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Review

Cyanobacterial toxins: A short review on phytotoxic effect in an aquatic environment

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Cyanobacteria are photosynthetic prokaryotes which frequently form blooms in eutrophic water bodies. Some species of cyanobacteria are able to produce toxins (cyanotoxins) that can cause aquatic environment and diverse organisms living there to be at a serious risk. One of the more serious impacts of eutrophication on aquatic ecosystems is the disappearance of submerged macrophytes and the shift to a phytoplankton-dominated state. Hence, cyanobacterial blooms may be of significant negative ecological impact. This may represent a sanitary risk due to toxin bioaccumulation and biotransfer through the food chain. So, with the increasing number of new researches made on this subject, we propose this paper to review clearly many recent and original reports that have demonstrated the effects of cyanotoxins on some biological and physiological pathways in different aquatic plants.

Key words: Cyanotoxins, microcystins, aquatic plants, eco-physiological, sanitary risk.

INTRODUCTION

Eutrophication of water bodies may lead to excessive growth of cyanobacterial blooms that are common in many lakes and rivers all over the world (Skulberg et al., 1984; Carmichael, 1992; Codd, 1995). Many of the cyanobacteria forming blooms are known to produce different types of toxins including neurotoxins, hepatotoxins, cytotoxins and lipopolysaccharide (LPS) endotoxins, which can be of a variety of human and animal health, ecological and aesthetic concerns (Carmichael, 1997). Cyanotoxins can have adverse effects on animals like humans and other mammals including sheep, cattle and horses, birds (Carmichael, 1992, 2001; Onodera et al., 1997), fish (Liu et al., 2002; El Ghazali et al., 2009), invertebrates (Delaney and Wilkins, 1995) including zooplankton (Rohrlack et al., 2001) and vegetable resources (aquatic and terrestrial plants) (Saqrane et al., 2007, 2008, 2009a).

Although the toxic effects of cyanotoxins on animals have been examined extensively, little research has focused on their effects on aquatic vegetable resources.

Aquatic plants have been investigated to a lesser extent but as part of the aquatic ecosystem, they are potentially exposed to higher levels of cyanobacterial toxins. Abe et al. (1996) reported that, when cyanobacterial blooms occur, the abundance of submerged plants decreases and the diversity of aquatic plant communities are reduced. Since the past investigations (Abe et al., 1996) the phytotoxic effect of cyanotoxins is a subject that interests many researchers in the whole world. However, many questions remain to be clarified to protect the quality freshwater and highlight the sanitary risk due to toxin bioaccumulation and biotransfer through the food chain.

In our last review (Saqrane and Oudra, 2009), we have well discussed the eventual cyanotoxins (microcystins) phytotoxicity as the major agricultural impacts induced by the use of contaminated water for terrestrial plants irrigation. The harmful effect of cyanobacterial toxins on agricultural plants has been reviewed. However, the focus of the present review will be especially on aquatic plants considered as aquatic vegetable resources. Many recent works made on microcystins toxicity on diverse aquatic plants will be documented in relation to sanitary

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risk, and ecological and physiological impacts.

ECOPHYSIOLOGICAL EFFECTS

Plant/cyanobacteria allelopathy

According to Rice (1984), “Allelopathy is the direct or indirect (harmful or beneficial) effects of a plant (including microbes) on another plant through the release of compounds that escape into the environment”. Allelopathy is not limited to terrestrial ecosystems; it can also exist in aquatic ecosystems. Although allelopathy has been extensively studied in terrestrial ecosystems, it has not been investigated as thoroughly in aquatic ecosystems.

In aquatic environment, many authors have observed a decrease in the abundance and diversity of macrophyte species in the presence of cyanobacterial blooms in some eutrophic lakes (e.g. Harper, 1992). Based on field observations, Yamasaki (1993) demonstrated that cyanobacterial allelopathic effects associated with toxic *Microcystis* blooms caused reduced shoot length, dry weight, and nutrient and oxygen absorption in *Phragmites australis.* Others investigations revealed that cyanotoxins have clear allelopathic effects on aquatic plants, including reductions in growth, chlorophyll contents, and photosynthetic capacity as well as changes in plant pigment composition (Weiss et al., 2000; Pflugmacher, 2002). Similar allelopathic effects of microcystin extract containing a mixture of MC-RR and MC-WR were confirmed on *Lemna gibba* (Saqrane et al., 2007). Similar results have been reported on other species of the *Lemna* genus (Weiss et al., 2000; Mitrovic et al., 2004; Leblanc et al., 2005; Jang et al., 2007) or others macrophyte plants (e.g. Rowmanowska- Duda and Tarczynska 2002; Chen et al., 2004). It was recently also reported that, when toxic *Microcystis* coexist with duckweed (*Lemna japonica*) reciprocal allelopathic interactions occur (Jang et al., 2007). In another study, Weiss et al. (2000) exposed *Lemna minor* to MC-RR for 6 days and found growth inhibition at concentrations higher than 3 mg/L. Generally; all these studies accepted the hypothesis of the potential allelopathic effect of cyanobacterial extract and cyanotoxins on aquatic plants. However, Leblanc et al. (2005) reported that microcystins from the toxic cyanobacterium *Microcystis aeruginosa* do not appear to have an allelopathic effect on the common aquatic macrophyte *L. gibba*.

Effects of cyanotoxins on photosynthetic activity and chlorophyll content

Impaired photosynthesis of aquatic plants by cyanobacterial toxins was detected in several species. Wiegand et al. (2002) reported that photosynthesis in *Vesicularia dubyana* was inhibited by MC-LR. For *L. minor*, it was shown that an effect of MC-RR on photosynthesis only becomes apparent above saturating light intensities (Weiss et al., 2000). In the same study a significant decrease of chlorophyll *a* and *b* as well as total carotenoids was shown in *L. minor* after exposure to 5 mg/L MC-RR for 6 days (Weiss et al., 2000). For *L. gibba*, it was observed that a chronic exposure to microcystins extract generates an important negative effect on the chlorophylls content (Saqrane et al., 2007). During the time of exposure (6 days) the chlorophyll (*a* + *b*) content of the cells, showed a net decrease and there was a significant difference compared to the control with a maximum decrease of 65% at 0.3 mg/l. It seems that photosynthetic pigment contents were dose dependent. In the MC-contaminated culture, *L. gibba* underwent a sharp biomass and pigmentation decrease (yellowish fronds of treated culture). Thus, toxicity may consequently affect both biology and physiology of the tested duckweed (Saqrane et al., 2007).

In a recent review, Barbica et al. (2006), report that, when present in sufficient concentrations, microcystins induce various metabolic and morphological changes (for example, leaf necrosis) in plants. Other cyanobacterial compounds such as fisherellin also caused a decrease in the photosynthetic capacity (Srivatava et al., 1998). According to Pflugmacher (2002), the macroalgae *Cladophora* sp., the submergent macrophyte *Myriophyllum spicatum* and the emergent macrophyte *Phragmites australis* all showed a significant inhibitory effect on photosynthesis after exposure to 0.5 µg/l MC-LR. This inhibitory effect was even more pronounced with 90% inhibition. Moreover, a clear switch between the ratio of *Chl a* and *Chl b* was caused in *Ceratophyllum demersum* by MC-LR. In control plants and plants exposed to 0.1 µg/l there was always more *Chl a* than *Chl b*. After exposure to a MC-LR concentration of 0.5 µg/L and higher, a sharp increase in *Chl b* was found and the *Chl a* level decreased (Pflugmacher, 2002).

Promotion of oxidative stress, detoxification and biotransformation process

It was shown previously that oxygen radicals are generated during plant metabolism, especially in the plants exposed to environmental stresses, and they need to be scavenged for maintenance of normal growth (Gueta-Dahan et al., 1997). A large body of evidence has accumulated from various plant systems showing that environmental stresses alter the amounts and the activities of enzymes involved in scavenging oxygen radicals (Gueta- Dahan et al., 1997). Reactive oxygen species may inactivate enzymes and lead to oxidative damage of DNA. Oxidative stress only occurs if the level of reactive oxygen species exceeds the antioxidative capacity of the plant. The peroxidase activity and phenol levels are two parameters associated oxidative stress caused by oxygen itself or by reactive oxygen species. They may prevent oxidative damage, such as lipid

peroxidation and DNA or enzyme oxidation, to the cell. Oxidative stress may play a significant role in the pathogenesis of microcystins toxicity in animals and humans (Ding et al., 2000, 2001; Zegura et al., 2003). Recently, more studies are known about the potential oxidative stress, caused by microcystins and other cyanotoxins types in plants (Pflugmacher, 2004; Pflugmacher et al., 2006).

Phenol compounds were accumulated, resulting of plant reaction defence to biotic and/or abiotic stress (Smith, 1996). According to our knowledge, to date, there is only one work reported about the possible effects of phenolic compounds as indicators of abiotic stress induced by cyanotoxins to aquatic plant (Saqrane et al., 2007). It was clearly demonstrated in *L. gibba* that phenolic compounds were increased in parallel to the toxin concentrations and the plant synthesized new phenolic compounds following exposure to microcystins extract (Saqrane et al., 2007). These results suggest that plants would improve their antioxidant abilities to combat microcystins induced oxidative injury. According to the results of this study, the changes in the peroxidase activity and phenol content may be associated with detoxication in *L. gibba* plant. Preliminary results suggest that in the possible biotransformation of microcystins (MC-LR and/or MC-RR) by *L. gibba in vivo* formation of an intermediate metabolite may occur which could be like a "microcystin-glutathione-conjugate (Saqrane et al., 2007). During cyanobacterial bloom development the microcystins are mainly endotoxins within the cyanobacterial cells. However, during senescence and lysis of the blooms these toxins are released into the surrounding medium and come into contact with many aquatic organisms including aquatic plants.

Wiegand et al. (2002) showed that the peroxidase of *Vesicularia dubyana* was strongly inhibited by MC-LR at 50 µg/l, but not by microcin SF608 at the same concentration. A higher concentration of microcin SF608 (500 µg/l) caused a nearly total inhibition. Mitrovic et al. (2004) reported that anatoxin-a induced a significant increase of peroxidase activity at 25 µg/ml for *L. minor* plant. Microcystins can also induce the oxidative stress in "*in vitro*" cultured rat and fish cells (Ding et al., 1998, 2000; Li et al., 2003). The findings suggest that peroxidase and phenols may take part in the process in which plants react against the stress, that is, exposure to microcystins. Microcystin stress, in addition to its known components of toxicity, may also be an oxidative stress, which may contribute to its deleterious effects. It has been reported that for many organisms, the major biotransformation pathways of the cyanobacterial toxins involve glutathione-related conjugates (Pflugmacher et al., 1998; Beattie et al., 2003). The intracellular tripeptide glutathione acts as a co-substrate for the biotransformation enzymes glutathione S-transferases (Wiegand and Pflugmacher, 2005). The biotransformation of cyanobacterial toxins has been shown in several aquatic plants to follow the same pathway as in animals (Pflugmacher et al., 1998, 2001).

The possible pathways of detoxication of the cyanobacterial toxin MC-LR were clearly demonstrated by Pflugmacher (2004) in the aquatic plant *Ceratophyllum demersum*. These authors reported that after 24 h of plant exposure to 5.0 µg/l of MC-LR, the production of a glutathione-MC-LR conjugate, with mass of m/z 1302, occurred. Its production was verified by high performance liquid chromatography (HPLC) followed by MALDI-TOF. The chemical reaction conjugating glutathione to MC-LR was the first step of the detoxication process which leads to an increase of the glutathione reductase activity and to a depletion of the total intracellular glutathione pool (Pflugmacher et al., 1998, 2001, 2004). During the biotransformation processes of microcystins, the toxin and the toxin conjugate are broken down and the formation of reactive oxygen species (ROS) might occur (Pflugmacher, 2004). These biochemical mechanisms were also reported for other aquatic organisms such as *Artemia salina*, fish and mussels (Wiegand et al., 1999; Beattie et al., 2003). Further, the detoxication products and the parent toxin have also been detected in urine, feces and bile of fish and mussels (Sahin et al., 1996; Williams et al., 1997; Amorim and Vasconcelos, 1999). As it is well described in Wiegand and Pflugmacher (2005), the binding mechanisms of MC to glutathione increases the water solubility of the toxin, aiding excretion in animal cells or in the case of plants, deposition in the vacuole or binding to the cell walls. Further metabolic steps recycle two amino acids of the glutathione, revealing a MC-LR-cysteine-conjugate (Pflugmacher et al., 2001).

Detection of microcystins contamination and oxidative stress induced

In spite of the high variability of the stress factors in the environment, the presence of toxic cyanobacteria and their toxins in aquatic ecosystem could also present a source of stress to aquatic and sub-aquatic plants (Pflugmacher, 2004). Using this approach, many studies were reported in the literature using biochemical parameter indicators such as the enzymatic activity of peroxidase and of superoxide dismutase in some aquatic plant such as the free floating plant *L. minor*, the filamentous macroalga *Chladophora fracta* and *Vesicularia dubyana* (Mitrovic et al., 2004; Wiegand et al., 2002). Other biochemical parameters, such as phenolic compounds and peroxidase activity, were used to quantify oxidative stress caused by cyanotoxins in *L. gibba* (Saqrane et al., 2007). Pflugmacher (2004) reported that superoxide dismutase, glutathione peroxidase, ascorbate peroxidase and dehydroascorbate reductase may be used as indicators of MC-LR stress induced in the aquatic macrophyte *C. demersum*. In

recent years, chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant ecophysiologists. However, to the best of our knowledge, the use of variable fluorescence as a biophysical indicator of cyanotoxins stress has been rarely reported, particularly in aquatic plants. Only one work was recently published to test the potential use of Chl-fluorescence as a simple biophysical method to evaluate stress induced by cyanotoxins in the aquatic plant *L. gibba,* following exposure to microcystins (10-300 µg/ml equivalent MC-LR) during 5 h under laboratory conditions (Saqrane et al., 2009b). For terrestrial plants, also a single work that was done to evaluate the physiological state of the photosynthetic apparatus of *Brassica oleracea var. italica* and *Sinapis alba* exposed to various cyanotoxin concentrations (1 to 10 µg/L of microcystins) (Järvenpää et al., 2007).

In general, phenolic compounds were accumulated, which is a result of plant defence reaction to biotic and/or abiotic stress (Smith, 1996). These results indicated that microcystins cause stress to *L. gibba* (Saqrane et al., 2007). Nevertheless, the determination of these compounds require quite a long time for extraction and quantification procedures. It was not possible to estimate the stress induced in the first hours after exposure especially at low concentrations (<0.07 µg/ml). On the contrary, chl-fluorescence measurements can be easily and promptly determined. It is a non-invasive and highly sensitive, fast and easy to perform method to determine stress. The same has been previously concluded by Ferrante and Maggiore (2007) when they compared chlorophyll fluorescence measurements to anthocyanins, carotenoids and phenolic compounds determination as means to evaluate the quality status of leafy vegetables such as the lettuce *Valeriana*. Saqrane et al. (2009b) has clearly demonstrated the Chl-fluorescence sensitivity to cyanotoxins stress induced in the aquatic plant *L. gibba*. So, it could be applied to monitor the aquatic ecosystem quality using *Lemna* as the biotest organism for water quality assessment under aquatic environment preservation programs.

BIOACCUMULATION AND SANITARY RISK

In recent years, research on the uptake and effects of microcystins on aquatic plants has emerged. It was reported that microcystin-LR can be accumulated by *C. demersum*, *Elodea canadensis*, *Vesicularia dubyana*, and *P. australis*, and that toxin transfer along the aquatic food chain may occur (Pflugmacher et al., 1998, 1999; Pflugmacher, 2004, 2001). In *L. gibba* cells, microcystins were detected following 12 days of exposure (Saqrane et al., 2007). Experiments were carried out with a range of microcystins levels, obtained from toxic *Microcystis* culture extracts containing MC-RR and MC-WR (0.075-0.3 µg/ml equiv. MC-LR) (Saqrane et al., 2007). This work confirms the MC uptake and accumulation in treated plant. The plant had a microcystins content of 2.24 µg/g dry weight after being exposed to 0.3 µg/ml MC for 12 days. It appears that MC accumulation in duckweed was dose-dependent. MC-RR may be accumulated by *L. gibba*. Nevertheless, MC-WR was not detected in the plant extracts. This accumulation was microcystin-variant and dose-dependent. In contrast, an unknown variant was also detected. It may be a degradation product of MC-RR or MC-WR. In this situation, several possible hypotheses could be presented: in the uptake process, *L. gibba* could select the toxin variants; it is also possible that MC-WR could be absorbed and bio-transformed in plant. The potential effects of MC-RR uptake in aquatic plants were also studied by Yin et al. (2005). Over 5 days of exposure to a medium containing 10 mg/l MC-LR, *L. minor* accumulated MC-LR to a concentration of around 0.11 µg/g (0.29 µg/g with 20 mg/l). The corresponding value in *Chladophora fracta* was 0.04 µg/g (Mitrovic et al., 2005).

As it is clearly demonstrated in Yin et al. (2005), microcystin-RR could accumulate in seedlings of *V. natans*. Toxin levels in the roots were 1.3 and 48.7 times higher than those in the leaves at 1 and 16 days, respectively, which was quite different from *E. canadensis*, whose leaves took up more toxin than did the roots (Pflugmacher et al., 1998).

Yin et al. (2005) reviews that, when microcystin-RR was used at the low concentration of 0.0001 mg/l for a 7 day exposure, the amount of microcystin-RR taken up by *V. natans* leaves was 0.3 ± 0.02 ng/g FW, about 10 times that taken up by *P. australis* leaves exposed to concentrations of 0.0005 mg/l for 7 days (Pflugmacher et al., 2001). It was reported that accumulation ability would be strengthen with an increase of the surface area/volume ratio (Gobas, 1991). The accumulation ability of *V. natans* leaves is between *E. canadensis* and *P. australis*, which is presumably because of their different surface area/volume ratios (Yin et al., 2005).

Aquatic plants are a food source for many animals such as fish, birds, and crabs. Uptake of microcystins and other cyanobacterial toxins in edible plants could expose plant consumers to toxicologically relevant concentrations of the compounds. Consequently, microcystins enter the food chain (Smith and Haney, 2006). Only few studies have found that MC can be transferred along the food web. Ibelings et al. (2005) studied the distribution of MC in the food web of Lake IJsselmeer and found that transfer of MC within the food web takes place, despite no evidence for biomagnification. Smith and Haney (2006) examined MC concentrations in three levels of an aquatic food web (phytoplankton, zooplankton, and sunfish) and found evidence for the direct transfer of MC from zooplankton to sunfish and the subsequent accumulation of toxin in the liver tissue. Ibelings and Chorus (2007) indicated that toxin content at each trophic level is dependent on biodilution and on the bioaccumulation and biotransformation capacity of the various organs.

Recently, El Ghazali et al. (2009) was studied the

relationship between different MC profiles taken up by carp larvae via contaminated *Artemia* nauplii and MC accumulation and effects in fish. In this study, the intake of MC did not affect the survival of *C. carpio* larvae, but we found evidence for the direct transfer of MC from *Artemia salina* nauplii to fish and the subsequent accumulation of toxin in the larvae tissue.

CONCLUSION

All physiological and biological effects induced in aquatic plants following exposure to microcystins, confirm that in aquatic ecosystems toxic cyanobacterial blooms naturally coexisting with plants could induce an important negative ecological impact. Thus, it could involve a risk to all parts of the food chain including aquatic animals. Also, bioaccumulation and biotransfer of toxins through the food chain may represent a serious sanitary risk. So, more attention should be paid to the behaviour of microcystins in the food chain and its possible hazards to human and animal health.

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