We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the top 1% of most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Risk Assessment of Cyanobacteria and Cyanotoxins, the Particularities and Challenges of *Planktothrix* spp. Monitoring

Catarina Churro\textsuperscript{1,2}, Elsa Dias\textsuperscript{1} and Elisabete Valério\textsuperscript{2}

\textsuperscript{1}Laboratório de Biologia e Ecotoxicologia, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, \textsuperscript{2}Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

1. Introduction

Cyanobacteria are a diverse well adapted group of organisms that presents amazing morphological diversity. Cyanobacteria can be unicellular or colonial (filamentous, spherical or amorphous) (Fig. 1). Since cyanobacteria have cells larger than normal bacterial cells and behavior more similar to algae, they were classified under the microalgae for a long time and acquire the name of blue-green algae or Cyanophyta (Whitton & Potts 2000). Cyanobacteria is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from their coloration (cyano = blue). The vegetative cell wall is of Gram-negative type and in some species the peptidoglycan layer is considerably thicker than in other bacteria. Many unicellular and filamentous cyanobacteria possess an “envelope” outside the lipopolysaccharide (LPS) “outer membrane”, which is called: sheath, glycocalyx, or capsule, and depending on the consistency, gel, mucilage or slime. The sheaths of cyanobacteria are predominantly polysaccharide, but a part of its weight may be polypeptides, and depending on the species, some types of sugar residues may be involved (Castenholz, 2001).

Fig. 1. Optical microscopy photographs of cyanobacteria presenting different morphologies. The arrow indicates the heterocyst cell in *Anabaena circinalis.*
Cyanobacteria are autotrophs and possess all the photosynthetic pigment (chlorophyll $a$, carotenoids, allophycocyanin, phycobilins, phycoerythrins) except chlorophyll $b$ (Castenholz, 2001). Prochlorophytes are also cyanobacteria that contain chlorophyll $a$ and $b$, but, opposing to other cyanobacteria, lack phycobiliproteins (Castenholz, 2001). Cyanobacteria have the ability to use low light intensities effectively, since they are able to produce the accessory pigments needed to adsorb light most efficiently in the habitat in which they are present, providing them a great advantage for the colonization of a wide range of ecological niches (van den Hoek et al., 1995; WHO, 1999). Phycobiliprotein synthesis is particularly susceptible to environmental influences, especially light quality. The chromatic adaptation is largely attributable to a change in the ratio between phycocyanin and phycoerythrin in the phycobilisomes. The photosynthetic pigments are located in thylakoids that are free in the cytoplasm near the cell periphery (Fig. 2). Cell colours vary from blue-green to violet-red due to the chlorophyll $a$ masking by the carotenoids and accessory pigments. The pigments are involved in phycobilisomes, which are found in rows on the outer surface of the thylakoids (Fig. 2) (WHO, 1999). Cyanobacteria are also able of storing essential nutrients and metabolites within their cytoplasm. Prominent cytoplasmic inclusions such as glycogen and cyanophycin granules (polymers of the amino acids arginine and asparagine), polyphosphate bodies, carboxysomes (containing the primary enzyme for photosynthetic CO$_2$ fixation, ribulose 1,5-bisphosphate carboxylase-oxygenase: RuBisCO) and gas vacuoles (Fig. 2) can be observed by electron microscopy. The occurrence of fimbriae (pili) is abundant in many cyanobacteria with varying patterns. Some filamentous forms are also able of gliding (sliding) (van den Hoek et al., 1995; WHO, 1999; Castenholz, 2001).

Fig. 2. Cyanobacteria cell structure. (A)Transmission electron micrographs showing the ultrastructure of an Anabaena circinalis vegetative cell; (B) Schematic diagram of a cyanobacterial vegetative cell. S: external 4-layered cell wall; OM: outer membrane; PL: peptidoglycan layer; CM: cytoplasmic membrane; CW: cell wall; E: cell envelope; TH: thylakoid; PB: phycobilisome; CY: cytoplasm; GV: gas vesicle; GG: glycogen granules; N: nucleoplasmic region; C: carboxysome; PP: polyphosphate granule; CP: cyanophycin granule; LP: lipid droplets (adapted from van den Hoek et al., 1995; Castenholz, 2001).
Cyanobacteria can be found in the most diverse environments like hot springs, salt marshes, soils, fresh, brackish, and marine waters (Sze, 1986). In sum, cyanobacteria are ubiquitous oxygenic photosynthetic prokaryotes.

2. Why the surveillance on cyanobacteria?

Cyanobacteria are common constituents of the phytoplankton in aquatic environments. In optimal conditions these phytoplanktons can develop massively and form blooms, becoming the dominant organism in the water column and creating serious problems in water quality (Cood, 2000; Vasconcelos, 2006). The water quality deterioration produced by cyanobacterial blooms includes foul odours and tastes, deoxygenation of bottom waters (hypoxia and anoxia), fish kills, food web alterations and toxicity. Other threatening characteristic of these organisms is their ability to produce toxins that affects other living organisms and humans (Carmichael, 2001). The capacity of mass development together with the ability to produce potent toxins enlightens the importance of implementing regular monitoring programs for cyanobacteria and cyanotoxins in freshwater environments, in order to minimize potential health risks to animal and human populations that results from exposure through drinking and recreational activities. The implementation of surveillance programs on cyanobacteria involves understanding the ecophysiology of cyanobacteria, bloom dynamics, conditions that promote blooms, production of toxins and their impact in human and animal health (McPhail & Jarema, 2005).

Cyanobacteria possess some ecstrategies that allows them to overcome other organism and become dominant. In general there are four constraints on cyanobacteria growth as pre-requisites for bloom enhancement: light, nutrients, temperature and stability of the water column. Cyanobacteria requires low light intensities for growth, compared with algae, which provides competitive advantages in lakes which are turbid due to growth of other phytoplankton. They also have a higher affinity for uptake phosphorous and nitrogen than many other photosynthetic organisms and they have a substantial storage capacity for phosphorous (Mur et al., 1999). Some genera like Anabaena, Aphanizomenon, Cylindrospermopsis, Nodularia and Nostoc have specialized cells (heterocysts) (Fig. 1) for nitrogen fixation and blooms of these genera can often be related with periodic nitrogen limitation. This means that they can compete other phytoplankton under conditions of phosphorous and nitrogen limitation (Briand et al., 2003; Sunda et al., 2006).

The success of some cyanobacteria is also due to the presence of gas vacuoles that provide buoyancy regulation. During water stratification conditions cyanobacteria can migrate in the water column, accessing light in the surface layers and nutrients near the sediment. During photosynthesis, carbohydrates are accumulated which makes them heavy and sinking away from light and when the carbohydrates are respired, buoyancy is restored. As large colonies sink faster than small ones or single cells, genera like Microcystis, Anabaena, Aphanizomenon and Nodularia have scum-forming strategies (Vance, 1965; Mur et al., 1999). Cyanobacteria also produce active substances that inhibits the growth of competing algae and grazers that feed upon them, which can also promote cyanobacteria proliferation (Briand et al., 2003; Granéli & Hansen, 2006; Sunda et al., 2006; van Apeldoorn et al., 2007; Figueredo et al. 2007).

As a consequence of the characteristics mentioned above the cyanobacterial cells numbers in water bodies vary seasonally. In temperate regions, seasonal successions of organisms belonging to different phytoplankton taxa are often observed. Whereas at the beginning of
| Toxin                        | Taxon                                      | LD_{50}^* (i.p., mouse) of pure toxin | Primary target in mammals | Mechanism of action                                                                 |
|------------------------------|--------------------------------------------|--------------------------------------|---------------------------|-------------------------------------------------------------------------------------|
| **Hepatotoxins**             |                                            |                                      |                           |                                                                                     |
| Microcystins (~80 variants)  | *Microcystis* spp.  *Planktothrix* spp.    | 25 to ~1000µg/kg bw                  | Liver                     | Multi-pathway process. MCs inhibit the serine/threonine protein phosphatases type 1  |
| (Cyclic heptapeptides)       | *Oscillatoria*, *Nostoc*, *Anabaena* spp.  |                                      |                           | and type 2A (PP1/PP2A) and induces oxidative stress leading to a cascade of events   |
|                              | *Anabaenopsis*, *Hapalosiphon*, *Snowella* |                                      |                           | responsible for the MC cytotoxic and genotoxic effects in animal cells               |
|                              | *Woronichinia*, *Aphanocapsa*              |                                      |                           |                                                                                     |
| Nodularin (9 variants)       | *Nodularia spumigena*                      | 30-50 µg/kg bw                       | Liver                     | Similar to MCs                                                                       |
| (Cyclic pentapeptide)        |                                            |                                      |                           |                                                                                     |
| **Cytotoxin**                |                                            |                                      |                           |                                                                                     |
| Cylindrospermopsin (3 variants) | *C. raciborskii*, *Umezakia natans*, *Aph. ovalisporum*, *Rhaphidiopsis curvata*, *Anabaena bergii*, *Aphanizomenon Lanyhlya* | 200 - 2100 µg/kg bw/d, 200 µg/kg bw/5-6 d | Liver, kidneys, lungs, heart | Inhibition of glutathione (GSH) and protein synthesis, as well as the inhibition of |
| (Guanidine alkaloid)         |                                            |                                      |                           | cytochrome P450                                                                     |
| **Dermalotoxins**            |                                            |                                      |                           |                                                                                     |
| Aplysiaotoxin (phenolic bislactone) | *Lynghia* Planktothrix Schizothrix          | 107-117 µg/kg                        | Skin                      | Inflammatory agent, protein kinase C activator                                       |
| Debromoaplysiaotoxin (phenolic bislactone) | *Lynghia*                              | 107-117 µg/kg                        | Skin                      | Inflammatory agent, protein kinase C activator                                       |
| Lyngbiatoxin-A (Alkaloid)    | *Lynghia*                                  | 250 µg/kg (7LD100)                   | Skin                      | Inflammatory agent, protein kinase C activator                                       |
Table 1. Cyanotoxins detected and correspondent taxa from which have been isolated, as well as their primary target in mammals. Based on the information from Chorus et al., 2000; Charmichael, 2001; Codd et al., 2005; Stewart et al., 2006; van Apeldoorn et al., 2007; Bláha et al., 2009; Valério et al., 2010; Mihali et al., 2009. * - the dose needed to kill 50% of exposed animals.

| Toxin                          | Taxon                                      | $LD_{50}^{*}$ (i.p., mouse) of pure toxin | Primary target in mammals | Mechanism of action                                      |
|-------------------------------|--------------------------------------------|------------------------------------------|----------------------------|----------------------------------------------------------|
| Neurotoxins                   |                                            |                                          |                            |                                                          |
| Anatoxin-a (5 variants)       | *Anabaena* spp., *Oscillatoria*            |                                          | 250 µg/kg bw               | Post-synaptic neuromuscular junction                      |
| (Tropane-related alkaloids)   | *Cylindrospermum* *Microcystis*            |                                          |                            | Agonism of the muscular and neuronal nicotinic acetylcholine receptor |
|                               | *Aphanizomenon* *Planktothrix*             |                                          |                            |                                                          |
| Homaoanatoxin-a (alkaloid)    | *Planktothrix* *Oscillatoria*              |                                          | 250 µg/kg bw               | Post-synaptic neuromuscular junction                      |
|                               | *Anabaena* *Raphidiopsis* *Phormidium*     |                                          |                            | Same as Anatoxin-a                                       |
| Anatoxin-a(s) (Guanidine methyl phosphate ester) | *Anabaena* *Aphanizomenon* |                                          | 40 µg/kg bw                | Post-synaptic neuromuscular junction                      |
|                               |                                            |                                          |                            | Irreversible inhibition of acetylcholinesterase          |
| Saxitoxin (20 variants)       | *Anabaena* *Aphanizomenon*                 | 10–30 µg/kg bw                          | Axons                      | Blockage of the sodium or calcium channels of the nerve axon membranes |
| (Carbamate alkaloids)         | *C. raciborskii* *Lyngbya* *Planktothrix*   |                                          |                            |                                                          |
the summer a great variety of microalgae and cyanobacteria usually co-exist in the same 
water body, towards the end of summer this diversity may drop drastically as the result of 
the mass development of the cyanobacterial communities (blooms) (Sze, 1986). These 
blooms may be formed by a consortium of cyanobacteria producing different amounts of 
toxins at different rates, with the same bloom-forming species having both toxigenic and 
non-toxigenic strains, indistinguishable by morphological examination. Cyanobacterial 
blooms are complex and can develop in a rather sudden and unpredictable way.

3. Cyanotoxins

Cyanobacteria are able to produce secondary metabolites that present a vast diversity of 
structures and variants. Most of cyanobacterial secondary metabolites are alkaloids, or 
possess peptidic substructures synthesised by NRPS (non-ribosomal peptide synthesis, 
involving peptide synthetases) or NRPS/PKS (involving peptide synthetases and polyketide 
synthases) hybrid pathways (Valério et al., 2010).

Cyanotoxins are usually classified according to their target in mammals, being divided in 
hepatotoxins (liver damaging), neurotoxins (nerve damaging), cytotoxins (cell damaging) 
and toxins responsible for allergic reactions (dermatotoxins), presenting several kinds of 
mechanisms of action. A considerable number of these different types of toxins have been 
isolated from cyanobacteria, belonging to different taxa, as summarized in Table 1.

4. Cyanobacteria/cyanotoxins risk assessment

Risk assessment consists in the identification and determination of quantitative or 
qualitative value of risk related to the exposure to a given hazard, taking into account 
possible harmful effects on individuals or populations exposed to that hazard and all the 
possible routes of exposure. The risk assessment process includes four steps: the hazard 
identification, hazard characterization, exposure assessment, establishment of dose–effect 
and dose–response relationships in likely target individuals and populations (Duffus et al., 
2007). A schematic representation of the steps involved in risk assessment of cyanotoxins is 
depicted in Fig. 3.

The scientific knowledge on cyanotoxins still does not enable to correctly assess the risk of 
human exposure to toxic cyanobacteria. Many toxicological aspects remain to clarify, 
epidemiological data are insufficient and the exposure assessment is a very complex task.

The human exposure to cyanobacterial cells and/or its toxins may occur through water 
swallowing or inhalation during recreational activities such as swimming, canoeing, 
sailboarding and paddling, through the intake of contaminated drinking water and through 
hemodialysis treatment.

Most episodes of human illness related with cyanobacteria/cyanotoxins resulted from an 
acute intoxication through the exposure routes mentioned above (for review see Chorus et 
al., 2000; Duy et al., 2000; van Apeldoorn et al., 2007), such as the following examples:

Example 1 – Symptoms after exposure through recreational activity: nausea, abdominal 
pain, fever, dyspnea, respiratory distress, atypical pneumonia and hepatotoxicosis with a 
significant increase of hepatic damage biomarkers (Giannuzzi et al., 2011);
Example 2 – Symptoms after exposure during hemodialysis treatment: weakness, muscular pain, nausea, vomiting, neurologic symptoms (head pain, vertigo, deafness, blindness and seizures), increase of hepatic damage biomarkers, hepatomegaly, hepatic failure and death (reviewed in Pouria et al, 1998).

Besides the acute effects mentioned above, few papers report the association between the ingestion of water contaminated with microcystins and the increase of hepatocarcinoma (Yu, 1995; Ueno et al., 1996) and colorectal cancer (Zhou et al, 2002) in human populations supplied with untreated- or ineffective-treated water.

Laboratorial studies have demonstrated that, in fact, microcystins, nodularins and cylindrospermopsin are genotoxic (reviewed in Zégura et al., 2011) and the carcinogenic
potential of these toxins have been postulated (Gehringer, 2004; Kinnear, 2010). However, there are still many uncertainties that difficult an unequivocal conclusion about this issue.

The problem of chronic effects are particularly relevant in the case of continuous exposure to low levels of cyanotoxins, even at residual levels, that are not detected by the conventional methods employed in the monitoring procedures. Moreover, the scientific and analytical limitations hinder the complete determination of the toxicological properties of cyanotoxins, and the correct assessment of human exposure to cyanotoxins, as well as lack to provide epidemiological evidence that could confirm the chronic effects of cyanotoxins on human health. Therefore, although the surveillance programs can somehow protect against the cyanotoxins acute effects, risk assessment procedures should be developed and implemented, particularly in what concerns to chronic exposure to cyanotoxins.

During the last decade, the WHO has been regularly reviewing the public health significance of cyanobacteria occurrence in freshwater and developed guidelines for drinking and recreational water environments (WHO, 1998, 2003). This organization recommends that the approach to developing guidelines for cyanobacteria in freshwater should consider:

- the occurrence of cyanobacteria in general (in addition to their toxins) as part of the hazard, because it is not clear that all known toxic components have been identified and irritation symptoms reported may be caused by these unknown substances;
- the particular hazard caused by the well-known cyanotoxins; and
- the hazard associated with the potential of scums formation, which increase the local hazard concentration.

WHO (2003) has divided the health effects into two categories:

- Symptoms associated with skin irritation and allergic reactions resultant from dermal exposure to unknown cyanobacterial substances, and
- Potentially more severe effects due to the exposure to high concentrations of already known cyanotoxins, particularly microcystins (the most commonly found and more studied cyanotoxins).

Given the two types of severity of the symptoms, the WHO considered that the establishment of a single guideline value was not appropriate and, therefore, it has defined several guideline values associated with increasing severity and probability of impact of cyanobacteria/cyanotoxins in health at three levels for bathing waters (Table 2) and guideline values for cyanotoxins in drinking water (see 4.1).

Cyanotoxin analysis will generally be required in one of the following circumstances (WHO, 1999):

1. Action Level 1 status (i.e. > 2000 cells mL\(^{-1}\)) predominated by Microcystis aeruginosa, or when concentrations of other potentially toxic taxa (see Table 1) exceed 15,000 cells mL\(^{-1}\).
2. Action Level 2 status where numbers of a cyanobacterial taxa not previously recorded as toxic exceed 100,000 cells mL\(^{-1}\) (recommended toxicity analysis by mouse bioassay or comparative method).

A brief summary of the steps that must be taken into account, when performing cyanobacteria monitoring, are presented in Fig. 4.
| WHO guideline levels | Cyanobacterial cells and chlorophyll levels                                                                 | Health risks                                                                                                                                                                                                 | Recommended action                                                                 |
|----------------------|-------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Low                  | < 20,000 of total cyanobacterial cells mL⁻¹ OR < 10 μg L⁻¹ chlorophyll-a with dominance of cyanobacteria OR < 2.5 mm³ L⁻¹ cyanobacterial biomass | Short term adverse health outcomes unlikely                                                                                                                                                    | Continue monitoring                                                                 |
| Moderate             | 20,000 - 100,000 of total cyanobacterial cells mL⁻¹ OR 10 - 50 μg L⁻¹ chlorophyll-a with dominance of cyanobacteria OR 2.5 - 12.5 mm³ L⁻¹ cyanobacterial biomass | Short term adverse health outcomes, e.g. skin irritations, gastrointestinal illness, probably at low frequency                                                                 | Add signs to indicate MODERATE alert level - increased health risk for swimming and other water contact activities |
| High                 | Cyanobacterial scum formation in contact recreation areas OR > 100,000 of total cyanobacterial cells mL⁻¹ OR > 50 μg L⁻¹ chlorophyll-a with dominance of cyanobacteria OR > 12.5 mm³ L⁻¹ cyanobacterial biomass | Short term adverse health outcomes such as skin irritations or gastrointestinal illness following contact or accidental ingestion Severe acute poisoning is possible in worst ingestion cases | Immediate action to prevent contact with scums Add signs to indicate HIGH alert level - warning of danger for swimming and other water contact activities |

Table 2. WHO guideline values for safe practice in managing bathing waters that may contain cyanobacterial cells, according to the level of probability of adverse health effects (WHO, 2003).

4.1 Derivation of guideline values

Characterization of human hazards usually relies mainly on animal studies, or incidents from which quantitative estimates of the hazards to humans can be extrapolated.

Few studies in rodents and pigs enabled to estimate the tolerable daily intake (TDI) of some cyanobacterial toxins (Duy et al., 2000; Falconer et al., 1999; Humpage and Falconer, 2003).

Usually, studies with different quantitative animal dosing data, with follow-up over extended periods (preferably over the lifetime of the animal being tested) are necessary to estimate a no-observed-adverse-effect level (NOAEL), or at least a lowest-observed-adverse-effect-level (LOAEL).

For drinking water, the TDI for cyanotoxins can be estimated as:

\[
\text{TDI} = \frac{\text{NOAEL or LOAEL}}{\text{UF}} \tag{1}
\]

www.intechopen.com
Where, TDI units are mg/kg body wt/day, or µg/kg body wt/day, and UF is the product of uncertainty factors, e.g.

$$UF = \begin{cases} 
10 \text{ (intra-specific variations)} \\
10 \text{ (inter-specific variations)} \\
10 \text{ (less-than-lifetime study)} 
\end{cases}$$

(2)

Fig. 4. Organizational chart of the steps involved in cyanobacteria risk management (adapted from Bartram et al. 1999).
Additionally, it may be also necessary consider a UF of 5 if the LOAEL is used and a UF of 3, if tumor promotion is considered (Codd et al., 2005).

The guideline value (GV; µg/L water) can be calculated as:

\[ GV = \frac{(TDI \times \text{body wt} \times AF)}{C} \]  

(3)

Where body weight is usually assumed to be 60 kg for a human adult and AF is the allocation factor, which is the proportion of daily exposure arising from drinking water ingestion. Because some oral exposure may occur via food or dietary supplements or other route, therefore, an AF of 0.8 (80% of total intake) is assumed for drinking water. Finally, C is the volume of drinking water consumption per day, assumed to be 2 L for an adult (Codd et al., 2005; van Apeldoorn et al., 2007).

4.2 Guidelines for microcystins

The drinking water guideline for microcystins was determined from a sub-cronic study (Fawell et al., 1993) with mice orally administered with microcystin-LR (since it is one of the most toxic and frequent microcystin variant and for which more information is available). In this study a NOAEL of 40 µg/kg bw was derived and a TDI of 0.040 was calculated using an uncertainly factor of 1000 (10 for intra-specific variations, 10 for inter-specific variations and 10 for limitations in the database). The resulting guideline value, using an allocation factor of 0.80 for total microcystin-LR (free plus cell bound), was aprox. 1 µg/L in drinking water.

A similar TDI for microcystins was obtained (0.067 vs. 0.040) from a study with pigs using freeze-thawed Microcystis cells containing quantified microcystins (Falconer et al., 1994). These resulted in similar GVs: 1 µg/L for mice vs. 1.61 µg/L for pigs.

For safety reasons, the World Health Organization (WHO) has adopted the lowest value (1 µg/L) as the GV for microcystin in drinking water for adults (WHO, 1998). However, if tumour-promoting actions of microcystins are also considered, then an additional UF of 3 for this hazard must be used, thus originating a GV of about 0.3 µg/L (Codd et al., 2005).

The Australian guideline is 1.3 µg/L for total microcystin. This slightly differs from the WHO provisional guideline of 1 µg/L microcystin-LR due to the use of a different average body weight for an adult (70 kg vs. 60 kg) and different Allocation Factor (0.9 vs. 0.8).

4.3 Guidelines for nodularin

No NOAEL can be derived for nodularin(s) due to the absence of suitable toxicological data. However, since nodularin(s) and microcystin-LR have identical mechanisms of action, the guideline value determined for MC-LR (1 µg/L) can also be used for nodularin(s).

4.4 Guidelines for anatoxin-a

A NOAEL of 98 µg/kg has been derived from a 28-day gavage study using mice (Fawell et al., 1999). If a uncertainly factor (UF) of 1000 (10 for intra-specific variations, 10 for inter-
specific variations and 10 for limitations in the database) is used, a TDI of 0.1 µg/kg bw can be reached. Svreck & Smith (2004) have suggested a guideline limit of 3.0 µg/L.

4.5 Guidelines for anatoxin-a(S)

There are no sufficient data to derive an NOAEL or LOAEL and, consequently, insufficient data to determine a TDI for anatoxin-a(S). However, in the Guidelines for Drinking-Water Quality Management for New Zealand 2005, a Maximum Acceptable Values (MAVs) for anatoxin-a(S) of 1.0 µg/L is suggested (Chorus, 2005).

4.6 Guidelines for cylindrospermopsin

According to the 90-day study of Shaw et al. (2000) using drinking water in mice a NOAEL of 150 µg/kg bw was obtained. A second study with mice administered by gavage with cylindrospermopsin for 11-weeks from Humpage and Falconer (2003) resulted on a NOAEL of 30 µg/kg bw. If a uncertainty factor (UF) of 1000 (10 for intra-specific variations, 10 for inter-specific variations and 10 for limitations in the database) is used, a TDI of 0.03 µg/kg bw can be calculated. Considering the “standard” adult body wt of 60 kg and a 0.9 AF, a GV of 0.81 is obtained, leading the authors to propose a Guideline Value of 1 µg/L (Humpage and Falconer, 2003).

4.7 Guidelines for saxitoxin

There are no attempts to determine a NOAEL or LOAEL and thus calculate a TDI for saxitoxin, because the range of lowest concentration where adverse effects were observed varies greatly. Given the different susceptibilities of person, it has been difficult to decide which uncertainty factor should be also used (van Appeldoorn et al., 2007).

Although there are no official guidelines, Australia considers a GV of 3 µg STX eq/L of drinking water, which was based on the data from marine shellfish toxicity (van Appeldoorn et al., 2007).

4.8 Guidelines for aplysiatoxin and lyngbyatoxins

There are no sufficient data to derive an NOAEL or LOAEL and thus calculate a TDI for these toxins.

The members of the population presenting greatest risk when exposed to cyanotoxins are children because of their water intake: body weight ratio, which is higher than that of adults (Falconer, 1999). Also the people having already certain pathologies may be more susceptible to the intake of the toxins (Falconer, 1999).

Ideally, the guidelines values established should protect against acute and chronic effects derived from the contact with cyanobacteria and their toxins, although, such it was stated above, the knowledge on the chronic effects of cyanotoxins still presents many gaps. The guideline values determined/suggested for each known cyanotoxin are summarized in Table 3.
| Toxin            | Drinking water guideline values       | Countries using the GV                  | References                                      |
|------------------|---------------------------------------|-----------------------------------------|-------------------------------------------------|
| MC-LR            | 1.0 µg/L (most generally accepted)    | Brazil, Czech Republic, Denmark, France, Great Britain, Greece, Italy, New Zealand, Poland, Portugal, Spain, South Africa, Spain, U.S.A. | Chorus, 2005; Codd et al., 2005; van Apeldoorn et al., 2007 |
| MC-LR            | 1.3 µg/L                               | Australia, Canada                       | Chorus, 2005; van Apeldoorn et al., 2007        |
| Nodularin        | No guideline, however, hazard assessment can be guided by that for microcystins 1.0 µg/L | New Zealand                             | Fitzgerald et al., 1999; Chorus, 2005; van Apeldoorn et al., 2007 |
| Anatoxin-a       | 3.0 µg/L (no official guideline)      | New Zealand                             | Codd et al., 2005; Svrcek & Smith, 2004; Chorus, 2005 |
|                  | 6.0 µg/L                               | New Zealand                             |                                                 |
| Homoanatoxin-a   | 2.0 µg/L                               | New Zealand                             | Chorus, 2005                                    |
| Anatoxin-a(S)    | Nd                                    | New Zealand                             | Chorus, 2005                                    |
|                  | 1.0 µg/L                               | New Zealand                             |                                                 |
| Cylindrospermopsin | 1.0 µg/L (suggested)                  | Canada, New Zealand                     | Humpage & Falconer, 2003; Svrcek & Smith, 2004; Chorus, 2005 |
|                  | 15.0 µg/L                              | Brazil                                  |                                                 |
| STX              | 3.0 µg STX eq/L                        | Australia, Brazil, New Zealand          | Svrcek & Smith, 2004; Chorus, 2005; Codd et al., 2005 |
| Aplysiatoxins    | nd                                    |                                        |                                                 |
| Lyngbyatoxins    | nd                                    |                                        |                                                 |

Table 3. Guideline values (GV) estimated for cyanobacterial toxins in drinking water. Nd - not determined.
5. Challenges and gaps in *Planktothrix* spp. risk assessment and management

Usually, *Microcystis* is the genus that occurs more frequently and is usually considered the main responsible for the production of microcystins. However, there is another emergent genus that has also the ability to produce microcystins, which is *Planktothrix*.

The cyanobacteria of the genus *Planktothrix* have a planktonic life style, occur in solitary filaments and lack sheaths, heterocysts and akinetes. Formerly classified into the genus *Oscillatoria*, *Planktothrix* now represents a well-defined independent genus based in phylogenetic and morphologic characteristics and comprises 13 species (Komárek & Komárkova 2004). Similar to other cyanobacteria, *Planktothrix* can achieve high cellular densities in water forming blooms that unbalance the ecosystem and it can also produce several types of cyanotoxins, namely microcystins, homoanatoxin-a, anatoxin-a, aplysiatoxins, saxitoxins, anabaenopeptins, (Luukkainen et al., 1993; Erhard et al., 1999; Kouzminov, 2001; Viaggiu et al., 2004; Wood, 2005; Kosol et al., 2009), thus threatening humans and animals.

From the 13 species described, *Planktothrix rubescens* and *Planktothrix agardhii* are the most studied and common species reported to cause water related problems. A summary of *Planktothrix* occurrence in European lakes where they form recurrent blooms and the associated toxicity found is presented in Table 4.

Unlike other cyanobacteria, *P. agardhii* and *P. rubescens* are well adapted to very low light intensities and this characteristic provides to them several advantages. For *P. agardhii* it allows them to grow in waters with high turbidity, in which it can be homogeneously dispersed throughout the epilimnion in eutrophic waters having a competitive advantage upon other phytoplankton species. For *P. rubescens* the low light intensity requirements together with the high content of the red pigment phycoerythrin enables it to growth in the metalimnetic layer in thermally stratified waters away from the phototic surface zone (Mur et al., 1999; Bright & Walsby, 2000). Furthermore, these two species have different irradiance tolerances; *P. agardhii* is more tolerant to high irradiance than *P. rubescens*, what is related with their occurrence in different ecological niches in the water column and inhabit in different types of water systems (Oberhaus et al., 2007). Therefore *P. agardhii* grows well in the upper part of the water column of shallow eutrophic lakes, however it can also grow at several depths along the water column (Halstvedt et al., 2007). On the other hand, *P. rubescens* is well adapted in forming metallicm populations of deep stratified lakes in spring and summer and when the lake loses its thermal stratification in the winter, it can be dispersed through the entire water column (Bright & Walsby, 2000; Briand et al., 2005). *Planktothrix* also has different water temperature tolerances when compared to other cyanobacteria, making them organisms that can be easily found in subalpine lakes or in temperate regions during winter, so *Planktothrix* blooms may persist all year around and not only during summer or spring where temperatures and light irradiance are higher. *P. agardhii* has been found viable under ice covers (Sivonen & Jones 1999; Oberhaus et al., 2007). Since both species occupies different water niches they can coexist in the same water body forming surface and deep layer blooms, although this coexistence is rare it has been reported (Davis & Walsby, 2002; Halstvedt et al., 2007).

Regarding the risk management measures that are usually followed to overcome the presence of cyanobacteria and cyanotoxins in the freshwater, *Planktothrix* has some particularities that need to be taken into account. One of them is *Planktothrix*'s ability to
| Country       | Lake          | Maximum depth (m) | Trophic status | Species            | Toxins detected | References                                                                 |
|--------------|---------------|-------------------|----------------|--------------------|-----------------|-----------------------------------------------------------------------------|
| Austria      | Mondsee       | 68                | Mesotrophic    | *P. rubescens*     | ---             | Kurnayer et al. 2004; Kurnayer & Gumpenberger 2006                        |
|              | Irrsee        | 32                | Mesotrophic    | *P. rubescens*     | ---             | Kurnayer et al. 2004; Kurnayer & Gumpenberger 2006                        |
|              | Afritzer      | 2.5               | Mesotrophic    | *P. rubescens*     | ---             | Kurnayer & Gumpenberger 2006                                              |
|              | Wörthersee    | 85.2              | Mesotrophic    | *P. rubescens*     | ---             | Kurnayer & Gumpenberger 2006                                              |
| Czech Republic | Bílá Lhota   | 1                 | Hypertrophic   | *P. agardhii*      | ---             | Pouličková et al. 2004                                                    |
| England      | Blelham Tarn  | 14.5              | Eutrophic      | *P. rubescens, P. agardhii* | ---             | Davis & Walsby 2002; Davis et al. 2003                                    |
|              | Elmhurst      | Shallow           | ---            | *P. agardhii*      | 3.2 µL⁻¹ MC-LR (a,c) | Akcaalan et al. 2006                                                     |
|              | Bassenthwaite | 21                | Mesotrophic    | *P. agardhii*      | 27-41 pg filament⁻¹ MC-LR (b,c) | Akcaalan et al. 2006                                      |
| Estonia      | Verevi        | 11                | Hypertrophic   | *P. agardhii*      | ---             | Nöges & Kangro 2005; Kangro et al. 2005                                   |
| Finland      | Vargunslet    | 32                | Oligotrophic   | *P. agardhii*      | 11.1 µg L⁻¹ MC-RR (a,b,d) | Lindholm et al. 1999                                              |
| France       | Bourget       | 145               | Mesotrophic    | *P. rubescens*     | Max. 6.7 µL⁻¹ MC-LR (a,b,d) | Briand et al. 2005; Jacquet et al. 2005                                 |
|              | Viry-Châtillon| 5.3               | Hypertrophic   | *P. agardhii*      | Max. 7.4 µg L⁻¹ MC-LR (a,d) | Max. 34.5 µg L⁻¹ MC-LR (a,d) | Catherine et al. 2008; Yéprémiyan et al. 2007 |
| Germany      | Ammersee      | 81.1              | Mesotrophic    | *P. rubescens*     | Mean 0.43 ± 0.06 µg L⁻¹ MC-LR (a,c) | Ernst et al. 2009                                              |
|              | Stolpsee      | Shallow           | Eutrophic      | *P. agardhii*      | 1.81 µg L⁻¹ MC-LR (a,d) | Mbedi et al. 2005                                               |
|              | Breiter Luzin | 58.3              | Mesotrophic    | *P. rubescens*     | 2.31 µg L⁻¹ MC-LR (a,d) | Mbedi et al. 2005                                               |
|              | Langer See    | 3.8               | ---            | *P. agardhii*      | ---             | Rückert et al. 1997                                                   |
|              | Wolziger See  | 13                | ---            | *P. agardhii*      | ---             | Rückert et al. 1997                                                   |
|              | Lebbiner See  | 4                 | ---            | *P. agardhii*      | ---             | Rückert et al. 1997                                                   |
| Greece       | Ziros         | 56                | Oligotrophic   | *P. rubescens*     | Max. 199 µL⁻¹ MC-LR (a,c) | Vareli et al. 2009                                               |
| Hungary      | Balaton       | 3.2³⁵             | Eutrophic      | *P. agardhii*      | ---             | Horti et al. 2007                                                   |
Table 4. Lakes were *Planktothrix* spp. has been reported to form recurrent blooms. (a) Bloom Sample/Environmental sample, (b) Filaments isolated from bloom samples, (c) anti-Adda ELISA Kit, (d) HPLC, (e) protein phosphatase 2A inhibition assay (PP2A), (f) Mean depth., (---) Information not available.
establish populations at several depths in the water column that allows them to access nutrients located near the bottom and still have enough light for photosynthesis, making them able to form blooms away from the surface. This unique characteristic of *Planktothrix* may possess a problem for the water monitoring authorities, since their bloom may be overlooked by surface monitoring inspection (Sivonen & Jones 1999). Furthermore, *Planktothrix* blooms may co-occur with other cyanobacterial surface blooms what can also be misleading in water monitoring. Generally cyanobacteria blooms are expected to occur in highly nutrient rich waters during summer or spring months (Chorus et al., 2000). The responsible agencies for the reservoirs monitoring often restrict or increases to normal level the water inspection and water sampling frequency. *Planktothrix* species such as *P. rubescens* occurs in low nutrient oligotrophic waters forming perennially blooms that can prevail for many years. Furthermore since nutrients are not a limiting factor for *P. rubescens* it has been reported the lodging and development of population of this species after restoration lake activities and decrease in nutrient input since it improves trophic level and increases water transparency (Jacquet et al., 2005; Legnani et al., 2005; Ernst et al., 2009). So, in lakes were *Planktothrix* species occur the surveillance must be during all year (Utkilen et al., 1999; Naselli-Flores et al., 2007). Other important feature is that *Planktothrix* may contain higher microcystins content per cell, when compared with other microcystins producers; and that the proportion of toxic strains is higher in *Planktothrix* blooms than for example *Microcystis* blooms, this may result in the occurrence of high toxin concentrations in water without scum formation (Falconer et al., 1999; Briand et al., 2008; Ernst et al., 2009).

6. *Planktothrix* spp. occurrence in Portugal

*Planktothrix* species can be commonly found in Portuguese freshwater reservoirs. Some of the species reported are *P. mougeoti/P. isothrix* from a wastewater treatment plant in the north of Portugal (Vasconcelos & Pereira 2001, Martins et al. 2010), *P. rubescens* from Beliche reservoir in the South of Portugal (Paulino et al. 2009a) and *P. agardhii* and *P. pseudoagardhii* isolated from several reservoirs in the center and south of Portugal that are maintained in laboratory cultures (Paulino et al. 2009b). However, their occurrence is more pronounced in the center and south of Portugal where it has been increasing and causing problems in some water reservoirs over the last years, such as the deep layer *P. rubescens* bloom with associated microcystin production reported by Paulino et al. 2009. Another example is the particular case of a drinking water reservoir located in the center of Portugal that has been monitored over the last eight years and where a continuous *Planktothrix* spp. bloom persists since 2006 (Fig. 5).

As it can be depicted from Fig. 5 high *Planktothrix* cell concentrations started to appear in the reservoir in 2006 and microcystin concentration increased significantly since 2007. Furthermore, the microcystin concentrations in raw water does not correlate will *Planktothrix* cell numbers, since a high cell concentration does not indicate the presence of high microcystin concentrations and high concentrations of microcystins are not directly associated with high cell densities. This is probably because distinct strains/species of this genus with distinct ability to produce microcystins may occur together. In fact, a natural cyanobacterial population is usually a consortium of toxic and nontoxic strains, and this is believed to be the reason why the population toxicity can vary over time and between samples (WHO, 1999).
As it can be seen by this monitoring data, *Planktothrix* can suddenly reach high cell densities and dominate the phytoplankton community presenting cell densities values close to total phytoplankton concentration. The figure also shows that *Planktothrix* can form perennial blooms but during this time no visible scum formation was observed within the reservoir. It is still unknown why this bloom of toxic *Planktothrix* persists for 5 years in this reservoir and the answer to this issue will be certainly an important contribution to the knowledge of cyanobacteria ecotoxicology. Since the beginning of this *Planktothrix* bloom this reservoir has been under strict vigilance: monitoring sampling is regular, cellular composition/densities and microcystin content in the samples are always screened and the water treatment plant efficiency analysed to avoid any possible harmful effect on the population. Nevertheless, due to the persistence of high cell densities and high toxin contents occasionally observed, the reservoir represents a potential risk for human and wildlife. Therefore, studies must be performed in order to understand the factors underlying the bloom appearance, persistence and toxicity and to access the risk that this reservoir represents to human health, in order to apply measures to prevent and manage the risk of *Planktothrix* occurrence in the reservoir and to restore the quality of this water-supply.

7. Conclusion

The risk of human exposure to toxic cyanobacteria is very difficult to assess because many scientific issues remain to be clarified, such as the toxicological properties of cyanotoxins and their real impact on human health. Nevertheless, the establishment of several guidelines for the most common toxins and the establishment of surveillance programs have contributed to minimize the human exposure to toxic cyanobacteria. However, particular attention should be taken for those species, such as *Planktothrix*, that develop particular strategies to adapt, survive and proliferate in freshwater environments. Therefore, the monitoring programs in water reservoirs where *Planktothrix* species occur must have into account that samples should be taken at several depths, microcystin concentration should be accessed constantly and the water system should be monitored regularly throughout the
years since perennial persistence of *Planktothrix* may occur. In water capture for potable water treatment plants the selection of water off-take depth is important and the infrastructures must be equipped with multiple off-takes. In water reservoirs where *Planktothrix* species occurs, certain particularities must be taken into account (Fig. 6) in order to implement the most adequate risk assessment procedures, monitoring programs and preventive measures to protect public health from cyanotoxin occurrence in freshwater supplies.

Fig. 6. Schematic representation of the steps involved in *Planktothrix* risk management.
8. Acknowledgments

We acknowledge the Ph.D research grant SFRH/BD65706/2009 to Catarina Churro from Fundação para a Ciência e a Tecnologia (Portugal) and the research grant BIC/04/DSA/2008 attributed to Elsa Dias by Instituto Nacional de Saúde Dr. Ricardo Jorge (Portugal).

9. References

Akcaalan, R.; Young, F.M.; Metcalf J.S.; Morrison, L.F.; Albay, M. & Codd, G.A. (2006) Microcystin analysis in single filaments of Planktothrix spp. in laboratory cultures and environmental blooms. Water Research, Vol.40, No.8, pp. 1583-1590.

Almodovar, A.; Nicola, G. G. & Nuevo, M. (2004) Effects of a bloom of Planktothrix rubescens on the fish community of a Spanish reservoir. Limnetica, Vol.23, No.1-2, pp. 167-178.

Al-Tebrineh, J.; Gehringer, M.M.; Akcaalan, R. & Neilan, B.A. (2011) A new quantitative PCR assay for the detection of hepatotoxicogenic cyanobacteria. Toxicon Vol.57, No.4, pp. 546-554.

Bartram, J.; Burch, M.; Falconer, I.R.; Jones, G. & Kuiper-Goodman, T. (1999) Chapter 6. Situation Assessment, Planning and Management. In: Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. I. Chorus, Bartram, J. (Ed), WHO.

Bettinetti, R.; Morabito, G. & Provini, A. (2000) Phytoplankton assemblage structure and dynamics as indicator of the recent trophic and biological evolution of the western basin of Lake Como (N. Italy). Hydrobiologia, Vol.435, pp. 177–190.

Bláha, L.; Babica, P. & Maršíález, B. (2009) Toxins produced in cyanobacterial water blooms - toxicity and risks. Interdisciplinary Toxicology, Vol.2, No.2, pp. 36-41.

Briand, E.; Gugger, M.; Francois, J.C.; Bernard, C.; Humbert, J.F. & Quiblier, C. (2008) Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming Planktothrix agardhii (Cyanobacterium) population. Applied and Environmental Microbiology, Vol.74, No.12, pp. 3839-3848.

Briand, J.; Jacquet, S.; Bernard, C. & Humbert J. (2003) Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. Veterinary Research Vol.34, No.4, pp. 361-377.

Briand, J.F.; Jacquet, S.; Flinois, C.; Avois-Jacquet, C.; Maisonnette, C.; Leberre, B. & Humbert, J.F. (2005) Variations in the microcystin production of Planktothrix rubescens (cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. Microbial Ecology, Vol.50, No.3, pp. 418-28.

Bright, D.I. & Walsby A.E. (2000) The daily integral of growth by Planktothrix rubescens calculated from growth rate in culture and irradiance in Lake Zürich. New Phytologist, Vol. 146, pp. 301-316.

Buzzi, F. (2002). Phytoplankton assemblages in two sub-basins of Lake Como. Journal of Limnology, Vol.61, No.1, pp. 117-128.

Carmichael, W.W. (2001) Health effects of toxin-producing cyanobacteria: “The CyanoHABs”. Human and Ecological Risk Assessment, Vol.7, No.5, pp. 1393-1407.
Castenholz, R. W. (2001) Phylum BX. Cyanobacteria. In Bergey’s Manual of Systematic Bacteriology. D. R. Boone, Castenholz, R.W. (Ed). Springer, New York, 2001, pp. 473-599.

Catherine, A.; Quiblier, C.; Yepremian, C.; Got, P.; Groleau, A.; Vincon-Leite, B.; Bernard, C. & Troussellier, M. (2008) Collapse of a Planktothrix agardhii perennial bloom and microcystin dynamics in response to reduced phosphate concentrations in a temperate lake. FEMS Microbiology Ecology, Vol. 65, No.1, pp. 61-73.

Chorus, I.; Falconer, I.R.; Salas, H.J. & Bartram, J. (2000) Health risks caused by freshwater cyanobacteria in recreational waters. Journal of Toxicology and Environmental Health, Part B, Vol.3, No.4, pp. 323-347.

Codd, G.A. (2000) Cyanobacterial toxins, the perception of water quality, and the prioritization of eutrophication control. Ecological Engineering, Vol.16, No.1, pp. 51-60.

Codd, G.A.; Morrison, L.F. & Metcalf, J.S. (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology, Vol.203, No.3, pp. 264–272.

Davis, P.A. & Walsby, A.E. (2002) Comparison of measured growth rates with those calculated from rates of photosynthesis in Planktothrix spp. isolated from Blelham Tarn, English Lake District. New Phytologist, Vol. 156, pp. 225–239.

Duy, T.N.; Lam, P.K.S.; Shaw, G.R. & Connell, D.W. (2000) Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. Reviews of Environmental Contamination & Toxicology, Vol.163, pp. 113-186.

Erhard, M.; von Döhren, H.; Jungblut, P. R. (1999). Rapid identification of the new anabaenopeptin G from Planktothrix agardhii HUB 011 using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry, Vol. 13, No.5, pp. 337–343.

Ernst, B.; Hoefer, S.J.; O’Brien, E. & Dietrich, D.R. (2009) Abundance and toxicity of Planktothrix rubescens in the pre-alpine Lake Ammersee, Germany. Harmful Algae, Vol.8, No.2, pp. 329-342.

Falconer, I. (1999) An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. Environmental Toxicology, Vol.14, No.1, pp. 5 - 12.

Falconer, I.; Bartram, J.; Chorus, I.; Kuiper-Goodman, T.; Utkilen, H.; Burch M.; Codd G.A.(1999) Chapter 5. Safe levels and safe practices In: Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. I. Chorus, Bartram, J. (Ed), WHO.

Falconer, I.R.; Burch, M.D.; Steffensen, D.A.; Choice, M. & Coverdale, O.R. (1994) Toxicity of the blue-green alga (cyanobacterium) Microcystis aeruginosa in drinking water to
growing pigs, as an animal model for human injury and risk assessment. *Environmental Toxicology and Water Quality*, Vol.9, No.2, pp. 131–139.

Fawell, J.K.; James, C.P. & James, H.A. (1993) *Toxins from Blue-Green Algae: Toxicological Assessment of Microcystin-LR and a Method for its Determination in Water*. Foundation for Water Research, Marlow, England.

Fawell, J.K.; Mitchell, R.E.; Everett, D.J. & Hill, R.E. (1999) The toxicity of cyanobacterial toxins in the mouse: I Microcystin-LR. *Human & Experimental Toxicology*, Vol.18, No.3, pp. 162-167.

Figueroedo, C.; Giani, A. & Bird, D. (2007) Does allelopathy contribute to *Cylindrospermopsis raciborskii* (Cyanobacteria) bloom occurrence and geographic expansion? *Journal of Phycology* Vol.43, pp. 256-265.

Fitzgerald, D.J.; Cunliffe, D.A. & Burch, M. D. (1999) Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. *Environmental Toxicology*, Vol.14, No.1, pp. 203-209.

Gehringer, M.M. (2004) Microcystin-LR and okadaic acid-induced cellular effects: a dualistic response. *FEBS Letters*, Vol.557, No.1-3, pp. 1–8.

Giannuzzi, L.; Sedan, D., Echenique, R. & Andrinolo, D. (2011) An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina. *Marine Drugs*, Vol.9, pp. 2164-2175; doi:10.3390/md9112164.

Granéli, E. & Hansen, J. (2006) Allelopathy in Harmful Algae: A mechanism to compete for resources? In: *Ecology of Harmful Algae*. E. Granéli & Turner J. (Ed.). Ecological studies Vol. 189. pp. 189-201.

Halstvedt, C.B., Rohrlack, T.; Andersen, T.; Skulberg, O. & Edvardsen, B. (2007) Seasonal dynamics and depth distribution of *Planktothrix* spp. in Lake Steinsfjorden (Norway) related to environmental factors. *Journal of Plankton Research*, Vol.29, No.5, pp. 471-482.

Honti, M.; Istvánovics, V.; Osztóics, A (2007) Stability and change of phytoplankton communities in a highly dynamic environment—the case of large, shallow Lake Balaton (Hungary). *Hydrobiologia*, Vol. 581(1), pp. 225-240.

Humpage, A.R. & Falconer, I.R. (2003). Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. *Environmental Toxicology*, Vol.18, No.2, pp. 94-103.

Jacquet, S; Briand, J.-F.; Leboulanger, C.; Avois-Jacquet, C.; Oberhaus, L.; Tassin, B.; Vinçon-Leite, B.; Paolini, G.; Druart, J.-C.; Anneville, O. & Humbert, J.-F. (2005) The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae*, Vol.4, No.4, pp. 651-672.

Kangro, K.; Laugaste, R.; Nõges, P.; Ott, I. (2005). Long-term changes and seasonal development of phytoplankton in a strongly stratified, hypertrophic lake. *Hydrobiologia* Vol. 547, pp. 91-103.

Kinnear, S. (2010) *Cylindrospermopsis*: a decade of progress on bioaccumulation research. *Marine Drugs*, Vol.8, No.3, pp. 542-564.

Komárek, J. & Komárková, J. (2004) Taxonomic review of the cyanoprokaryotic genera *Planktothrix* and *Planktothricoides*. *Czech Phycology*, Vol.4, pp. 1-18.
Kosol, S.; Schmidt, J. & Kurmayer, R. (2009) Variation in peptide net production and growth among strains of the toxic cyanobacterium *Planktothrix* spp. *European Journal of Phycology*, Vol.44, No.1, pp. 49-62.

Kurmayer, R. & Gumpenberger, M. (2006) Diversity of microcystin genotypes among populations of the filamentous cyanobacteria *Planktothrix rubescens* and *Planktothrix agardhii*. *Molecular Ecology*, Vol.15, No.12, pp. 3849-3861.

Kurmayer, R.; Christiansen, G.; Fastner, J. & Borner, T. (2004) Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. *Environmental Microbiology*, Vol.6, No.8, pp. 831-841.

Legnani, E.; Copetti, D.; Oggioni, A.; Tartari, G.; Palumbo, M. T. & Morabito, G. (2005). *Planktothrix rubescens*’ seasonal dynamics and vertical distribution in Lake Pusiano (North Italy). *Journal of Limnology*, Vol.64, No.1, pp. 61-73.

Lindholm, T.; Öhman, P.; Kurki-Helasmo, K.; Kincaid, B. & Meriluoto, J. (1999) Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. *Hydrobiologia*, Vol.397, pp. 109–120.

Luukkainen, R.; Sivonen, K.; Namikoshi, M.; Fardig, M.; Rinehart, K.L.; Niemela, S.I. (1993) Isolation and Identification of eight Microcystins from thirteen *Oscillatoria agardhii* strains and structure of a new Microcystin. *Applied and Environmental Microbiology*, Vol. 59, No. 7, pp. 2204-2209.

MacPhail, R.C. & Jarema, K.A. (2005) Prospects on behavioral studies of marine and freshwater toxins. *Neurotoxicology and Teratology*, Vol. 27, No.5, pp. 695-699.

Manganelli, M.; Scardala, S.; Stefanelli, M.; Vichi, S.; Mattei, D.; Bogialli, S.; Ceccarelli, P.; Corradetti, E.; Petrucci, I.; Gemma, S.; Testai, E. & Funari, E. (2010) Health risk evaluation associated to *Planktothrix rubescens*: An integrated approach to design tailored monitoring programs for human exposure to cyanotoxins. *Water Research*, Vol.44, No.5, 1297-1306.

Mankiewicz-Boczek, J.; Gagala, I.; Kokocinski, M.; Jurczak, T. & Stefaniak, K. (2011) Perennial toxigenic *Planktothrix agardhii* bloom in selected lakes of Western Poland. *Environmental Toxicology*, Vol.26, No.1, pp. 10-20.

Martins, J.; Peixe, L.; Vasconcelos, V. (2010) Cyanobacteria and bacteria co-occurrence in a wastewater treatment plant: absence of allelopathic effects. Water Science Technology, Vol. 62I(8), pp. 1954-1962.

Mbedi, S.; Welker, M.; Fastner, J. & Wiedner, C. (2005) Variability of the microcystin synthetase gene cluster in the genus *Planktothrix* (Oscillatoriales, Cyanobacteria). *FEMS Microbiology Letters*, Vol.245, No.2, pp. 299-306.

Mihali, T.K.; Kellmann, R. & Neilan, B.A. (2009) Characterisation of the paralytic shellfish toxin biosynthesis gene clusters in *Anabaena circinalis* AWQC131C and *Aphanizomenon* sp. NH-5. *BMC Biochemistry*, Vol. 10, No.8, DOI:10.1186/1471-2091-10-8.

Montealegre, R. J.; Verreth, Steenbergen, K.; Moed, J.; Machiels, M. (1995) A dynamic simulation model for the blooming of *Oscillatoria agardhii* in a monomictic lake. *Ecological Modelling* Vol. 78, pp. 17-24.

Morabito, G.; Ruggiu, D. & Panzani, P. (2002) Recent dynamics (1995-1999) of the phytoplankton assemblages in Lago Maggiore as a basic tool for defining www.intechopen.com
association patterns in the Italian deep lakes. *Journal of Limnology*, Vol.61, No.1, pp. 129-145.

Mur, L.; Skulberg, O. & Utkilen, H. (1999) Chapter 2. Cyanobacteria in the environment. In: *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*. I. Chorus, Bartram, J. (Ed), WHO.

Naselli-Flores, L.; Barone, R.; Chorus, I.; Kumorayer, R. (2007). Toxic Cyanobacterial Blooms in Reservoirs Under a Semi-arid Mediterranean Climate: The Magnification of a Problem. *Environmental Toxicology*, Vol. 22, pp. 399-404.

Nõges, T. & Kangro, K. (2005). Primary production of phytoplankton in a strongly stratified temperate lake. *Hydrobiologia*, Vol.547, pp. 105-122.

Oberhaus, L.; Briand, J.F.; Leboulanger, C.; Jacquet, S. & Humbert, J. F. (2007) Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. *Journal of Phycology*, Vol.43, No.6, pp. 1191-1199.

Paulino, S.; Sam-Bento, F.; Churro, C.; Alverca, E.; Dias, E.; Valério, E. & Pereira, P. (2009) The Estela Sousa e Silva Algal Culture Collection: a resource of biological and toxicological interest. *Hydrobiologia*, Vol.636, No.1, pp. 489-492.

Paulino, S.; Valério, E.; Faria, N.; Fastner, J.; Welker, M.; Tenreiro, R. & Pereira, P. (2009a) Detection of *Planktothrix rubescens* (Cyanobacteria) associated with microcystin production in a freshwater reservoir. *Hydrobiologia*, Vol.621, No.1, pp. 207-211.

Pouliskova, A.; Hasler, P. & Kitner, M. (2004) Annual cycle of *Planktothrix agardhii*(Com.) Anagn. & Kom. Nature Population. *International Review of Hydrobiology*, Vol.89, No.3, pp. 278-288.

Pouria, S.; Andrade, A.; Barbosa, J.; Cavalcanti, R.L.; Barreto, V.T.S.; Ward, C.J.; Preiser, W. & Poon, G.K. (1998) Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *The Lancet*, Vol.352,No.9121, pp. 21-26.

Rücker, J.; Wiedner, C.; Zippel, P. (1997) Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes. *Hydrobiologia*, Vol. 342/343, pp. 107-115.

Rudi, K.; Skulberg, O.M. & Jakobsen, K.S. (2005) 16S rDNA analyses of the cyanobacterial microbiota through the water-column in a boreal lake with a metalimnic *Planktothrix* population. *Preparative Biochemistry & Biotechnology*, Vol.35, No.4, pp. 301-312.

Salmaso, N. (2000). Factors affecting the seasonality and distribution of cyanobacteria and chlorophytes: a case study from the large lakes south of the Alps, with special reference to Lake Garda. *Hydrobiologia* Vol. 438, pp. 43-63.

Salmaso, N. (2002). Ecological patterns of phytoplankton assemblages in Lake Garda: seasonal, spatial and historical features. *Journal of Limnology*, Vol.61, No.1, pp. 95-115.

Salmaso, N. (2010) Long-term phytoplankton community changes in a deep subalpine lake: responses to nutrient availability and climatic fluctuations. *Freshwater Biology*, Vol.55, No.4, pp. 825-846.

Shaw, G.R.; Seawright, A.A.; Moore, M.R. & Lam, P.K.S. (2000) Cylindrospermopsin, a cyanobacterial alkaloid: evaluation of its toxicologic activity. *Therapeutic Drug Monitoring*, Vol.22, No.1, pp. 89-92.
Sivonen, K. & Jones, G. (1999) Chapter 3. Cyanobacterial Toxins. In: Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. I. Chorus, Bartram, J. (Ed), WHO.

Stefaniak, K.; Kokocinski, M. & Messyasz, B. (2005) Dynamics of Planktothrix agardhii (Gom.) Anagn. et Kom. blooms in polimictic lake Laskownickie and Grylewskie (Wielkopolska region) Poland. Oceanological and Hydrobiological Studies, Vol.34, No.3, pp. 125-136.

Stewart, I.; Webb, P.; Schluter, P. & Shaw, G. (2006) Recreational and occupational field exposure to freshwater cyanobacteria - a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. Environmental Health: A Global Access Science Source, Vol.5, No.1, pp. 6. doi:10.1186/1476-1069X-1185-1186.

Sunda, W.; Granelli, E. & Gobler, C. (2006) Positive feedback and the development and persistence of ecosystem disruptive algal blooms. Journal of Phycology Vol.42, pp. 963-974.

Svrcek, C. & Smith, D.W. (2004) Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. Journal of Environmental Engineering and Science, Vol.3, No.3, pp. 155-185.

Sze, P. (1986) Prokaryotic Algae (Cyanophyta, Prochlorophyta). In A Biology of the Algae. WCB Publishers, pp. 19-34.

Ueno, Y.; Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Watanabe, M.F.; Park, H.D.; Chen, G.C.; Chen, G. & Yu, S.Z. (1996) Detection of microcystins, a blue-green algal hepatotoxins, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis, Vol.17, No.6, pp. 1317-1321.

Utkilen, H.; Fastner J.; Bartram J. (1999) Chapter 11. Fieldwork: site inspection and sampling. In: Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. I. Chorus, Bartram, J. (Ed), WHO.

Valério, E.; Chaves, S. & Tenreiro, R. (2010) Diversity and Impact of Prokaryotic Toxins on Aquatic Environments: A Review. Toxins Vol.2, No.10, pp. 2359-2410.

van Enden, H.P.; Egmond, H.P.; Speijers, G.J.A. & Bakker, G.J. (2007) Toxins of cyanobacteria. Molecular Nutrition & Food Research, Vol.51, No.1, pp. 7-60.

van den Hoek, C.; Mann, D.G. & Jahns, H.M. (1995) Algae. An introduction to phycology. Cambridge University Press.

Vance, B. (1965) Composition and succession of cyanophycean water blooms. Journal of Phycology Vol. 1, pp. 81-86.

Vareli, K.; Briasoulis, E.; Filidis, G. & Sainis, I. (2009) Molecular confirmation of Planktothrix rubescens as the cause of intense, microcystin—Synthesizing cyanobacterial bloom in Lake Ziros, Greece. Harmful Algae, Vol.8, No.3, pp. 447-453.

Vasconcelos, V. (2006) Eutrophication, toxic cyanobacteria and cyanotoxins: when ecosystems cry for help. Limnatica Vol.25, No.1-2, pp. 425-432.

Vasconcelos, V.M. & Pereira, E. (2001) Cyanobacteria diversity and toxicity in a wastewater treatment plant (Portugal). Water Research, Vol. 35, No.5, pp. 1354-1357.
Viaggiu, E.; Calvanella, S.; Mattioli, P.; Albertano, P.; Melchiorre, S. & Bruno, M. (2003) Toxic blooms of *Planktothrix rubescens* (Cyanobacteria/Phormidiaceae) in three waterbodies in Italy. *Algological Studies*, Vol.109, No.1, pp. 569-577.

Viaggiu, E.; Melchiorre, S.; Volpi, F.; Di Corcia, A.; Mancini, R.; Garibaldi, L.; Crichigno, G. & Bruno, M. (2004) Anatoxin-a toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. *Environmental Toxicology*, Vol.19, No.3, pp. 191-197.

Whitton, B.A. & Potts, M. (2000) Chapter 1. Introduction to the cyanobacteria. In *The ecology of cyanobacteria*. B.A Whitton, Potts, M. (Ed). Kluer Academic Publishers, Netherlands, pp. 1-11.

WHO (1998) *Guidelines for drinking water quality*, 2nd ed., Addendum to Vol. 2, Health criteria and other supporting information. World Health Organization: Geneva, Switzerland.

WHO (1999) Chapter 2. Cyanobacteria in the environment. In *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. I. Chorus, Bartram, J. (Ed). World Health Organization.

WHO (2003) *Guidelines for Safe Recreational Water Environments. Coastal and Freshwaters*. World Health Organization: Geneva, Switzerland, Vol.1, pp. 136–158.

Yepremian, C.; Gugger, M.F.; Briand, E.; Catherine, A.; Berger, C.; Quiblier, C. & Bernard, C. (2007) Microcystin ecotypes in a perennial *Planktothrix agardhii* bloom. *Water Research*, Vol.41, No.19, pp. 4446-4456.

Yu, S.Z. (1995) Primary prevention of hepatocellular carcinoma. *Journal of Gastroenterology and Hepatology*, Vol.10, No.6, pp. 674-82.

Zegura, B.; Štraser, A. & Filipič, M. (2011) Genotoxicity and potential carcinogenicity of cyanobacterial toxins – a review. *Mutation Research*, Vol.727, No.1-2, pp. 16–41.

Zhou, L.; Yu, H. & Chen K. (2002) Relationship between microcystin in drinking water and colorectal cancer. *Biomedical and Environmental Sciences*, Vol.15, No.2, pp. 166-71.
Risk assessment is a critical component in the evaluation and protection of natural or anthropogenic systems. Conventionally, risk assessment is involved with some essential steps such as the identification of problem, risk evaluation, and assessment review. Other novel approaches are also discussed in the book chapters. This book is compiled to communicate the latest information on risk assessment approaches and their effectiveness. Presented materials cover subjects from environmental quality to human health protection.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Catarina Churro, Elsa Dias and Elisabete Valério (2012). Risk Assessment of Cyanobacteria and Cyanotoxins, the Particularities and Challenges of Planktothrix spp. Monitoring, Novel Approaches and Their Applications in Risk Assessment, Dr. Yuzhou Luo (Ed.), ISBN: 978-953-51-0519-0, InTech, Available from: http://www.intechopen.com/books/novel-approaches-and-their-applications-in-risk-assessment/risk-assessment-of-cyanobacteria-and-cyanotoxins-the-particularities-and-challenges-of-planktothrix-
