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Assessment of specific antibodies as biological indicators of human chronic exposure to microcystins.

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ABSTRACT

Cyanobacteria can produce potent natural toxins known as cyanotoxins. Blooms of cyanobacteria, produced mainly as result of the pollution of water bodies with excessive amounts of phosphorus, represent a severe environmental problem; not only do they affect the normal equilibrium of the aquatic ecosystem but may also affect animal and human health. The occurrence of algal blooms have been increasing globally (it has been recently reported in at least 100 countries) and it has been considered by WHO as an emerging public health issue. The toxic effects of cyanotoxins have been thoroughly demonstrated in laboratory experiments, however, the effects on humans and the extent of these effects have been more difficult to assess. Epidemiological research is difficult as there are no specific symptoms or routine biomarkers to diagnose intoxication with cyanotoxins, in particular those cases associated with chronic exposure. The objectives of this study were to assess the exposure of a population settled near a lake with recurrent cyanobacteria blooms and to investigate the presence of biological markers of chronic exposure to cyanotoxins, in particular the microcystins (MCs). We first investigated the exposure of the population to cyanobacteria by using a questionnaire on how the population used the water and by analyzing water samples for the presence of cyanobacteria and total microcystins (TMCs). Secondly, we investigated the presence of biological indicators by analyzing the biochemical and immunological parameters in sera of the exposed population. The questionnaires and the water analyses revealed that the population under study (n = 47) is exposed to several exposure routes. The biochemical analyses of the sera showed the alteration of at least one hepatic enzyme in 25% of the exposed people, but this cannot be associated solely to MCs exposure. On the contrary, the immunological analyses, which included microcystin-LR specific antibodies IgE and IgG, showed significant differences between the exposed and non-exposed groups. The presence of MCs specific antibodies confirms the exposure to MCs. We propose the study of specific antibodies as a non-complex biomarker to detect chronic exposure to the toxin and to assist epidemiological studies.

1. Introduction

Cyanobacteria or blue green algae are prokaryotic microorganisms that inhabit natural waters. They can potentially produce toxins (cyanotoxins) and cause severe adverse health effects on humans and animals, in particular, when cyanobacteria develop massively on the surface of water bodies used as recreational areas or as sources for drinking water. Massive developments of cyanobacteria, known as algal blooms, represent a severe global issue within environmental and human health fields. A recent review (Harke et al., 2016) indicated a geographic expansion of this phenomenon, with algal blooms recorded in at least 108 countries and WHO recognized it as an emerging health issue (WHO, 2003a and b; Sauvé and Desrosiers, 2014). In Argentina, in particular, toxic cyanobacteria blooms have been identified in at least 13 out of 23 provinces and different water bodies (rivers, lakes and estuaries) (Aguilera et al., 2018). Microcystins (MCs) are a group of cyanotoxins (cyclic heptapeptides) with hepatotoxic characteristics. There are more than 100 variants or congeners of MCs (Testai et al., 2016). The most common are Microcystin-LR (MC-LR), Microcystin-RR (MC-RR) and Microcystin-LR (MC-YR). The MC-LR has been by far the most studied variant. It is a potent hepatoxin which can also damage other organs such as the lungs, the kidneys and the brain (McLellan and Manderville, 2017). Human exposure to MCs occurs through ingestion, inhalation, dermal contact and more rarely intravenous contact via hemodialysis

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(Meneely and Elliott, 2013; Testai et al., 2016). People may be exposed to the toxins in an acute and/or chronic manner. Human intoxications are not easy to diagnose because they present symptoms common to other pathologies on one hand, and because it is difficult to identify the link between exposure and health damage on the other. Nonetheless, some cases of acute intoxication associated with MCs have been documented (Jochimsen et al., 1998, Giannuzzi et al., 2011; Vidal et al., 2017). The effects associated with human long-term and low-level chronic exposure are even more difficult to identify (Weirich and Miller, 2014, Meneely and Elliott, 2013). One of the reasons is the short half-life time of the toxin which makes it unlikely to find the parent toxin in biological fluids after a certain time (67% of MC-LR concentrates in the liver within 60 min (Robinson et al., 1991)). Another reason is that the analytical techniques for toxin detection in body fluids are not simple. Different techniques have been used to find the toxin in human serum (Hilborn et al. 2005, 2013; Chen et al., 2009; Li et al., 2011). For example, Hilborn et al. (2005) reported a simple way of measuring the toxin in blood using ELISA but the time needed for sample preparation (extraction, concentration and clean up), the overestimation of some specific MC congener concentrations, and the fact that MC spiking may be required to detect low levels of MCs in human blood are major issues for the routine use of this method (Heussner et al., 2014a and b). Chen et al., (2009) detected MCs in blood of chronically exposed fishermen using LC/MS. Again, a drawback of this method is the laborious methodology used for toxin extraction, low reproducibility, especially with low concentrations of MCs, the expertise needed to run the equipment, as well as the cost involved. As no simple method is so far available, some researchers investigated the association of chronic exposure to MCs with epidemic liver and colorectal cancers to assess the impact of cyanobacteria blooms on health in China, Florida, Eastern Europe and Canada (Ueno et al., 1996; Zhou et al., 2002; Fleming et al., 2002; Svircev et al., 2009; Li et al., 2011; Svircev et al., 2017). However, while these studies may provide evidence of a link between MCs in water and chronic health problems, these studies may have failed to identify either the etiology of the disease in blood (liver and colorectal cancer can be caused by other agents such as HBV, HBC, alcohol consumption and aflatoxins in food) or the time of exposure, or even fail to consider confounding factors (Testai et al., 2016; Buratti et al., 2017).

It is therefore complicated to identify the link between exposure and health damage, particularly in cases of chronic exposure. To understand the importance of human exposure to microcystins, the ideal method would be a simple, highly sensitive and non-invasive method of analysis measuring either the target analyte (MCs) in blood, urine, hair or feces (Meneely and Elliott, 2013) or some specific biomarker (Van der Merwe, 2013).

In previous studies, we investigated the presence of MCs in a lake (San Roque Reservoir) with more than 15 years of recurrent cyanobacteria blooms. This lake is used as a source of water by a small population located on its shores (Ruibal Conti et al., 2005; Ruibal Conti et al., 2007; Ruiz et al, 2010). In this work we developed a questionnaire and investigated the exposure routes and the overall health status of the nearby residents. We also developed a method and investigated the presence of MC-LR specific antibodies (IgE and IgG) in the serum of those exposed. We propose that specific antibodies against MC-LR may be used as biochemical markers related to microcystin exposure and may serve as a practical, specific method to use for large epidemiological studies in exposed populations.

2. Materials and methods

2.1. Sampling site and study population

Over the last three decades, San Roque Lake, located in Córdoba, Argentina has been having recurrent toxic cyanobacteria blooms mainly composed of *Microcystis spp*. and *Dolichospermum spp*. (Amé et al., 2003; Ruibal Conti et al., 2005; Ruiz et al., 2013; Galanti et al., 2013). On the coast of the lake, in an area near the dam, there is a small settlement of around 114 inhabitants with a median residence time of 20 years. There is also a precarious and rural school where children receive kindergarten and primary education. Through three survey campaigns, the research team collected data about age, body height, body weight, medical history, dwelling place and use of lake water. The surveyed population was composed by 9 adult males, 18 adult females, 12 children (3–12 years old) and 8 teenagers (13–18 years old). Also, blood samples were collected. We considered this surveyed population (n = 47) as the Exposed Group (EG). A consent form was read to each person who agreed to participate as volunteer. In case of children, parents of participants gave written informed consent before the study. Questionnaires and the consent form were approved by the ethical committee of the Clínica Universitaria Reina Fabiola (Provintial Registry on Health Research RePIS Nº820). The research was conducted progressively from 2003 to 2009.

2.2. Collection of water samples and analysis by a commercial ELISA kit

Total microcystins (TMCs), phytoplankton and bacterial analyses were performed to the most relevant water sources. Water samples were collected monthly (over 33 consecutive months) from three sites: surface of the lake, tap water from the school and a site named by locals as “water spring” (name given by locals to a leak in the wall of the dam). Samples were collected in plastic bottles. Samples for MCs analyses were taken as a subsample of the bottle collected for phytoplankton analyses. TMCs were determined by immuno-enzymatic assay (ELISA Kit, Envirologix USA) after a pretreatment of three thaw-freeze cycles and filtration through acetate-cellulose membrane filters (pore size 0.45 µm; Ø = 47 mm). Total, Thermotolerant Coliform bacteria and *E coli* were done following the Standard Methods (APHA, 1998).

2.3. Analyses of serum biochemical indices

Venipuncture system was used by the trained research members to collect blood samples from volunteers. Approximately 10 mL of blood was drawn per subject and was left to clot. The clotted blood was centrifuged for 10 min to yield serum. Serum determinations were performed by Synchron Clinical System LX20 (Beckman-Coulter Diagnosis, Fullerton, CA). Blood was extracted from two groups: the exposed group (EG, residents near the lake as mentioned in section 2.1) and a non-exposed group (N-EG, allergic people with gastrointestinal disorders that attended the Reina Fabiola Clinic located in Córdoba City, 35 km from the lake). In both groups we tested the presence antibodies IgE and IgG specific for MC-LR using an ELISA method developed by this research group. Additionally, in the EG we measured the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma – glutamyltransferase (GGT), total bilirubin (TBIL) and creatinine (CREA). This was done to assess the general health status of the liver in the exposed population EG (n = 47).

2.4. Analysis of serum specific antibodies against MC-LR by development of ELISA method

Briefly, microtiter plates (Nunc, Rochester, NY) were coated with MC-LR (Sigma) antigens (50 µg/mL) in carbonate-bicarbonate buffer (pH 9,6) incubated 2 h at 37 °C and overnight at 4 °C. The unbound polypeptides were removed by washing five times with PBS-0.1% (vol/vol) Tween 20. Plates were then blocked for 1 h with 1% (wt/vol) soya milk/PBS and incubated for 2 h with serum 1:5 diluted (for IgE antibodies) or serum 1:100 diluted (for IgG antibodies) in 1% (wt/vol) soya milk/PBS. Plates were then washed five times with PBS-0.1% Tween 20 and incubated with a 1:1000 dilution of peroxidase conjugated goat anti-human IgE and IgG (Sigma) for 1 h at 37 °C. Plates were washed as

described above and bound secondary antibodies were allowed to react with the substrate o-phenylenediamine (Sigma) for 30 min. The reaction was stopped by adding 4N H_2SO_4 and the optical density was measured at 490 nm in a microplate reader (Bio-Rad, Richmond, CA). In order to determine the levels of antibodies in the EG and NEG groups, the optical densities of these groups were compared to the optical densities of a control group (CG: Control Group, n = 10). The control group used for the ELISA assay involved sera of healthy workers of a factory, who showed no clinical symptoms and attended the clinic to undergo a general check-up. A sample was considered positive if the optical density was two or more standard deviations above the mean of a normal control sera (CG sera). The assays were performed in duplicate. A positive or negative result appeared in the same patient samples with an interassay CV of 5%.

2.5. Statistical analysis

For the data treatment, the following considerations were followed. Data reported as "below detection limit" (0.16 μgL^{-1} for MCs) were treated as zero. Comparisons between mean concentration of antibodies of exposed group and non-exposed group were performed using Welch's ANOVA and Tukey's test ($\alpha = 0.05$). The R Core Team (2014) software was used for the statistical analyses.

3. Results

3.1. Quality of the water used by local residents

The surveys revealed that local residents collect water from multiple sources. For domestic purposes (washing, cleaning and personal hygiene) they use water from the lake which is pumped to a tank or collected in buckets. For consumption they use water delivered by the local Council in trucks and from what they call a "water spring" (which is actually a leakage of lake water through the wall of the dam). The surveys also indicated that 48% of people drink water from the "water spring", 72% wash with water from the lake and 60% use the lake for recreation (swimming and fishing).

Analyses of water sampled from the local school, the "water spring" and the lake indicated the presence of phytoplankton, MCs and coliforms. Water from the school is pumped from the lake to a tank and it is used for cooking, cleaning and consumed only occasionally. On the other hand water from the "water spring" is consumed regularly. The latter is not in an accessible place, but it is collected and consumed by local residents because it is clear and tastes good. Lake water is used for recreational activities especially in summer. A summary of water quality data is presented in Table 1 (detailed information in supplemental material).

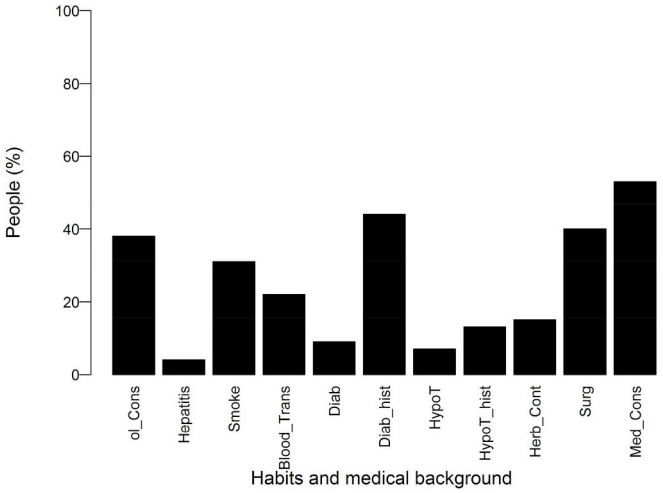

Fig. 1. Population's habits and medical background (ol_Cons = alcohol consumption, Blood_Trans = blood transfusion, Diab = diabetes, Diab_hist = diabetes history, HypoT = hypothyroidism, HypoT_his = hypothyroidism history, Herb_Cont = herbicides contact, Surg = surgery, Med_Cons = medicine consumption).

3.2. Population general health condition and serum biochemical indicators

3.2.1. General characteristics of population health condition: questionnaire results

The most common symptoms that emerged from the surveys were itching, scaling, hives, rashes, allergies (in armpits and arms), in mucosa: ear infections (otitis) and conjunctivitis, and at a gastrointestinal level, nausea, vomiting, cramps and diarrhea. There is a high incidence of diabetes (8.9%) and people with antecedents of diabetes (44%). Only 2 individuals claimed to have had hepatitis (4.4%) (Fig. 1). Parasitosis other than Chagas (trypanosimiasis), were not specifically reported. Other reported illnesses were: cholecystitis, renal failure and hypothyroidism.

Regarding population habits, 31% of the population smoked, 37.8% consumed some kind alcoholic beverage, 15% are in contact with herbicides and 22% consume fish caught from the lake.

3.2.2. Serum biochemical indicators

Levels of hepatic enzymes (ALT, AST, GGT, ALP) in serum are routinely used to assess the general conditions of liver function. As such, they have been used in several works to explore the association between exposure to MCs and health damage. In this line, we studied the levels of enzymes in the EG. The proportion of people with at least one abnormal level of enzymes were 25%, being mainly women (12/47; 2 men and 10 women). ALT and AST were outside the normal range

Table 1
Summary of water uses and water analyses.

Type of Water	Cyanobacteria Genera	Presence of TMCs	Coliforms (total and thermotolerant)	Use of Water
School water (school water tank is filled with lake water)	*Dolichospermum sp. Microcystis sp.* *Oscillatoria sp.* *Lyngbya sp.*	66% of samples (n = 32) Max. conc. = 3.0 μgL^{-1} Ave. conc. = 0.7 μgL^{-1}	Above provincial drinking water guideline values.	Cooking, cleaning and occasional consumption.
Water spring (leak in the dam wall)	*Microcystis sp.* *Oscillatoria sp. Chroococcus sp.* *Lyngbya sp.*	59% of samples (n = 29). Max. conc. = 2.3 μgL^{-1} Ave. conc. = 0.6 μgL^{-1}	Above provincial drinking water guideline values.	Consumption
Lake water (surface up to ~3 m depth)	*Microcystis sp.* *Dolichospermum sp.*	39% of samples (n = 33). Max. conc. = 4.3 μgL^{-1} Ave. conc. = 0.3 μgL^{-1}	Above provincial drinking water guideline values[a].	Recreation

[a] Note: from Ruibal-Conti (1996), Rossen et al. (2008), Brandalise et al. (2011).

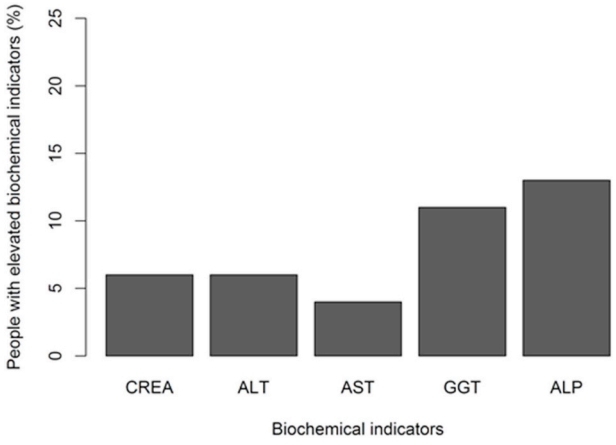

Fig. 2. Percentage of people in the exposed group (EG) with a concentration of biochemical indicators above normal levels.

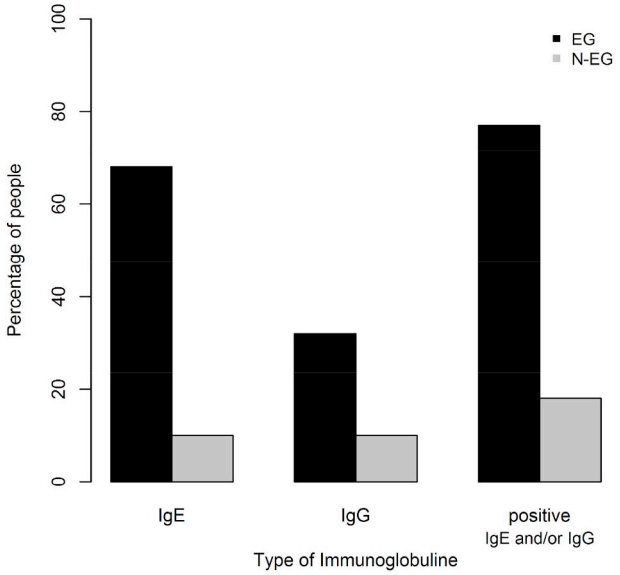

Fig. 3. Percentage of people with presence of antibodies against MC-LR. Comparison between the exposed (EG) and non-exposed (N-EG) groups. (Positive = serum with at least one Ig positive).

between 4 and 6%, while GGT and ALP, were altered in 11% and 13% of the population, respectively (Fig. 2).

The hepatic enzymes were not equally altered in the different age groups (Table 2); while the adult group presented alterations in all the enzymes, the teen group presented alterations only in GGT and the children mainly in ALP (33%) and AST (8%). CREA levels were within the normal range for most of the individuals (high levels were detected in some adults and some children) and TBIL was normal in all individuals.

In relation to other biochemical indicators the presence of eosinophilia was the most relevant indicator. Eosinophilia was detected in 14.5% of the studied group (3 adults, 1 teen and 3 children). Other parameters of the leukocyte formula were normal (suppl. Material: tengo que citarlo y agregarlo al material sumplementario).

3.2.3. *Presence of anti-MC-LR antibodies in serum of local residents*

The presence of antibodies specific for MC-LR was investigated (Fig. 3). Anti-MC-LR IgE and IgG isotypes were investigated by ELISA in serum samples of exposed residents (EG = exposed group) and in non-exposed people (N-EG = non-exposed group). The highest percentage of immunoreactive sera in the EG group corresponds to the IgE isotypes in 32 of 47 cases (68%) followed by IgG in 15 of 47 cases (39%). Sera from subjects not exposed (N-EG) were positive in 3 of 28 cases (11%) for IgE and IgG. A total of 77% of the exposed population had at least one type of antibody positive. There were significant differences between the exposed vs. non exposed residents at the $p < 0.05$ for IgE and for IgG (Welch's ANOVA; Table 3 and Fig. 4). The highest levels of antibody concentrations were the isotype IgE in exposed residents.

Table 3
Summary of Welch's ANOVA test for comparison of mean level of antibodies IgE and IgE in sera of EG, N-EG and CG.

	F	Num df	Denom df	p-value
IgE	15.527	2	47	6.366 e-6
IgG	13.496	2	27	8.548 e-6

Table 2
Summary of biochemical indicators assessed in sera of the exposed group.

Bioch. Ind.	Normal range	Proportion of subjects with indicators above normal level.			
		Total Population	Adults	Teens	Children
CREA (mg%)	♂ = 0.73–1.40 ♀ = 0.61–1.12 Teens ♂/♀ = 0.50–1.07 children ♂/♀ = 0.30–0.75	$\frac{3}{47}$ (6)	$\frac{2}{27}$ (7)	–	$\frac{1}{12}$ (8)
ALT ($U.L^{-1}$)	♂/♀ = up to 41	$\frac{3}{47}$ (6)	$\frac{3}{27}$ (11)	–	–
AST ($U.L^{-1}$)	children & teens ♂/♀ = up to 48 ♂/♀ = up to 38	$\frac{2}{47}$ (4)	$\frac{1}{27}$ (4)	–	$\frac{1}{12}$ (8)
GGT ($U.L^{-1}$)	♂ = 11-50 ♀ = 7–32 children & teens ♀ = 4–15 children & teens ♂ = 6-24	$\frac{5}{47}$ (11)	$\frac{4}{27}$ (15) (only ♀)	$\frac{1}{8}$ (13)	–
ALP($U.L^{-1}$)	children (2–8 yrs) = 140-740 children-teens (9–15 yrs) = 95-1060 teens-adults (16–21 yrs) = 65-705 adults = 70-300	$\frac{6}{47}$ (13)	$\frac{2}{27}$ (7)	–	$\frac{4}{12}$ (33)

Note: Numbers between brackets are percentages which are shown as rounded values. Total n = 47, adult: n = 27, teen: n = 8, children: n = 12.
♂ = adult male.
♂/♀ = either male or female adult.
Teen ♂/♀ = 13–18 years old, male or female.
Child ♂/♀ = 0–12 years old, male or female.

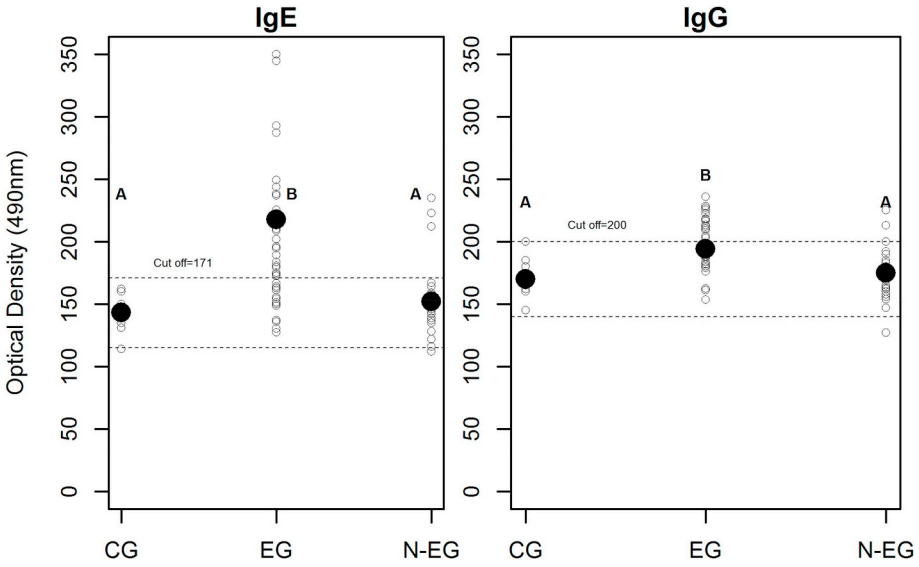

Fig. 4. Level of antibodies against MC-LR for each isotype (IgE and IgG) in exposed (EG,n = 47) and non-exposed group (N-EG, n = 28). Results expressed as Optical Densities (OD). A sample was considered positive if the OD was two or more Standard Deviation (SD) above the mean of the normal control sera (CG, n = 10) of ELISA assay. (cut-off: CG mean + 2 SD). Cut-off IgE: 171, Cut-offIgG: 200. The black dot represents the mean of each group.

4. Discussion

We first investigated the exposure of a population to toxic cyanobacteria. The results of the questionnaires and water analyses indicated that the population under study is exposed to MCs through different and combined exposure routes (oral, dermal and inhalational exposure). Oral exposure occurs through the consumption of water and fish. People consume water from what they call the “water spring”. In this water MCs were detected in nearly 60% of the samples. In 47% of the positive samples, MCs concentrations were above 1 $\mu g.L^{-1}$ which is the guideline value suggested by WHO (WHO, 2017) and by the Provincial guidelines for drinking water (MAAySP-SRH, 2016). In addition, the survey questionnaires revealed that people occasionally consume fish from the lake. Samples of fish were not collected during this study, but previous studies reported an accumulation of MCs in muscle of fish caught in this lake. An average concentration of 0.05 µg MC-RR per gram of fresh weight was found in muscle of *Odontesthes bonariensis*, a fish commonly caught for consumption (Cazenave et al., 2005). In the questionnaires, people were unable to indicate precisely how frequently they eat fish from the lake, but taking into consideration the people's low income and their proximity to the lake, we may consider they may ate fish at least twice a week.

Considering that an average person of 70 Kg, consumes 2 L of water per day with an average MC concentration of 0.6 $\mu g.L^{-1}$ and eats 200 g of fish per day twice a week, we calculated a total daily intake of 0.06 µg of MC per kg of body weight per day. This value is slightly above the limit of the tolerable daily intake (TDI) recommended by WHO (2017) (TDI = 0.04 µg per kg body weight per day). Therefore, in average, this population is exposed to chronic doses of MCs. However, it should be noted that 42% of the study population are children. As children can be most at risk due to the combination of smaller size, risky behaviors and developmental stage (Weirich and Miller, 2014; Stewart et al., 2006), some countries have suggested guideline levels lower than 1 $\mu g.L^{-1}$. This is the case for the USA, which suggests 0.3 $\mu g.L^{-1}$ for pre-school children (under 8 years old) as they drink proportionally more water per body weight (U.S. EPA, 2015). Taking into account these criteria, children of the population under study are at greater risk as all the positive samples for MCs were above 0.3 $\mu g.L^{-1}$.

Dermal and inhalational contacts were also identified as important exposure routes. In summer, people use the lake for bathing, particularly children. Over the study period, MCs concentration found in the lake water ranged from < 0.16 to 4.3 $\mu g.L^{-1}$. WHO (WHO, 2003b) and the recently issued National Guidelines for recreational waters (MSN, 2016) do not recommend a single guideline value of MC for recreational waters; instead, a series of guideline values associated with incremental severity and probability of health effects is defined at three levels of cyanobacteria cells/mL. For the lowest risk level (20,000 cell/mL), a concentration range of MCs from 2 to 4 $\mu g.L^{-1}$ is estimated and in the case of a highly toxic strain the concentration may rise up to 10 $\mu g.L^{-1}$ (Falconer et al., 1999). Previous works have shown that the concentration of MCs in this lake can be as high as 100 $\mu g\,L^{-1}$ (Ruibal Conti et al., 2005; Ruiz et al., 2013); therefore people could be exposed at higher risk. Recreational exposure may lead to episodes of acute intoxication, especially in children (Vidal et al., 2017). From the questionnaires, it seems that cases of acute intoxication have not occurred (or may have not been identified) in this area; only cases of rashes and skin irritation were mentioned.

Both acute and chronic intoxication cause liver damage. Although the damage pattern of the liver is different in each case (Sedán et al., 2015), both types of intoxication are associated to elevated liver enzymes. Therefore, we secondly investigated serum enzyme levels. Previous papers have described that in patients affected by acute intoxication, serum enzymes rose 8-fold and total bilirubin 4-fold (Jochimsen et al., 1998; Pouria et al., 1998; Giannuzzi et al., 2011). In our study the magnitude of the enzyme increment was moderate, about 3-fold at most. This coincides with cases of human chronic and subchronic exposure reported in China, Brazil and Australia (Chen et al., 2009; Hilborn et al., 2013; Falconer, 2005). It should be mentioned that in these studies and in our work, it cannot be disregarded that factors other than MCs, for example alcohol consumption and self-medication, could also be responsible for the increment in the hepatic enzymes. Regarding the proportion of people with abnormal serum enzymes found in our work (25%), it was within those registered in other case studies of chronic exposure to cyanobacteria for example in Chen et al., (2009) and Li et al., (2011). We also observed that while the adult group presented alterations in all the enzymes, children had mainly abnormal levels of ALP (accompanied with elevated AST in one case). This is in agreement with the study performed by Li et al., (2011), who found significant differences in ALP and AST between an exposed and a non-exposed group of children. ALP is a less specific marker of liver injury than GGT, ALT and AST, so the fact that adults have more altered enzymes would support the idea that the longer the exposure, the more affected the liver would be, leading to more types of abnormal levels of hepatic enzymes.

The association between MCs and elevated hepatic enzymes is not easy to determine. In the cases studied in China (Chen et al., 2009; Li

et al., 2011) and Brazil (Hilborn et al., 2013) the toxin was found in blood; however, the association between levels of MC in serum and serum enzyme levels was different in China and Brazil. While positive association was found in China, in Brazil the association was not significant. In a more recent study, however, evidence of MCs in serum as an independent risk factor for Hepatocellular Carcinoma (HCC) was demonstrated by Zhen et al. 2017).

As the detection of MCs in blood samples is complex using instrumental methods (Chen et al., 2009) and not completely reliable using enzymatic methods (Heussner et al., 2014a), we thirdly studied the presence of specific antibodies against MCs as potential markers of cyanotoxicosis. We found that an important percentage of the population shows high levels of specific antibodies, especially the isotype IgE. This is in agreement with the fact that symptoms such as rashes, skin irritation and eye itching were observed in the residents, especially newcomers to the area, school teachers for example. These symptoms recede in time and become milder. This suggests that the body may adjust to a long term contact with cyanobacteria, where the antibodies may take on a protective role and simulate a natural immunization. We also observed that 100% of those with elevated enzymes had IgE antibodies. Unfortunately, we were unable to measure levels of hepatic enzymes and levels of specific antibodies simultaneously in all the volunteers, therefore we were limited at the time to explore the association between levels of hepatic enzymes and levels of antibodies. However, the importance of IgE isotype has been described in other pathologies such as Hydatidosis as indicator of the severity of the infection. In fact, a progressive decrease in the levels of antibodies is observed after the extirpation of the hepatic cyst (Manterola et al., 2007) and similar results were observed post treatment with Albendazole (Vildózola et al., 2012; Vildózola Gonzales et al., 2015). In allergic bronchopulmonary aspergillosis, the specific IgE is used as a marker to follow the pathology (Romero et al., 2009). On the other hand, in the autoimmune uveitis the IgE antibodies specific for S retin specific protein are detected in a high percentage of patients (Muiño et al., 1999). Furthermore, IgE Galectin-1 antibodies were significantly higher in uveitic patients with poor evolution; the opposite (a decrease of IgE levels) was observed in patients with a positive response to the treatment in which significant levels of these IgE antibodies were detected (Romero et al. 2006).

The use of chromatography and ELISA for toxin dosage in sera underestimates the degree of exposure as these methods detect only free MCs (non-conjugated) and lose reproducibility when toxin concentration is low (chronic exposure). In contrast, antibodies are directly associated with the exposure to the free toxin and their detection in sera may give a better idea of the level of exposure.

Health consequences of human chronic exposure to MCs has been difficult to demonstrate as the few available epidemiological studies are limited by their study design, poor measures of exposure, potential co-exposure to other contaminants, and the lack of control for confounding factors (Rastogi et al., 2014; Buratti et al., 2017). The main contribution of this work is that the presence of antibodies could be a useful exposure biomarker for epidemiological studies.

5. Conclusion

To our knowledge this is first study to investigate the presence of MCs specific antibodies (IgE and IgG) in sera of a population exposed to naturally occurring cyanobacteria. We propose the study of antibodies as a potential non-complex method to detect biological evidence of human exposure to MCs and to assist epidemiological studies. This will consequently help to promote measures to prevent the development of cyanobacteria blooms and thus increase human safety. It remains for future studies to confirm the method with a greater study group, to determine the role that antibodies play, to investigate the presence of other types of antibodies such as IgM and IgA and to explore the association between levels of hepatic enzymes and levels of antibodies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.03.071.

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