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Freshwater Phytoplankton of Argentina

Problems on drinking water related to toxigenic Cyanobacteria: some cases studied in Argentina

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With 4 figures

Abstract: The massive presence of harmful Cyanobacteria in freshwaters has been acknowledged since 1878 when the death of a series of farm animals associated with a bloom of *Nodularia spumigena* was registered in Australia. In Argentina, these phenomena are known since 1944 when in the Bedetti shallow lake (Province of Santa Fe) about 1000 farm ducks died as a consequence of the ingestion of water after a mixed bloom of various species of blue green algae developed.

The anthropic impact on aquatic ecosystems favours eutrophication and, combined with rising temperatures due to global climate change, promotes the development and expansion of harmful algal blooms. Cyanobacterial blooms are now widely recognised as a serious water quality problem with regard to both recreational and drinking water. In Argentina, several toxigenic Cyanobacteria have been reported and associated with blooms, being the most common genera *Microcystis* and *Dolichospermum* (*Anabaena*), while the most common cyanotoxins detected are microcystins.

In this article we present four case studies related with the presence of toxigenic Cyanobacteria in drinking water, involving *Snowella lacustris, Microcystis aeruginosa* and species of *Dolichospermum* (*Anabaena*).

Keywords: toxigenic Cyanobacteria, drinking water.

Introduction

The constant rise in human population leads to an increase of certain activities which have a major impact on freshwater environments, causing their deterioration. Human activities include the discharge of wastewater, impounding of rivers, off-river storage impoundments and other activities that alter the original conditions of natural waters and force the aquatic systems through the incorporation of an excess of nutrients and organic matter (Carmichael 2008). Anthropogenic pressures lead to 'cultural eutrophication' which remarkably alters the

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natural characteristics of ecosystems and involves also the decrease of water quality for the associated fauna and flora, causing, among other consequences, a reduction and loss of biodiversity. In relation to man, eutrophication processes are a real problem and have serious consequences: landscape deterioration is accompanied by a reduction in the aesthetic and recreational value; the processing costs for water purification increase significantly and the decrease in water quality causes serious sanitary problems such as the development of appropriate conditions for the settlement of disease vectors (Echenique & Aguilera 2009).

Despite the acknowledgement of the eutrophication phenomena started around 1950, it was not until about four decades later that these begun to be associated with the massive proliferation of planktonic Cyanobacteria, in many cases producers of harmful substances of diverse chemical nature. Health problems attributed to the presence of toxins and offensive tastes and odours in drinking water have been reported worldwide (Perovich et al. 2008, Bartram et al. 1999). The term "off-flavor" is used to describe the accumulation of odorous compounds within water or tissue produced from biological origins. In the case of potable water, consumer perception of the quality of drinking water is typically influenced by odor and taste. Some Cyanobacteria species are able to liberate smelly volatile metabolites such as geosmine or the metilisoborneol. Although these compounds are not considered toxic (Falconer 1998, Falconer et al. 1999) or have shown no mutagenic effects (Dionigi et al. 1993), they tend to generate breathing and digestive disorders in vulnerable people. A recent review of Cyanobacterial odorous and bioactive metabolites provides a thorough listing of known off-flavor producing Cyanobacteria species (Smith et al. 2008). Such odors are a major impediment to the growth of freshwater aquaculture industry, and have a negative effect on recreational water since the smell is disgusting and similar to that produced by some organochlorine pesticides. Even though it has been suggested that the presence of such metabolites might be related to the generation of cyanotoxins, a direct relation between both situations has not been established. On the other hand, the absence of smell does not guarantee the absence of Cyanobacteria (Falconer et al. 1999, Brena & Bonilla 2009)

Cyanobacterial blooms and cyanotoxins have been linked to the deaths of wild and domestic animals all over the world. They constitute a health-risk for human beings worldwide via recreational or drinking water through the production of a range of toxic compounds – hepato-, neuro- and dermatotoxic compounds (Carmichael 2008). The first world reference related to toxic aspects of Cyanobacteria in freshwater bodies refers to the death of both wild and domestic animals, after these drank water from the Alexandrina lake (Australia), where a bloom of *Nodularia spumigena* developed (Francis 1878). The register of similar events has increased both as regards the number of responsible species as well as frequency, intensity and geographical extension (Hudnell & Dortch 2008). Acute illnesses and death in human populations following exposure to cyanotoxins-contaminated water have been reported worldwide (Carmichael & Falconer 1993) and in Argentina (Giannuzzi et al. 2011).

The first register of human death caused by cyanotoxins was reported in 1996 in a clinic in Caruaru (Brazil) where renal dialysis patients exposed to MC-LR contaminated water had liver failure initially, and finally died. Microcystins were detected in blood and liver samples of poisoned patients and water analysis confirmed the presence of microcystins and cylindrospermopsins in the activated carbon used in water purification. Water samples from the reservoir which provided the city and the clinic with water, indicated dominance of toxigenic

Cyanobacteria (Carmichael 1996, Jochimsen et al. 1998, Falconer 1998, Kuiper-Goodman et al. 1999, Azevedo com. pers.).

The first cyanotoxic event recorded in Argentina dates back to 1944 in the Bedetti shallow lake (Santo Tomé, Santa Fe). After *Anabaena* sp. bloom exposure, about 1000 farm ducks and series of wild animals died as a consequence of water ingestion. Although the toxic compound could not be isolated and completely *c*haracterized, a bioactive metabolite produced by *Anabaena* sp. was considered the cause of the animal deaths (Mullor 1945). In 1954, cyanobacterial blooms with fish kills occurred in shallow lake Laguna de Monte, Province of Buenos Aires (Ringuelet et al. 1955). Phytoplankton analyzes confirmed that the phenomenon had been produced by *Microcystis flos-aquae*, *Dolichospermum* (*Anabaena*) *circinalis* and *Anabaena inaequalis*, all cyanotoxic taxa. After several tests, a direct relation between phytoplankton density and the death of fish was found.

Posteriorly and since the 80's, several blooms have been observed in rivers, reservoirs, lakes, coastal lagoons and estuaries from North to South Argentina (Azevedo 2005). On one hand, problems associated with blooms in freshwaters and their ecological consequences were described in detail for different aquatic systems (Meichtry de Zaburlin 2002, De León & Chalar 2003, Zalocar de Domitrovic & Forastier 2005, Ruibal et al. 2005, Echenique et al. 2008, O'Farrell et al. 2012). On the other hand, extensive reviews of the occurrence of cyanobacterial blooms and their harmful aspects were performed regionally (Zalocar de Domitrovic & Forastier 2005) and for the entire Argentine territory (Pizzolón et al. 1997, Pizzolón et al. 1999, Echenique & Aguilera 2009). These surveys show that the assessment of the presence of toxigenic Cyanobacteria progressively took place in the last decades in several research centers. Toxicology tests and analytic studies of the toxins found in freshwater increased as well as the study of the toxicological effects on the biota and the impact on recreational environments and drinking water supplies. Finally, toxic strains characterization and phylogenetic analysis using molecular techniques on natural populations and cultures began (Oteiza et al. 2007).

Although several toxigenic species of Cyanobacteria have been reported and associated with algal blooms in Argentina, the most common genera are *Microcystis* and *Dolichospermum* (*Anabaena*), and the most common cyanotoxins detected are microcystins. As regards the presence of volatile compounds, geosmine produced by species of *Dolichospermum, D. spiroides* and *D. circinalis* was registered several times in water supplies of the cities of San Luis and Bahía Blanca (Silva et al. 1995, Echenique et al. 2003, Echenique et al. 2006). *Dolichospermum* species are frequently mentioned as responsible for these types of disorder in different parts of the world (Slater & Block 1983, Falconer et al. 1999, González et al. 2001, Busso 2009).

The answer to an environmentally-related health issue, such as a toxic cyanobacterial blooms, implicates and involves not only scientists and researchers. Governments have ultimate responsibility for safeguarding public health and their role should be to minimise the damage and to assess the circumstances leading to cyanobacterial problems. They should also assist with public awareness activities. Unfortunately, in the field of water supply, there has not been a serious recognition of the general need to ensure availability and safety of drinking water in Argentina. Besides, there is an important lack of communication between government and industry sectors (e.g., water suppliers) which is especially detrimental in managing cyanotoxin issues. Public meetings and workshops, in which key issues were discussed, started to be held in Argentina since 1994. Most meetings focused mainly in analyzing the problems caused by *Microcystis aeruginosa* harmful blooms in the Río de la Plata basin (Buenos Aires). At the same time, a law modification was proposed in order to take into consideration the harmful aspects generated by the presence of toxigenic Cyanobacteria in water drinking supplies.

During 2004, researchers from many parts of the world established the first global network for the risk management of cyanobacterial blooms and cyanotoxins in water resources. In this way, CYANONET (website: http://www.cyanonet.org.) was created as a part of UNESCO's International Hydrology Programme-V. Some Argentinean researchers took part in this project as country representatives who presented a report which provided an initial assessment of the occurrence and impacts of cyanobacterial blooms and cyanotoxins as well as risk management responses available in Argentina (Codd et al. 2005).

In this article we will present four case studies related with the presence of toxigenic Cyanobacteria in Argentina, involving *Snowella lacustris, Microcystis aeruginosa* and species of *Dolichospermum* (*Anabaena*). Figure 1 shows the areas where these studies were carried out.

Results

Case A

Blooms of toxic species of *Dolichospermum,* principally *D. circinalis*, have been reported several times since 1978 in the Limay River Basin northwestern Patagonia, Argentina (Guarrera et al. 1981, Guarrera et al. 1995, Echenique 1987, Alcalde et al. 1996, Alcalde et al. 1998). With the aim of detecting the presence of cyanobacterial harmful blooms, regional authorities established a monitoring programme in October 2001. The study comprised mainly sectors of the Río Limay basin downstream from the Arroyito reservoir and near Neuquén city (Fig. 1, Case A).

Dolichospermum blooms were registered during the study period. Species determination was impossible since *D. circinalis*, *D. spiroides* and *D. lemmermannii* are a group of morphologically similar species which coexist in the basin. Considering such similarity and the absence or low frequency of akinetes, it was decided to identify these three toxic species as *Dolichospermum* spp. "complex". In the Limay River stations, this complex presented a significant development during the warm months, mainly from November to January, with values that fluctuated between 1100 and 3500 cells. ml^{-1}, reaching a maximum in December 2004, in the Neuquen City station (15516 cells. ml^{-1}). Densities over 1000 cells. ml^{-1} were registered even in February, extending in occasions to April (1368 cells. ml^{-1} en 06/04/2009) (Fig. 2). The population dynamics of *Dolichospermum* spp. "complex" was determined and it was possible to define the critical growth periods as well as the frequency of occurrence of this "complex". Considering its cyclic blooms and based on cellular density, a contingency water supply plan was adapted from Australian procedures (Carmichael & Falconer 1983, Bartram et al. 1999) where water treatment plant operators and managers used a gradual response to the onset and progress of a cyanobacterial bloom. Accordingly, treatment companies of the region were warned to take adequate measures in order to reassure the supply of drinking water reducing the health risks associated with cyanobacterial contamination (Alcalde et al. 1998, Labollita 2007).

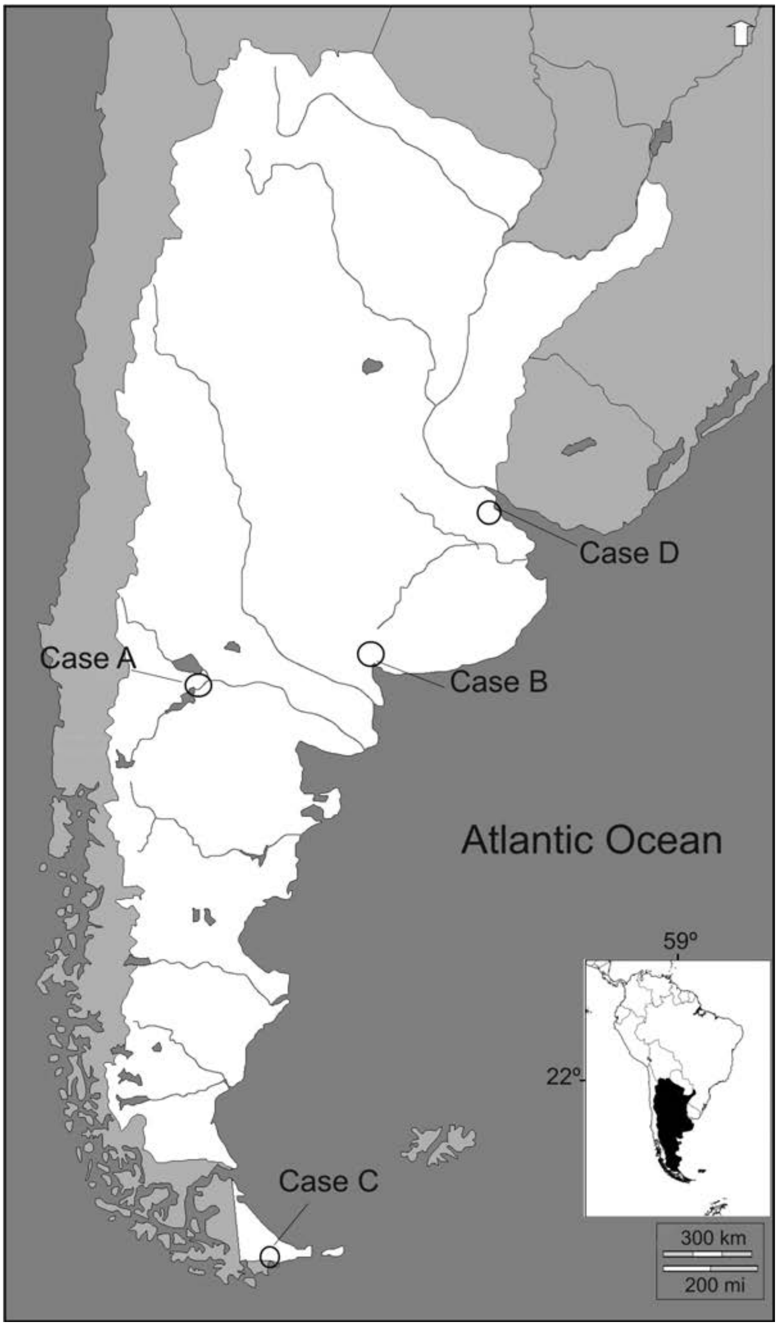

Fig. 1. Map of Argentine and areas of study. Case A: Río Limay Basin; Case B: Paso de las Piedras Reservoir; Case C: Fagnano Lake; Case D: Río de la Plata Basin.

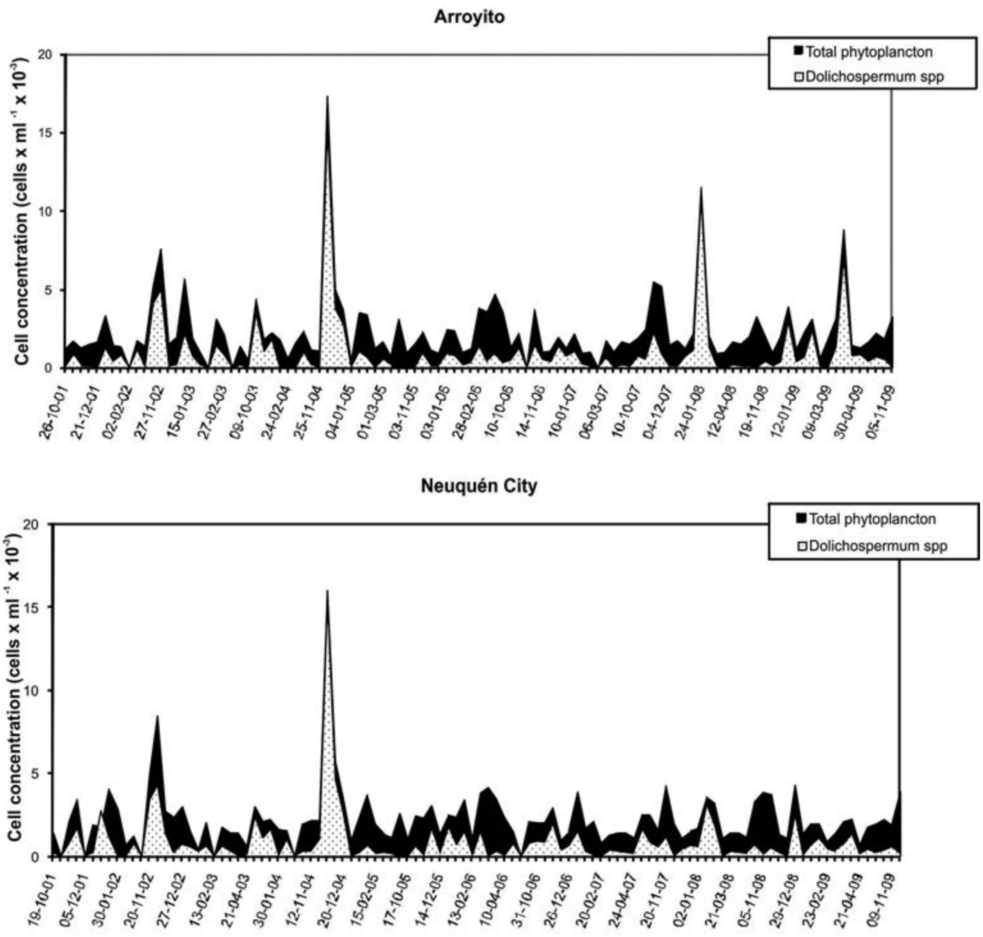

Fig. 2. Density variation of *Dolichospermum* spp. complex and of the total of phytoplankton in the Limay River at the Arroyito and Neuquen City (October 2001–December 2009)

Acute toxicity was evaluated in mice from blooms in the Exequiel Ramos Mexía Reservoir located upstream the sites here analyzed at the Limay River basin. The death of tested mice occurred within 70 minutes after the intraperitoneal injection. Given the animals behavior, cyanobacterial neurotoxins were considered to be the cause of the deaths (Alcalde et al. 1996).

Case B

Offensive tastes and odours were detected in tap water of Bahía Blanca City (Buenos Aires province) in April 2000. Such alterations coincided with skin and respiratory problems in the

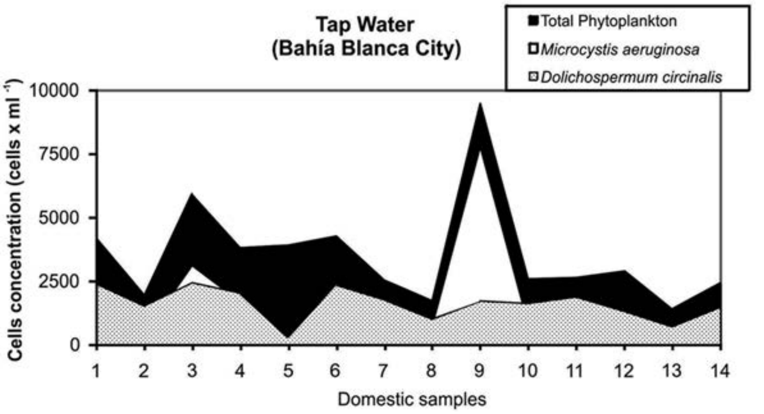

Fig. 3. Cellular density of *Dolichospermum circinalis*, *Microcystis aeruginosa* and total phytoplankton (cells. ml^{-1}) registered in domestic tap water samples (Bahía Blanca, Argentina).

population. As a consequence, water samples were taken from Paso de las Piedras Reservoir (38º22'S; 61º18'W) (Fig. 1, Case B), at the input and output points of two water treatment plants and at particular houses of the city.

At the reservoir samples, cell density of total phytoplankton fluctuated between 49440 and 84832 cells. ml^{-1}, being *Dolichospermum circinalis* the dominant species (values between 48320–84032 cells. ml^{-1}). *Microcystis aeruginosa* and *D. circinalis* were detected in high concentrations at inlet and outlet samples of water treatment plants. Concentration at the outlet was even higher than at the inlet, revealing a proliferation of Cyanobacteria during the purification processes (Echenique et al. 2006), thus revealing the inefficiency of the treatment plant in removing the Cyanobacteria. Tricomes of *D. circinalis,* fragmented colonies of *M. aeruginosa* and other planktonic organisms were also detected in domestic samples (Fig. 3). Given the results, tap water was not fit for consumption since the current legislation in Buenos Aires province points out that phytoplankton must be absent in drinking water.

Geosmine was also detected in the reservoir, filters and mainly in tap water. This metabolite is synthesized by *D. circinalis* (Slater & Block 1983, Silva et al. 1995, González et al. 2001) and since this species was present in every sample, it was assumed as the main responsible for the strong smells registered. Typical health signs of consumption of cyanotoxins such as nausea, cephalalgia, gastroenteritis and allergic reactions in skin, eye sores, and even asthma, could not be associated with any cyanobacterial toxin as the acute toxicity tests performed in mice indicated an absence of cyanotoxins in the water of the region (Echenique et al. 2006). In any case, mouse bioassays are able to detect levels of toxins related with degrees of acute toxicity but they are not sensitive enough to detect concentrations of toxins which represent chronic toxicity risks (Echenique et al. 2003).

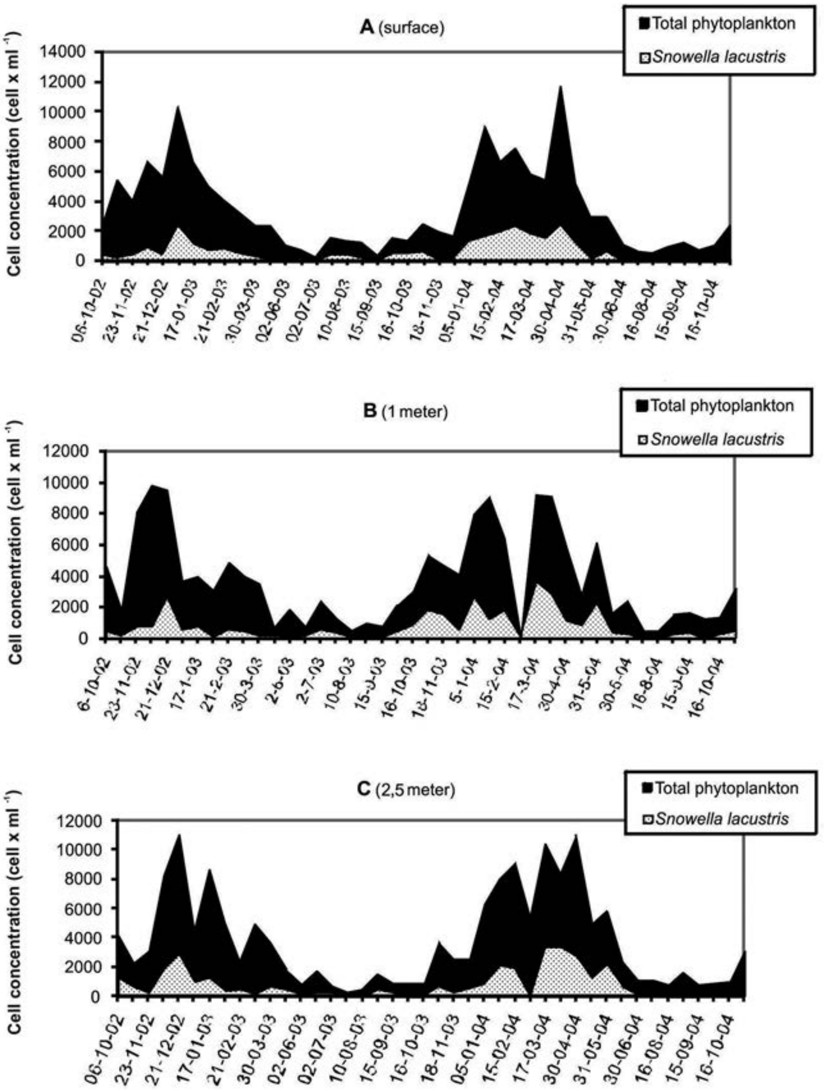

Fig. 4. Temporal variation in *Snowella lacustris* and total phytoplankton densities at three levels of the water column. A: surface; B: 1 meter; C: 2.5 meters (Data not published).

Case C

Short fragments and single cells of phytoplankton species were detected in tap water of Tolhuin City (Tierra del Fuego province), at the beginning of 2002. *Snowella lacustris,* the dominant species, was found in the extraction wells, in the net, and even in the storage tanks (cisterns). This fact resulted highly confusing since the water was extracted from boreholes.

The authorities of Tierra del Fuego evaluated the possibility of modifying the source of supply, choosing the Fagnano Lake (54º35'S, 68º00'W) as the new water supply. It was suggested the building of a purification plant. A routine monitoring plan was carried out from October 2002 to November 2004 in the Fagnano Lake (Fig. 1, Case C), near Tolhuin city. Samples were taken at three levels of the water column to estimate phytoplankton density and optimize the depth level of water taking. During this period, *Snowella lacustris* and *Woronichinia naegeliana* were detected, both considered as potential producers of toxins (Skulberg et al. 1983, Sivonen et al. 1990, Pizzolon 1996, Falconer 1998). *S. lacustris* densities were superior to 1000 cells. ml^{-1} in December 2002 and January 2003, with average values close to 2000 cells. ml^{-1} at the three considered depths. Similar values were registered again from November 2003, extending to the first months of the fall of 2004, a fact which coincides with the periods of greater availability of nutrients, associated with the thaw. No significant differences were observed as regards the behavior of *S. lacustri*s or the total phytoplankton at the designated levels of the water column (Fig. 4).

The obtained results were considered for the design of the water treatment plant which should guarantee tap water with the characteristics required by law (Bleta et al. 2007). Although toxicological analysis were not made, phytoplankton data allowed for the recognition of the taxa with floating characteristics, which was taken into account to optimize the sedimentation and coagulation processes. It was possible to establish and specify the technical characteristics and necessary procedures for the effective physical removal of phytoplankton (Bernazeau 1998) and the information was also used to define the type and the dose of coagulants (Bernhardt & Clasen 1991).

Case D

Cyanobacterial blooms in the Río de la Plata Estuary (eastern Buenos Aires province) are frequent and generally occur in summer. In most cases *Microcystis aeruginosa* is responsible for the formation of non-specific, widespread blooms with high cell densities (Guarrera 1950, Gómez & Bauer 2000). This phenomenon occurs in areas where human activity is intense, especially near urban centers, where anthropogenic inputs of domestic and industrial discharges have been identified as the primary cause for the eutrophication of this water body. Reports of people suffering of gastrointestinal disorders after bathing and swimming are quite frequent but have not been published and, in fact, there are no epidemiological records available. Events of massive fish mortality associated to algal blooms are also typical.

From December 2004 to January 2006 monitoring of the Río de la Plata was performed in an area near to La Plata City, where water is pumped and directed to the water treatment plant (Fig. 1, Case D). Samples were collected on the surface layer in different sectors where *Microcystis aeruginosa* was detected as the main cyanotoxic species. Population density fluctuated between 0 and 51000 cells. ml^{-1}. Even if the most significant development of this species was extended from the beginning of February to middle April, the peak was registered about middle March. The lowest densities were observed between early August and early December 2005 (Andrinolo et al. 2007).

Cells were concentrated by centrifugation from a *M. aeruginosa* bloom and were analyzed by mouse bioassay and by high-performance liquid chromatography with Diode-array and

MS detector. The samples indicated high hepatotoxicity and, in accordance, high levels of microcystins, with microcystin-LR and a variant of microcystin with a molecular ion $[M+H]^+$ = 1037,8 m/z as major components. The total toxin content in these samples was 0.94 $\mu g\ mg^{-1}$ and 0.69 $\mu g\ mg^{-1}$ of lyophilized cells.

As a consequence of *Microcystis aeruginosa* blooms in the coastal area of the Río de la Plata Estuary, the efficiency of the treatment plant was evaluated, focusing on its capacity for cyanotoxins removal (December 2004 and April 2005). To this end, water samples were collected from the river and in the area where the water taking is located. On the other hand, tap water samples were collected in 13 houses of the supply net. In the river, some *M. aeruginosa* strains reached toxicities above 6 mg of microcystins gr^{-1} of dry weight, placing them among the most toxic ones described in the literature (Falconer 1998). As regards home water samples, microcystins were present in 10 out of 13 water samples (Echenique et al. 2008). Toxin content was higher than the guidance levels proposed by the WHO, indicating a high toxicological risk (WHO 1998, Falconer 1998, Falconer et al. 1999, Kuiper-Goodman et al. 1999).

Given these results, it is evident that blooms of toxic strains of *Microcystis aeruginosa* in the Argentinean coast of the Río de la Plata Estuary are of both sanitary and environmental concern. The risk for human consumption of microcystins is high due to the fact that conventional water treatment processes may not be effective enough to remove microcystins.

Discussion

Nowadays the occurrence of harmful algal blooms has increased its frequency, severity, and duration as well as the amount of toxic species and cyanotoxins reported. Anthropogenic eutrophication has produced changes in water quality, and cyanobacterial harmful blooms are often a symptom of the resulting changes from human activities.

In Argentina, the adverse impacts on water supplies due to cyanobacterial blooms are usually underestimated and the reports on these impacts are mainly related to massive fish mortality. Although the loss of scenic quality and consequent reduction of recreational activities have been described in some regions in Argentina, the ecological impacts (e.g., the effects on aquatic biodiversity, bioaccumulation of cyanotoxins through the food chain) have been poorly estimated and described.

Harmful aspects associated with Cyanobacteria have not been seriously considered by the authorities; there have been some exceptions such as the cases here presented. The consequences of toxic cyanobacterial bloom occurrence are underestimated or not evident to those who are responsible for the environment and water quality control. The management actions or implementation of preventive plans and remedial measures are only taken into account when serious events occur. Besides, it is common to observe that these actions are restricted to a few weeks surrounding the event.

One of the most outstanding examples is the lack of legislation at national level regarding the presence of Cyanobacteria and its toxins, both in the supply sources and in the tap water. The Argentinean guideline for water quality is now under revision and there is a strong mobilization of scientific researchers and some governmental authorities to include Cyanobacteria and cyanotoxins as a new parameter to be considered. Although management actions to monitor Cyanobacteria and cyanotoxins in water resources and to control their negative impacts have been poorly developed in Argentina, there is a possibility to extend and increase the recognition and awareness of the occurrence of Cyanobacteria and their toxins, and to more widely provide further management tools to avoid the development and adverse effects of cyanobacterial proliferation and cyanotoxins.

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