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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A B S T R A C T

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.

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

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

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 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	

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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

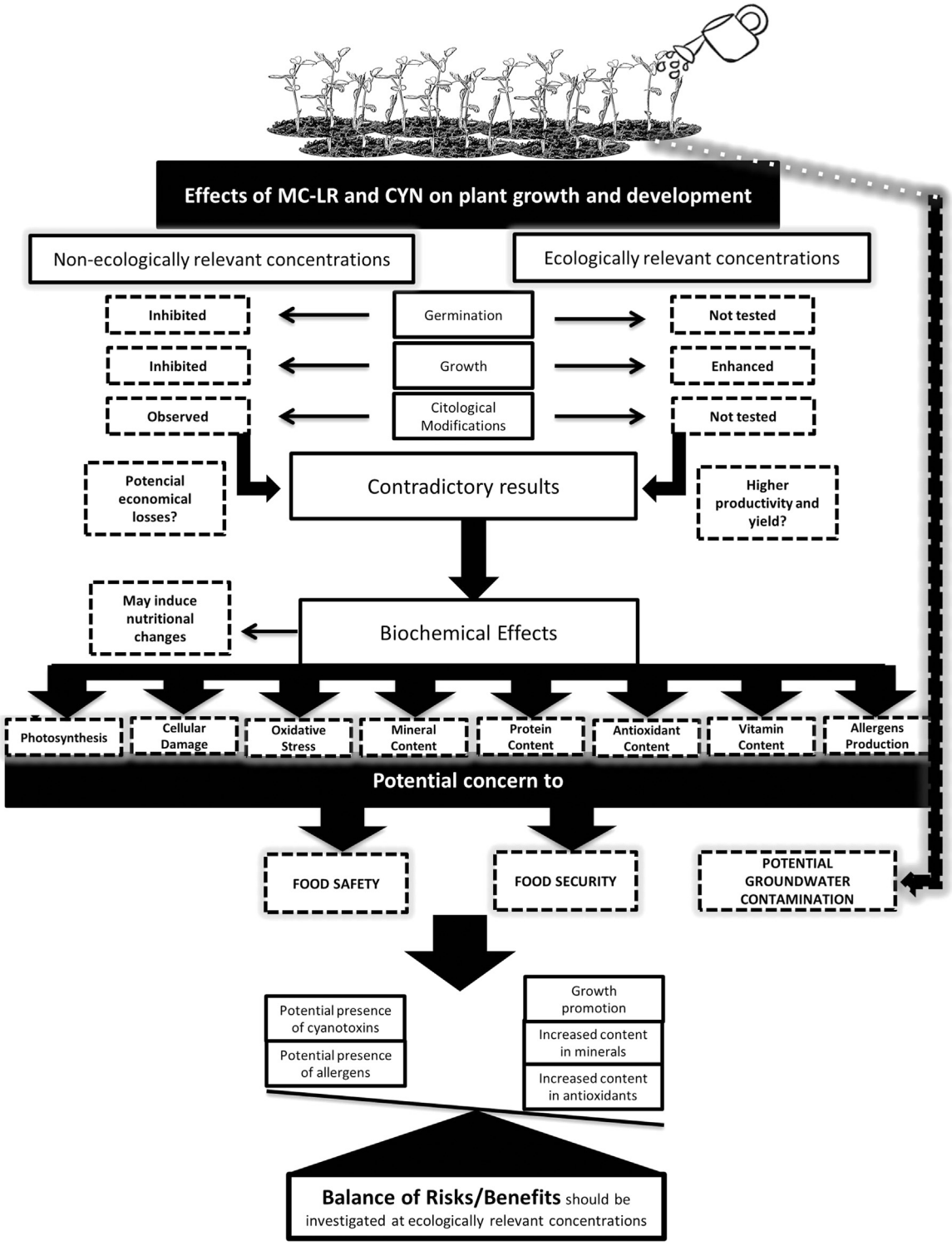

Fig. 1. Balance of the effects of MC-LR and CYN on plant growth, development and biochemical processes as well as human health implications considering ecologically relevant concentrations.

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.

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