Risk Assessment of Cyanobacteria and Cyanotoxins, the Particularities and Challenges of *Planktothrix* spp. Monitoring

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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 *Anabena 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).
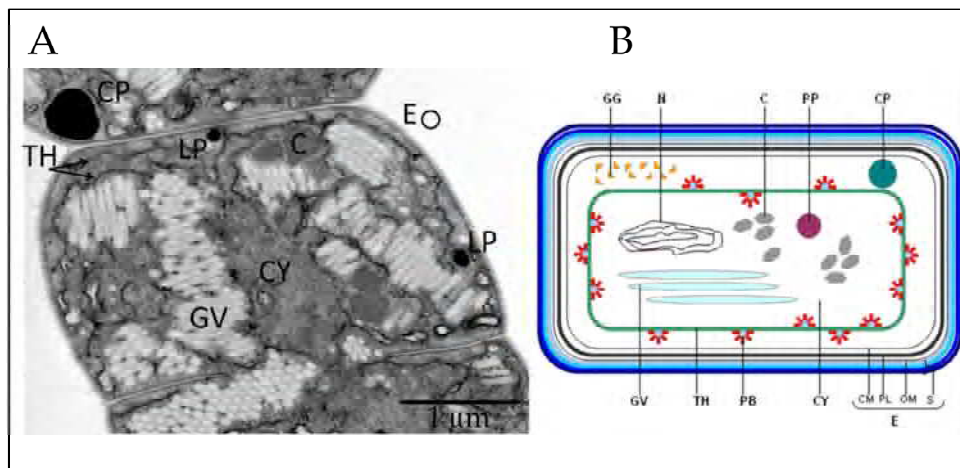

Fig. 2. Cyanobacteria cell structure. (A)Transmission electron micrographs showing the ultrastructure of an *Anabena circinalis* vegetative cell; (B) Schematic diagram of a cyanobacterial vegetative cell. S: external 4-layered cell wall; OM: outer membrane; PL: peptidoglycan layer; CM: cytoplasmic membrane; 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 ecostrategies 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) (Cyclic heptapeptides)	*Microcystis* spp. *Planktothrix* spp. *Oscillatoria* *Nostoc* *Anabaena* spp. *Anabaenopsis* *Hapalosiphon* *Snovella* *Woronichinia* *Aphanocapsa*	25 to ~1000µg/kg bw	Liver	Multi-pathway process. MCs inhibit the serine/threonine protein phosphatases type 1 and type 2A (PP1/PP2A) and induces oxidative stress leading to a cascade of events responsible for the MC cytotoxic and genotoxic effects in animal cells
Nodularin (9 variants) (Cyclic pentapeptides)	*Nodularia spumigena*	30–50 µg/kg bw	Liver	Similar to MCs
Cytotoxin				
Cylindrospermopsin (3 variants) (Guanidine alkaloid)	*C. raciborskii* *Umezakia natans* *Aph. ovalisporum* *Raphidiopsis curvata* *Anabaena bergii* *Aphanizomenon* *Lyngbya*	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 cytochrome P450
Dermatotoxins				
Aplysiatoxin (phenolic bislactone)	*Lyngbia* *Planktothrix* *Schizothrix*	107–117 µg/kg	Skin	Inflammatory agent, protein kinase C activator
Debromoaplysiatoxin (phenolic bislactone)	*Lyngbia*	107–117 µg/kg	Skin	Inflammatory agent, protein kinase C activator
Lyngbiatoxin-A (Alkaloid)	*Lyngbia*	250 µg/kg (?LD100)	Skin	Inflammatory agent, protein kinase C activator

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Toxin	Taxon	LD$_{50}$* (i.p., mouse) of pure toxin	Primary target in mammals	Mechanism of action
Neurotoxins				
Anatoxin-a (5 variants) (Tropane-related alkaloids)	*Anabaena* spp. *Oscillatoria Cylindrospermum Microcystis Aphanizomenon Planktothrix*	250 µg/kg bw	Post-synaptic neuromuscular junction	Agonism of the muscular and neuronal nicotinic acetylcholine receptor
Homoanatoxin-a (alkaloid)	*Planktothrix Oscillatoria Anabaena Raphidiopsis Phormidium*	250 µg/kg bw	Post-synaptic neuromuscular junction	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) (Carbamate alkaloids)	*Anabaena Aphanizomenon C. raciborskii Lyngbya Planktothrix*	10–30 µg/kg bw	Axons	Blockage of the sodium or calcium channels of the nerve axon membranes

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 Apeldoom et al., 2007; Bláha et al., 2009; Valério et al., 2010; Mihali et al., 2009. * - the dose needed to kill 50% of exposed animals.

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 allergenic 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 Apeldoom 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 heamodialysis treatment: weakness, muscular pain, nauseas, 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).

Toxicological studies

Compound structure-activity relation

Epidemiological studies

Hazard Identification

Source

Dose of exposure

Exposure assessment

Exposure pathways

LOAEL (Lowest Observed Adverse Effect Level)

Duration of exposure

Dose-response assessment

Pattern of exposure

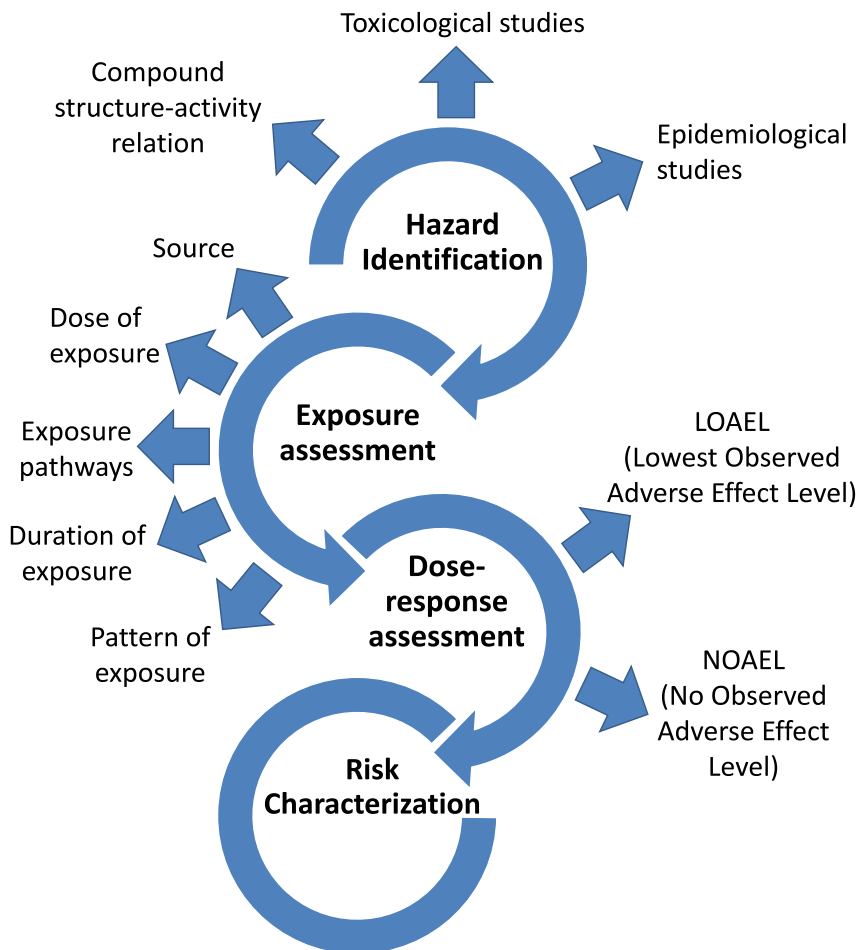NOAEL (No Observed Adverse Effect Level)

Risk Characterization

Fig. 3. Organizational chart of the steps involved in risk assessment (adapted from Dolah et al. 2001).

Besides the acute effects mentioned above, few papers reports 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 Zĕgura 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 resultants 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 mm^3 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 mm^3 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 mm^3 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:

$$TDI = \frac{NOAEL \text{ or } LOAEL}{UF} \qquad (1)$$

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

$$UF=1000 \begin{Bmatrix} 10 \text{ (intra-specific variations)} \\ 10 \text{(inter-specific variations)} \\ 10 \text{ (less-than-lifetime study)} \end{Bmatrix} \qquad (2)$$
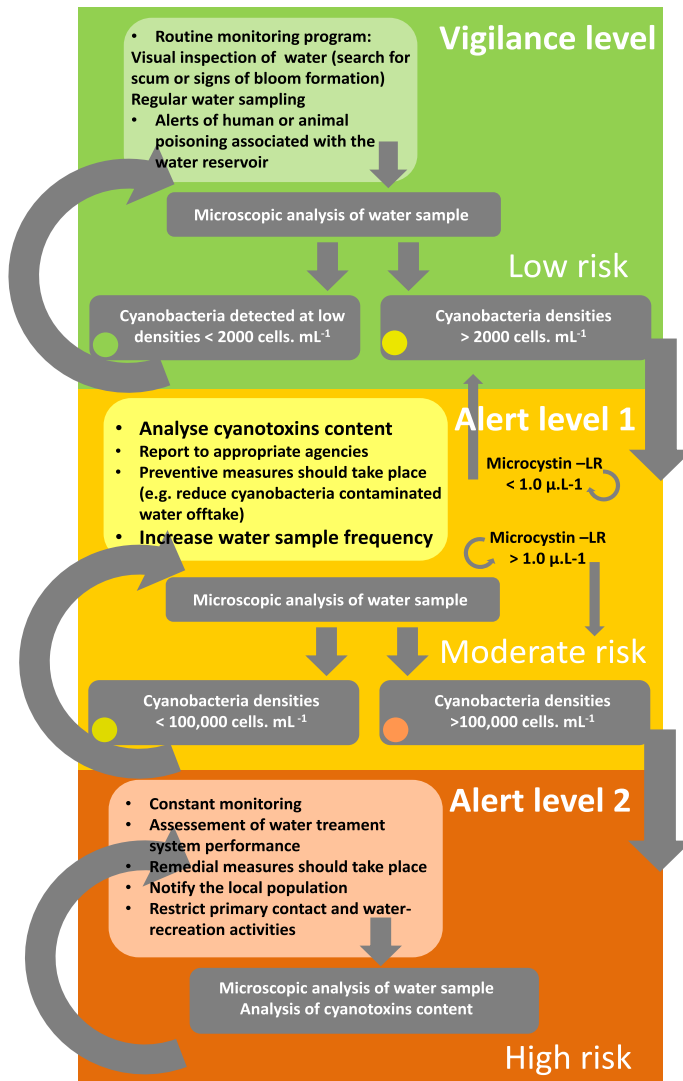

Fig. 4. Organizational chart of the steps involved in cyanobacteria risk management (adapted from Bartram et al. 1999).

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:

$$GV = \frac{(TDI) \times body\ wt \times AF}{C} \tag{3}$$

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 Alocation 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. Svrcek & 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 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 0.03 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 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		Fitzgerald et al., 1999; Chorus, 2005; van Apeldoorn et al., 2007
	1.0 µg/L	New Zealand	
Anatoxin-a	3.0 µg/L (no official guideline)		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		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 to 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 metalimnic 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 cyanobateria, 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

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Country	Lake	Maximum depth (m)	Trophic status	Species	Toxins detected	References
Austria	Mondsee	68	Mesotrophic	*P. rubescens*	---	Kurmayer et al. 2004; Kurmayer & Gumpenberger 2006
	Irrsee	32	Mesotrophic	*P. rubescens*	---	Kurmayer et al. 2004; Kurmayer & Gumpenberger 2006
	Afritzer	2.5	Mesotrophic	*P. rubescens*	---	Kurmayer & Gumpenberger 2006
	Wörthersee	85.2	Mesotrophic	*P. rubescens*	---	Kurmayer & 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
	Elmhirst	Shalow	---	*P. agardhii*	3.2 $\mu.L^{-1}$ 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*	---	Nõges & Kangro 2005; Kangro et al. 2005
Finland	Vargsundet	32	Oligotrophic	*P. agardhii*	11.1 $\mu g.L^{-1}$ MC-RR [a,d]	Lindholm et al. 1999
France	Bourget	145	Mesotrophic	*P. rubescens*	Max. 6.7 $\mu.L^{-1}$ MC-LR [a,d]	Briand et al. 2005, Jacquet et al. 2005
	Viry-Châtillon	5.3	Hypertrophic	*P. agardhii*	Máx. 7.4 $\mu g.L^{-1}$ MC-LR [a,e] Máx. 34.5 $\mu g.L^{-1}$ MC-LR [a,d]	Catherine et al. 2008, Yéprémian et al. 2007
Germany	Ammersee	81.1	Mesotrophic	*P. rubescens*	Mean 0.43 ± 0.06 $\mu g.L^{-1}$ MC-LR [a,c]	Ernst et al. 2009
	Stolpsee	Shalow	Eutrophic	*P. agardhii*	1.81 $\mu g.L^{-1}$ MC-LR [a,d]	Mbedi et al 2005
	Breiter Luzin	58.3	Mesotrophic	*P. rubescens*	2.31 $\mu.L^{-1}$ MC-LR [a,d]	Mbedi et al 2005
	Langer See	3.8	---	*P. agardhii*	---	Rücker et al. 1997
	Wolziger See	13	---	*P. agardhii*	----	Rücker et al. 1997
	Lebbiner See	4	---	*P. agardhii*	----	Rücker et al. 1997
Greece	Ziros	56	Oligotrophic	*P. rubescens*	Máx. 199 $\mu.L^{-1}$ MC-LR [a,c]	Vareli et al. 2009
Hungary	Balaton	$3.2^{(f)}$	Eutrophic	*P. agardhii*	---	Honti et al. 2007

Country	Lake	Maximum depth (m)	Trophic status	Species	Toxins detected	References
Italy	Pusiano	24.3	Eutrophic	P. rubescens	---	Legnani et al. 2005
	Como	410	Mesotrophic	P. rubescens	---	Bettinetti et al. 2000; Buzzi 2002
	Maggiore	370	Oligo-mesotrophic	P. rubescens	---	Morabito et al. 2002
	Fiastrone			P. rubescens		Viaggiu et al. 2003
	Spino	8		P. rubescens	12.13 ng.mg Anatoxin-a [a,d]	Viaggiu et al. 2003, Viaggiu et al. 2004
	Gerosa	50	Oligotrophic	P. rubescens	Máx. 1.94 µg.L^{-1} MC-LR [a,c]	Manganelli et al. 2010
	Pozzillo	50	Meso-eutrophic	P. rubescens	34 mg.L^{-1} MC-LR [a,d]	Naselli-Flores et al. 2007
	Prizzi	44	Meso-eutrophic	P. rubescens	7 µg L^{-1} MC-LR [a,d]	Naselli-Flores et al. 2007
	Nicoletti	36	Mesotrophic	P. rubescens		Naselli-Flores et al. 2007
	Garcia	43	Meso-eutrophic	P. rubescens	---	Naselli-Flores et al. 2007
	Garda	350	Oligo-mesotrophy	Planktothrix sp. P. rubescens	---	Salmaso 2000; Salmaso 2010
Netherlands	Vechten	11.9	---	P. agardhii	---	Montealegre et al. 1995
Norway	Steinsfjorden	24	Mesotrophic	P. rubescens P. agardhii	---	Halstved et al. 2007; Rudi et al. 2005
Poland	Bytynskie	3.5[f]	Eutrophic	P. agardhii	15.8 µ.L^{-1} MC-LR [a,e]	Mankiewicz-Boczek et al. 2009
	Lubosinskie	2.6[f]	Eutrophic	P. agardhii	21.9 µ.L^{-1} MC-LR [a,e]	Mankiewicz-Boczek et al. 2009
	Laskownickie	7.4	Hypertrophic	P. agardhii	---	Stefaniak et al. 2005
	Grylewskie	6.5	Hypertrophic	P. agardhii	---	Stefaniak et al. 2005
Portugal	Beliche	52	Eutrophic	P. rubescens	1.1 µg.L^{-1} MC-LR [a,c]	Paulino et al. 2009a
Spain	El Atazar	100	Oligo-mesotrophic	P. rubescens	---	Almodóvar et al. 2004
Switzerland	Zürich	143	Mesotrophic	P. rubescens	---	Bright & Walsby 2000; Kurmayer & Gumpenberger 2006
	Hallwilersee	48	Mesotrophic	P. rubescens		Kurmayer & Gumpenberger 2006
Turkey	Spanca	55	Oligo-Mesotrophic	P. rubescens	18.4-66.1 pg.filament^{-1} MC-LR [b,c]	Akcaalan et al. 2006
	Iznik	70	Mesotrophic	P. rubescens	29.2-114 pg.filament^{-1} MC-LR [b,c]	Akcaalan et al. 2006
	Küçükçekmece	20	---	P. agardhii	Nontoxic [a]	Al-Tebrineh et al. 2011
	Sapanca	52	---	P. rubescens	Mean 8.4 ± 0.5µL^{-1} MC-LR [a,d]	Al-Tebrineh et al. 2011

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 depts. 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 overlook 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 restricts or increases to normal level the water inspection and water sampling frequency. *Plantothrix* 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. mougeotii/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).
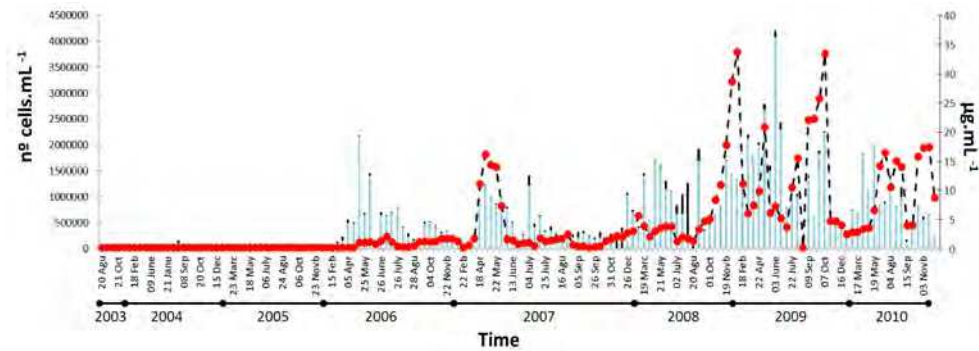
Fig. 5. *Planktothrix* occurrence in a freshwater reservoir located in the center of Portugal and microcystin-LR concentration in raw water over the same sampling period (--•-- microcystin concentration in $\mu g.mL^{-1}$, light blue bars represent *Planktothrix* spp. cell concentration in $n^{o}cells.mL^{-1}$, black bars represent total phytoplankton cell concentration in $n^{o}cells.mL^{-1}$).

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 begining 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 harmfull 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 wild life. 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

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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

9. References

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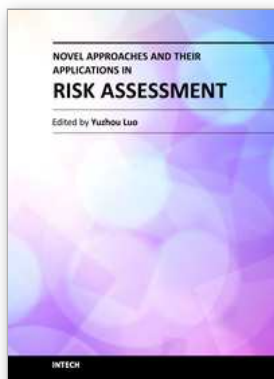
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Catarina Churro, Elsa Dias and Elisabete Valério (2012). Risk Assessment of Cyanobacteria and Cyanotoxins, the Particularities and Challenges of Planktothrix spp. Monitoring, Novel Approaches and Their Applications in Risk Assessment, Dr. Yuzhou Luo (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-

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