC-LR and CYN bioaccessibility and therefore the risk of human exposure. Thus, a lack of recognition of the likelihood of these hazards is an important factor for the late development of risk management strategies. The risk analysis can also be extended to an evaluation of the benefits and risks of potentially contaminated vegetable consumption in an attempt to balance the use/restriction of MC-LR- and CYN-contaminated water for irrigation (Fig. 1). It is well recognized in the epidemiological literature that the regular consumption of vegetables has been correlated with lower risks of human chronic diseases (Boeing et al., 2012), and they are a convenient source of nutrients of fundamental importance, such as water, soluble fibers, vitamins (C, K, B2, and folic acid), minerals and phytochemicals (carotenoids). The public health impact of restrictions on the amount or type of vegetables consumed will depend on the other foods that then would substitute them, and this substitution should be appropriately weighed with the severity associated with exposure to cyanotoxins (e.g., liver cancer). Nevertheless, if it is proven that the use of water containing low concentrations of cyanotoxins does not represent a risk to consumers, a possible proactive measure to address this challenge is the addition of non-contaminated water to dilute the cyanotoxin concentrations to values that are considered risk-free. This method may be especially relevant in countries with intense water scarcity or that have no alternative to the use of cyanotoxin-contaminated water.

## 6. Conclusion

The increasing occurrence of toxic cyanobacterial blooms creates important challenges for agricultural productivity and public health.

The current literature shows that both cyanobacterial toxins, MC-LR and CYN, can cause toxic effects in agricultural plants, especially at the biochemical level. Furthermore, MC-LR can accumulate in a wide range of agricultural plants, and the predicted human exposure would be higher than the TDI proposed by WHO. However, because most of these studies have been performed using cyanotoxin concentrations that are higher than those usually found in the environment and in hydroponic conditions, these effects can be overestimated in some studies because the accumulation of MC-LR and CYN seems to be dependent on the exposure concentration, and their uptake by plants can be reduced due to the adsorptive effects of soil particles and the potential biodegradation, photolysis and hydrolysis. In addition, at environmentally relevant conditions, the growth and nutritional value (antioxidant and mineral content) of some plants are enhanced as a mechanism to cope with oxidative stress. However, the potential tolerance of plants can increase their susceptibility for accumulating these cyanotoxins and allergenic proteins following long-term exposure. Therefore, further studies should be developed in this field. Furthermore, there is not sufficient information on the persistence and lifetime of these cyanotoxins in agricultural soils. This lack of information is more remarkable for CYN, and this subject should be explored in the future because of the leaching potential to groundwater. Notably, there is a need to survey the groundwater regarding the presence of these cyanotoxins, especially in the areas where contaminated water is used for irrigation. Although no appreciable risks were found at ecologically relevant concentrations, it cannot be assumed with the current data that the use of contaminated water for agricultural irrigation is free of risks to either plant productivity or public health. Irrigation water monitoring programs should be initiated, and when the concentrations of MC-LR and CYN are higher than 100 μg/L, dilutions should be made to avoid major risks. A *risk-benefit analysis* would also be a valuable tool to understand the real impacts of MC-LR and CYN on agriculture and public health. Additionally, future research should also investigate the potential effects of interactions between cyanotoxins and other chemicals when present together.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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# Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in…

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Review

# Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops

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## HIGHLIGHTS

- Phytotoxic effects of cyanotoxins on agricultural plants have been updated.
- We report mechanisms of cyanotoxins and target molecules in vegetable organisms.
- The effects of cyanotoxins in the terrestrial environment is particularly scarce.
- We describe fate of cyanotoxins in aquatic and soil ecosystems.
- We examine bioaccumulation of cyanotoxins in vegetable foods.

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## ABSTRACT

The occurrence of harmful cyanobacterial blooms in surface waters is often accompanied by the production of a variety of cyanotoxins. These toxins are designed to target in humans and animals specific organs on which they act: hepatotoxins (liver), neurotoxins (nervous system), cytotoxic alkaloids, and dermatotoxins (skin), but they often have important side effects too. When introduced into the soil ecosystem by spray irrigation of crops they may affect the same molecular pathways in plants having identical or similar target organs, tissues, cells or biomolecules. There are also several indications that terrestrial plants, including food crop plants, can bioaccumulate cyanotoxins and present, therefore, potential health hazards for human and animals. The number of publications concerned with phytotoxic effects of cyanotoxins on agricultural plants has increased recently. In this review, we first examine different cyanotoxins and their modes of actions in humans and mammals and occurrence of target biomolecules in vegetable organisms. Then we present environmental concentrations of cyanotoxins in freshwaters and their fate in aquatic and soil ecosystems. Finally, we highlight bioaccumulation of cyanotoxins in plants used for feed and food and its consequences on animals and human health. Overall, our review shows that the information on the effects of cyanotoxins on non-target organisms in the terrestrial environment is particularly scarce, and that there are still serious gaps in the knowledge about the fate in the soil ecosystems and phytotoxicity of these toxins.



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## 1. Introduction

In light of global climate change, and particularly measurable rises in global temperature, as well as increased fluxes of certain nutrients (i.e., nitrates, phosphates) brought either by agricultural run-off or by sewage treatment plants and other anthropogenic sources, it has been suggested that cyanobacteria, including toxin-producing taxa, may be increasing in abundance, and thus represent an emerging human and environmental health concern (For review see in O'Neil et al., 2012). The presence of such toxins has been reported throughout the world and it appears that liver-toxic microcystins are more commonly found in 40–75% cyanobacterial blooms (Sivonen and Jones, 1999). The contamination of surface waters by these cyanotoxins can cause water quality problems for fisheries, aquaculture, farming, and sanitary hazard for human and animals. Humans are exposed to cyanobacteria toxins through many routes, including drinking water, recreational contact, and health food products made from cyanobacteria, and food chain. In recent years, several cyanobacterial toxins were investigated in regard to their ability to enter the food chain *via* freshwater seafood (Ibelings and Chorus, 2007; Ettoumi et al., 2011), however, their ability to enter the food chain *via* agricultural crops has not been thoroughly investigated to date. Although no case of poisoning by these products has been reported in the literature, this eventuality must not be ignored. Indeed, a recent epidemiological study showed that the excessive incidence of amyotrophic lateral sclerosis in the population of the islands of Guam in the Pacific was linked to a consumption of the seeds of cycas contaminated by a neurotoxin, β-methylamino-L-alanine (BMAA), produced by a species of cyanobacteria of the genus *Nostoc* living in symbiosis in the roots of this plant (Banack and Cox, 2003; Cox et al., 2003; Murch et al., 2004; Steele and McGeer, 2008). This last cited fact is gaining importance since plants could in a direct or indirect manner contribute to food chain cyanotoxin's transfer, and by the way constitute a potent health risk source. Indeed, numerous studies reported that both submerged and emergent aquatic plants have been shown to absorb microcystins from low external concentrations (Pflugmacher et al., 1998, 2001; Yin et al., 2005; Saqrane et al., 2007). In terrestrial plants, Codd et al. (1999) reported that spray irrigation of commercial lettuce (*Lactuca sativa*) plants with water containing *Microcystis* resulted in colonies and single cells of the cyanobacterium being lodged on the leaves 10 d after the last irrigation. MC-LR was present at 2.5 mg $kg^{-1}$ dry weight (DW) in the central leaves, 0.833 mg $kg^{-1}$ (DW) in the distal zone of mature leaves, and 0.094 mg $kg^{-1}$ (DW) in the basal zone of mature leaves. The last study indicated that toxins were absorbed by the plant as the central leaves would have been protected from irrigation. Similar conclusions were reached for rice (*Oryza sativa*) and rape (*Brassica napus*) by Chen et al. (2004). Therefore, the accumulation of cyanotoxins in the terrestrial food chain is at present remains more worrying and the proposed quality limits are rare, indeed, many aspects concerning these toxins are particularly scarce, notably those relative to the fate of cyanotoxins in the soil ecosystems and their toxicity and bioaccumulation on agricultural crops.

There have been several reviews of the intensification and global expansion of harmful cyanoabcterial blooms in terms of abundance, geographic extent, factors that may be promoting this expansion, and prevention and management of cyanobacteiral blooms and their toxins, as well as effects on aquatic ecosystem health and transfer on food webs (Wiegand and Pflugmacher, 2005; Ibelings and Chorus, 2007; Paerl and Huisman, 2009; Aráoz et al., 2010; Kinnear, 2010; Merel et al., 2010; Jančula and Maršálek, 2011; O'Neil et al., 2012). However, the purpose of this review is to: (1) Highlight important findings of the last decade of modes of actions of cyanotoxins in humans and mammals and occurrence of target biomolecules in vegetable organisms; (2) Describe the fate of cyanotoxins in aquatic and soil ecosystems and focus in their phytotoxicity; and (3) Emphasize bioaccumulation of these toxins in vegetable foods and its consequences on animals and human health.

## 2. Cyanotoxins and their producers

Recent research suggests that eutrophication and climate change are two processes they may promote the proliferation and expansion of harmful cyanobacterial blooms in freshwater, estuarine, and marine ecosystems. These microorganisms are known to biosynthesize a wide range of chemical classes of secondary metabolites such as peptides, macrolides, and glycosides (Patterson et al., 1994; Namikoshi and Rinehart, 1996) possessing a number of bioactivities: antiviral (Patterson et al., 1993, 1994), antifungal (Patterson et al., 1994), cytotoxic (Patterson et al., 1991), enzymatic inhibitor (Honkanen et al., 1995), antineoplastic (Moore, 1996), and allelopathic (Pushparaj et al., 1998). However, some of these cyanobacterial secondary metabolites encompass a diversity of alkaloid and peptide cyanotoxins which have been suggested to both pose threats to human and environmental health worldwide (Hawkins et al., 1985; Carmichael and Falconer, 1993; Kuiper-Goodman et al., 1999; Sivonen and Jones, 1999; Hitzfeld et al., 2000; Ettoumi et al., 2011). Toxic cyanobacteria that have been involved in such incidents belong essentially to the genera *Microcystis*, *Anabaena, Aphanizomenon, Planktothrix, Oscillatoria, Cylindrospermopsis* and less often *Gomphosphaeria*, *Coelosphaerium, Gloeotrichia*, *Nodularia* and *Nostoc* (Hawkins et al., 1985; Sivonen and Jones, 1999). The cyanotoxins are essentially endotoxins which can be released in the environment following a cellular lyse (Codd et al., 1989) or following treatment of cyanobacterial blooms with algaecides (Kenefick et al., 1993). They can be classified into four families according to the organs on which they act: neurotoxins (nervous system), hepatotoxins (liver), cytotoxins (several organs: liver, kidneys, adrenal glands, small intestine), and dermatotoxins (irritant toxins).

Cyanobacterial neurotoxins are divided in three groups: anatoxins (anatoxin-a, homoanatoxin-a, and anatoxin-a(s)), saxitoxins,

and the neurotoxic amino acid L-beta-N-methylamino-L-alanine (BMAA). Anatoxins and the BMAA are specific of cyanobacteria, while, saxitoxins are also synthesized by some marine dinoflagellates and associated with the human disease paralytic shellfish poisoning or PSP (Falconer, 1991; Carmichael, 1994; Kaebernick and Neilan, 2001). By contrast to the other neurotoxins which production depends on the phylogeny of the species, the BMAA can be produced by almost all groups of cyanobacteria from freshwater, brackish, and marine environments (Cox et al., 2005; Banack et al., 2007). Hepatotoxins are divided into two groups: Microcystins (MCs), cyclic heptapeptide hepatotoxins (MW 900–1200), that are regarded as the most frequently occurring and widespread of the cyanotoxins with more than 80 MC variants already reported (Sivonen and Jones, 1999; Cox et al., 2005; del Campo and Ouahid, 2010); and nodularins (MW 800–900) composed of five amino acids with only nine different natural analogs have been characterized (De Silva et al., 1992; Namikoshi et al., 1993; Rinehart et al., 1994; Codd et al., 2005). The hydrophilic alkaloid cytotoxin, cylindrospermopsin (MW 415) has been identified in the freshwater cyanobacteria *Cylindrospermopsis raciborskii* (Ohtani et al., 1992), *Umezakia natans* (Harada et al., 1994), *Aphanizomenon ovalisporum* (Sivonen and Jones, 1999), *Anabaena* sp. (Schembri et al., 2001), and *Raphidiopsis* sp. (Li et al., 2001). Today, only two congeners of cylindrospermopsin have been identified: 7-epicylindrospermopsin and deoxycylindrospermopsin. The freshwater cyanobacterial irritant toxins such as lipopolysaccharides (LPS), or endotoxins as they are commonly called, are major components of the cell wall in most Gram-negative bacteria including cyanobacteria (Jann and Jann, 1984; Mayer and Weckesser, 1984; Kaya, 1996; Stewart et al., 2006).

## 3. Modes of actions in humans and mammals and occurrence of target biomolecules in vegetable organisms

### 3.1. Neurotoxins

Anatoxin-a is a potent postsynaptic depolarizing neuromuscular blocking agent that affects both nicotinic and muscarinic acetyl cholineacetylcholine receptors (Carmichael et al., 1979; Spivak et al., 1980). It acts as a depolarizing neuromuscular blocking agent mimicking the action of acetylcholine. However, this neurotoxin is not degraded by the acetylcholinesterase, and consequently its action on the muscular cells does not stop and, due to being stimulated, these cells are blocked and thereby resulting to muscle paralysis (Carmichael, 1994; Lilleheil et al., 1997). When the respiratory muscles are affected, the insufficient oxygenation of the brain engenders convulsions and the oppression (Carmichael, 1994; Humpage et al., 1994). The $LD_{50}$ (lethal dose resulting in 50% deaths) of this neurotoxin is 200 μg $kg^{-1}$ (mouse, i.p.) (Carmichael et al., 1979; Skulberg et al., 1992). Homoanatoxin-a is a homologue of anatoxin-a, that was reported to be a potent nicotinic agonist (Wonnacott et al., 1992). It enhances the release of acetylcholine from peripheral cholinergic nerves through opening of endogenous voltage dependent neuronal L-type calcium channels (Aas et al., 1996; Lilleheil et al., 1997). Despite the similarity in their names, anatoxin-a(s) and anatoxin-a are not structurally related and exhibit different physiological properties. Anatoxin-a(s) belongs to the organophosphate class of neurotoxins and it acts as an irreversible inhibition of acetylcholinesterase at the nerve synapse (Mahmood and Carmichael, 1986, 1987). The $LD_{50}$ (mouse, i.p.) of this toxin is about 20–40 μg $kg^{-1}$ (Mahmood and Carmichael, 1987; Matsunaga et al., 1989; Carmichael et al., 1990). In animals, the mechanisms of action of PSP toxins (saxitoxins) are based on the blockage of sodium conductance in axons (Kao et al., 1967; Henderson et al., 1973). They so inhibit the transmission of the electric activity and prevent the liberation of the acetylcholine (Nishiyama, 1968). Their toxicity is more important than that of anatoxins with a $LD_{50}$ (mouse, i.p.) in the same conditions for the saxitoxin of 10 μg $kg^{-1}$ (Gorham and Carmichael, 1988). Saxitoxins can also bind to calcium ($Ca^{+2}$) and $K^+$ channels, interfering with the speed of opening and closing of these channels, which can in turn lead to alteration in the influx of ions to the cell (Wang et al., 2003; Su et al., 2004). In addition, the $Na^+$-channel blockage may alter the selective permeability of the membrane and may change the flow of ions, leading to damage to cellular homeostasis (Hille, 1992; Jablonski et al., 2007). Concerning the neurotoxic amino acid (BMAA), it acts in mammals as a glutamate agonist at AMPA, kainite and NMDA receptors (Spencer et al., 1986, 1987; Andersson et al., 1997; Seawright et al., 1999). Consequently, it increases the intracellular concentration of calcium in neurons and induces neuronal activity by hyperexcitation (Brownson et al., 2002). To our knowledge, no data regarding the toxicity of cyanobacterial neurotoxins in higher plants have been reported. However, interfering of some of them such as saxitoxins with the speed of opening and closing of $Na^+$, $Ca^{2+}$ and $K^+$ channels could modify ions transport in plant cells. For example, a modification of sodium signals can modify osmotic pressure in cells or the assimilation of $CO_2$ for C4 plants (Brownell and Crossland, 1972). While sodium extrusion in animal cells and microorganisms (including yeast) is directly energized by ATP hydrolysis ($Na^+$-ATPases), these $Na^+$ pumps are absent from higher plants (Horie and Schroeder, 2004).

### 3.2. Hepatotoxins

Cyanobacterial hepatotoxins type microcystin-LR are generally not able to penetrate vertebrate cell membranes and therefore, require uptake via the bile acid transport system present in hepatocytes and cells lining the small intestine (Runnegar et al., 1991). As a result of this, toxicity of these cyanotoxins is restricted to organs expressing the organic anion transporter on their cell membranes such as the liver (Fischer et al., 2005). However, in vegetable cells one relatively unexplored question regarding these toxins concerns the mechanism of uptake, particularly the variants that would be predicted to be membrane impermeable based on polarity. They may cross cell membranes of plants by other mechanisms, including diffusion or by root absorption. Pflugmacher et al. (2001) have been reported that when the emergent reed plant *P. australis* was exposed to 0.5 μg of $^{14}C$-labeled microcystin-LR $L^{-1}$ for 3 d, it demonstrated a rapid uptake (since 0.5 h) of the toxin. The main uptake route appeared to be in the stem and rhizome, from which the toxin is transported into the higher parts of the plant to the leaves. Uptake directly through the leaves may also occur by direct contact of small plants or by the lowest leaves of a plant with surface water and with upper leaves by wave and spray contact (Pflugmacher et al., 2001). Once in both vertebrate and vegetable cells, microcystins and nodularins have been shown to be potent and specific inhibitors of protein phosphatases 1 and 2A, and this inhibition accounts for their extreme toxicity (MacKintosh et al., 1990; Kurki-Helasmo and Meriluoto, 1998; Hastie et al., 2005). Those proteins are involved in several physiological and molecular processes in higher plants (Sheen, 1993; Takeda et al., 1994). Indeed, numerous studies reported that microcystins have several perturbatory effects on plant physiology and metabolism, when sufficient levels of toxin enter the plant cells (MacKintosh et al., 1990; Siegl et al., 1990; Sheen, 1993; Yamasaki, 1993; Smith et al., 1994; Takeda et al., 1994; Abe et al., 1996; Smith, 1996; Kurki-Helasmo and Meriluoto, 1998; Weiss et al., 2000; McElhiney et al., 2001; Pflugmacher, 2002; Romanowska-Duda and Tarczyńska, 2002; Gehringer et al., 2003; Chen et al., 2004, 2011; Mitrovic et al., 2005; Saqrane et al., 2007, 2008; Stüven and Pflugmacher, 2007; Järvenpää et al.,

2007; Jang et al., 2007; Peuthert et al., 2008; Máthé et al., 2009; Huang et al., 2009; El Khalloufi et al., 2011, 2012; Jámbrik et al., 2011; Perron et al., 2012). On the other hand, several studies have also reported that these hepatotoxins induce oxidative stress in mammal cells (Žegura et al., 2003; Botha et al., 2004; Bouaïcha and Maatouk, 2004; Puerto et al., 2010). Therefore, their toxicity on aquatic plants seems to be also more linked to the induction of oxidative stress manifested by elevated reactive oxygen species (ROS) production and malondialdehyde (MDA) content (Lefevre et al., 1950; Pflugmacher, 2004; Hu et al., 2005; Leflaive and Ten-Hage, 2007).

### 3.3. Cytotoxins

The alkaloid cylindrospermopsin (CYN) is known as a general cytotoxin that blocks protein synthesis in mammal cells (Runnegar et al., 1995; Froscio et al., 2001, 2003). Implications of this effect can be also observed in vegetable cells. In fact, Froscio et al. (2008) reported that CYN was shown to inhibit the eukaryotic protein synthesis apparatus with similar potency in plant and mammalian cell extracts, IC50 of 334 nM in wheat germ extract and 110 nM in reticulocyte lysate. Metcalf et al. (2004) also showed that CYN inhibited pollen germination in tobacco plants (*Nicotiana tabacum*), with partial inhibition of protein production in the germinating pollen tubes following exposure to 138 μg $mL^{-1}$ of toxin.

## 4. Environmental concentrations of cyanotoxins in freshwaters and fate in aquatic and soil ecosystems

### 4.1. Environmental concentrations of cyanotoxins

The occurrence of cyanobacterial toxins was reported throughout the world in surface waters, where hepatotoxic microcystins are more commonly found in 50–75% cyanobacterial blooms (Ettoumi et al., 2011). Data on environmental concentrations of cyanotoxins have been compiled and reviewed in numerous papers (Sivonen and Jones, 1999; Falconer and Humpage, 2006; Van Apeldoorn et al., 2007; Messineo et al., 2009). In this review, we give a summary on environmental concentrations focusing on irrigation waters with the ultimate aim to relate them to phytotoxicological data. Cyanotoxins are intracellular toxins contained within living cells, depending on both the nature of the toxin and the growth stage (Jungmann et al., 1996; Orr and Jones, 1998; Park et al., 1998a,b; Sivonen and Jones, 1999). They are only released into the water, to form dissolved toxin, during cell senescence or cell death and lysis or through water treatment processes such as algaecide application, rather than by continuous excretion (James and Fawell, 1991; Gupta et al., 2001; Babica et al., 2006). The highest total (intracellular plus dissolved) cyanotoxin levels have been found in blooms and scums. For example, total MC concentrations in surface waters vary from trace to several milligrams per liter, being strongly influenced by the occurrence of these forms of cyanobacterial biomass. In surface waters used as irrigation source, total MC concentrations of 4–50 μg $L^{-1}$, up to 6500 μg $L^{-1}$, have been reported in multiple locations, including but not limited to the Morocco (Oudra et al., 2001), Tunisia (El Herry et al., 2008), India (Prakash et al., 2009), Turkey (Gurbuz et al., 2009), and Finland (Spoof et al., 2003), but much higher levels up to 29 000 μg $L^{-1}$ in Algeria (Nasri et al., 2008) (Table 1). It should be noted, however, that these very high concentrations of cyanotoxins would be from scums or from very dense cyanobacterial biomass. In the field, water samples with more than 1 μg $L^{-1}$ total MCs, dissolved fraction did not comprise more than 10% of the combined intra and extracellular pool (Lindholm and Meriluoto, 1991; Jones and Orr, 1994; Tsuji et al., 1996; Ueno et al., 1996; Lahti et al., 1997). As well in some laboratory studies, where both intracellular and extracellular cyclic peptide toxins and STXs have been measured, it is generally the case that in healthy log phase cultures, less than 10–20% of the total toxin pool is dissolved in the culture medium (Sivonen et al., 1990; Lehtimaki et al., 1997; Negri et al., 1997; Rapala et al., 1997). On the contrary, CYN may often be found at higher levels in dissolved form than within cells, as it readily leaks from cells under normal growth conditions (Norris et al., 2001; Falconer and Humpage, 2006; Wörmer et al., 2008). For example, Shaw et al. (1999) found that in two instances of *A. ovalisporum* blooms around 80% of the total toxin content of the water was in free solution. Recently, Messineo et al. (2009) reported that in several Italian lakes of different characteristics and human uses, extracellular concentrations of total CYN varied from non-detectable values up to 126 μg $L^{-1}$. However, limited or no information is available about the proportion of dissolved form with respect to the total level for the cyanobacterial neurotoxins.

### 4.2. Fate in aquatic and soil ecosystems

Once they enter in aquatic and soil ecosystems, cyanotoxins can be removed according to various processes such as photochemical degradation by UV, adsorption in particles in suspension or onto sediments, and biodegradation (Tsuji et al., 1994; Rapala et al., 1994; Lahti et al., 1996; Chiswell et al., 1999; Welker and Steinberg, 1999; Kaebernick and Neilan, 2001; Mazur-Marzec et al., 2006; Wörmer et al., 2008; Burns et al., 2009; Klitzke et al., 2010, 2011; Thirumavalavan et al., 2012). However, the four groups of cyanotoxins: hepatotoxins, neurotoxins, cytotoxins, and dermatotoxins, exhibit quite different chemical stabilities in these ecosystems. Hepatotoxin cyclic peptide cyanotoxins, microcystins and nodularins, are extremely stable compounds and may persist in aquatic systems for weeks after being released from the cells (Jones and Orr, 1994; Chen et al., 2008; Edwards et al., 2008). According to other studies, these toxins in natural conditions could persist for several months or years (Harada et al., 1996; Sivonen and Jones, 1999). However, numerous studies reported that photochemical degradation by sunlight UV and exposure to degrading bacteria may speed up their removal from the water (Bourne et al., 1996; Heresztyn and Nicholson, 1997; Sivonen and Jones, 1999; Park et al., 2001; Song et al., 2009; Ho et al., 2012). The photodegradation of MCs in full sunlight can take as little as two weeks or longer than six weeks, depending on the presence of water-soluble cell pigments (Tsuji et al., 1994; Welker and Steinberg, 2000). More recently, Thirumavalavan et al. (2012) showed in a laboratory experiment that the presence of humic acid and turbidity affected the photo-degradation process. Additionally, in sea water the rate of nodularin photolysis can be accelerated by the presence of some cell components and humic substance (Welker and Steinberg, 1999). Conversely, during the benthic phase, the photodegradation of these cyanotoxins is expected to be almost negligible due to low radiation penetration (Wörmer et al., 2010). In fact, Welker and Steinberg (2000) found that the half-life of MCs in the deep lakes is longer than the season of cyanobacteria growth, what suggests that the photolysis is significant only for shallow lakes. The alkaloid cytotoxin, CYN, is relatively stable in the dark; however, in sunlight and in the presence of cell pigments degradation occurs quite rapidly with more than 90% within 2–3 d (Chiswell et al., 1999). The neurotoxin, anatoxin-a, is also relatively stable in the dark, but it undergoes rapid photochemical degradation in sunlight particularly in alkaline conditions, even in the absence of cell pigments (Stevens and Krieger, 1991; Smith and Sutton, 1993). However, no data are available for other cyanobacterial neurotoxins and LPS dermatotoxins.

Cyanotoxins can also be retained on suspended particles or onto sediments in aquatic systems. Wörmer et al. (2011) showed the

**Table 1**
Overview of some published cyanobacterial toxin concentrations from various countries. Concentrations are presented in μg $g^{-1}$ dry weight (DW) or else in μg $L^{-1}$ as indicated.

| Country | Location | Use | Type | Concentrations (μg $L^{-1}$ or μg $g^{-1}$ dw*) | Reference |
|---|---|---|---|---|---|
| Algeria | L. Oubeira | ¤ | microcystin-LR | 3–29,163 | Nasri et al. (2008) |
| Argentina | R. San Roque | $, £ | microcystin-LR | 920 | Conti et al. (2005) |
| | – | – | microcystin-LR | 48.6 | Giannuzzi et al. (2011) |
| Australia | R. of drinking water | $ | saxitoxin | 30 | Orr et al. (2004) |
| | L. Julius | $, £, ¤ | cylindrospermospin | $2 \times 10^6$ | Saker and Griffiths (2001) |
| | R. Cania | $, £, ¤ | cylindrospermospin | 18.9 | McGregor and Fabbro (2000) |
| | L. Cobaki Village | $, £, ¤ | cylindrospermospin | 101.4 | Everson et al. (2011) |
| | Narrung Channel | $ | nodularin-R | 1.6 | Heresztyn and Nicholson (1997) |
| | L. Coolmunda | £ | microcystin-LR | 12 | Stewart et al. (2006) |
| | L. Wivenhoe | £ | cylindrospermospin | 1–2 | |
| Brazil | L. Bolonha | $ | microcystin | 1.25 | Vieira et al. (2005) |
| | Sao Paulo | $, £ | microcystin | 0.5–100 | Nobre (1997) |
| | Parana | $ | microcystin | 0.2–6.6 | Hirooka et al. (1999) |
| | D. Itaipu | £ | microcystin | 6,4–10 | |
| | R. Tapacura | $ | saxitoxin | 52 | Molica et al. (2005) |
| | R. Armando Ribeiro Goncalves | $ | microcystin-LR | 8.8 | Costa et al., 2006 |
| | | | saxitoxin | 3.14 | |
| China | R. Haimen | $ | microcystin-LR | 1,556 | Ueno et al. (1996) |
| | L. Taihu | $, £, ¤ | microcystin-LR | 34.2 | Liu et al. (2011) |
| Denmark | L. Knud so | – | homoanatoxin-a | 2,300* | Henriksen (1996) |
| | | – | homoanatoxin-a | 800* | |
| | | – | homoanatoxin-a | 60* | |
| | L. Ravn so | – | homoanatoxin-a | 2,300* | |
| | L. Salten Langso | – | homoanatoxin-a | 20* | |
| | L. Agerso | – | saxitoxin | 37* | Kaas and Henriksen (2000) |
| | L. Bastrup so | – | saxitoxin | 6.4* | |
| | L. Hvideso | – | saxitoxin | 85.1–182.5** | |
| | L. Vissiggaard so | – | saxitoxin | 224.1* | |
| Finland | Prästträsket | $, £, ¤ | microcystin-LR | 42 | Spoof et al. (2003) |
| | Södra Slemmern | $, £, ¤ | nodularin-R | 0.2 | |
| | Högskär | $, £, ¤ | nodularin-R | 0.5 | |
| France | La Loue | £ | anatoxin-a | 8,000* | Gugger et al. (2005) |
| | L. Champs-sur-marne | £ | saxitoxin | 4.8–6.7 | Ledreux et al. (2010) |
| Germany | 20 water bodies | £ | anatoxin-a | 0.39–6.7 | Bumke-Vogt et al. (1999) |
| | 55 water bodies | – | microcystin-LR | 10 | Fastner et al. (1999) |
| | – | £ | microcystin-LR | 36 | Ueno et al. (1996) |
| | Berlin water bodies | £ | microcystin-LR | 0.14–119 | Fromme et al. (2000) |
| Greece | 33 water bodies | – | microcystin-LR | 50–1,600* | Cook et al. (2004) |
| Ireland | L. Caragh | $, £ | anatoxin-a | 112–444 | James et al. (1997) |
| | | $ | homoanatoxin-a | 1.4 | Furey et al. (2003b) |
| | L. Lough Sillan | $ | homoanatoxin-a | 24 | Furey et al. (2003a,b) |
| | R. Innincarra | $ | homoanatoxin-a | 34 | Furey et al. (2003b) |
| | L. Lough Key | $ | homoanatoxin-a | 12 | |
| | L. Corbally | $, £ | anatoxin-a | 60–100* | James et al. (1997) |
| Italy | R. Monteleone | $ | microcystin | 226 | Messineo et al. (2009) |
| | L. Albano | £ | cylindrospermospin | 126 | |
| Japan | L. Inbanuma | $, £ | microcystin | 52 | Ueno et al. (1996) |
| | L. Suwa | – | microcystin | 3.61 | Park et al. (1998a,b) |
| Kenya | L. Baringo | ¤ | anatoxin-a | 0.05–0.21 | Ballot et al., 2004 |
| | L. Nakuru | – | anatoxin-a | 5–223* | |
| | L. Baringo | ¤ | anatoxin-a | 0.05–0.21* | Ballot et al. (2003) |
| | L. Bogoria | – | anatoxin-a | 10–18* | Krienitz et al. (2003) |
| | | – | anatoxin-a | 0.3–9* | Ballot et al. (2004) |
| | L. Simbi | – | microcystin-LR | 19.7–39 | Ballot et al. (2005) |
| | L. Sonachi | – | microcystin-LR | 1.6–12* | |
| | L. Norivasha | $, ¤ | microcystin-LR | 0.041 | Krienitz et al. (2013) |
| Morocco | R. Lalla takerkoust | $, £, ¤ | microcystin | 73* | Oudra et al. (2001) |
| | | ¤ | microcystin | 95.4 | El Ghazali et al. (2011) |
| Netherlands | L. 't Joppe | $, £ | microcystin-LR | 2.5 | Kardinaal et al. (2007) |
| | L. Volkerak | $, £ | microcystin-LR | 7 | |
| | L. Kinselmeer | $, £ | microcystin-LR | 18 | |
| New Zealand | L. Waitawa | – | microcystin-LR | 28,000 | Wood et al. (2006b) |
| | L. Horowhenua | – | microcystin-LR | 16,291 | |
| | L. Ngaroto | – | microcystin-LR | 1,535 | |
| | L. Taupo | – | microcystin-LR | 708 | |
| | Neuma Pond | – | microcystin-LR | 22,58 | |
| | L. Rotoiti | ¤ | microcystin-LR | 10–760 | Wood et al. (2006a) |
| | L. Rotoehu | ¤ | microcystin-LR | 23 | |
| Portugal | – | $, £ | microcystin | 13.7 | Ueno et al. (1996) |
| Poland | R. Sulejow | $, £ | microcystin-LR | 1.17 | Gagala et al. (2010) |
| | L. Bninskie | £ | microcystin-LR | 1.87 | |
| S. Africa | D. Nhanganzwane | – | microcystin | 23,718 | Oberholster et al. (2009b) |
| | D. Makhohlolo | – | microcystin | 0.317 | |
| | L. Krugersdrift | – | microcystin | 43.7 | Oberholster et al. (2009a) |

(*continued on next page*)

**Table 1** (*continued*)

| Country | Location | Use | Type | Concentrations ($\mu g\ L^{-1}$ or $\mu g\ g^{-1}$ dw*) | Reference |
|---|---|---|---|---|---|
| S. Korea | R. Younglang | £ | anatoxin-a | 417* | Park et al. (1998b) |
| | Jangsong | $ | anatoxin-a | 1444* | |
| Serbia | L. Ludös | $, £ | microcystin-LR | 362.68 | Svirčev et al. (2007) |
| | R. Celije | | microcystin-LR | 650 | Svirčev et al. (2009) |
| Spain | R. Santillana | $ | microcystin | 9.99–55.02 | Carrasco et al. (2006) |
| | R. Valmayor | $ | microcystin | 1.2 | |
| | R. Picadas | $ | microcystin | 1.3 | |
| | R. Oros | $, ¤ | microcystin | 1.6* | Aboal and Puig (2005) |
| | R. Cenajo | ¤ | microcystin | 3* | |
| Tunisia | D. Lebna | ¤ | microcystin-LR | 5.485 | El Herry et al. (2008) |
| Turkey | L. Kovada | $, £, ¤ | microcystin-LR | 0.73–48.5 | Gurbuz et al. (2009) |
| United States | L. Pinto | £ | microcystin-LR | 100 | Miller et al. (2010) |
| | San Francisco estuary | ¤ | microcystin-LR | 0.02 | Lehman et al. (2007) |
| | L. Doctors | £ | microcystin-LR | 1 | Stewart et al. (2006) |
| | L. Seminole | £ | anatoxin-a | 1 | |
| | – | – | cylindrospermospin | 100 | Falconer and Humpage 2006 |
| | St Johns river | £ | microcystin-LR | 0.1–31 | Williams et al. (2007) |
| | | | cylindrospermospin | 0.07–1.6 | |
| | L. Bufalo Springs | £ | microcystin-LR | 0.41–1.78 | Billam et al. (2006) |
| | L. Ransom Canyon | £ | microcystin-LR | 0.44–1.08 | |

About location: L. for lake, R. for reservoir, D. for dam. About use of water: ($) for drinking supply, (£) for recreational activities, and (¤) for agriculture (irrigation and pasture) and aquaculture. "–" absence of information.

* Concentrations are presented in $\mu g\ g^{-1}$ dry weight.

great importance of sedimentation processes in the fate of MCs in freshwaters with an amount of toxin associated to settling particles to be in the range of $mg\ d^{-1}\ m^{-2}$. But other studies reported that no more than 20% of toxins can be adsorbed on sediments (Rapala et al., 1993; Lahti et al., 1996). Furthermore, it was suggested that the removal of cyanotoxins in this process was the result of both adsorption and biodegradation (Lahti et al., 1996). Therefore, biodegradation would appear to be the main fate for most cyanotoxins in aquatic systems and the relative performance of this process would be very site specific and dependent upon local sediment characteristics and microbial activity. It was recently reported that the data generated in laboratory and field studies strongly indicate that, in shallow lakes, low persistence and natural eliminations of MCs are due to biodegradation; suggesting that sediments play a crucial role in biodegradation by continuously supplying toxin-degrading bacteria to the water column (Chen et al., 2008, 2010; Mazur-Marzec et al., 2009). However, in deep sediments, biodegradation might be limited due to anoxic conditions (Holst et al., 2003; Grützmacher et al., 2002, 2010) and sediments only bring nutrients for bacteria responsible of cyanotoxins biodegradation. Degradative heterotrophic bacteria of hepatotoxic cyanotoxins (MCs and NOD), and cytotoxins (CYN) have been found in various media, such as water columns (Jones and Orr, 1994; Cousins et al., 1996; Christoffersen et al., 2002; Hyenstrand et al., 2003; Lemes et al., 2008; Mazur-Marzec et al., 2009; Chen et al., 2010), sediments (Rapala et al., 1994; Holst et al., 2003), sewage effluents (Lam et al., 1995) or soils (Miller et al., 2001; Grützmacher et al., 2002), with specific enzymatic pathways well characterized (Bourne et al., 1996; Okano et al., 2009; Zhang et al., 2010). Several previous studies have been indicated that MCs can be degraded by aquatic bacteria identified as pertaining especially to the genus *Sphingomonas* (Bourne et al., 1996; Harada et al., 2004; Ishii et al., 2004; Maruyama et al., 2006; Manage et al., 2009). Therefore, a microcystin-degrading gene cluster, *mlr*A, B, C and D was identified in these microorganisms, sequenced and the degradation process was proposed (Bourne et al., 2001; Saito et al., 2003; Imanishi et al., 2005). In the last two decades, several other species of bacteria capable of degrading peptidic cyanotoxins were identified, *Sphingomonas* sp. strain ACM-3962 (Jones et al., 1994), *Paucibacter toxinivorans* (Rapala et al., 2005), *Sphingosinicella microcystinivorans* (Maruyama et al., 2006), *Burkholderia* sp. (Lemes et al., 2008). The most toxic congener, Microcystin-LR, was also found susceptible to breakdown by *Sphingomonas*, which initiated ring-opening and the production of a linear compound 200 times less toxic (Bourne et al., 1996). Recently, Ho et al. (2012) identified another bacterium strain (TT25) whose genome is similar to *Sphingopyxis* sp. that it is able to degrade MCs. The ability of these all species to degrade other congeners of MCs and NODs was investigated and revealed that peptides with the Adda–Arginine bond were successfully degraded while MC-LF, with Adda–Phenyalanine bond and 6(z)-Adda-MC-LR and 6(z)-Adda-MC-RR were not significantly degraded (Imanishi et al., 2005). Another Japanese *Sphingomonas* isolate, 7CY, was shown to degrade a wider range of MCs, including MC-LR, -RR, -LY, -LW, and -LF but it was unable to degrade NOD-Har a NOD analog where arginine is replaced by homoarginine (Ishii et al., 2004). Biodegradation has also been shown to be an important process for the removal of the alkaloid cytotoxin, CYN, from contaminated water (Chiswell et al., 1999; Senogles et al., 2002). By contrast, a laboratory study investigating biodegradation of CYN with bacterial communities from two water bodies in Spain, one having frequent exposure to CYN, the other rarely, has been shown that biodegradation of this toxin by an active microbial community does not take place during a 40-d (Wörmer et al., 2008). A recent study demonstrated that CYN was degraded by indigenous microbial flora in waters with a history of *Cylindrospermopsis* blooms (Smith et al., 2008). Despite isolation of many bacteria from CYN enriched cultures, only a single isolate (*Delftia* sp.) capable of degrading CYN has been obtained (Smith, 2005). However, for cyanobacterial neurotoxins there are few reports on their persistence and biodegradation compared to cyanobacterial heptotoxins, although the increasing occurrence of these toxins in surface waters. A recent study indicated that saxitoxins (STXs) are predisposed to bacterial degradation during passage through bioactive treatment plant (Kayal et al., 2008). However, this study showed that structural modification during the biological treatment resulted to decrease of the predominant C-toxins variants and an increase in GTX2 and GTX3 which are more toxic than the C-toxins. Early work by Kiviranta et al. (1991) reported the isolation of a *Pseudomonas* sp. capable of rapid degradation of anatoxin-a, with a rate of 6–30 $mg\ mL^{-1}$ per 3 d. A later study reported by Rapala et al. (1994) has been shown the removal of anatoxin-a by microbial populations isolated from water and sediments of a eutrophic, oligotrophic, and humic lake. In conclusion, the period of photodegradation of cyanotoxins is relatively long in

comparison to the degradation caused by the microbial activity. Recently, Hu et al. (2012) found that the *Bacillus* sp. strain EMB is able to completely remove 2.99 mg $L^{-1}$ of MC-RR and 2.15 mg $L^{-1}$ of MC-LR within 24 h. However, the biodegradation speed of cyanotoxins in aquatic ecosystems can be influenced by the initial concentration and nature of toxins (Edwards et al., 2008; Ho et al., 2012) and by additional factors such as the water temperature (Park et al., 2001; Ho et al., 2007a,b; Smith et al., 2008; Hoefel et al., 2009) and the bacterial community composition within the water body; not only the types of organisms present, but also their abundance (Hoefel et al., 2009; Ho et al., 2012). Hoefel et al. (2009) have demonstrated a direct relationship between the abundance of degrading organisms and the rate of degradation of MC-LR. Furthermore, although MCs are degraded by most of bacteria species, it seems that a lasting day's delay or weeks are necessary before the degradation is introduced. This result was in agreement with the conclusion of Hyenstrand et al. (2003) indicating that bacteria species have to adapt themselves at first to the cyanobacteria metabolites before the degradation of MCs becomes effective. Indeed, the results of this last study indicate a weaker degradation of the MC-LR in May compared with September where the occurrence of cyanobacteria is higher. Similarly, Smith et al. (2008) found that CYN was degraded by indigenous microbial flora in waters with a history of *Cylindrospermopsis* blooms.

The physicochemical fate and the environmental concentrations of cyanotoxins in soil have been the subject of a range of recent studies. Several classes of these toxins have been detected in field soils, and the sorption behavior and degradation and transfer to vegetables have been studied to a large extent (Morris et al., 2000; Miller et al., 2001; Chen et al., 2006b; Bibo et al., 2008; Sathishkumar et al., 2011). The use of water from sources containing cyanobacterial blooms and toxins for spray irrigation of terrestrial plants, including food crop plants presents both a harmful effect on growth and development of plants and on soil ecosystems and potential health hazards through several exposure routes, including uptake into the food chain and accumulation of toxins on the external surfaces of edible plant material. Questions, therefore, arise about the persistence of total cyanotoxins (dissolved and within the cyanobacterial cells) when reach the soil ecosystem to produce phytotoxic effects. Once reach the soil ecosystem, cyanotoxins persist in the environment, depending on the efficiency of degradation (i.e., photolysis, hydrolysis and bacterial degradation). Microcystins can persist in agriculture soils for relatively long times, with a half-life ranging between 6 and 17.8 d (Chen et al., 2006b). Jones et al. (1995) reported that scums of *M. aeruginosa* that dry on the shores of lakes may contain high concentrations of MCs for several months. Recently, Metcalf et al. (2012) found that MCs were detected in herbarium specimens of cyanobacteria which had been collected from aquatic and terrestrial environments in 11 countries throughout the world, dried, and stored at ambient temperatures in the dark for up to 170 years. Microcystins were also detected by HPLC and ELISA assays in desert crust samples from Qatar at concentrations between 1.5 and 53.7 ng $g^{-1}$ dry weight (Metcalf et al., 2012). Thus, the persistence of these toxins within dried cyanobacterial cells for long period suggests that they will be released back into the soil when re-immersed by irrigation water, particularly when cyanobacterial blooms are used in some countries as an organic fertilizer (Chen et al., 2006a,b). However, as mentioned above for aquatic ecosystems, adsorption on sediments and specially exposure to degrading bacteria may also speed up their removal from the soil.

The information on the adsorption of cyanotoxins in agriculture soil ecosystems is particularly scarce. However, adsorption of cyanobacterial hepatotoxins was measured in several batch studies to determine the applicability of bank filtration as an efficient removal strategy of these toxins from drinking water. For example, in batch experiments Miller et al. (2001) studied the adsorption of cyanobacterial hepatotoxins, MC-LR and NOD, in five soils with different physicochemical properties collected from regions around South Australia. They found that the soils with the high clay and/or organic carbon contents had the higher toxins adsorption coefficients. In similar experiments, Miller and Fallowfield (2001) found that the soils with the highest organic carbon content (2.9%) and the highest clay content (16.1%) were the most effective at removing these toxins in batch experiments. However, the sandy soil (98.5% sand) was incapable of the removal of cyanotoxins. This finding was supported by Morris et al. (2000) who reported that the clay content and its quality may be more important for the adsorption than other soil characteristics. However, Eynard et al. (2000) suggested that soil was unable to protect groundwater from cyanotoxins that originated from surface waters. Thus, it seems that cyanotoxins sorption in soils is low and could potentially result in their high bioavailability to soil organisms and plants. In several studies, it seems that the major dissipation process for cyanotoxins in soil ecosystems is mainly via microbial degradation (Miller and Fallowfield, 2001; Chen et al., 2006b). In fact, numerous soil bacteria as *Arthrobacter* sp., *Brevibacterium* sp. and *Rhodococcus* sp. are able to breakdown MCs (Manage et al., 2009). Bourne et al. (2001) observed the same thing with *Sphingomonas* sp. that possesses a gene cluster involved in the degradation of MC-LR. Furthermore, Falconer et al. (1983) and Lambert et al. (1996) conclude that sand filtration alone is unable to remove dissolved cyanotoxins. However, slow sand filters can be expected to remove 99% of dissolved cyanotoxins (Keijola et al., 1988; Grützmacher et al., 2002). This can be explained by the formation of a biofilm on top of the filter that it allows for some biodegradation of cyanotoxins in slow sand filtration. No data are available for other cyanoabcterial toxins such as neurotoxins and dermatotoxins, but some degradation may be expected, again depending on the chemical conditions of soil. In conclusion, the scarce results on the fate of cyanotoxins in soil ecosystems are very variable, which do not allow affirming with certainty the necessary time for a complete disappearance of these toxins. This variability ensues partially from used methods (e.g. studies led in laboratory with non environmental concentrations of toxins and in free-soil systems). Therefore, the fate of cyanotoxins in soil ecosystems will require more studies before we are capable to formulate an opinion on their persistence and uptake into the food chain.

## 5. Phytotoxicity effects of cyanotoxins

The information on the effects of cyanotoxins on non-target organisms in the terrestrial environment is particularly scarce. However, despite the impressive amount of information on their toxicity on mammals compiled during the last two decades, there are still serious gaps in the knowledge about the phytotoxicity of these toxins. The phytotoxic effects of cyanotoxins on higher plants were firstly focused on aquatic photoautotrophic organisms (algae and macrophytes) that are naturally exposed to cyanotoxins (Harper, 1992; Papke et al., 1997; Weiss et al., 2000; Yu et al., 2000; Ikawa et al., 2001; Pietsch et al., 2001; Mitrovic et al., 2004; Ha and Pflugmacher, 2013). Since few years, scientists were also interested by the effect of these toxins on terrestrial plants because, irrigation waters from sources containing cyanobacterial blooms and toxins are generally used without treatment for spraying agricultural crops and plants that might, therefore, induce a food chain contamination with a considerable health risk and potential economic losses.

### 5.1. Neurotoxins

The cyanobacterial neurotoxins have not received more research attention than have cyanobacterial hepatotoxins. This is a consequence of the many livestock deaths caused by cyanobacte-

rial species producing hepatotoxic microcystins and their more widespread occurrence rather than species producing neurotoxins (Ettoumi et al., 2011). In addition, the recent inclusion of microcystin-LR as a toxic chemical in the World Health Organisation (WHO) drinking water guidelines has further accelerated investigation of the toxic effects of microcystins on mammals and vegetables rather than cyanobacterial neurotoxins. Therefore, there are only few studies reported in the literature on the effects of cyanoabcterial neurotoxins on crops and plants. Mitrovic et al. (2004) were exposed the free-floating aquatic plant *L. minor* and the filamentous macroalga *Chladophora fracta* to anatoxin-a at 0.1–25 μg $L^{-1}$ under laboratory conditions for 4–7 d. They found in both organisms significantly increase of peroxidase activity after 4 d exposure at 25 μg $L^{-1}$ but not at lower concentrations. After 7 d exposure to this neurotoxin significant increase of GST activity and reduction of photosynthetic oxygen production were observed at 5 and 20 μg $L^{-1}$ but not at lower concentrations in *L. minor*. In addition, Ha and Pflugmacher (2013) reported that this alkaloid neurotoxin at an environmentally relevant concentration (15 μg $L^{-1}$), induced phytotoxic effects on the submerged aquatic macrophyte *Ceratophyllum demersum*, mediated by oxidative stress. Recently, Esterhuizen-Londt et al. (2011) investigated in *in vitro* study the effect of BMAA at different environmentally concentrations (0.5, 1, 5, 50 and 100 μg $L^{-1}$) for 24 h on the oxidative stress responses of the macrophyte *C. demersum*. The most pronounced effects found were activity-inhibiting effects on all the oxidative stress response enzymes at all exposure concentrations. However, enzymes not related to oxidative stress response were not affected by the BMAA in these experiments. For other neurotoxins, the literature search did not yield any results.

### 5.2. Hepatotoxins

The effects of cyanoabcterial toxins on photoautotrophic organisms have been most intensively studied for MCs, in line with their abundance and their mode of action. First experiments were focused on the ability of these hepatotoxins to act as general allelopathic compounds against planktonic microalgae, macroalgae and macrophytes in aquatic ecosystems. The allelopathic effects of *Aphanizomenon* and other cyanobacteria bloom formers on chlorophyte species are early documented in several studies (Lefevre et al., 1950; Tassigny and Lefevre, 1971; Boyd, 1973). Subsequently, Ikawa et al. (2001) and Papke et al. (1997) observed that cyanobacterial metabolites can induce the growth inhibition of the green alga *Chlorella pyrenoidosa* and the photosynthesis of other cyanobacteria species, respectively. Similarly, Sukenik et al. (2002) found that *Microcystis* sp., a MCs producer, severely inhibited the growth of the freshwater dinoflagellate *Peridinium gatunense* in mixed laboratory cultures which was attributed to the excretion of allelopathic substances rather than to successful competition for nutrients. Hu et al. (2005) found that the growth of *Synechococcus elongatus* was reduced by 53.6% after 6 d of exposure to 100 μg $L^{-1}$ of MC-RR suggesting that oxidative stress manifested by elevated ROS levels and MDA contents might be responsible for the toxicity of MC-RR to this species. Moreover, Singh et al. (2001) demonstrated that MCs are strongly algicidal and point to the possibility that they may have an important role in establishment and maintenance of toxic blooms of *M. aeruginosa* in freshwater ecosystems. Valdor and Aboal (2007) demonstrated the inhibitory effect of both cyanobacterial extracts and pure MCs on the growth of microalgae. Bártová et al. (2010) examined effects of semipurified *Microcystis* extract containing MCs (0.2–20 nM) on age-induced cell differentiation of the filamentous cyanobacterium *Trichormus variabilis* and they found that heterocyst and akinete formation was significantly decreased after exposure to extract containing 2 or 20 nM of MCs within 10 d of exposure. Recently, Perron et al. (2012) evaluated the effect of four microcystins standards (variants MC-LF, -LR, -RR, -YR) at different concentrations (0.01–10 μg $mL^{-1}$) and 0.01, 0.1, and 1 μg $mL^{-1}$ equivalent microcystins extracted from *Microcystis aeruginosa* (CPCC299), which is known to produce mainly MC-LR, on the fluorescence of four green algae (*Scenedesmus obliquus* CPCC5, *Chlamydomonas reinhardtii* CC125, *Pseudokirchneriella subcapitata* CPCC37 and *Chlorella vulgaris* CPCC111) and how they can affect the flow of energy through photosystem II. Their results showed that MCs affect the photosynthetic efficiency and the flow of energy through photosystem II from 0.01 μg $mL^{-1}$ within only 15 min and that MC-LF was the most potent variant, followed by MC-YR, -LR and -RR.

It was also noticed that in eutrophic freshwaters dominated by cyanobacteria, a decrease in species diversity and in the growth of macrophytes often occurs (Harper, 1992; Weiss et al., 2000; Yu et al., 2000; Pietsch et al., 2001). Casanova et al. (1999) found that the abundance and the variety of macrophytes are reduced in the presence of cyanobacterial blooms. In 1986, Kirpenko showed for the first time the inhibition growth of water plants *Elodea* and *Lemna* by MCs isolated from a natural bloom. This allelopathic action was recently confirmed by Weiss et al. (2000) further to the coculture of the plant *Lemna minor* with the cells of *M. aeruginosa.* Moreover, Pflugmacher (2002) revealed that MC-LR induces allelopathic effects on the aquatic macrophytes such as *C. demersum* and *Myriophyllum spicatum*, resulting in growth inhibition, reduction in photosynthetic oxygen production, and changes in pigment pattern. Jang et al. (2007) found by examining cyanobacterial toxin production in response to direct exposure to an axenically cultured aquatic plant (*Lemna japonica* Landolt) using two toxic monoclonal strains of *M. aeruginosa* Küzing (NIES strains 103 and 107) that reciprocal allelopathic responses have been observed between these two species *Microcystis* and *Lemna*. In several other studies, it occurred that MCs have the potential to exert toxic effects on growth and physiological processes, which all might be related to the inhibition of protein phosphatase activity or oxidative stress in aquatic moss (Wiegand et al., 2002) and in higher aquatic plants such as *Lemna gibba* (Saqrane et al., 2007), *Lemna genus* (Mitrovic et al., 2005), *L. japonica* (Jang et al., 2007), *Spirodela oligorrhiz* (Romanowska-Duda and Tarczyńska, 2002), *Phragmites australis* (Yamasaki, 1993; Máthé et al., 2009; Jámbrik et al., 2011), and *C. demersum* (Pflugmacher, 2004).

There are also several indications that terrestrial plants, including food crop plants, can be altered by MCs present in irrigation waters, resulting principally to their serine/threonine phosphatases inhibition and reactive oxygen species (ROS) production. Sheen (1993) found that the marine phycotoxin okadaic acid, a potent inhibitor of serine/threonine protein phosphatases like MCs, efficiently blocks chlorophyll accumulation induced by light in etiolated maize leaves. It seems also that this phycotoxin blocks root hair growth and alter cortical cell shape of *Arabidopsis thaliana* L. at 3 nM (Smith et al., 1994). Takeda et al. (1994) found that okadaic acid and MC-LR, inhibitors of protein phophatases type 1 and 2A block the sugar-inducible gene expression in petioles of sweet potato *Ipomoea batatas*. Similarly, Siegl et al. (1990) reported that in *in vivo* these toxins prevented the light-induced activation of sucrose-phosphate synthase (SPS) that is generally activated by dephosphorylating by protein phosphatase 2A, and decreased sucrose biosynthesis and $CO_2$ fixation in spinach leaves. Yin et al. (2005) reported that MC-LR at 5 mg $L^{-1}$ is able to cause oxidative damage resulting in lipid peroxidation and decrease of glutathione GSH content and increases of superoxide dismutase (SOD) and catalase (CAT) activities on *A. thaliana* cells. Later, Stüven and Pflugmacher (2007) provide further evidence that cyanobacterial toxins as well as cyanobacterial crude extract containing MC-LR induce oxidative stress response in *Lepidium sativum* seedlings, manifested by lipid peroxidation, elevation of alpha- and beta-

tocopherol concentrations and elevated activities of antioxidative enzymes like the glutathione peroxidase, glutathione S-transferase and glutathione reductase. El Khalloufi et al. (2012) showed that 30 d exposure of *Lycopersicon esculentum* to a cyanobacterial crude extract containing 2.22–22.24 μg MCs $mL^{-1}$ caused enhancement on peroxidase activity and phenolic content indicated that the extract caused an oxidative stress. The exposure of rice plants (*Oriza sativa*) to toxic *M. aeruginosa* cyanobacterial extracts containing 50 μg MC-LR $L^{-1}$ resulted in a significant increase in the GST activity in leaves of this plant (Prieto et al., 2011). Therefore, by acting as protein phosphatase inhibitors and inducers of ROS production, MCs could be involved in several physiological and molecular processes in higher terrestrial plants.

### 5.3. Cytotoxic alkaloids

Cylindrospermopsin, a protein synthesis inhibitory cyanoabcterial cytotoxin also led to a clear growth inhibition and anatomy modification through the alteration of microtubules organization of the common reed *P. australis* at concentrations 0.5–40 μg $mL^{-1}$ (Beyer et al., 2009). Previous study demonstrated that CYN inhibited the growth of *Sinapsis alba* mustard seedlings at 18.2 μg $mL^{-1}$ (Vasas et al., 2002). Short term exposure of rice plants (*Oriza s.*) to toxic *A. ovalisporum* cyanobacterial extracts containing 0.13 μg CYN $L^{-1}$ can lead to an increase of oxidative stress (increase in GST and GPx activities). Moreover, longer exposure periods can lead to tissue necrosis (loss of tissue fresh weight) concomitant with the oxidative stress. In addition, the plant exposure to a mixture of *A. ovalisporum* and *M. aeruginosa* cell extracts containing 0.13 μg CYN $L^{-1}$ and 50 μg MC-LR $L^{-1}$, respectively, resulted in a significant increase in the GST and GPx activities, suggesting a synergistic effect of both extracts (Prieto et al., 2011).

## 6. Bioaccumulation of cyanotoxins in vegetable foods and consequences on animals and human health

In aquatic ecosystems, several studies have been reported the bioaccumulation of cyanotoxins in common aquatic vertebrates and invertebrates, including zooplankton, mollusks and crustaceans, and fish, which pose a potential risk to both animal and human health if such aquatic animals are consumed (Ibelings and Chorus, 2007; Ettoumi et al., 2011). However, their ability to enter the food chain *via* agricultural crops has not been thoroughly investigated to date. Questions, therefore, arise about the health significance of spray irrigation of crops with water from sources containing cyanobacterial blooms and toxins. Nevertheless, several studies have been shown the accumulation potential of cyanotoxins in aquatic vegetable organisms, suggesting that terrestrial plants, including food crop plants, can also take up these toxins. Mitrovic et al. (2005) reported that the filamentous alga *C. fracta* accumulates MC-LR at a rate of 8 ng $g^{-1}$ $d^{-1}$. In addition, few amounts of MCs were detected in *C. vulgaris* and *Scenedesmus quadricauda* cells only during the first 3 d of exposure, but not during the remaining period of the experiment, suggesting a possible biotransformation of MCs in these algae (Mohamed, 2008). The emergent reed plant *P. australis* showed an apparent distribution of MC-LR in the different parts of the plant, after exposure to this toxin at 0.5 μg $L^{-1}$ with highest uptake was detected in the stem and then the rhizome (Pflugmacher et al., 2001). In addition, *Lemna minor* has also been shown to accumulate MC-LR up to a concentration of 0.2887 ± 0.009 ng $mg^{-1}$ wet wt plant material, after 5 d of exposure to this toxin at 20 μg $L^{-1}$ with an accumulation rate equivalent to 58 ng $g^{-1}$ $d^{-1}$ (Mitrovic et al., 2005). However, Saqrane et al. (2007) reported that *L. gibba* could take up and biotransform microcystins. The chronic exposure of plant led to dose-dependent MCs accumulation which reached 2.24 μg $g^{-1}$ dry weight after being exposed to 0.3 μg $mL^{-1}$ of MCs (Saqrane et al., 2007). Recently, it has been shown that collected water chestnut (*Trapa natans*) from Lake Tai accumulated MCs at highest level up to 7.02 ng $g^{-1}$ dw (Xiao et al., 2009).

Terrestrial plants could be exposed to cyanobacterial toxins via the use of eutrophic water that may contain cyanobacterial blooms and toxins from irrigation and, therefore, they can take up cyanotoxins. Peuthert et al. (2007) have been reported that MC-LR could be absorbed by roots and be translocated from roots to shoots in seedlings of eleven agricultural plants. A second study by Crush et al. (2008) that used different species too, revealed a high level of MCs accumulation in lettuce (*L. sativa*) exceeding the tolerable daily intake of 0.04 μg $kg^{-1}$ of body weight $d^{-1}$ recommended by the World Health Organization (Sivonen and Jones, 1999). However, the most of these studies have been performed in hydroponic conditions where the roots have been in direct contact with the toxin solutions and can, therefore, overestimate the bioaccumulation rate. In our knowledge the only study reported in the literature that was realized in soil showed that MC concentrations in roots did not exceed the tolerably limit, however, the concentration of MCs in aerial parts of the plant are not determined (Järvenpää et al., 2007). Both the roots and shoots of rice were reported to accumulate MC-LR in a laboratorial study (Chen et al., 2004). In addition, a recent study by Chen et al. (2012) reported for the first time the accumulation of MC-LR in rice grains harvested from Lake Taihu in China. However, the concentration of MC-LR detected in rice grains was very low and thus may not pose a threat to human health currently. In addition to the possibility of internal accumulation of MCs, irrigation may lead to accumulation of toxins on the external surfaces of edible plant materials when the contaminated water dries on the plant surface between irrigation periods or when the water becomes trapped in the centers of, for example, salad plants. In fact, Codd et al. (1999) have been reported that colonies and single cells of *M. aeruginosa* and microcystins were retained by salad lettuce after growth with spray irrigation water containing the microcystin-producing cyanobacteria. Recently, Kittler et al. (2012) reported that treatment of *Brassica oleracea* var. *sabellica*, *Brassica juncea*, and *S. alba* under varying experimental conditions showed significant CYN uptake, with CYN levels ranging from 10% to 21% in the leaves compared to the CYN concentration applied to the roots (18–35 μg $L^{-1}$). These results suggest that crop plants irrigated with CYN-containing water may represent a significant source of this toxin within the food chain. However, further research is needed into the uptake and fate of microcystins and other cyanobacterial toxins by food plants and the persistence of these toxins in the edible plant materials.

## 7. Conclusion and future directions

This review has established that cyanobacterial cells and toxins can be associated with crop plants after spray irrigation with water containing these agents. Therefore, the use of water from sources containing cyanobacterial blooms and toxins for spray irrigation of crop plants may not only inhibit growth of plants, but also can induce a food chain contamination with a considerable health risk and potential economic losses. Several studies have been shown that cyanotoxins could be absorbed by roots, transported to shoots, and then be translocated to grains and/or fruits. Nevertheless, the concentration of MC-LR detected, for example, in rice grains was very low and thus may not pose a threat to human health currently. Cyanotoxins could be partially metabolized during the long distance transportation from roots to grains or fruits, which may resulted in the lower level of cyanobacterial hepatotoxins type

microcystins detected in rice grains. In addition, MCs could bind to serine/threonine phosphatases during transport and thus could also affect their accumulation in grains and fruits. Therefore, further investigations are needed into the uptake and fate of microcystins and other cyanobacterial toxins by food plants during the totally period of vegetative and fruit development.

However, there are gaps remaining concerning information on the future of cyanotoxins in soil in term of speciation, persistence, mode of degradation and impact on biological life in soils. The results of many existing tests and particularly laboratory studies on phytotoxicity of cyanotoxins are done in soil-free systems and using non realistic environmental concentration of toxins. Therefore, they are difficult to compare to field studies because both abiotic (*e.g.*, soil conditions) as well as biotic (composition of the degrading biological community) factors can influence the outcome of such studies. In order to assess the relevance of phytotoxicity of cyanotoxins and their bioaccumulation in crop plants in the terrestrial environment, further research seems thus appropriate.

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