Emerging health issues of cyanobacterial blooms

Maura Manganelli[(a)], **Simona Scardala**[(a)], **Mara Stefanelli**[(b)], **Francesca Palazzo**[(a)],
Enzo Funari[(a)], **Susanna Vichi**[(a)], **Franca Maria Buratti**[(a)] and **Emanuela Testai**[(a)]

[(a)]*Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy*
[(b)]*Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro,
Dipartimento Installazioni di Produzione e Insediamenti Antropici, Rome, Italy*

Abstract. This paper describes emerging issue related to cyanobacterial dynamics and toxicity and human health risks. Data show an increasing cyanobacteria expansion and dominance in many environments. However there are still few information on the toxic species fitness, or on the effects of specific drivers on toxin production. Open research fields are related to new exposure scenario (cyanotoxins in water used for haemodialysis and in food supplements); to new patterns of co-exposure between cyanotoxins and algal toxins and/or anthropogenic chemicals; to dynamics affecting toxicity and production of different cyanotoxin variants under environmental stress; to the accumulation of cyanotoxins in the food web. In addition, many data gaps exist in the characterization of the toxicological profiles, especially about long term effects.

Key words: toxic cyanobacteria, human health, climate change, toxins, risk assessment.

Riassunto *(Problemi sanitari emergenti legati a fioriture di cianobatteri)*. Questo articolo esamina problemi emergenti relativi alla dinamica e alla tossicità di cianobatteri in relazione ai rischi per la salute umana. È nota la crescente diffusione dei cianobatteri in molti ambienti. Tuttavia, ci sono tuttora poche informazioni sulle specie tossiche e sui fattori in grado di modulare la tossicità. Sono aree di ricerca emergenti: nuovi scenari di esposizione (cianotossine nelle acque usate per emodialisi e negli integratori alimentari); nuovi modelli di co-esposizione a cianotossine e tossine algali e/o prodotti chimici artificiali; dinamiche di tossicità e produzione delle diverse varianti di cianotossine in condizioni di stress; trasferimento di cianotossine nella catena alimentare. Inoltre, sono ancora presenti lacune nella caratterizzazione dei profili tossicologici di molte tossine, specialmente sugli effetti a lungo termine.

Parole chiave: cianobatteri tossici, salute umana, cambiamenti climatici, tossine, valutazione del rischio.
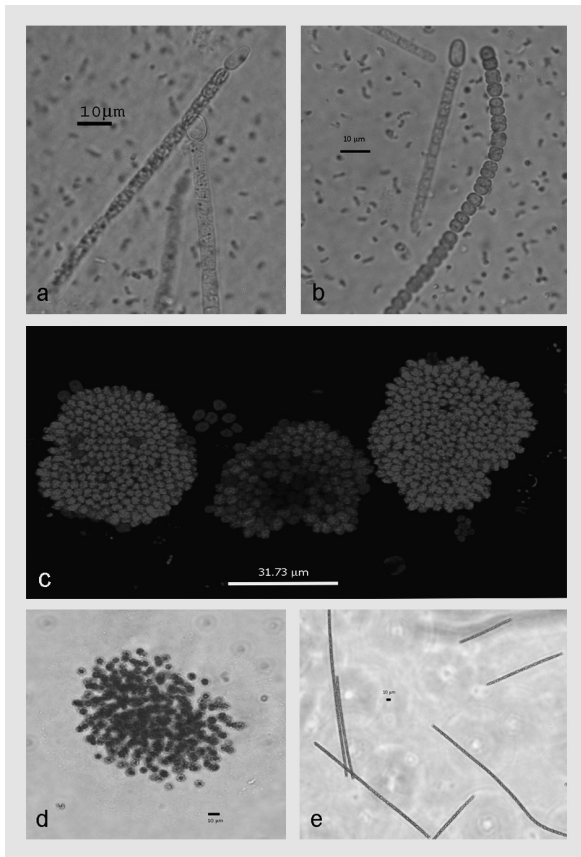
INTRODUCTION

Cyanobacteria are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches, from freshwater to hydrothermal vents, from desert rocks to Antarctic lakes *(Figure 1)*. They have been reported in freshwater lakes, basins, rivers, irrigation channels, brackish and sea waters, salty lakes, as pelagic or benthic organisms. Several cyanobacteria species produce toxins as secondary metabolites, which can impact on ecosystems, animal and human health [1, 2]. On the basis of toxin production, to which human can be exposed via different routes, WHO has listed cyanobacteria among the emerging health issues, although not considering them as emerging pathogens, since their possibility to infect human beings has never been reported so far.

Cyanobacteria are expanding geographically and now threaten the ecological integrity and sustainability of some of the world's largest and most resourceful water bodies, including Lakes Victoria (Africa), Erie (US-Canada), Okeechobee (Florida, USA), Taihu (China), Kasumigaura (Japan), the Baltic Sea in Northern Europe and the Caspian Sea in West Asia [3]. The present and possible future cyanobacterial bloom expansion can be attributed to nutrient over enrichment in watersheds with relevant human activities [3] and changing climatic conditions [4].

Predicting future scenarios is a major challenge to ensuring protection of human health. It is necessary to define the potential risk associated to cyanobacterial presence in different environment in relation to exposure routes. As a consequence, it will be possible to identify the appropriate management strategies to be adopted to protect water resources, mitigating the negative ecological and biogeochemical impacts and economic losses both in the short and long run. It has been estimated that only in the United Sates cyanobacterial blooms result in losses of recreational, drinking and agricultural water resources that are worth > $ 2 billion annually [5].

A lot of studies have been published on the occurrence of cyanobacteria and their ability to produce cyanotoxins in surface waters; they have been extensively reviewed in dedicated publications on both ecological and toxicological aspects [1, 2, 6, 7].

Address for correspondence: Maura Manganelli, Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: maura.manganelli@iss.it.

Fig. 1 | *Some examples of different morphologies.*
a) Cylindrospermopsis raciborskii *filaments; the Australian strain and the strain from North America produce cylindrospermopsins and saxitoxins, respectively; b)* Nostoc *sp. filaments, microcystins producer; c)* Woronichinia naegeliana*, unicellular colonies, microcystins producer; d)* Microcystis botrys *unicellular colonies, microcystins producer; e)* Planktothrix rubescens *filaments, microcystins producer (Photos by Stefanelli Mara).*

notoxins vs unknown ones. A number of data gaps can be identified in the toxicological profile of the different cyanotoxins known so far, as well as in the description of exposure scenarios for humans.

Although many data are available on cyanobacterial ecology, the most studied aspect so far, from the health perspective there are still many issues that have not been clarified and, most importantly, they can vary depending on the species and on the environmental area of occurrence. The knowledge of these aspects should be the solid scientific ground in developing models to better predict the occurrence of blooms and their toxicity, that would limit the monitoring activities saving human and economic resources, and consequently to identify efficient prevention measures.

This paper briefly summarizes the current knowledge on toxic cyanobacteria with specific focus on emerging issues and main gaps in the area of risk assessment. After the description of main habitats affected by toxic cyanobacteria bloom, other than the very well known freshwater rivers and lakes, exposure scenarios and effects on human health will be addressed. The impacts of climate changes on cyanobacteria diffusion and toxicity, and hence on human exposure, are addressed in Funari *et al.* [8], in this same journal issue.

DISTRIBUTION OF CYANOBACTERIA AND THEIR TOXICITY

Respect to the past, data are available indicating a wider distribution and increasing dominance of cyanobacteria in many environments. However there are much less clear indications on the fitness or diffusion of the toxic species, or on the effects of specific drivers on toxin production.

Cyanobacteria can bloom not only in euthrophic habitats, but also in oligotrophic environment thanks to various strategies. Some cyanobacteria, like *Anabaena*, and other filamentous ones, can resist in nitrogen (N) depleted waters because are diazotrophic, that is they can fix atmospheric nitrogen. For these organisms, phosphorous (P) seems the key nutrient for bloom formation, but they can still outcompete other organisms in environments with low dissolved P thanks to the formation of akinetes, sedimented in the bed of the water basin, which allow the organisms to get P from sediments

The number of papers on the issue has dramatically raised up in the last few years, after the increased occurrence of cyanobacterial blooms *(Figure 2)* and the awareness of their possible health impact, but also due to the continuously reported new findings. Indeed, the list of cyanobacterial species/strains appears to be far to come to an end, and at present it is not clear which is the proportion of known cya-

Fig. 2 | *Two blooms of* Microcystis aeruginosa *in temperate lakes.*

[9]. Otherwise, *Anabaena* and *Cylindrospermopsis raciborskii* can also use organic form of P [9]. *Microcystis aeruginosa* has a high affinity for dissolved inorganic phosphorous (DIP), which allows it to bloom in low DIP environment [9].

Other than high affinity nutrients systems and/or their own ectoenzymatic activity, cyanobacteria can utilize the nutrients mineralized by ectoenzymatic activity of other organisms. It has been demonstrated that cylindrospermopsin (CYN) produced by *Aphanizomenon ovalisporum* induces the release of alkaline phosphatase in other phytoplankton species. Under phosphorus limitation the production of CYN, coupled to a high affinity P uptake system, enables *Aphanizomenon* to take advantage of the enzymatically released P [10]. In a small oligotrophic lake in Central Italy, with undetectable inorganic and significant concentration of organic nitrogen, *Planktothrix rubescens* overcame nitrogen limitation thanks to bacterial amminopeptidase and chitinase activity [11], releasing small oligopeptides and organic compounds in the surrounding water. Therefore the strategic role of phytoplankton bacteria should be carefully considered, especially for oligotrophic water bodies not to underestimate the possible occurrence of cyanobacterial blooming episodes.

Marine planktonic cyanobacteria

Cyanobacteria occupy a wide range of niches in marine ecosystems in tropical and temperate regions, where they occur along eutrophic maritime coasts as well as in the oligotrophic open ocean representing more than 50% of phytoplankton biomass and carbon production [12]. According to Fristachi and Sinclair [13] the knowledge of adverse effects of cyanobacteria in the marine environments has only recently begun to be recognized. It is possible that in the future, as more information will be available, we will realize that the frequency of blooming and the impact for both human health and the environment are much higher.

Marine planktonic cyanobacteria are classified into three morphological and functional groups: unicellular (*Synechococcus, Prochlorococcus, Synechocystis, Aphanothece* and *Merismopedia*), non-heterocystous filamentous (*Lyngbya, Oscillatoria, Phormidium, Spirulina* and *Trichodesmium*), some of which can fix nitrogen, and heterocystous filamentous (*Anabaena, Aphanizomenon, Nodularia* and *Richelia*), all N_2-fixing organisms.

Among planktonic cyanobacteria, *Trichodesmium* is the most abundant and well studied diazotrophic cyanobacteria in the open ocean and in coastal water as well. It forms extensive blooms in oligotrophic, tropical, and subtropical oceans [14], usually characterized by a very stable water column and a deep upper mixed layer (about 100 m). The success of *Trichodesmium* in these low-nutrient water includes its capability to fix nitrogen, its natural buoyancy, the resistance to a high light regime, a relatively low growth rate [14] and the ability to utilize a wide range of organic phosphorus compounds [15]. Under calm conditions, filaments accumulate as surface blooms that are often large enough to be detected by satellite. *Trichodesmium* bloom production supports a large significant heterotrophic community, important for carbon and nitrogen fluxes and remineralization [14]. Some evidences of *Trichodesmium* spp. toxicity are available, related to more than one compound. Ramos *et al.* [16] found 0.1-1 μg g^{-1} microcystins (MCs) in a bloom of *Trichodesmium erythraeum*, which was recorded for the first time in 2004 in the Canary Islands Archipelago, during the warmest period recorded since 1912. Samples from a *Trichodesmium erythraeum* bloom off the Brazilian coast were analyzed for MCs, saxitoxins analogues and cylindrospermopsin [17]. MCs and STX were found in the range 10-302 μg g^{-1} MC-LR equivalent and 2-10 μg g^{-1} STX equivalent, respectively; samples resulted toxic to sea-urchin larvae, but did not show any acute toxicity in the mouse bioassay. *Trichodesmium* has also been known to produce the same toxins isolated from contaminated fishes involved in the ciguatera fish poisoning, an important food borne intoxication due to ingestion of fish contaminated with ciguatoxin [18]. Toxicological tests on lipid and water-soluble extracts from New Caledonia lagoon *Trichodesmium* spp. confirmed the production of ciguatoxin-like and neurotoxic-paralytic activity (PSP like toxin) [19]. Recently, the neurotoxic palytoxin and 42-hydroxy-palitoxin have been isolated and characterized from *Trichodesmium* bloom in New Caledonia [20]. The Authors suggest that the ingestion of fish contaminated by both palytoxin and ciguatoxin-like could lead to intoxication suffered from New Caledonian population. A novel cytotoxic toxin, trichotoxin, has been characterized from *Trichodesmium thiebautii*, from the Gulf of Mexico [21].

The planktonic species *Synechococcus* is ubiquitous in the oceans and nutrient rich marine environments, except for Arctic and Antarctic seawater [22]. It occurs in coastal areas and in the river plumes, where nutrients are abundant and salinity lower, together with *Prochlorococcus*, which is usually more abundant, except in upwelling areas. Few studies addressed toxicity in *Synechococcus* and *Synechocystis*. Analysis of crude and partially purified extracts from the two species from several Portuguese coastal sites revealed variable toxic effects on some marine invertebrates (*Artemia*, sea-urchin) [23]. High rate of migratory birds mortality in the Salton Sea, a lake that experiences a range of salinities from brackish to hypersaline in Southern California, has been at least partially associated to the presence of MCs produced by *Synechococcus* [24]. Recently, the presence of MCs in water and their accumulation in mussels in the Amvrakikos Gulf (Greece) was associated to the presence of *Synechococcus-Synechocystis* cells, as the only cyanobacteria in the analyzed community [25]. These results suggest that the risk associated to cyanobacteria in the seawater is probably not restricted only to filamentous species, but can be extended also to ubiquitous species like *Synechococcus*.

Coastal zone and brackish areas

The distribution of *Microcystis* which is a well known toxic freshwater genus, has now spread into several estuaries, with possible consequences on fishery production [26]. Indeed, *Microcystis* shows some salinity tolerance, with no significant variation in growth rate and toxin per cell quota up to 10 g salt L^{-1} [27]. *M. aeruginosa* can resist and produce toxins for one week to sudden exposure to salinities up to 17 g L^{-1}, although cell lysis causes an increase in extracellular quota of toxins of about 30% [27]. As reported in Funari *et al.* [8], heavy rainfall and floods have an important role in transporting cyanobacteria in estuaries and coastal waters, where many aquaculture plants are located, thus increasing the risk of exposure of edible organisms.

Toxic *Microcystis aeruginosa* and not toxic *M. wesenbergii* have been recently found in a shallow brackish lake (0.9% salinity) in China, following rising eutrophication conditions in the last few years, together with *Aphanizomenon* and *Anabaena* [28]. The brackish strains are not genetically different (16S rRNA, *mcyB*) from surrounding freshwater lakes, suggesting that the diffusion of *Microcystis* in the brackish waters is a recent event, and/or that the lake salinity does not exert a strong evolutionary pressure [28]. Even if the specific content of detected MCs in these strains is at present significantly lower than in other freshwater strains, it can become a future health problem at increasing densities/frequencies of blooming, since the basin represents an important fishery base [28].

Within Breton Sound estuary, Louisiana, during one year study (Sept 2007-Aug 2008) aimed to observe the effects of high and low river inputs on the phytoplankton community of the estuary, 5 toxin producing genera of cyanobacteria were observed, which were at least an order of magnitude more abundant than 4 genera of toxin producing dinoflagellate. The cyanobacteria genera included *Anabaena, Anabaenopsis, Microcystis, Raphidiopsis* and *Cylindrospermopsis*; they were most frequently observed in the outer portion of the estuary when the river input was low, and most abundant during warm season and at low nutrient concentration. Particulate and dissolved MCs were almost always detected throughout the estuary, in a range of concentrations 0.10-2.92 µg l^{-1} [29].

The northern reach of San Francisco Bay has been infested by an extensive bloom of the colonial form of Microcystis aeruginosa in 1999, and again in 2003, when 180 km of connected waterways was also covered by a bloom. Lower salinities associated with higher temperatures favored the high cyanobacterial density [30], whose origin was attributed to resident cells in sediment and seeded cells from tributaries and high streamflow. Microcystins were found along the waterways, with the highest concentrations in the transition zone, between fresh and brackish water, and in samples of zooplankton and clams, at levels of 0.7-3.5 µg g-dw^{-1} in the former and 0.02 µg g-dw^{-1} in the latter.

Benthic cyanobacteria

Besides planktonic cyanobacteria, benthonic species have also been described, producing cyanotoxins, although mechanisms and factors influencing their production are much less known than for planktonic species.

Cyanobacterial mats have been found in hot springs, a habitat characterized by harshness and extreme conditions, in which they can survive due to genetic adaptations [31]. Since most hot springs are accessible to the public worldwide, as recreational places for citizens and tourists and some are also used as cooking resources by rural communities, human exposure can easily occur. In a study of several hot springs in Saudi Arabia, 12 out of 17 isolated benthic species possessed lipopolysaccharide endotoxins (LPS) and 2 of them, *Oscillatoria limosa* and *Synechococcus lividus*, produced MCs at concentration ranging from 468 to 512.5 µg g^{-1} [32]. Dissolved MCs (5.7 µg l^{-1}) were also found in water. In hot springs on the shore of the alkaline Lake Bogoria in Kenia, dominated by *Phormidium terebriformis*, *Oscillatoria willei*, *Spirulina subsalsa* and *Synechococcus bigranulatus*, MCs and ATX are considered a possible cause for the deaths of Lesser Flamingos [33].

Toxic benthic species are well represented also in freshwater environments. Two southeast Queensland populations of *Lyngbya wollei*, a common freshwater mat-forming species, have been found to produce CYN and deoxyCYN at significant level [34], whereas in Southern United States the same species is known to produce STX [35]. In California some unidentified benthic filamentous cyanobacteria were isolated from four drinking water reservoirs, producing high concentration of MC-LR per carbon unit (1.15 to 4.15 µg mg^{-1} C^{-1}) [36]. Several toxic species of cyanobacteria (*Anabaena subcylindrica, A. variabiles, Calothrix parietina, Nostoc spongiaeforme, Plectonema boryanum, Phormidium corium* and *P. tenue*) were isolated from mats in the Nile River and in irrigation canals, producing MCs [37]. Sediments in Nile River and irrigation canals were also found to be contaminated by *M. aeruginosa* and MCs, suggesting a risk for the benthic fauna [38].

Anatoxin-a producing benthic species such as *Phormidium* sp. and *Oscillatoria* sp. have been identified as the causative agent of dog killing in different countries [39-42].

Benthic cyanobacteria are widespread also along maritime coasts, often forming visually epilithic growths and mats on rocks and sediments. The intertidal zone is dominated by taxa of filamentous non-heterocystous *(Lyngbya, Microcoleus, Phormidium, Schizothrix)* and heterocystous *(Rivula, Calothrix, Scytonema)* genera. The benthic coastal filamentous *Lyngbya maiuscula*, widespread in subtropical and tropical estuarine and coastal waters, is well known for the production of a variety of biologically active components, many of which highly toxic [43].

In the infra-littoral zone, a high biodiversity is found, especially near coral reefs, where the *Oscillatoria*-ocean

genera *(Oscillatoria, Hydrocoelum, Microcoleus)* are represented by many species. Recently, the production of homoanatoxin-a has been reported from mats of *Hydrocoelum lyngbyaceum* in New Caledonia, where giant clams intoxication has been reported, suggesting a possible relationship between the episodes and the presence of the neurotoxic cyanobacterium [44].

HUMAN HEALTH
EXPOSURE AND EFFECTS
Toxicological profiles of cyanotoxins

The presence of a cyanobacterial species or its blooming is not always synonymous of toxicity, which is rather associated to the ratio toxic vs nontoxic strain within the population and to dynamics through which this ratio is able to change in a very short time. A combination of temperature, light and nutrients, can affect, although with still unknown pattern and specific for each species, not only the success of toxic vs non-toxic strains and hence the total production of cyanotoxins in quantitative terms, but also the profile of cyanotoxin variants, which can be even more relevant from a toxicity perspective, considering their diverse toxicological potential [8].

A detailed description of the toxicological properties of cyanotoxins known so far can be found in extensive publications [1, 2, 6]. In the following a brief outline of their toxicological profile is depicted.

Among cyanotoxins, the most investigated group is that of hepatotoxins, which includes about 100 different congeners of cyclic epta-peptides termed microcystins (MCs), differing from each other for amino-acids substitutions in position 2 and 4 and other changes such as methylation/desmethylation. Microcystin mechanism of action is associated with specific inhibition of protein serine/threonine phosphatases (PP1 and PP2A), altering phosphorilation of cellular proteins involved in signal transduction [45]. Each congener is characterized by a different acute toxic potential and indeed, LD_{50}s are spread in a wide range of values (from 50 up to 1200 μg/kg) [1]. Recently, besides hepatotoxic effects, neurotoxic potential has been assessed for MC-LF and to a lesser extent to MC-LW and MC-LR [46]. The long-term effects, known for MC-LR, include tumour promoting activity, due to which IARC has classified MC-LR as possible human carcinogen (class 2B) [47]. In the absence of information on the other congeners, on the basis of the highest acute toxicological properties of MC-LR, concentration MC-LR equivalents are usually used as default value for the total concentration of all MC variants [2]; however, the extrapolation from the acute toxicity ranking among MC congeners to the chronic toxicity remains to be demonstrated.

Microcystins are produced mainly by *Microcystis* spp., *Planktothrix* spp. and some *Anabaena*. Within the same species, only some strains possess the gene for MCs biosynthesis: this is quite relevant since the toxicity of a cyanobacterial bloom is determined by its strain composition, *i.e.*, the relative share of toxic vs nontoxic genotypes: remarkable and rapid variations of the genotype ratio can occur even on a weekly scale [48]. In addition, it has been demonstrated that a mechanism of up- and down-regulation related to cell densities can regulate toxin production, so that the rate of toxin production can change in few hours [49]. Therefore MCs concentrations in water can significantly vary as the result of cyanobacterial population dynamics. The 6 variants of the penta-peptide nodularin (NOD), produced by the brackish-water species Nodularia spumigena, share the major toxicological feature with MCs.

Neurotoxins (*e.g.* anatoxins, anatoxin a(s) and saxitoxins), are produced by strains of the genera *Oscillatoria, Phormidium, Aphanizomenon* and *Anabaena*. All of them act on the neuromuscular system by blocking skeletal and respiratory muscles, causing death by respiratory failure. Anatoxins (ATX) are potent pre- and post-synaptic depolarizing agents, efficiently competing with acethylcoline for nicotinic receptors in neuromuscular junctions and in the central nervous system [50]. Anatoxin-a(s) irreversibly inhibits acetylcholinesterase (AChE) in the neuro-muscular junctions in the peripheral nervous system only. Saxitoxins (STXs) are a family of more than 30 natural alkaloids, synthesized by both freshwater cyanobacteria and by marine dinoflagellates by similar processes [51]: they block Na-channels in neuronal cells [52] and Ca^{++} and K^+ channels in cardiac cells, thus preventing the propagation of electrical transmission within the peripheral nerves and skeletal or cardiac muscles [53]. Based on the kind of intoxication, they are also called Paralytic Shellfish Poisoning (PSP).

Cylindrospermopsin (CYN) is produced by *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Umezakia natans*, or *Raphidiopsis curvata*. Its main target organs are the liver and kidney [54, 55], but its citotoxicity is common to other organs. Cylindrospermopsin has a late and progressive acute toxicity, associated to protein synthesis inhibition [56]. However, its metabolites, which have not been identified so far, very likely act with a different mechanism, involving interactions with DNA [57], as supported by CYN induced *in vitro* DNA damage in mouse primary hepatocytes [58] and in HepaRG cells [59].

In spite cyanotoxins represent an emergent health problem, the available toxicological information are limited, data on humans are very scant, and risk assessment is possible only in few cases, with huge degree of uncertainties [1]. In order to improve the reliability of quantitative risk assessment to protect human health, new toxicological information should become available. USEPA has indicated the study of relative contribution of different exposure routes to total exposure, the role of metabolism in toxic responses and detoxification, and particularly, the characteristics of human toxicokinetics, as major research needs [60]. Indeed, species-specific differences are very often attributable to differences in toxicokinetics processes, affecting the internal dose.

Other possible factors modulating the outcome of cyanotoxin exposure in humans are linked to differential exposure, or to the involvement of active transporters or enzymes catalysing cyanotoxin metabolism, characterized by genetic polymorphism, such as in the case of MC conjugated by human glutathione-S-transferases [61]. This could end up in different levels of expression and enzymatic activity within the human population, suggesting the presence of groups of people differently susceptible to cyanotoxin-induced effects.

Human health

Human exposure scenarios and hence health impacts are strictly related to the use of water bodies, generally associated to source of drinking water or for haemodyalisis purposes and recreational activities; the human exposure can be also indirect due to consumption of freshwater fish, crops and vegetables, or items of animal origins following the use of contaminated water for irrigation or in farming activities. Therefore appropriate monitoring programs (for either water and consumer products) should be planned and adopted depending on the cyanobacteria specie, the characteristic and the use of the water body, foreseeing intensification of controls after rain falls and floods, water evaporation associated to higher temperature and drought, and to thermal stratification, all factors expected to favor cyanobacterial bloom (Funari *et al.* 2012).

1. Drinking and recreational waters

These two sources for cyanotoxins human exposure have been considered from the very beginning and guidelines values have been set by WHO [62, 63] and risk assessment carried out [1] on the basis of toxicological information available for the most studied cyanotoxins. WHO defined guidance values have been adopted by some countries as specific regulatory framework or recommendations.

Episodes of acute/short term human intoxications due to drinking water consumption have been reported in some countries as a consequence of failure or inefficiency of water treatment [64]. Gastroenteritis and liver damages were among the most frequently reported diseases, although other effects have been reported as well. The chronic risk associated with repeated exposure to cyanotoxins in humans through drinking water is difficult to be demonstrated, as information from epidemiological studies is scarce and inconclusive. Indeed, the International Agency for Research on Cancer (IARC) concluded that it was not possible to associate the excess of hepatocellular carcinoma and of colorectal cancer reported in the available epidemiological studies with exposure to MCs [47].

In freshwater recreational settings, the available data show a range of diverse symptoms associated with exposure to cyanobacteria, like severe headache, pneumonia, fever, myalgia, vertigo and blistering in the mouth [1]. Some allergic responses to

cyanobacteria have also been published, possibly due to the action of cyanobacterial LPS endotoxins [65]. On the basis of available data, it seems possible to conclude that the risk of severe effects for bathers posed by ingestion of cyanotoxins is significant only when cyanobacteria bloom or form scums. Inhalation of aerosol is another possible route of human exposure. In a study, in which aerosols were collected using high and low volume air samplers for 4, 12 and 24 h, close to two lakes in New Zealand, experiencing blooms of *Nodularia spumigena* and *Microcystis*, levels of MCs (1.8 pg/m^3) and nodularin (16.2 pg/m^3) detected in the air did not appear to represent an acute or chronic hazard to humans [66]. However, aerosolized toxins should be considered when developing risk assessments for lakeside populations and recreational users where inhalation of cyanotoxins may be a secondary exposure source to a primary oral exposure [66].

2. Haemodyalisis

The most serious known episode associated to human exposure to cyanotoxins occurred in Brazil, where 56 out of 130 haemodialysed patients died after treatment with MC contaminated water [67]. Again in Brazil, in a later survey 0.2-0.96 µg/L MC were found in plasma of apparently asymptomatic patients following a bloom in the water body supplying some dialysis centers [68].

This route of exposure is probably underestimated over the world: indeed, the quality of water used for hemodialysis is not subjected to any mandatory regulations in most countries and cyanotoxin detection is not requested as a routine quality control. In addition, results obtained in Brazil revealed that the reverse osmosis system compulsory used in that country, did not prevent MC contamination of water used in the treatment of dialysis patients, although different reverse-osmosis membranes have been described as very efficient, showing retention rates in the range 96.7%-99.9% for two MC variants [69]. This could be tentatively attributed to the fact that the filters in the system are usually set to properly work at 20 °C ± 5 °C, operational temperatures that especially during summertime can be easily exceeded in many countries.

3. Fish, shellfish, and molluscs

No cases of human intoxications associated with consumption of aquatic organisms contaminated by cyanotoxin have been reported so far; however, this possibility does exist, since the number of reports about the presence of cyanobacteria in coastal and brackish waters is increasing. And indeed, the major emerging problem with respect to human exposure might come from transitional-, brackish- and coastal sea-waters, which are going to be strongly affected by increasing heavy rainfall and floods [8]. Edible organisms will be more often exposed to cyanotoxins which are not routinely monitored, according to the existing international and national regulations. This

pattern of exposure might be relevant particularly for edible aquatic organisms grown in aquaculture plants which are preferentially located in lagoons, brackish- and estuarine-waters.

Significant levels of cyanotoxins have been detected in aquatic organisms: maximum concentrations of 370, 2700 and 16 000 µg/kg have been reported in the edible parts of fish, crustaceans, and mussels, respectively [1]. Juvenile rainbow trout has been experimentally found to accumulate, in the whole body, anatoxin-a with a bio-concentration factor (BCF) of 30-47, based on fresh weight [70]. Fish viscera and the hepatopancreas of shellfish and mollusks usually contained higher levels than edible parts [71]. Significant levels of MCs have also been found in the tissues of the demersal blue crab Callinectes sapidus (up to 820, 65 and 105 µg MC/kg in hepatopancreas, viscera and muscle, respectively), during a bloom of toxic cyanobacteria in the hypereutrophic Lac des Allemands, that is the end-member of the Barataria estuary (Luoisiana) [72].

Marine cyanobacteria such as *Nodularia spumigena*, *Aphanizomenon flos-aquae*, and *Anabaena* sp. bloom widely in the Baltic Sea; in addition some other cyanobacteria like *Trichodesmium* are now described in temperate sea water, favoured by poleward shift [8]. Since cyanotoxins can accumulate in aquatic organisms tissues, and sea-food is not routinely monitored for cyanotoxins presence, the dietary exposure of consumers through sea food may be particularly relevant considering the size of the exposed population, and the reported levels of contamination. A recent survey of literature data on biomagnification factors of MCs, showed that biodilution (BCF < 1) is the main process in aquatic environment for all primary consumers except for zooplankton and zooplanktivorous fishes, thus highlighting the importance of fish diet in their relative ability to bioaccumulate cyanotoxins [73]. BCF is also related to the length of exposure, which makes more relevant the problem in environments where toxic organisms are constantly present [73]. Recently, the death of 21 sea-otters in Monterey Bay National Marine Sanctuary has been associated with the trophic transfer of MCs flown from three tributaries into the Ocean [74]. The authors found compelling evidence that the mammals death was caused by accumulation of the hepatotoxins through food web magnification. The same can occur in the human diet.

MCs have been found in marine waters and in *Mytilus galloprovincialis*, at a concentration ranging between 45 to 140 ng g^{-1} of whole mussel, during a one year monitoring program in the Mediterranean waters of the Amvrakikos Gulf (Greece) [25]. A maximum level of 105 µg MC/kg has been reported in the muscle of the blue crab *Callinectes sapidus*. Considering a daily consumption of 200 g meat in a meal, approximately 20 µg MC/kg bw would be ingested, which is comparable to the reference values for subchronic risk of 24 µg/kg bw per day for an adult weighing 60 kg [1]. This suggests the need of a more comprehensive data base related to potential human exposure in order to estimate the actual threat for human health from this source. The population living in the coastal areas or close to aquaculture plants, likely being strong seafood consumers, can be considered the most vulnerable ones and cyanobacteria pose a serious threat for the future.

Furthermore, in coastal waters (including estuarine and brackish areas), toxins produced by cyanobacteria can contaminate sea-food already exposed to toxins released by other marine organisms (*i.e.* harmful algae). Particular attention has been given so far to STX, since their presence in seafood has caused several episodes of severe intoxications and deaths in human beings, as reported in a comprehensive FAO Report [75]. Human fatalities due to ingestion of STX-contaminated seafood still occur in countries where prevention programs are not effectively implemented [76] and it has been estimated that more than 2000 human cases of food-borne PSP occur globally every year with a mortality rate of 15% [77]. The problem is not limited to STX, indeed sea-food can be contaminated by toxins produced by cyanobacteria and by tropical/subtropical species of harmful algae, which have been subjected to a similar poleward movement to temperate areas. The production of palytoxin-like compounds and 42-hydroxypalitoxin by *Trichodesmium* spp. in coastal areas where the presence of *Ostreopsis ovata* (producing the same toxins) has also been described is only one example. In addition different toxins have a similar mechanism of action (as MC and okadaic acid, both inhibitor of PP2A) and hence a possible interaction for toxicity is another aspect that deserves further consideration.

4. Products of animal origin and vegetables

Another possible impact on human health is due to residues in edible products of animal origin. Data available seem to indicate that repeated MC ingestion (up to 10 µg/L contained in an extract of *M. aeruginosa*), not toxic for cows and sheep, correspond to negligible residues in meat, milk and dairy products [78, 79]. Therefore, the risk associated to their consumption has not been considered significant. However, this seems to be valid for hydrophilic MC variants, which have a low potential for bioaccumulation, but information related to lipophilic MC congeners (likely showing a different elimination kinetics in the animal) or to other cyanotoxins are not available. Finally the possible consumption of animal viscera (liver and sometimes intestines), which is often neglected in exposure scenarios but frequent in some geographical areas or ethnic groups, can represent a problem which deserves special attention, due to the high levels of MCs which can be expected in these organs. Specific data on these issues are missing so far.

Consumption of vegetables, irrigated with contaminated waters, could represent an additional source of human exposure to cyanotoxin. It has

been reported that some vegetables can retain MCs present in irrigation water infested by blooms or scums, where the cyanotoxins levels was so high to be able to inhibit the plant growth and induce phytotoxicity [80, 81], lowering seed germination and seedling growth [82]. Detrimental effects on plants growth were observed also when plants were exposed for 30 consecutive days to water containing 0.5-4 µg L^{-1} MC-LR equivalents [83]. This poses a threat to the yield and quality of crops having an impact on food availability, but at the same time strongly limits the possibility for human exposure. The low concern posed by human exposure to cyanotoxins via vegetable consumption is supported by the observation that when watering broccoli and mustard seedlings with water containing MC concentrations typically found in natural surface waters (1-10 µg/L), the toxins were found only in the roots, at levels of no human health concern [84]. No information on other MC variants or cyanotoxins is available for the time being.

5. Food supplements

Food supplements, known as blue-green algae supplements (BGAS) are available on the market and over the internet in a variety of forms: tablets, powder, capsules consisting of extracts or dried powder, mainly derived from *Spirulina* spp. and *Aphanizomenon flos-aquae* grown in artificial ponds or collected directly from the natural environment. Producers claimed they are health-promoting natural products, used as a support in losing weight during hypocaloric diets, increasing alertness and energy and elevated mood for people suffering depression and for their supposed anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, hypocholesterolemic and hypotriglyceridemic properties and immune-stimulating functions [85-87]. In addition, BGAS are administered to children as an alternative, natural therapy to treat attention deficit hyperactivity disorders (ADHD) [88]. No clear comprehensive evaluation of the putative benefic effects resulting from their assumption has been carried out yet.

Spirulina sp. and *A. flos-aquae* can coexist with other potentially toxic strains of cyanobacteria which share the same habitat, as *Microcystis* sp.; the contamination of BGAS sold as nutraceuticals in different countries with MCs has been repeatedly shown [89-92], in many cases showing a substantial percentage of samples (approximately 40-70%) with MCs levels up to 35 µg/g per dry weight product [93], thus exceeding 1 µg/g MC-LR equivalents, the provisional guidance value set by the Oregon Dept. of Agriculture, starting from a provisional TDI of 0.04 µg/kg bw per day [63]. The presence of DNA belonging to *Microcystis* genus in all *A. flos-aquae*-derived contaminated samples, strengthens the hypothesis that *M. aeruginosa* harvested together with *A. flos-aquae* is responsible for the contamination [92].

Assuming a daily consumption of 4 g of BGAS, easily achieved through a consumption of 4-8 tablets/day, and a contamination level of 1 µg/g, a daily intake of 4 µg MCs/person would result, which would be al-most twice as high as the TDI value, posing a risk for the chronic consumer, independently from exposure to MCs from other possible sources, as contaminated water or fish. For moderate users, taking pills for a limited period of time, the risk would be relevant at higher levels of contamination, which on the other hand are quite frequent, as mentioned above.

The estimate of the actual exposure is difficult, since BGAS are perceived as safe 'natural' products and are consumed following individual programs, without any prescription nor indication for a specific daily dosage. Indeed, consumption of extremely high daily doses (up to 20 g) has been reported [93], for which even the possibility of acute hepatotoxicity can be expected at levels of contamination around 5 µg/g MC-LR equivalents [92].

To date neurotoxins have never been detected in BGAS [92, 94]; the presence of other cyanotoxins such as cylindrospermopsin has also been excluded [95], but data are scant.

ANIMAL HEALTH

Several poisoning episodes of livestock, wild and domestic animals have been associated with the occurrence of cyanobacterial blooms in surface waters used for drinking [96]. Although anecdotal reports dated many years ago, the issue has been raised as relevant only more recently, when a specific cause-effect relationship could be established, due to appropriate sampling schedules of both water and body fluids and investigative chemistry techniques.

Animals, especially caws and sheep can be exposed to extremely high levels of toxins in the presence of scums accumulating by lake or river side: in this condition they can ingest lethal doses contained in water volumes lower than their daily consumption. In addition it has been reported that animals of different species seem to drink preferentially waters contaminated by high cyanobacteria density rather than clean ones [97]. Cattle murrain, more than one hundred calves and heifers, has been documented in Switzerland alpine pastures [98]. The animals died by acute hepatotoxicosis, but presented also symptoms of neurotoxicity. The analysis of the highly oligotrophic water bodies in the pasture, showed an abundant community of mat-forming cyanobacteria, dominated by *Oscillatoria limosa* and *Oscillatoria tenuis*, and MCs were detected both in the mats and in the water [98].

A case of sheep mortality associated with PSP from the cyanobacterium *A. circinalis* has been reported in South Wales (Australia) [99]: the toxin was present in the small intestine of the dead animals. Interestingly, the toxin profile of the environmental sample indicated a high content of C-toxin (70%), a PSP variant with low toxicity, whereas in the small intestine the more toxic gonyautoxin was the dominant form (87%), clearly confirming the possibility of biotransformation within the organism between PSP forms with different toxicity.

Diagnosis of ATX and HTX poisoning in dogs after drinking and bathing in infested waters have been reported from Scotland [39], France [40], Ireland [41], and in the USA [42]. In most cases, dog poisoning was associated with neurotoxic cyanotoxins produced in rivers by benthic taxa, such as *Phormidium* sp. and *Oscillatoria* sp. These species readily form biofilms, attached as a sticky mass to surfaces, including dog hair during bathing. Exposure of dogs may be significantly increased by fur licking after immersing in a bloom.

Anatoxin-a(s) has been frequently associated with mass mortality of birds [100]. Anatoxins and MCs are also considered a contributing factor in the deaths of Flamingos in Kenia [33].

Besides the ethical and ecological problems related to animal welfare and protection, even when wild animals including birds are considered, such events have also economic consequences (*i.e.* on farming activities).

DATA GAPS AND RESEARCH NEEDS

In assessing the risk for the exposed population, the wide-spreading of cyanobacteria in environments other than freshwater as described above indicate that additional focus should be given to the exposure to toxins via food web, due to the expansion of cyanobacterial population in coastal and brackish waters, where many aquaculture plants are located. This scenario is likely to become more important, for the additional effects of climate changes, specifically the increase in frequency and intensity of heavy rainfall and floods [8].

Other emerging scenarios requiring additional focus, by characterising the levels of exposure in a more accurate way, are the case of haemodialysis, food supplements consumption, mat forming and marine cyanobacteria.

The possibility of changes in the toxicological profiles of the toxins produced by some species, under environmental stress, is an additional issue for potential concern. Indeed, both *C. raciborskii* and *Trichodesmium* species have been found to produce different toxins with different, and often unknown, toxicological profile, depending on the geographical areas: this is particularly important for these two subtropical species, due to their spreading into temperate regions, associated to their poleward shift [8]. As another example *Limnothrix redekei*, which is generally reported to produce MCs, is also able to produce another still unidentified water soluble toxin as it causes *in vitro* and *in vivo* effects different from those previously described for known cyanobacterial toxins [101, 102]. Since the genus *Limnothrix* occurs in a range of freshwater habitats that are used as sources of drinking water, the toxicological and chemical characterization of the toxin is an urgent need.

Recently, a research on benthic and pelagic cyanobacteria from Portuguese coasts, revealed that isolates of *Leptolyngbya*, *Oscillatoria* and *Phormidium*, as well as *Cyanobium* and *Synechococcus*, induced acute toxicity in nauplii of the brine shrimp *A. salina* [103]. Apparently, none of the known toxins has been detected, even if fragments of *mcyE* gene have been found in several isolates, indicating the possibility of new toxins produced by these organisms. The potential risk associated to these and other cyanobacterial species producing unknown toxins is of particular concern for human health, and suggest the need for unspecific biological methods to detect the potential toxicity of a bloom, not just linked to analytical detection of known molecules.

Furthermore, in assessing the risk, aggregate/cumulative exposure has to be considered, for those individuals that can be exposed to the same cyanotoxin via different routes of exposure (*i.e.* drinking water, contaminated food and food supplements) or to different toxins at the same time. Combined exposure to different cyanotoxins represents very likely the rule rather than the exception, since the same cyanobacteria may produce more than one toxin as in the case of MC variants and STX derivates, characterized by different toxicity in addition to the fact that blooms are very rarely characterized by the presence of a unique cyanobacterium. Furthermore, cyanobacteria can produce other types of biologically active peptides, such as cyanobactins, on which research has just started. These are small cyclic peptides recently found in cyanobacteria and reported to have pharmacological activities (*i.e.* antimalarial, antitumor) and produced through the proteolytic cleavage and cyclization of precursor peptides [104].

A model to predict the combined neurotoxic effects of binary and ternary mixtures of STX has been proposed, indicating that the most potent toxin is by far the most relevant component, whereas the less toxic derivatives should be order of magnitude more concentrated to contribute to the cumulative toxic potency [105]. In the case of MC variants, exposure is generally represented by a mixture of MC congeners: usually by adopting a conservative approach, acute toxicity is referred to MC-LR equivalents, since MC-LR is the most acutely hepatotoxic (when administered ip). An approach similar to the "toxicity equivalent factor" (TEF), used for polychlorinated dibenzo[p]dioxins (PCDD), has also been proposed for MCs and NODs [1, 106]. However, such an approach is generally based on data obtained after intraperitoneal treatment, which is poorly representative of the actual human exposure conditions, it is limited to acute toxicity and does not consider the possibility that some variants could also have targets other than the liver, such as the case of neurotoxic potential shown by LC-LF, and as a consequence should be used with caution.

Although accumulation of different cyanotoxins up to significant levels in aquatic organisms usually eaten by humans could be particularly relevant (especially for neurotoxins), the issue of exposure to cyanotoxins' mixture has not been sufficiently inves-

tigated so far. In coastal environments cyanotoxins will sum up to algal toxins, increasing the risk of intoxication of the exposed population, as described above for *Trichodesmium*.

Moreover, concomitant exposure with other chemicals may be of relevance, such as in the case of organophosphorus pesticides. Indeed, exposure to the insecticides could potentiate the ATX-s induced toxicity by inhibiting AChE activity also in the brain, not only in peripheral nervous system [107]. Combined and single effects have been reported associated with the exposure of pesticide carbaryl and toxic *Microcystis aeruginosa* on the life history of *Daphnia pulicaria* [108].

In addition to co-exposure among chemicals, rivers and lakes affected by cyanobacterial mats might promote the growth of autochtonous pathogens and pathogenic microorganisms like *Legionella*, which has been shown to use algal extracellular products as carbon and energy sources [109] and can proliferate in biofilm, in association with amoebae or protozoa or cyanobacteria [100]. This would increase the possibility of transmission of the related infections. This is also true for brackish-/sea-water, where heterotrophic bacteria communities associated with cyanobacteria include possible pathogenic taxa that need to be considered in assessing the risk for human health. Berg [111] found that most (90%) of the *Aeromonas* spp. associated to cyanobacteria in Baltic Sea, were positive for at least one of several factors of virulence. Blooms of the toxic cyanobacterium *Nodularia spumigena* are an ideal growing medium for several pathogenic microorganisms, including the deadly serotypes of *Vibrio cholerae* O1 and O139, and *V. vulnificus* [112], exposing people also during bathing activities. Decaying blooms of *Microcystis aeruginosa* can increase the concentration of *Vibrio* spp. in estuarine and brackish environment [113]. Short term experiments demonstrat-ed that pathogenic serotype *Vibrio* O139 can survive in saline microcosms in association with *Anabaena, Nostoc* and *Hapalosiphon* spp. [114].

Important open research fields and data gaps are related to new scenario of exposure and to the potential bioaccumulation of cyanotoxins in the food web, as well as to new patterns of co-exposure between cyanotoxins and anthropogenic chemicals and/or algal toxins or pathogenic microorganisms. However, data gaps are also present in the elucidation of dynamics affecting blooming and production of different cyanotoxin variants and the environmental factors able to influence these processes. The environmental factors responsible on a short time scale for sudden changes in the toxin genes expression, or on longer time scale for the dynamic of toxic/non toxic individuals; the physiological/ecological role of cyanotoxins production and factors triggering the active transport outside the cell and the inter-relationship between cyanotoxins and other biologically active peptides produced by cyanobacteria, are specific fields of research particularly needed to better predict the occurrence of blooms and their toxicity, and consequently to identify efficient prevention measures. In addition, many data gaps are also present in the characterization of the toxicological profiles, especially regarding long term effects. The study of these issues represents a key step for a better risk assessment and management, allowing the preparation of appropriate and efficient plans for prevention and human health protection.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

References

1. Funari E, Testai E. Human health risk assessment related to cyanotoxins exposure. *Crit Rev Toxicol* 2008;38(2):97-125. http://dx.doi.org/10.1080/10408440701749454

2. Chorus I, Bartram J (Ed.). *Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management.* London: E & FN Spon; 1999. http://dx.doi.org/10.4324/9780203478073

3. Paerl HW, Hall NS, Calandrino ES. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci Tot Environ* 2011;409(10):1739-45. http://dx.doi.org/10.1016/j.scitotenv.2011.02.001

4. Paerl HW, Huisman J. Blooms like it hot. *Science* 2008;320:57-8. http://dx.doi.org/10.1126/science.1155398

5. Dodds W, Bouska W, Eitzmann J, Pilger T, Pitts K, Riley A, *et al.* Eutrophication of US freshwaters: analysis of potential economic damages. *Environ Sci Technol* 2009;43:12-9. http://dx.doi.org/10.1021/es801217q

6. van Apeldoorn ME, van Egmond HP, Speijers GJA, Bakker DCE. Toxins of cyanobacteria. *Mol Nutr Food Res* 2007; 51:7-60. http://dx.doi.org/10.1002/mnfr.200600185

7. Neilan BA, Pearson LA, Muenchhoff J, Moffitt MC, Dittmann E. Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environ Microbiol* 2012:1-15. http://dx.doi.org/10.1111/j.1462-2920.2012.02729.x

8. Funari E, Manganelli M, Sinisi L. Impact of climate change on waterborne diseases. *Ann Ist Super Sanità* 2012;48(4):473-83.

9. O'Neil JM, Davis TW, Burford MA, Gobler CJ. The rise of harmful cyanobacteria blooms. The potential roles of eutrophication and climate change. *Harmful Algae* 2012;14(0):313-34. http://dx.doi.org/10.1016/j.hal.2011.10.027

10. Bar-Yosef Y, Sukenik A, Hadas O, Viner-Mozzini Y, Kaplan A. Enslavement in the water body by toxic *Aphanizomenon ovalisporum*, inducing alkaline phosphatase in phytoplanktons. *Curr Biol* 2010;20(17):1557-61. http://dx.doi.org/10.1016/j.cub.2010.07.032

11. Manganelli M, Scardala S, Stefanelli M, Vichi S, Mattei D, Bogialli S, *et al.* Health risk evaluation associated to *Planktothrix rubescens*: An integrated approach to design tailored monitoring programs for human exposure to cyanotoxins. *Water Res* 2010;44(5):1297-306. http://dx.doi.org/10.1016/j.watres.2009.10.045

12. Paerl HW. Marine plankton. In: Whitton BA, Potts M (Ed.). *The ecology of cyanobacteria*. New York/Boston/Dordrecht/London/Moscow: Kluver Academic Publishers; 2000. p. 121-48.

13. Fristachi A, Sinclair JL. Occurrence of cyanobacterial harmful algal blooms Workgroup Report. In: Hudnell HK (Ed.). *Cyanobacterial harmful algal blooms: state of the science and research needs*. Springer; 2008. p. 45-103. http://dx.doi.org/10.1007/978-0-387-75865-7_3

14. Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* 1997;276:1221-9. http://dx.doi.org/10.1126/science.276.5316.1221

15. Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, *et al.* Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. *Nature* 2006;439(7072):68-71. http://dx.doi.org/10.1038/nature04203

16. Ramos AG, Martel A, Codd GA, Soler E, Coca J, Redondo A, *et al.* Bloom of the marine diazotrophic cyanobacterium *Trichodesmium erythraeum* in the Northwest African Upwelling. *Mar Ecol Prog Ser* 2005;301:303-5. http://dx.doi.org/10.3354/meps301303

17. Proença LAO, Tamanaha MS, Fonseca RS. Screening the toxicity and toxin content of blooms of the cyanobacterium *Trichodesmium erythraeum* (Ehrenberg) in Northeast Brazil. *J Venom Anim Toxins Incl Trop Dis* 2009;15(2):204-15. http://dx.doi.org/10.1590/S1678-91992009000200004

18. Endean R, Monks SA, Griffith JK, Llewellyn LE. Apparent relationships between toxins elaborated by the cyanobacterium *Trichodesmium erythraeum* and those present in the flesh of the narrow-barred Spanish mackerel *Scomberomorus commersoni*. *Toxicon* 1993;31(9):1155-65. http://dx.doi.org/10.1016/0041-0101(93)90131-2

19. Kerbrat A-S, Darius HT, Pauillac S, Chinain M, Laurent D. Detection of ciguatoxin-like and paralysing toxins in *Trichodesmium* spp. from New Caledonia lagoon. *Mar Poll Bull* 2010;61(7-12):360-6. http://dx.doi.org/10.1016/j.marpolbul.2010.06.017

20. Kerbrat AS, Amzil Z, Pawlowiez R, Golubic S, Sibat M, Darius HT, *et al.* First evidence of palytoxin and 42-hydroxy-palytoxin in the marine cyanobacterium *Trichodesmium*. *Mar Drugs* 2011;9(4):543-60. http://dx.doi.org/10.3390/md9040543

21. Schock TB, Huncik K, Beauchesne KR, Villareal TA, Moeller PD. Identification of trichotoxin, a novel chlorinated compound associated with the bloom forming cyanobacterium, *Trichodesmium thiebautii*. *Environ Sci Technol* 2011;45(17):7503-9. http://dx.doi.org/10.1021/es201034r

22. Ferris MJ, Palenik B. Niche adaptation in ocean cyanobacteria. *Nature* 1998;396(6708):226-8. http://dx.doi.org/10.1038/24297

23. Martins R, Fernandez N, Beiras R, Vasconcelos V. Toxicity assessment of crude and partially purified extracts of marine *Synechocystis* and *Synechococcus* cyanobacterial strains in marine invertebrates. *Toxicon* 2007;50(6):791-9. http://dx.doi.org/10.1016/j.toxicon.2007.06.020

24. Carmichael WW, Li RH. Cyanobacteria toxins in the Salton Sea. *Saline Systems* 2006;2(1):5. http://dx.doi.org/10.1186/1746-1448-2-5

25. Vareli K, Zarali E, Zacharioudakis GSA, Vagenas G, Varelis V, Pilidis G, *et al.* Microcystin producing cyanobacterial communities in Amvrakikos Gulf (Mediterranean Sea, NW Greece) and toxin accumulation in mussels (*Mytilus galloprovincialis*). *Harmful Algae* 2012;15(0):109-18. http://dx.doi.org/10.1016/j.hal.2011.12.005

26. Lehman PW, Teh SJ, Boyer GL, Nobriga ML, Bass E, Hogle C. Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. *Hydrobiologia* 2010;637(1):229-48. http://dx.doi.org/10.1007/s10750-009-9999-y

27. Tonk L, Bosch K, Visser PM, Huisman J. Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. *Aquat Microb Ecol* 2007;46(2):117-23. http://dx.doi.org/10.3354/ame046117

28. Lin S, Shen J, Liu Y, Wu X, Liu Q, Li R. Molecular evaluation on the distribution, diversity, and toxicity of *Microcystis* (Cyanobacteria) species from Lake Ulungur – a mesotrophic brackish desert lake in Xinjiang, China. *Environ Monitor Assess* 2011;175(1):139-50. http://dx.doi.org/10.1007/s10661-010-1500-x

29. Czubakowski J. *Estuarine phytoplankton response to annual and manipulated river inputs*. Ph.D. Thesis. Louisiana State University and Agricultural and Mechanical College; 2010.

30. Lehman PW, Boyer G, Hall C, Waller S, Gehrts K. Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary, California. *Hydrobiologia* 2005;541(1):87-99. http://dx.doi.org/10.1007/s10750-004-4670-0

31. Stal L. Cyanobacterial mats and stromatolites. In: Whitton B, Potts M (Ed.). *The ecology of cyanobacteria*. Springer Netherlands; 2002. p. 61-120. http://dx.doi.org/10.1007/0-306-46855-7_4

32. Mohamed ZA. Toxic cyanobacteria and cyanotoxins in public hot springs in Saudi Arabia. *Toxicon* 2008;51(1):17-27. http://dx.doi.org/doi:10.1016/j.toxicon.2007.07.007

33. Metcalf JS, Morrison LF, Krienitz L, Ballot A, Krause E, Kotut K, *et al.* Analysis of the cyanotoxins anatoxin-a and microcystins in Lesser Flamingo feathers. *Toxicol Environ Chem* 2006;88(1):159-67. http://dx.doi.org/10.1080/02772240500491604

34. Seifert M, McGregor G, Eaglesham G, Wickramasinghe W, Shaw G. First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck. *Harmful Algae* 2007;6(1):73-80. http://dx.doi.org/10.1016/j.hal.2006.07.001

35. Carmichael WW, Evans WR, Yin QQ, Bell P, Moczydlowski E. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. *Appl Environ Microbiol* 1997;63:3104-10.

36. Izaguirre G, Jungblut A-D, Neilan BA. Benthic cyanobacteria (Oscillatoriaceae) that produce microcystin-LR, isolated from four reservoirs in southern California. *Water Res* 2007;41(2):492-8. http://dx.doi.org/10.1016/j.watres.2006.10.012

37. Mohamed ZA, El-Sharouny HM, Ali WSM. Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. *Toxicon* 2006;47(5):584-90. http://dx.doi.org/10.1016/j.toxicon.2006.01.029

38. Mohamed ZA, El-Sharouny HM, Ali WSM. Microcystin concentrations in the Nile River sediments and removal of microcystin-LR by sediments during batch experiments. *Arch Environ Contam Toxicol* 2007;52:489-95. http://dx.doi.org/10.1007/s00244-006-0140-1

39. Edwards C, Beattie KA, Scrimgeour CM, Codd GA. Identification of anatoxin-A in benthic cyanobacteria (blue-

green algae) and in associated dog poisonings at Loch Insh, Scotland. *Toxicon* 1992;30(10):1165-75.
http://dx.doi.org/10.1016/0041-0101(92)90432-5

40. Gugger M, Lenoir S, Berger C, Ledreux A, Druart J, Humbert J, *et al.* First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. *Toxicon* 2005;45:919- 28.
http://dx.doi.org/10.1016/j.toxicon.2005.02.031

41. James K, Crowley J, Hamilton B, Lehane M, Skulberg O, Furey A. Anatoxins and degradation products, determined using hybrid quadruple time-of-flight and quadruple iontrap mass spectrometry: forensic investigations of cyanobacterial neurotoxin poisoning. *Rapid Commun Mass Spectrom* 2005;19:1167-75.
http://dx.doi.org/10.1002/rcm.1894

42. Puschner B, Hoff B, Tor ER. Diagnosis of Anatoxin-a Poisoning in Dogs from North America. *J Veter Diagnostic Invest* 2008;20(1):89-92.
http://dx.doi.org/10.1177/104063870802000119

43. Osborne NJT, Webb PM, Shaw GR. The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environ Internat* 2001;27(5):381-92.
http://dx.doi.org/10.1016/S0160-4120(01)00098-8

44. Méjean A, Peyraud-Thomas C, Kerbrat AS, Golubic S, Pauillac S, Chinain M, *et al.* First identification of the neurotoxin homoanatoxin-a from mats of *Hydrocoleum lyngbyaceum* (marine cyanobacterium) possibly linked to giant clam poisoning in New Caledonia. *Toxicon* 2010;56(5):829-35.
http://dx.doi.org/10.1016/j.toxicon.2009.10.029

45. Gehringer MM. Microcystin-LR and okadaic acid-induced cellular effects. A dualistic response. *FEBS Lett* 2004;557:1-8.
http://dx.doi.org/10.1016/S0014-5793(03)01447-9

46. Feurstein D, Stemmer K, Kleinteich J, Speicher T, Dietrich DR. Microcystin congener- and concentration-dependent induction of murine neuron apoptosis and neurite degeneration. *Toxicol Sci* 2011;124(2):424-31.
http://dx.doi.org/10.1093/toxsci/kfr243

47. International Agency for Research on Cancer. *Ingested nitrate and nitrite, and cyanobacterial peptide toxins.* Geneva: WHO; 2010.

48. Kurmayer R, Kutzenberger T. Application of real-time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium *Microcystis* sp. *Appl Environ Microbiol* 2003;69(11):6723-30.
http://dx.doi.org/10.1128/AEM.69.11.6723-6730.2003

49. Wood SA, Rueckert A, Hamilton DP, Cary SC, Dietrich DR. Switching toxin production on and off: intermittent microcystin synthesis in a *Microcystis* bloom. *Environ Microbiol Rep* 2011;3(1):118-24.
http://dx.doi.org/10.1111/j.1758-2229.2010.00196.x

50. Campos F, Durán R, Vidal L, Faro L, Alfonso M. *In vivo* effects of the anatoxin-a on striatal dopamine release. *Neurochem Res* 2006;31:491-501.
http://dx.doi.org/10.1007/s11064-006-9042-x

51. Stüken A, Orr R, Kellmann R, Murray S, Neilan B, Jakobsen K. Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates. *PLoS ONE* 2011;6(5):e20096.
http://dx.doi.org/10.1371/journal.pone.0020096

52. Kao CY. Paralytic shellfish poisoning. In: Falconer IR (Ed.). *Algal toxins in seafood and drinking water*. San Diego, CA.: Academic Press; 1993. p. 75-86.

53. Wang J, Salata J, Bennett P. Saxitoxin is a gating modifier of hERG KC channels. *J Gen Physiol* 2003;121:583-98.
http://dx.doi.org/10.1085/jgp.200308812

54. Falconer IR, Hardy SJ, Humpage AR, Froscio SM, Tozer GJ, Hawkins PR. Hepatic and renal toxicity of the blue-green alga (cyanobacterium) *Cylindrospermopsis raciborskii*

in male Swiss albino mice. *Environ Toxicol* 1999;14:143-50.
http://dx.doi.org/10.1002/(SICI)1522-7278(199902)14:1<143::AID-TOX18>3.3.CO;2-8

55. Seawright A, Nolan C, Shaw G, Chiswell R, Norris R, Moore M, *et al.* The oral toxicity for mice of the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska). *Environ Toxicol* 1999;14:135-42.
http://dx.doi.org/10.1002/(SICI)1522-7278(199902)14:1<135::AID-TOX17>3.0.CO;2-L

56. Terao K, Ohmori S, Igarashi K, Ohtani I, Watanabe M, Harada K, *et al.* Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. *Toxicon* 1994;32:833-43.
http://dx.doi.org/10.1016/0041-0101(94)90008-6

57. Froscio SM, Humpage AR, Burcham PC, Falconer IR. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. *Environ Toxicol* 2003;18:243-51.
http://dx.doi.org/10.1002/tox.10121

58. Humpage A, Fontaine F, Froscio S, Burcham P, Falconer I. Cylindrospermopsin genotoxicity and cytotoxicity: role of cytochrome P-450 and oxidative stress. *J Toxico Environ Health: Part A* 2005;68(9):739-53.
http://dx.doi.org/10.1080/15287390590925465

59. Bazin E, Mourot A, Humpage A, Fessard V. Genotoxicity of a freshwater cyanotoxin, cylindrospermopsin, in two human cell lines: Caco-2 and HepaRG. *Environ Molecular Mutagen* 2010;51(3):251-9.
http://dx.doi.org/10.1002/em.20539

60. Pegram RA, Humpage AR, Neilan BA, Runnegar MT, Nichols T, Thacker RW, *et al. Cyanotoxins Workgroup Report. 2008.*
http://dx.doi.org/10.1007/978-0-387-75865-7_15

61. Buratti FM, Scardala S, Funari E, Testai E. Human Glutathione Transferases catalyzing the conjugation of the hepatoxin Microcystin-LR. *Chem Res Toxicol* 2011;24:926-33.
http://dx.doi.org/10.1021/tx2000976

62. World Health Organization. *Coastal and fresh waters.* Geneva: WHO; 2003.

63. World Health Organization. *Recommendations.* Geneva: WHO; 2004.

64. Falconer IR. Health problems from exposure to cyanobacteria and proposed safety guidelines for drinking and recreational water. In: Codd GA, Jefferies TM, Keevil CW, Potter E (Ed.). *Detection methods for cyanobacterial toxins.* London: Royal Society of Chemistry; 1994. p. 3-10.
http://dx.doi.org/10.1533/9781845698164.1.3

65. Stewart I, Schluter P, Shaw G. Cyanobacterial lipopolysaccharides and human health - a review. *Environ Health* 2006;5:7.
http://dx.doi.org/doi:10.1186/1476-069X-5-7

66. Wood SA, Dietrich DR. Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes. *J Environ Monit* 2011;13(6):1617-24.
http://dx.doi.org/10.1039/c1em10102a

67. Azevedo S, Carmichael W, Jochimsen E, Rinehart K, Lau S, Shaw G, *et al.* Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology* 2002;181:441-6.
http://dx.doi.org/10.1016/S0300-483X(02)00491-2

68. Soares R, Yuan M, Servaites J, Delgado A, Magalh~aes V, Hilborn E, *et al.* Sub-lethal exposure from microcystins to renal insufficiency patients in Rio de Janeiro, Brazil. *Environ Toxicol* 2006:95-103.
http://dx.doi.org/10.1002/tox.20160

69. Neumann U, Weckesser J. Elimination of microcystin pep-

tide toxins from water by reverse osmosis. *Environ Toxicol Water Qual* 1998;13(2):143-8.
http://dx.doi.org/10.1002/(SICI)1098-2256(1998)13:2<143::AID-TOX5>3.0.CO;2-7

70. Osswald J, Azevedo J, Vasconcelos V, Guilhermino L. Experimental determination of the bioconcentration factors for anatoxin-a in juvenile rainbow trout *(Oncorhynchus mykiss)*. *Proc Intern Acad Ecol Environ Sci* 2011;1(2):77-86.

71. Ibelings BW, Chorus I. Accumulation of cyanobacterial toxins in freshwater 'seafood' and its consequences for public health: A review. *Environ Pollut* 2007;150:117-92.
http://dx.doi.org/10.1016/j.envpol.2007.04.012

72. Garcia AC, Bargu S, Dash P, Rabalais NN, Sutor M, Morrison W, *et al.* Evaluating the potential risk of microcystins to blue crab *(Callinectes sapidus)* fisheries and human health in a eutrophic estuary. *Harmful Algae* 2010;9(2):134-43.
http://dx.doi.org/10.1016/j.hal.2009.08.011

73. Kozlowsky-Suzuki B, Wilson AE, Ferrão-Filho AdS. Biomagnification or biodilution of microcystins in aquatic foodwebs? Meta-analyses of laboratory and field studies. *Harmful Algae* 2012;18(0):47-55.
http://dx.doi.org/10.1016/j.hal.2012.04.002

74. Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, Tinker MT, *et al.* Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. *PLoS ONE* 2010;5(9):e12576.
http://dx.doi.org/10.1371/journal.pone.0012576

75. Food and Agriculture Organization of the United Nations. *Marine biotoxins.* Rome: FAO; 2004.

76. Batorèu MCC, Dias E, Pereira P, Franca S. Risk of human exposure to paralytic toxins of algal origin. *Environm Toxicol Pharmacol* 2005;19:401-6.
http://dx.doi.org/10.1016/j.etap.2004.12.002

77. Hallegraeff G. Harmful algal blooms: a global overview. In: Hallegraeff GM, Anderson DM, Cembella AD (Ed.). *Manual on harmful marine microalgae.* Paris: UNESCO; 1995. p. 1-22.

78. Feitz A, Lukondeh T, Moffitt M, Burns B, Naido D, Vedova J, *et al.* Absence of detectable levels of cyanobacterial toxin (microcystin-LR) carry-over into milk. *Toxicon* 2002;40(8):1173-80.
http://dx.doi.org/10.1016/S0041-0101(02)00123-X

79. Orr PT, Jones GJ, Hunter RA, Berger K. Exposure of beef cattle in sub-clinical doses of Microcystis aeruginosa: toxin bioaccumulation, physiological effects and human health risk assessment. *Toxicon* 2003;41:613-20.
http://dx.doi.org/10.1016/S0041-0101(03)00006-0

80. Chen J, Song L, Dai J, Gan N, Liu Z. Effects of microcystins on the growth and the activity of superoxide dismutase and peroxidase of rape *(Brassica rapus L.)* and rice *(Oryza sativa L.)*. *Toxicon* 2004;43:393-400.
http://dx.doi.org/10.1016/j.toxicon.2004.01.011

81. Crush JR, Briggs LR, Sprosen JM, Nichols SN. Effect of irrigation with lake water containing microcystins on microcystin content and growth of ryegrass, clover, rape, and lettuce. *Environ Toxicol* 2008;23(2):246-52.
http://dx.doi.org/10.1002/tox.20331

82. Kós P, Gorzo G, Suranyi G, Borbely G. Simple and efficient method for isolation and measurement of cyanobacterial hepatotoxins by plant tests *(Sinapis alba L.)*. *Anal Biochem* 1995;225(1):49-53.
http://dx.doi.org/10.1006/abio.1995.1106

83. Saqrane S, Ouahid Y, El Ghazali I, Oudra B, Bouarab L, del Campo FF. Physiological changes in *Triticum durum, Zea mays, Pisum sativum* and *Lens esculenta* cultivars, caused by irrigation with water contaminated with microcystins: A laboratory experimental approach. *Toxicon* 2009;53(7-8):786-96.
http://dx.doi.org/10.1016/j.toxicon.2009.01.028

84. Järvenpää S, Lundberg-Niinistö C, Spoof L, Sjövall O, Tyystjärvi E, Meriluoto J. Effects of microcystins on broccoli and mustard, and analysis of accumulated toxin by liquid chromatography-mass spectometry. *Toxicon* 2007;49:865-74.
http://dx.doi.org/10.1016/j.toxicon.2006.12.008

85. Rodriguez-Hernandez A, Ble-Castillo JZ, Juarez-Oropeza MA, Diaz-Zagoya JC. *Spirulina maxima* prevents fatty liver formation in CD-1 male and female 611 mice with experimental diabetes. *Life Science* 2001;69:1029-37.
http://dx.doi.org/10.1016/S0024-3205(01)01185-7

86. Samuels R, Mani UV, Iyer UM, Nayak US. Hypocholesterolemic effect of Spirulina in patients with hyperlipidemic nephrotic syndrome. *J Med Food* 2002;5(96).
http://dx.doi.org/10.1089/109662002760178177

87. Jensen GS, Ginsberg DI, Drapeau C. Blue-green algae as an immuno-enhancer and biomodulator. *J Am Med Assoc* 2001;3:24-30.

88. Lindermann B. *Complicated child? Simple options.* Ramona, CA, USA: Ransom Hill Press; 1995.

89. Gilroy DJ, Kauffman KW, Hall RA, Huang X, Chu FS. Assessing potential health risks from microcystin toxins in bluegreen algae dietary supplements. *Environ Health Perspect* 2000;108:435-9.

90. Saker ML, Jungblut AD, Neilan BA, Rawn DF, Vasconcelos VM. Detection of microcystin synthase genes in health food supplements containing the freshwater cyanobacterium *Aphanizomenon flos-aquae. Toxicon* 2005;46:555-62.
http://dx.doi.org/10.1016/j.toxicon.2005.06.021

91. Jiang Y, Xie P, Chen J, Liang G. Detection of the hepatotoxic microcystins in 36 kinds of cyanobacteria *Spirulina* food products in China. *Food Add Contam* 2008;25:885-94.
http://dx.doi.org/10.1080/02652030701822045

92. Vichi S, Lavorini P, Funari E, Scardala S, Testai E. Contamination by *Microcystis* and microcystins of blue-green algae food supplements (BGAS) on the italian market and possible risk for the exposed population. *Food Chem Toxicol* 2012;50:4493-9.
http://dx.doi.org/10.1016/j.fct.2012.09.029

93. Dietrich D, Hoeger SJ. Guidance values for microcystins in water and cyanobacterial supplement products (blue green algal supplements): a reasonable or misguided approach? *Toxicol Appl Pharmacol* 2005;203:273-89.
http://dx.doi.org/10.1016/j.taap.2004.09.005

94. Dietrich DR, Fischer A, Michel C, Hoeger SJ. Toxin mixture in cyanobacterial blooms-a critical comparison of reality with current procedures employed in human health risk assessment. *Adv Exp Med Biol* 2008;619:885-912.
http://dx.doi.org/10.1007/978-0-387-75865-7_39

95. Liu H, Scott PM. Determination of the cyanobacterial toxin cylindrospermopsin in algal food supplements. *Food Add Contam* 2011;28:786-90.
http://dx.doi.org/10.1080/19440049.2010.501824

96. Stewart I, Seawright A, Shaw G. Cyanobacterial poisoning in livestock, wild mammals and birds - an overview. *Adv Exp Med Biol* 2008;619:613-37.
http://dx.doi.org/10.1007/978-0-387-75865-7_28

97. Codd G, Edwards C, Beattle K, al. e. Fatal attraction to cyanobacteria? *Nature* 1992;359:110-1.
http://dx.doi.org/10.1038/359110b0

98. Mez K, Beattie K, Codd G, Hanselmann K, Hauser B, Naegeli H, *et al.* Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. *Europ J Phycol* 1997;32(2):111-7.
http://dx.doi.org/10.1017/S0967026297001224

99. Negri A, Jones G, Hindmarsh M. Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium *Anabaena circinalis. Toxicon* 1995;33:1321-9.
http://dx.doi.org/10.1016/0041-0101(95)00068-W

100. Ibelings B, Havens K. Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. *Adv Exp Med Biol* 2008;619:675-732.
http://dx.doi.org/10.1007/978-0-387-75865-7_32

101. Bernard C, Froscio S, Campbell R, Monis P, Humpage A, Fabbro L. Novel toxic effects associated with a tropical Limnothrix/Geitlerinema-like cyanobacterium. *Environ Toxicol* 2011;26(3):260-70.
http://dx.doi.org/10.1002/tox.20552

102. Humpage A, Falconer I, Bernard C, Froscio S, Fabbro L. Toxicity of the cyanobacterium Limnothrix AC0243 to male Balb/c mice. *Water Res* 2012;46:1576-83.
http://dx.doi.org/10.1016/j.watres.2011.11.019

103. Frazão B, Martins R, Vasconcelos V. Are known cyanotoxins involved in the toxicity of picoplanktonic and filamentous North Atlantic Marine cyanobacteria? *Marine Drugs* 2010;8(6):1908-19.
http://dx.doi.org/10.3390/md8061908

104. Leikoski N, Fewer DP, Jokela J, Alakoski P, Wahlsten M, Sivonen K. Analysis of an inactive cyanobactin biosynthetic gene cluster leads to discovery of new natural products from strains of the genus *Microcystis. PLoS ONE* 2012;7(8): e43002.
http://dx.doi.org/10.1371/journal.pone.0043002

105. Llewellyn LE. The behaviour of mixture of paralytic shellfish toxins in competitive binding assay. *Chem Res Toxicol* 2006;19:661-7.
http://dx.doi.org/10.1021/tx050277i

106. Wolf HU, Frank C. Toxicity assessment of cyanobacterial toxins mixtures. *Environ Toxicol* 2002;17:395-9.
http://dx.doi.org/10.1002/tox.10066

107. Cook WO, Beasley VR, Dahlem AM, Dellinger JA, Harlin KS, Carmichael WW. Comparison of effects of anatoxina(s) and paraoxon, physostigmine and pyridostigmine on mouse brain cholinesterase activity. *Toxicon* 1988;26:750-3.
http://dx.doi.org/10.1016/0041-0101(88)90282-6

108. Cerbin S, Kraak MHS, Voogt Pd, Visser PM, Donk EV. Combined and single effects of pesticide carbaryl and toxic *Microcystis aeruginosa* on the life history of *Daphnia pulicaria. Hydrobiologia* 2010;643(1):129-38.
http://dx.doi.org/10.1007/s10750-010-0130-1

109. Tison DL, Pope DH, Cherry WB, Fliermans CB. Growth of Legionella pneumophila in association with blue-green algae (cyanobacteria). *Appl Environ Microbiol* 1980;39(2):456-9.

110. Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S (Ed.). *Legionella and the prevention of legionellosis*. Geneva: WHO; 2007.

111. Berg KA. *Heterotrophic bacteria associated with cyanobacteria in recreational and drinking water*. Ph.D Thesis. Helsinki: University of Helsinki; 2009.

112. Eiler A, Gonzalez-Rey C, Allen S, Bertilsson S. Growth response of *Vibrio cholerae* and other *Vibrio* spp. to cyanobacterial dissolved organic matter and temperature in brackish water. *FEMS Microbiol Ecol* 2007;60(3):411-8.
http://dx.doi.org/10.1111/j.1574-6941.2007.00303.x

113. Turner JW, Good B, Cole D, Lipp EK. Plankton composition and environmental factors contribute to *Vibrio* seasonality. *ISME J* 2009;3(9):1082-92.
http://dx.doi.org/10.1038/ismej.2009.50

114. Islam MS, Mahmuda S, Morshed MG, Bakht HBM, Khan MNH, Sack RB, *et al.* Role of cyanobacteria in the persistence of *Vibrio cholerae* O139 in saline microcosms. *Can J Microbiol* 2004;50:127-31.