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Global changes and the new challenges in the research on cyanotoxin risk evaluation

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ABSTRACT

Global changes and the new challenges in the research on cyanotoxin risk evaluation

Global changes comprehend a series of changes in populations, climate, economy, atmospheric and oceanic circulations, water cycle, pollution, biodiversity among many others that have significant impacts in the eutrophication of aquatic ecosystems and consequently on the occurrence of bloom forming toxic cyanobacteria and on their toxins. The development of sensitive and specific techniques together with an increased effort in the research related to cyanobacteria and cyanotoxins has contributed to a significant increase on the knowledge of these toxins. The understanding of the dynamics of cyanotoxins along food chains and about their biological activity allows us to estimate environmental and human health risks. Nevertheless, there is still much to do in what concerns with effective management measures and risk communication concerning cyanotoxins. Scientists, environmental and health technicians as also politicians and common population should all be involved so as to minimize animal and humans intoxications due to cyanotoxins. In this paper we discuss also the challenges for the scientists working on cyanotoxins and also the future needs in terms of research so as to minimize risks.

Key words: Global changes, cyanobacteria, cyanotoxins, risk evaluation, new challenges.

RESUMEN

Los cambios globales y los nuevos desafíos de la investigación y de la evaluación del riesgo de las cianotoxinas

Los cambios globales incluyen un conjunto de cambios en la población, el clima, la economía, la circulación atmosférica y oceánica, el ciclo del agua, la contaminación, la biodiversidad, entre muchos otros, que tienen un impacto significativo en la eutrofización de los sistemas acuáticos y por lo tanto en la aparición de floraciones de cianobacterias y sus toxinas. El desarrollo de técnicas cada vez más sensibles y específicas, junto con un mayor esfuerzo en las investigaciones relacionadas con las cianobacterias y cianotoxinas, han contribuido en las últimas décadas a un aumento significativo en el conocimiento de estas toxinas. Por otra parte, una mejor comprensión de la dinámica de las cianotoxinas a lo largo de las cadenas tróficas y de su actividad biológica nos va a permitir comprender mejor los riesgos desde el punto de vista de la salud humana y del medio ambiente. Sin embargo, todavía hay mucho terreno por explorar incluyendo el desarrollo de una gestión eficaz y la comunicación de los riesgos asociados con cianotoxinas. La participación de científicos y técnicos en las áreas de medio ambiente y de la salud, de los políticos y de la población en general, es crucial para disminuir las intoxicaciones en humanos y en animales. En esta comunicación se analizan los desafíos para los científicos que trabajan con cianotoxinas así como las necesidades futuras de investigación para minimizar los riesgos.

Palabras clave: *Cambios globales, cianobacterias, cianotoxinas, evaluación del riesgo, nuevos desafíos.*

INTRODUCTION

Cyanobacteria are very ancient organisms being responsible for the establishment of an oxygen rich atmosphere in the Earth about 2.4 Ga ago since they were the first photosynthetic organisms colonizing our planet (Bekker *et al*., 2004). In fact, nowadays they are responsible for a high percentage atmosphere of the oxygen production in the oceans due to picoplanktonic forms such as *Prochlorococus* spp. (Chisholm *et al*., 1988). They were the precursors of algae and plants, as we know them now, because they were the precursors of the chloroplasts in those autotrophic organisms (Raven & Allen, 2003). Being part of the phytoplankton they are on the base of many aquatic trophic chains and may occur in a variety of environments, from temperate lakes and reservoirs (Bumke-Vogt *et al.,* 1999) to rivers (Aboal *et al.,* 2005), from the Antarctica (Hitzfeld *et al.*, 2000) to desert environments (Metcalf *et al.*, 2012). They can live as planktonic or benthic organisms (Vasconcelos, 2006) or in symbiosis together with organisms such as fungi (Kaasalainen *et al*., 2012), plants (Pereira *et al*., 2009), sponges (Alex *et al*., 2013) and corals (Lesser *et al*., 2004).

Their occurrence in high densities –blooms– in aquatic ecosystems has been increasingly registered in the last decades due to eutrophication, mainly in freshwater ecosystems (Sivonen & Jones 1999), but also increasing reports in brackish and marine systems (Stal *et al*., 2003; Miller *et al*., 2010; Kerbrat *et al*., 2011). Global changes, lead by and increasing human population growth in special concentrated close to aquatic ecosystems, by increased touristic travelling, damming of rivers and fertilizer application has led to increased pressure on aquatic ecosystems. At the same time, global warming is expected to increase temperature in the next 100 years by 2-5 °C (US national Research Council, 2006). This increase in temperature is crucial for cyanobacteria development and will have a serious impact in the bloom development, its timing and duration as also on the occurrence of new invasive species that can colonize environments at higher latitudes.

Cyanobacteria blooms are frequently associated with toxin production. In fact, the toxicity of a bloom depends on several factors. First, the percentage of toxin producing strains over non-toxin producing ones. In a certain ecosystem not only varies the density and diversity of cyanobacteria species along time and throughout the years (Vasconcelos *et al*., 2011) as also the amount of toxin producing strains (Sabart *et al*., 2010). Toxicity of a bloom depends a lot on this rate and also

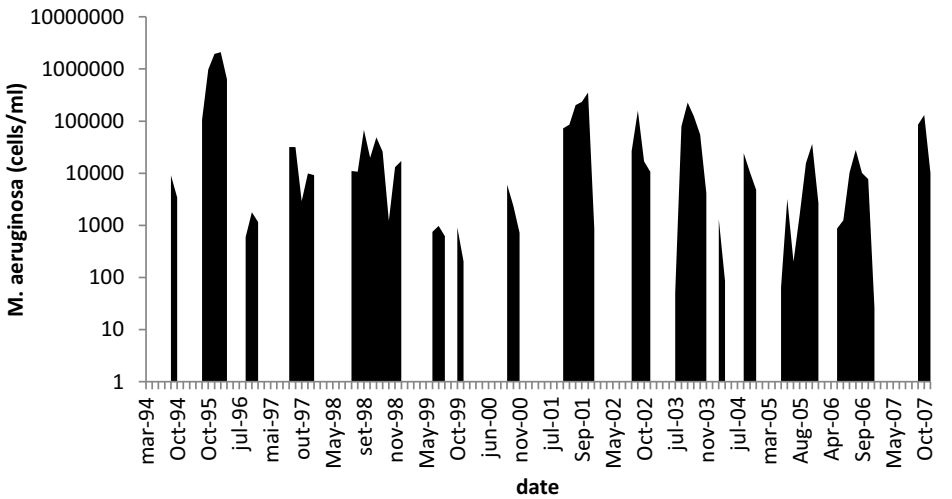

Figure 1. *Microcystis aeruginosa* (cells/ml) in Aguieira reservoir during the period 1994-2007 (Vasconcelos *et al*., 2011). *Variación de la densidad de* Microcystis aeruginosa *(células/ml) en el embalse de Aguieira para el período 1994-2007 (Vasconcelos* et al.*, 2011).*

on the growth rate of the toxin producing population. Cyanobacteria produce toxins at different rates depending on the growth phase of their populations. Exponential phase is usually that where cyanotoxins production is maximized (Rapala *et al*., 1997). Nevertheless all these factors are not the only ones that condition the toxin production so monitoring is essential to better assess the hazard and predict the risks. In a 14 year monitoring study done in a temperate reservoir in Portugal, Aguieira, it was shown that cyanobacteria occurrence may vary along years from 20 to 100% of the total phytoplankton density (Vasconcelos *et al*., 2011). In this reservoir *M. aeruginosa* was the dominant species and its cell density varied from a maximum of 10^3 to 10^6 from 1994 to 2007 (Fig. 1).

Cyanobacteria toxins are diverse not only in terms of chemical entity as also in their physiological mode of action (Fig. 2). Until now, the smallest cyanotoxin is the neurotoxin amino acid BMAA and the largest the palytoxin, previously only known to be produced by marine dinoflagelates. Several hypotheses have been launched to explain why cyanobacteria produce toxins (allelopathy, grazing deterrents, nutrients reserve). In the last decades, the amount of papers published on the diverse cyanotoxins has been increasing with microcystins (MC) as the winners with cylindrospermopsin (CYN) as the second runner (Fig. 3).

PERSPECTIVES

Cyanotoxins have been regarded as allelopathic compounds with several studies showing negative effects on several levels of biological organization of phytoplanktonic species (Keating, 1977; Leflaive & Ten-Hage 2007). Nevertheless, many of these works were done with extracts or with toxins at unreasonable and not ecologically relevant concentrations. In a work done with strains producers and non producers of MC,

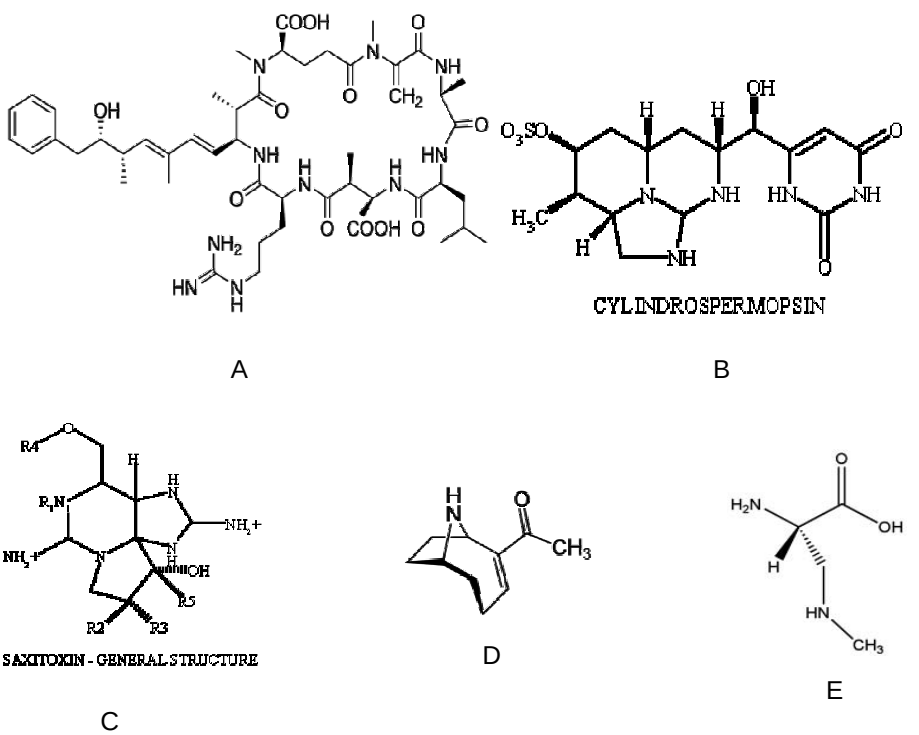

Figure 2. Cyanobacterial toxins diversity: A. Microcystin, B. Cylindrospermopsin, C. Saxitoxin, D. Anatoxin-a, E. BMAA. *Diversidad de cianotoxinas: A. Microcistina, B. Cilindrospermopsina, C. Saxitoxina, D. Anatoxina-a, E. BMAA.*

Vasconcelos & Almeida (2008) have shown that the most severe effects were produced by non MC producing strains. More recently, Pinheiro *et al.* (2013) have shown that pure MC does not affect negatively the growth of three species of phytoplankton, *Chlamydomonans reihardtii, Chlorella vulgaris* and *Nanochloropsis* sp. In fact, *C. reinhardtii* was able to grow better when exposed to MC concentrations of 0.4 to 1.6 mg MC/l. This work showed similar effects using CYN indicating clearly that these toxins do not act as allelopathic compounds.

The effect of cyanotoxins in plants seems to be more pronounced. Cyanotoxins may inhibit germination, roots and aerial parts of plants development and also may be translocated from the soil/water to roots, leaves and fruits. One of the most common plants used in ecotoxicological assays, *Lemna gibba*, shown frond inhibition when exposed to MC (Saqrane *et al*., 2007). This is concentration dependent and toxicity can also be shown by a decrease in chlorophyll content with increasing MC exposure concentrations. Other plants may react differently accordingly to the strain of cyanobacteria used and on the plant species. Grass species may be more resistant to cyanobacteria extracts as shown by a work done using extracts of MC and non MC cyanobacteria species (Pereira *et al*., 2009). *Festuca rubra* and *Lolium perene* germination was not significantly inhibited by several extracts of MC and non MC producing *M. aeruginosa* strains. On the contrary, lettuce –*Lactuca sativa*– germination was significantly inhibited by MC strains on a concentration dependent manner (Pereira *et al*., 2009). Other agricultural plants also have shown different patterns of inhibition, with peas (*Pisum sativum*) as being severely inhibited, corn (*Zea mays*) and wheat (*Triticum durum*) at a middle range, and lentil (*Lens esculenta*) as being barely affected (Saqrane *et al*., 2008). These type of data are very important for farmers because it allows them to understand which species will suffer less impact with cyanotoxins contaminated water, making the right choices taken into account the available water quality.

Zooplankton and other organisms that may contact directly with cyanobacteria and their toxins, can also be affected by them. Although initially it was postulated that cyanotoxins would be substances produced by cyanobacteria to prevent grazing (DeMott, 1999) soon it was found that this was not the case. Some toxins such as microcystins seem to affect negatively *Daphnia* (Rohrlack *et al*., 2005) but to have an effect cyanobacteria have to be ingested, because cyanotoxins are usually not excreted to the media. A true allelopathic substance is liberated in the media so as to cause its effect. On the other side, work done with toxin and non-toxin producing strains of *C. raciborskii* showed that a CYN + caused evident toxicity on *Daphnia*, a –CYN also did it at a slightly lower scale (Nogueira *et al*., 2004). This clearly shows that cyanobacteria may produce other substances, apart from the known toxins, that can act as allelopathic towards zooplankton.

Fish are also affected especially at early development stages. Adults seem to be more resistant and also are more able to escape from a bloom. El Ghazali *et al.* (2009) showed that zebra fish embryos exposed to MC extracts had malformations such edema, bent and curving tail, and causing a sever impact on the development and survival of the organisms. Osswald *et al.* (2007) using anatoxin-a cyanobacteria also proved that this toxin at ecologically relevant concentrations was able to cause death at cell densities of 10^7 cells/ml and in another experiment, malformation in carp embryos, causing skeletal malformations, characterized by bent tail/body axes (Osswald *et al*., 2009).

Impact of cyanotoxins in environment is not restricted to acute or chronic in the different populations. Cyanotoxins can also be accumulated by many organisms, leading to a potential transfer of those toxins along food chains and eventually reaching humans. Not all organisms have the same ability to accumulate toxins and not all toxins are accumulated at the same rate. Mollusks, and among them bivalves, are those that have the highest accumulation rates (Martins & Vasconcelos, 2009). This is understandable since these organisms are effective samplers of the environment, filtering and retaining contaminants. Among cyanotoxins, BMAA and MC seem to

be those with higher rates of accumulation (Vasconcelos, 1995; Amorim & Vasconcelos, 1999; Baptista *et al*., 2014), being followed by CYN (Saker *et al*. 2004), STX (Pereira *et al*., 2004) and anatoxin-a (Osswald *et al*. 2008). Interestingly, gastropods have also a capacity to accumulate cyanotoxins (Martins & Vasconcelos, 2011) and this is due mainly to the fact that they can graze on benthic cyanobacteria and these are also known to produce the toxins (Aboal *et al.*, 2005). Crustaceans and fish can also accumulate cyanotoxins but at lower rates. In the case of crustaceans, we may point out *Litopenaeus vannamei* (Zimba *et al*., 2006), *Macrobrachium nipponensis* (Chen & Xie, 2005) and *Procambarus clarkii* (Vasconcelos *et al*., 2001) with maximum MC accumulations of 55, 12.4 and 9.9 μg/g respectively. Fish accumulate toxins mostly in their gastrointestinal organs and much less in the muscles, usually the edible part (Martins & Vasconcelos, 2011). So in the case of fish human intoxications seem to be less probable if only muscle is consumed.

In the last 5 decades, work on toxins from cyanobacteria focused on those well known compounds with toxic properties targeting mammals. Nevertheless, the results of ecotoxicological assays using non-toxin producing species and the establishment of new molecular and chemical tools allowed scientist to focus on new molecules with interest. New and emerging toxins and new interesting bioactive molecules have been found, such as BMAA and palytoxin in the first case, and cyanobactins in the second one.

BMAA is a neurotoxin non proteinogenic amino acid discovered in the 1967 due to the case of high incidence of neurodegenerative disease such as Amyotrophic Lateral Sclerosis (ALS) in Guam (Vega & Bell, 1967). High levels of BMAA were found in patient brains and the origin was found to be a *Nostoc* species, symbiotic of *Cycas* roots, a plant used for human consumption. The bioaccumulation of the BMAA in *Cycas* flour and also on bats that feed on it and were the eaten by humans established the uptake route of this toxin. More recently it was found that BMAA also exists in free-living cyanobacteria in fresh, brackish water and marine environments. Brand *et al*. (2010) showed that BMAA can also be biomagnified in Florida Bay food chains and Jonasson *et al*. (2010) pointed out that transfer of BMAA in the Baltic Sea suggest pathways for human exposure. Levels of BMAA found in estuarine cyanobacteria

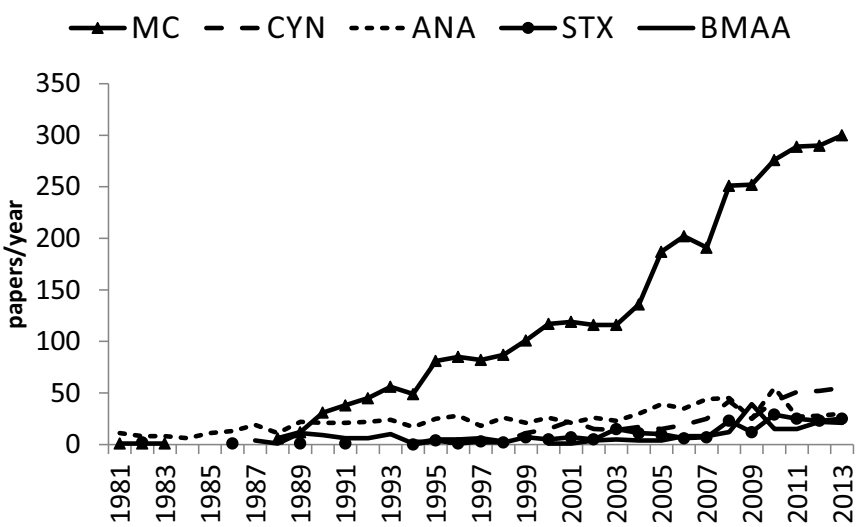

Figure 3. Number of papers published from 1981 till 2013 on microcystins (MC), cylindrospermopsin (CYN), anatoxin-a (ANA), saxitoxins (STX), BMAA (source of data-SCOPUS). *Número de artículos publicados desde 1981 hasta 2013 sobre microcistinas (MC), cilindrospermopsina (CYN), anatoxina-a (ANA), saxitoxinas (STX) y beta-N-metilamino-L-alanina (BMAA).*

isolated from Portuguese estuaries (Cianca *et al*., 2012) reveal that biomagnifications may occur in those ecosystems, where filter feeding mollusks are abundant. In fact, laboratorial experiments with the mussel *M. galloprovincialis* showed that levels up to 40 μg BMAA/g may be accumulated (Baptista *et al*., 2014).

New molecules isolated from cyanobacteria are being published every year and many more are being isolated and characterized, not all being regarded as toxins. cyanobactins are cyclic peptides, being reported all over the world and being produced by freshwater as well as by marine cyanobacteria (Sivonen *et al*., 2010; Donia *et al*., 2008). The bioactivity of these molecules has been studied, being reported anticancer activity (Table 1). Cyanobactin gene clusters have been described, and recently Sivonen *et al.* (2010) have reported those from seven distantly related cyanobacteria. The design of degenerated primers has allowed us to screen LEGE cyanobacteria culture collection revealing interesting potential in cyanobacterial strains from marine and freshwater ecosystems belonging to different orders (Martins *et al*., 2013). Phylogenetic analysis of those genes revealed the potential occurrence of new cyanobactins.

A bioassay guided work on the potential allelopathic effects of a freshwater cyanobacterium, lead us to discover new compounds that act synergistically and may be useful to control phytoplankton blooms (Leão *et al*., 2010). Portoamide are cyclic amides that have 4 variants, being the effect maximized when two of the variants 1 and 2 act synergistically. These portoamides seem also potential anticancer substances since they tested positively in lung cancer cell toxicity assay (Leão *et al*., 2010).

The development of sensitive and specific techniques together with an increased effort in the research related to cyanobacteria and cyanotoxins has contributed to a significant increase on the knowledge of these toxins. Chemical techniques have evolved much in the last years making the use of more precise and sensitive techniques such as LC-MS more affordable. LC-MS is nowadays fundamental since most of the cyanotoxins have not commercial standards available and so it is important to attribute a mass to each peak revealed in the chromatogram. MALDI-TOF is also a powerful technique, with the advantage of requiring low preparation of the sample but it still not on the route of many laboratories, being useful on new toxin discovery and proteomic analysis (Campos *et al*., 2012). The development of biosensors that allow real time detection and quantification of cyanotoxins is a need because it will help environmental and human health authorities to efficiently manage situations were cyanotoxins may constitute and hazard.

Molecular methods are also being used for the early warning detection not only cyanobacteria blooms but also the potential occurrence of cyanotoxins. Multiplex PCR may simultaneously detect several cyanobacterial species but also the cyanotoxin producing genes (Saker *et al*., 2007; Valério *et al*., 2010; Barón-Sola *et al*., 2012). On

Table 1. Bioactivity of some selected cyanobactins produced by Cyanobacteria. *Bioactividad de algunas cianobactinas producidas por cianobacterias.*

Compound	Bioactivity	Reference
Patellamide	Cytotoxic, antineoplastic	Ireland *et al*., 1982
Microcyclamide A	Moderate cytotoxicity against P388 murine leukemia cells	Ishida *et al*., 2000; Ziemert *et al*., 2008
Trunkamide	Cytotoxic, multidrug reversing activity	Caba *et al*., 2001; Donia *et al*., 2008
Trichamide	No effects found (tested for cytotoxic, antifungal, antibacterial and antiviral activities)	Sudek *et al*., 2006

the other side, qPCR may allow us to quantify gene copies that identify a certain cyanobacteria or toxin producing gene, being a very sensitive method if conveniently validated with laboratorial experiments (Moreira *et al*., 2011;Churro *et al*., 2012).

CONCLUSIONS

Although we have improved methods to detect and quantify cyanotoxins, new challenges will arise from the global changes that will expose human populations to emergent toxins and invasive cyanobacteria species. Monitoring of cyanobacteria and toxins via molecular, chemical and biological methods is needed to prevent hazards. Microcystins and cylindrospermopsin may be the main hazards but emergent toxins such as BMAA and new molecules still being characterized should not be neglected. New routes on intoxication need to be studied, in special agricultural products and marine organisms that are currently only monitored for Paralytic Shellfish Poisoning (PSP), Diarrheic Shellfish Poisoning (DSP) and Amnesic Shellfish Poisoning (ASP) marine toxins.

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