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# **Cyanobacterial Toxins Emerging Contaminants in Soils: A Review of Sources, Fate and Impacts on Ecosystems, Plants and Animal and Human Health**

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Additional information is available at the end of the chapter

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## **Abstract**

In the last few decades, there has been a growing interest in the occurrence of cyanotoxins and their potential toxicity in the aquatic environment. However, the used of dried toxic cyanobacteria cells as fertilizer or the used of surface water contaminated with cyanotoxins for agricultural crops irrigation can be source of soil contamination. In addition, surface waters presenting dense toxic blooms of cyanobacteria and used for agricultural practices are not controlled and are often used without prior treatment. Once in soil, cyanotoxins may be transported again to water bodies by leaching, runoff and drainage processes or can be accumulated in soils and, therefore, may cause contamination of vegetation by absorption from soils or by surface pollution of plants. In addition to possible effects on human health, elevated levels of cyanotoxins in soils can negatively affect plant vigour, animal health, microbial processes and overall soil health. Consequently, the focus of this chapter of soil contamination is cyanotoxins as contaminants of emerging concern in the soil, identifying sources of contamination, determining their fate and effects in the soil, and understanding their bioaccumulation in agricultural plants used for feed and food and consequences on animal and human health.

**Keywords:** cyanotoxins, microcystins, soil, fate, phytotoxicity, plant, bioaccumulation

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## **1. Introduction**

The occurrence of toxic cyanobacterial blooms has become increasingly frequent throughout freshwater bodies in the world. To date, factors identified as contributing towards their global

expansion included increased nutrient inputs, transport of cells or cysts via anthropogenic activities and/or migratory birds, increased aquaculture production and/or overfishing, altering food webs and permitting harmful species to dominate algal communities [1, 2]. It has also been shown that an increase in surface water temperatures and CO<sub>2</sub> concentrations due to changing global climate could play a role in the proliferation of cyanobacterial blooms [3–6] and may also affect the strain composition within a cyanobacterial community and consequently change the concentration of cyanotoxins, such as microcystins [7, 8]. The problems associated with cyanobacterial blooms in fresh waters are diverse, from the environment asphyxiation due to excessive consumption of oxygen to purely aesthetic problems in recreational areas when the blooms are a colourful and often smelly scum on the surface of the water [9]. To these problems possibly affecting the economic development of specific areas, productions of cyanotoxins as secondary metabolites can represent a human and animal health threat [10]. Humans can be indeed exposed to cyanotoxins through both direct routes, including contamination of drinking and recreational waters, and indirect routes, including food supplements made from cyanobacteria or through consumption of contaminated food after toxin accumulation in fish, shellfish and other aquatic organisms, as well as in vegetables after using contaminated water for irrigation [11]. In the case of use of surface waters contaminated by cyanotoxins for the supply of drinking water, the potential health risks are managed at the level of the treatment station. In general, a strengthening of clusters of treatment and a complete operation and correct of this station would avoid any risk of contamination of the drinking water [12–20]. By contrast, the raw water used in irrigation often comes from a natural water body or an artificial pond for agricultural

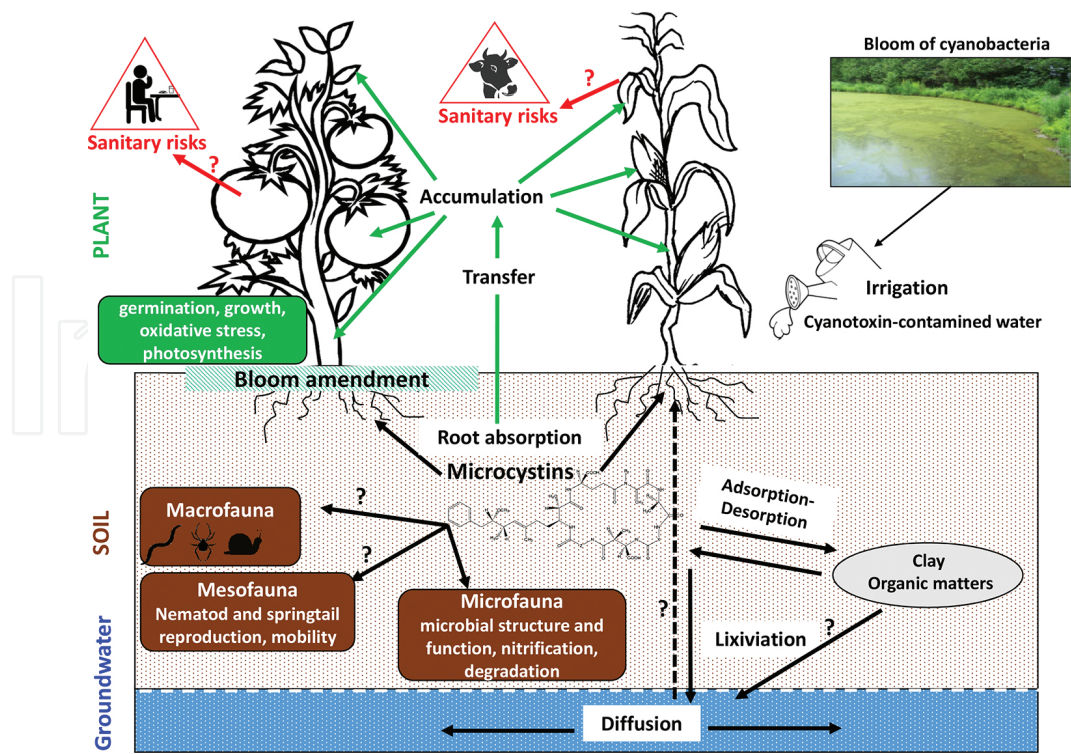


Figure 1. Schematic microcystins fate process in soil-plant systems and their impacts on human and animal health.

purposes and is not subject to any control or supervision. Consequently, the presence of cyanotoxins in irrigation water may cause toxic effects in the biological activity of the soil and in edible plants presenting therefore, a threat to animals and humans health (**Figure 1**). In fact, many studies have shown that the presence of microcystins in the irrigation water can have a considerable impact on the germination, growth and development of cultivated plants (reviewed in Ref. [10]). However, the fate of these toxins in the soil and their effects on the microfauna (protozoa, nematodes) and the microflora (bacteria, fungi and algae) of cultivated soils are scarce. This chapter aims to provide a current description of knowledge of cyanotoxins present in the irrigation water and their effects on soil and consequences on animals and public health.

## 2. Cyanotoxins and their producers

Some cyanobacteria species belong essentially to the genera *Microcystis*, *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Oscillatoria*, *Cylindrospermopsis* and less often *Gomphosphaeria*, *Coelosphaerium*, *Gloeotrichia*, *Nodularia* and *Nostoc* are known to biosynthesize a diversity of alkaloid and peptide cyanotoxins that have been suggested to pose threats to human and environmental health worldwide [9, 21–24]. These cyanotoxins are essentially endotoxins that can be released in the environment following a cellular lyse during the senescence phase [25] or following treatment of cyanobacterial blooms with algacides [26]. They can be classified into four families according to the organs on which they act: hepatotoxins (liver), neurotoxins (nervous system), cytotoxins (liver and kidneys) and dermatotoxins (irritant toxins). Hepatotoxins are divided into two groups: microcystins, cyclic heptapeptide hepatotoxins (MW 900–1200), that are regarded as the most frequently occurring and widespread of the cyanotoxins with more than 100 MC variants already reported [27, 28] and nodularins (MW 800–900) composed of five amino acids with only nine different natural analogues have been characterized [29–32]. Both microcystins and nodularins are water-soluble molecules and their cyclic structure provides them a high chemical stability [22]. Their toxicity resulted on a potent and specific inhibition of serine/threonine protein phosphatases [33]. They have also known to induce oxidative stress [34]. Cyanobacterial neurotoxins (for review, see Ref. [35]) are divided into three groups: anatoxins that are neuromuscular junction blocking agents [35], saxitoxins that block nerve cell voltage-gated sodium channels [36] and the unusual non-protein neurotoxic amino acid L-beta-N-methylamino-L-alanine (BMAA) that has been associated to the neurological disorder amyotrophic lateral sclerosis/Parkinsonism dementia complex (ALS/PDC) among the indigenous Chamorro people of Guam and other Marianas islands [37]. Its neurotoxicity may be mediated via glutamate regulation [38]. 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 [39]. Cytotoxins are represented by the hydrophilic alkaloid cytotoxin, cylindrospermopsin (MW 415), that has been first isolated from the filamentous *Cylindrospermopsis raciborskii* [21], and further from other species *Aphanizomenon ovalisporum* [40, 41], *Anabaena bergii* [42], *Umezakia natans* [43] and *Raphidiopsis curvata* [44]. It inhibits the synthesis of protein, resulting in a wide spread necrosis

of the tissues of many organs such as liver and kidneys [45–47]. Two structural variants of cylindrospermopsin (7-epicylindrospermopsin and deoxycylindrospermopsin) have been characterized so far from bloom samples and isolated strains of cyanobacteria [41, 44, 48]. The dermatotoxins, irritant toxins such as lipopolysaccharides (LPS) commonly known endotoxins, are major components of the cell wall in most Gram-negative bacteria including cyanobacteria. They can elicit irritant and allergenic responses in human and animal tissues with contact [49–51].

### 3. Sources and occurrence of cyanotoxins in the soil

The main source of contamination of soils by cyanotoxins is by using cyanotoxin-contaminated water for agricultural purposes. Among the cyanobacterial toxins, microcystins are the most widespread group with microcystin-LR (MC-LR) the more toxic and the main congener detected in freshwaters [10]. Recently, concerns are also focused in the increasing occurrence of the cytotoxic cylindrospermopsin in temperate areas [52]. However, cyanobacterial neurotoxins are less reported in the literature and studies regarding their effects on organisms of soils and plants are relatively scarce. The concentrations of microcystins in the surface water are generally comprised between 1 and 100  $\mu\text{g L}^{-1}$  [10] and the use of this microcystin-contaminated water for agricultural purposes has already been reported in several countries such as Morocco [53], Finland [54], Spain [55], New Zealand [56], Algeria [57], Australia [58], Tunisia [59], Turkey [60], Saudi Arabia [61], India [62], China [63] and Guatemala [64]. In addition to the contamination of soils by dissolved cyanotoxins and with the strong occurrence of cyanobacterial blooms worldwide, a strong quantity of cyanobacterial biomass (from thousand to million tons) is removed from water and discharged directly into croplands and forest land without another treatment [65]. This alternative represents a possible source of soil pollution with cyanotoxins. Another source of soil contamination by cyanotoxins consists of direct application of cyanobacterial biomass as an organic fertilizer as in China [66, 67]. In fact, since 1970s, the cyanobacteria were known for their interest in rice culture, as a biofertilizer. In wetland rice and wheat crops, free living cyanobacteria allowed nitrogen fixation to supplement soil nitrogen [68–70]. Cyanobacterial and rhizobacterial associations are used with the objective to increase soil fertility and crop yields, but the cyanobacteria and their secondary metabolites represent also interesting properties and can be involved as natural biocide or bio-control agents (see review in Ref. [71]). In a recent study Han et al. [72], they related the use of algae waste as an organic fertilizer after composting. This process can allow the degradation of 90–95% of the total microcystins containing in cyanobacteria between 1 and 35 days [73, 74]. The microbial degradation of cyanotoxins, during composting, may be due to the diversity of microorganisms present, the conditions of composting and the type of cyanotoxins present in the bloom, as observed for microcystins by Dawson [75] and Kormas and Lymperopoulou [76]. In addition, several studies reported that the presence of cyanotoxins in *biological soil crust* (biocrust) samples in arid soils can be considered as another source of cyanotoxin-contaminated soils [77–79].



## 4. Fate and transport of cyanotoxins in agricultural systems

### 4.1. Persistence in the soil and adsorption in particles

The most abundant cyanotoxins, microcystins, have a cyclic structure that provides a high chemical stability in the environment. Once these toxins are present in the soil, they can be removed according to various processes such as photochemical degradation by UV and biodegradation by some bacteria species [10, 80–84]. The photochemical degradation of microcystins can last from 2 to 6 weeks [85, 86] in freshwater. But in the soil, this process was not studied, however as observed in water it depends on the adsorption on soil particles that is more important than in water. In fact, numerous studies on sediments and soil particles showed that the adsorption induced a diminution of photochemical degradation of microcystins [83]. The time of total degradation of MC-RR in cropland should be about 6 days according Bibo et al. [87], whereas Chen et al. [67] founded a relatively long time of microcystins persistence with a half-life ranging between 6 and 18 days. Another study, where the scums of *Microcystis aeruginosa* were dried on the shores of lakes revealed the persistence of high concentrations of microcystins for several months [88]. The results obtained by Miller et al. [89], on five soils with different physicochemical properties, showed the role of clay and organic carbon contents for microcystin-LR (MC-LR) and nodularin (NOD) adsorption. In fact, Miller and Fallowfield [90] found in batch experiments that the soil with the highest concentrations of organic carbon and clay content 2.9 and 16.1%, respectively, was the most effective at removing these toxins in comparison to the sandy soil. These results were supported by Morris et al. [91] works, who reported that sandy soil (98% sand) was incapable of removing microcystins; however, they confirmed the role of clay content and the clay quality for their adsorption. Consequently, the adsorption in soil particles depends on soil properties and the quantities of cyanotoxins brought. A laboratory study on cropland soil showed an adsorption of MC-RR from 3750 to 30,000  $\mu\text{g kg}^{-1}$  [87]. In pound used to stock cyanobacterial bloom, the concentrations of adsorbed microcystins attained 65–200  $\mu\text{g kg}^{-1}$  DW, whereas in China crop fields, the concentration after amendment was 6  $\mu\text{g kg}^{-1}$  DW [65]. In addition, Chen et al. [67] reported that the adsorption mechanism of microcystins in soil is also due to chemical binding with the metal ions on the surface of particles. Therefore, with the possible adsorption onto soil particles, microcystins could be accumulated in soil for long times. Indeed, Corbel et al. [92] detected microcystins in soil in concentrations ranging from 1.3 to 3.9  $\mu\text{g MC-LR equivalent kg}^{-1}$  (dry weight) after 90 days of silty-soil irrigation with water containing dissolved cyanobacterial extract containing 100  $\mu\text{g equivalent MC-LR L}^{-1}$ . These results corroborate with an earlier study done by the same research team where they reported that the half-life of  $^{14}\text{C}$ -MC-LR exceeded 60 days in the same agricultural soil [93]. However, in this last study the authors reported that only less than 14% of  $^{14}\text{C}$ -MC-LR were adsorbed in soil particles, suggesting that a part of this toxin could be biodegraded [93]. In fact, it seems that the major dissipation process of microcystins in the soil is mainly via microbial degradation [67, 90, 94, 95]. Cylindrospermopsin can persist in the water for long periods because it has a very low photodegradation rate under natural conditions [81]. However, all the studies performed with

cylindrospermopsin were carried out in a soil-free cultivation system, and therefore the persistence of this toxin in agricultural soils was not considered.

#### 4.2. Transport and uptake into biota and infiltration in groundwater

As described above regarding the microcystins adsorption in cropland soils, it is suggested that the adsorption of these toxins is generally low, which can therefore potentially result in their higher bioavailability for plants and the groundwater contamination due to infiltration into the soil. Consequently, Eynard et al. [96] suggested that the soil was unable to protect groundwater contamination by microcystins. Chen et al. [67] reported that microcystins can migrate from the surface to deeper layers of the soil following precipitation, leading to possible groundwater contamination. In a recent study, Corbel et al. [93] showed that when the radiolabeled  $^{14}\text{C}$ -MC-LR was introduced in a column of silty-sand agricultural soil, it underwent a weak microbial mineralization under aerobic conditions and therefore the large amounts of the toxin remained in soil aqueous extracts. In addition, the authors reported that the lixiviation of this toxin by  $\text{CaCl}_2$  was even stronger than soil application was recent. These results were confirmed by other environmental measures, where microcystins were found in groundwater [61, 65]. For example, Chen et al. [65] found a concentration of  $2.5 \mu\text{g L}^{-1}$  in lixivate water that was higher than the WHO recommendation in drinking water ( $1 \mu\text{g L}^{-1}$ ). The risk associated with the underground stock in water is the long-time persistence of toxins, in result of low microbial degrading activity. In fact, Holst et al. [97] did not detect any degradation of microcystins in groundwater maintained under oxic and anoxic conditions after a 100-day period. The toxins present in the soil solution are also available for soil organisms' uptake such as plants. For example, Pflugmacher et al. [98] demonstrated a rapid uptake of  $^{14}\text{C}$ -MC-LR by aquatic plant (*Phragmites australis*). A recent study established the transfer of MC-LR from agricultural soil contaminated with radiolabeled MC-LR ( $18 \text{ mg } ^{14}\text{C}\text{-MC-LR kg}^{-1}$ ) to tomato seedling, with a final concentration of  $6 \mu\text{g MC-LR g}^{-1} \text{ FW}$  [93]. Several other studies reported an uptake of microcystins by plant roots and a presence of these toxins in shoots and leaves after culture on sand or agricultural soils [61, 65, 92, 99–102]. Concerning the other cyanotoxins, less detected in the surface waters, the soil-plant transfer data are scarce. However, Prieto et al. [103] reported the uptake of cylindrospermopsin by the roots of *Oryza sativa* plants. In a recent study, Contardo-Jara et al. [104] reported the transfer of the neurotoxin  $\beta$ -N-methylamino-L-alanine by *Triticum aestivum* in roots and shoots after irrigation with contaminated water at  $100 \mu\text{g L}^{-1}$ .

## 5. Impacts of cyanotoxins on soil organisms

### 5.1. Microorganisms

Secondary, metabolites produced by cyanobacteria seem to have several activities as antiviral, antifungal and antibacterial [71]. In aquatic environments, several studies revealed an inhibition of bacterial growth after 8 days of exposure to cyanobacterial extract containing microcystins or pure microcystin standards [105]. In the same way, Giaramida et al. [106]

reported that the exposure to cyanobacterial extract containing microcystins induced changes in structure and physiology of bacterial communities. The measure of arylsulfatase, phosphatase, urease and  $\beta$ -D-glucosidase activities in the soil, after irrigation with cyanobacterial extract of *M. aeruginosa* (PCC7820) diluted between 5 and 100  $\mu\text{g}$  equivalent MC-LR  $\text{L}^{-1}$  during 14 or 90 days, revealed an absence of the alteration of the activity of these enzymes linked to sulphur, phosphorus and nitrogen mineralization and cellulose degradation, respectively [107, 108]. In contrast, these studies revealed a stimulation of the potential of nitrification that was positively correlated to an increase in the abundance of ammonia-oxidizing bacteria, whereas the ammonia-oxidizing archaea were not impacted. In a recently study, El Khalloufi et al. [109] highlighted the effects of cyanobacterial extract containing microcystins on soil microorganisms from the rhizosphere of *Medicago sativa*. The authors exposed *M. sativa* to 100  $\mu\text{g}$  equivalent MC-LR  $\text{L}^{-1}$  during 30 days at three times a week and a pyrosequencing analysis was further established to characterize the bacterial community of the rhizosphere. The results revealed fluctuations with an increase in *Betaproteobacteria* and a decrease in *Gammaproteobacteria* proportion. Furthermore, cyanobacterial extract containing microcystins used for irrigation seemed to be toxic towards *Actinobacteria*, *Gemmatimonas*, *Deltaproteobacteria* and *Gammaproteobacteria*, however other groups as *Clostridia*, *Opitutae* and bacteria related with *Betaproteobacteria*, were stimulated [109]. However, Lahrouni et al. [110, 111] reported that rhizobia-*Vicia faba* symbiosis was not impacted by microcystins. Nevertheless, several studies revealed the presence of heterotrophic bacteria in the soil containing a microcystin-gene cluster, *mlrA*, B, C and D essential for degradation of microcystins [89, 94, 95, 112]. For example, some species of the proteobacteria belonging to the genera *Sphingomonas*, *Methylobacillus* and *Paucibacter* are known to degrade microcystins [10, 76]. Additionally, Jia et al. [113] showed that a fungus, *Trichaptum abietinum*, was able to degrade microcystins. However, no studies have yet examined the effects of cylindrospermopsin and neurotoxins in soil microorganisms.

## 5.2. Invertebrates

The impact of cyanotoxins on aquatic invertebrates was well documented (for review, see Ref. [114]). However, the effects of these toxins on soil invertebrates are scarce. The effects of microcystins on soil nematods *Ceanorhabditis elegans* were studied by Li et al. [115, 116] and Holajjer et al. [117]. After exposure to 1  $\mu\text{g}$  MC-LR  $\text{L}^{-1}$ , a reduction in lifespan, a delay of development, an increase in generation time, a decrease in brood size, a suppression of locomotion behaviour and a decrease in *hsp-16-2-gfp* expression were observed [115]. In addition, the neurotoxicity of MC-LR was demonstrated in *C. elegans* with significant severe defects of chemotaxis to NaCl and diacetyl, and thermotaxis [116]. Therefore, the application of toxic cyanobacteria in soil may reduce nematode infestation and finally increase plant yield (see review in Ref. [117]). Concerning the macrofauna, and to the best of our knowledge, only one study was reported in the literature on the survival and reproduction of the springtail *Folmiosa candida* after application to the soil of a cyanobacterial biomass containing different concentrations of microcystins from 21 to 3662  $\mu\text{g g}^{-1}$  DW [118]. The results showed no adverse effects on survival and reproduction when the ratio cyanobacterial biomass/soil attained 4 g  $\text{kg}^{-1}$  DW soil.



### 5.3. Plants

The phytotoxicity of cyanotoxins was observed on aquatic plants but in the last years several studies investigated this field for terrestrial plants. As described in the review of Corbel et al. [10], the phytotoxicity of neurotoxins and cytotoxic alkaloids is less studied in comparison to microcystins. In laboratory conditions, several studies reported that the rate of germination of several plants decreased with an EC50 of 11 mg eq. MC-LR L<sup>-1</sup> for *Triticum durum* [107, 119] and an EC50 comprised between 16 and 20 mg eq. MC-LR L<sup>-1</sup> for tomatoes [120]. In these conditions, generally, the germination was impacted by microcystins for concentrations upper than 1 mg eq. MC-LR L<sup>-1</sup> and responses differed according the sensitivity of plants. Indeed, Corbel et al. [107] highlighted the higher sensitivity of wheat in comparison with tomato and lettuce seeds. Chen et al. [121] reported that the rice seed were more resistant than the rape ones. In addition, Corbel et al. [107] reported that a crude extract of cyanobacteria containing microcystins induced a significant decrease in the radicle lengths of MicroTom and Saint-Pierre tomatoes plants for concentrations higher than 5 and 20 mg eq. MC-LR L<sup>-1</sup>. Similar results, showing an inhibition of 44% of root growth, were obtained after exposition of *Triticum aestivum* exposed to 0.5 µg MC-LR L<sup>-1</sup> [122]. Chen et al. [121] reported that high concentrations of MC-LR (>2 mg L<sup>-1</sup>) inhibited root elongation, crown roots formation and lateral root formation from primordia for rice plants. In an earlier study, Gehringer et al. [123] observed a decrease in root and leaf biomasses of *M. sativa* with 5 and 10 µg eq. MC-LR L<sup>-1</sup>. By contrast, other studies demonstrated that pure MC-RR at environmental concentrations (<10 µg L<sup>-1</sup>) accelerated the rape growth of some plants [87]. In a recent study based on tomato irrigation for 14 days by cyanobacterial extract containing concentrations from 5 to 100 µg eq. MC-LR L<sup>-1</sup>, Corbel et al. [107] showed similar results with an enhancement of aerial biomasses, whereas the root biomasses were not impacted by these treatments. In the same way, a chronic exposure with an experiment of duration 90 days revealed a stimulation of tomato growth during the first 40 days post-germination [124]. In addition to the toxicity of microcystins linked to the specific inhibition of serine/threonine protein phosphatases [33], the increase in antioxidant defences induced by these toxins suggests that oxidative stress is also a major mechanism contributing to their phytotoxicity (reviewed in Ref. [10]). Yin et al. [125] reported that the exposure of *Arabidopsis thaliana* cells to MC-LR at 5 mg L<sup>-1</sup> induced a lipid peroxidation, a decrease in glutathione GSH content and increase in superoxide dismutase (SOD) and catalase (CAT) activities. Stüven and Pflugmacher [126] reported also that microcystins induced oxidative stress response in *Lepidium sativum* with an elevation of alpha- and beta-tocopherol concentrations and an increase in the activity of antioxidative enzymes (glutathione peroxidase, glutathione S-transferase and glutathione reductase). Peuthert et al. [99] observed lipid peroxidation in both the roots and shoots of several agricultural plants (*Pisum sativum*, *Cicer arietinum*, *Vigna radiata*, *Phaseolus vulgaris*, *Glycine max*, *M. sativa*, *Lens culinaris*, *T. aestivum* and *Zea mays*) that were exposed to MC-LR, either purified or in crude extract. Finally, the presence of microcystins in irrigation waters can imply modifications in the plant metabolism, notably on the photosynthesis. A study of Saqrane et al. [127] showed a decrease in chlorophyll concentrations in *Z. mays* and *L. esculenta* leaves after chronic exposure by irrigation for 30-day period to 4.2 and 2.1 mg eq. MC-LR L<sup>-1</sup>, respectively. Consequently, the photosynthesis activity was disrupted as indicated by chlorophyll fluorescence. Similar results and conclusions were

obtained by El Khalloufi et al. [120] when they exposed tomato plants with 22 mg eq. MC-LR L<sup>-1</sup>. In contrast, in a recent study Corbel et al. [124] reported that chronic irrigation of tomato plants for a period of 90 days with lower concentrations (from 5 to 100 µg eq. MC-LR L<sup>-1</sup>) did not induced a modification of chlorophyll-*a* and *b* concentrations or disturbed the photosynthesis metabolism. However, in a study performed by Gutiérrez-Praena et al. [102] in which tomato plants were exposed to MC-LR at 100 µg L<sup>-1</sup>, 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. [128] in rice plants exposed to 13 µg MC-LR L<sup>-1</sup>. The contradictory results obtained in these different studies may be associated with differences in the microcystin concentrations used in each study and the nature of the toxin pure or present in a cyanobacterial crude extract. Furthermore, some studies have demonstrated that MC-LR can be responsible for changes in the mineral content of plants; in which the macro-mineral content of the roots is increased after exposing the plants to MC-LR in a concentration-dependent manner [120, 127, 129]. However, Freitas et al. [130] and Lahrouni et al. [129] reported that the exposure of *Lactuca sativa* and *V. faba*, respectively, to purified MC-LR and MC-LR contained in a cyanobacterial crude extract produced a decrease in the mineral content of the leaves. Compared to the different effects of microcystins on plants described above, the effects due to the alkaloid cylindrospermopsin (CYN) exposure is poorly documented. This cyanotoxin seems like microcystins to induce oxidative stress in plants [103, 131, 132]. For example, in a recent study Freitas et al. [130] reported that CYN induced in time- and concentration-dependent manner an increase in the GST activity in the roots of lettuce plants. However, the glutathione peroxidase (GPx) activity was significantly decreased in both the roots and the leaves of the same plant exposed to 100 µg CYN L<sup>-1</sup> for 5 days. In the same study Freitas et al. [130] reported also 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. In addition, in another study [133] reported a significant increase in the abundance of proteins involved in photosynthesis in lettuce plants exposed to CYN.

## 6. Bioaccumulation of cyanotoxins in agricultural plants and consequences on human and animal health

Humans were exposed to cyanobacteria toxins through many routes, including drinking water, recreational contact and health food products made from cyanobacteria, and food chain. While some of these routes are well enough informed the others are them less, notably that corresponding to the consumption of crop plants. Although, no case of poisoning by these products has been reported worldwide, 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 [134].

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. Therefore, the accumulation of cyanotoxins in cultivated plants could transform them into vectors of exposure as much for the herbivorous animals that for humans. However, it's important to notify that most of the published results on cyanotoxin's transfer on plants have been performed in hydroponic conditions, which can overestimated the availability of toxins to the root system. In addition, and as indicated previously the soil particles can adsorb microcystins, reducing therefore, their bioavailability for the plants' uptake. For example, recently, Kanzo et al. [135] reported that in hydroponic conditions, microcystins were able to accumulate in the roots, stems and leaves of *Brassica rapa* after exposure to 100 and 1000 µg MC-LR L<sup>-1</sup>. However, in the same plant when cultivated in a soil system no accumulation was detected after exposure to the same concentrations of MC-LR.

Nevertheless, the ability of microcystins and cylindrospermopsin to accumulate in the tissues of different agricultural plants has been reported in the literature, and it was recently reviewed by Corbel et al. [10]. Microcystins have been detected in tissues of terrestrial plants [92, 93, 104, 122, 136, 137], indicating that they can be absorbed and transported in plants although their transport mechanism is unclear yet. However, the ability of absorbing microcystins and their accumulation in different tissues was variable among different plant species and depends on toxins' concentrations [99, 107, 127]. For example, Järvenpää et al. [138] reported that microcystins were detected on roots (a non-edible plant tissue for human but can be for animal) but not detected in leaves of mustard and broccoli. Furthermore, numerous studies concerning accumulation of cyanotoxins in agronomic plants growing in the soil were reported in radish roots, leaves of arugula and dill [61], in rice grains [65], in leaves of lettuce and cabbage [61, 139], in leaves and stems of water spinach [139] and in fruits and seeds of tomato and pepper [64]. However, a recent study based on the use of <sup>14</sup>C-labelled MC-LR showed that tomato fruits did not accumulate the toxin [92].

## 7. Conclusion and future directions

The occurrence of toxic cyanobacterial blooms, in surface waters that can be used without treatment for irrigation in agricultural purposes, has become increasingly frequent worldwide. With this increased awareness, research has been recently focused towards the fate of cyanotoxins in soils and health risk due to their potential transfer and accumulation in plants. Although there is much basic information on the concentrations of cyanotoxins found in freshwaters, there are very significant gaps in our knowledge of their effects on the biological activity of the soil and their bioaccumulation, and the role of detoxication and covalent binding in the agricultural plants irrigated with cyanotoxin-contaminated water. The great majority of the studies published recently were performed in hydroponic conditions and focused on microcystins (MCs) and specifically on a single MC variant (MC-LR) out of the almost more than 100 variants known and with high no relevant environmental concentrations. To protect consumers from the adverse effects of MCs, the WHO proposed a provisional upper limit in drinking water of 1 µg/L for the most toxic congener MC-LR and a tolerable daily intake (TDI)

of 0.04 µg/kg body weight (bw). The available data on the phytotoxicity of microcystins indicate that their concentrations in edible tissues of various agricultural plants can exceed the WHO-TDI guideline. Consequently, more information on this aspect is urgently needed for risk assessment purposes such as

- The fate of cyanotoxins in agricultural soils and the biochemical, physiological and ecological processes that control their trophic transfer in different plants remain to be clarified.
- Furthermore, even the provisional guidelines that exist for MCs in water are only recommendations, and policy will not only need to clarify acceptable levels but also address to monitor and enforce these guidelines. As such, improvements, validation and standardization of methods for chemical analysis of MCs—towards effective monitoring and enforcement in agriculture food webs—will be crucial.
- Acceptable levels for foodborne cyanotoxins are based entirely on data from waterborne toxins and are not likely to be accurate in terms of exposure through agriculture foods; therefore, reliable exposure scenario and more good quality data should be collected before robust conclusions on the health risks.

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