Herrera *et al.*
Sustainable Environment Research (2023) 33:44
https://doi.org/10.1186/s42834-023-00202-w

Sustainable Environment Research

RESEARCH

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Reduction of the toxin microcystin-LR with different types of sediments

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Abstract

Microcystis aeruginosa blooms in water bodies, evidencing a high risk of exposure to human health due to the release of toxins, which affects water quality. Implementing physical, chemical, and microbial control methods requires an integrated understanding of cyanotoxin dynamics, especially their relationship with sediments. Consequently, sediment obtained from three stations of the Riogrande II reservoir (Antioquia, Colombia) was analyzed to determine the adsorption and removal capacity of the microcystin-LR (MC-LR). For this purpose, the sediment was subjected to different treatments to select the one with the highest MC-LR removal capacity. Furthermore, the effectiveness and stability of adsorption removal process were evaluated by analyzing mechanical processes such as aeration, sonication, and agitation. The dried sediment showed the highest reduction in toxin concentration (93%) after 24 h, followed by washed sediment (91%) and sterilized sediment (81%).

On the other hand, the sediment was fractionated into silts and clays; the latter was the least effective. Finally, the fine and half silts were better adsorbents of the toxin, acting similarly over time. Initially, the utilization of sediment that has been dried by sunlight could be a complementary alternative to reinforce MC-LR control methodologies in water bodies.

On the one hand, MC-LR desorption assays showed that aeration of the sediment for 30 min caused a release of up to 96% of the adsorbed compound. At the same time, the effect of sonication and agitation was less intense. However, the absorption process must be fast to avoid efficiency losses due to desorption since a high percentage of the toxin was spontaneously desorbed from the sediment in two days.

Keywords *M. Aeruginosa*, Reduction, Sediments, Adsorption, Microcystin-LR

1 Introduction

Blooms in water bodies are produced mainly by algae and cyanobacteria cells, including *Microcystis aeruginosa*. This species is characterized by the production of microcystin-type toxins, of which microcystin-LR (MC-LR) is the most toxic. This substance causes mortality in fish and other animals and accumulates throughout the food chain. In addition, direct effects in humans have also been reported, such as allergic reactions and liver and kidney damage [1]. MC-LR has also been detected in various environments, including water, sediments, and plants [2, 3].

The high chemical stability and low biotransformation of these toxins in aquatic environments are additional reasons to consider them a public health threat since microcystins toxins are resistant to ozone and UV radiation, among others [4]. Likewise, normal water treatment processes, including coagulation, sedimentation, and disinfection, have demonstrated a limited effect on removing microcystins during periods of intense blooming [5].

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In various cases, expensive methods with limited effectiveness are often used, creating the need for less costly approaches such as the one presented in this paper [6]. Thus, the World Health Organization established the lowest reference value for MC-LR in drinking water at 1.0 μg L^{-1} [7]. Finally, blooming generates economic losses for aquaculture and tourism sector.

Instead, sediments are considered the foremost destination of microcystins in the environment; they act as significant receptors for environmental pollutants due to their high adsorption capacity by organic matter [8]. In this way, they play a fundamental role in the fate of the cyanobacterial toxins in drinking water [9]. In recent years, it has been proposed that sediments control cyanotoxins in water bodies through adsorption processes [10–12], which can occur through reversible physical phenomena and irreversible chemical processes due to hydrophobic or electrostatic interactions, cation exchange, and the formation of hydrogen bonds, depending on the type of toxins and the characteristics of the sediment [13].

This research was carried out to study the reduction of the toxin MC-LR with different sediments and generate information to evaluate the possibility of using them as detoxification filters in water treatment. However, it is still necessary to know deeply about the physicochemical processes involved and the stability of the adsorption. Previously, we found high adsorption of MC-LR in the sediments in a static way [10]. Now we aim to evaluate the adsorption capacity of MC-LR in three sediments of the Riogrande II reservoir (Antioquia, Colombia). Therefore, now sediments were submitted to several treatments like washing, drying, sterilization, and fractionation to determine their adsorption capacity under several physical and chemical transformations. In addition, the stability of this adsorption process was established in conventional detoxification methods such as aeration, agitation, and sonication.

2 Materials and methods

2.1 Sampling site

The Riogrande II reservoir (Antioquia, Colombia) is between 75°32'30" − 75°26'10" W and 6°33'50" − 6°28'07" N. It became operational in 1991, has a total volume (2,270 m above sea level) of 240 Mm^3, and covers an average area of 1,214 ha. The multiple uses of Riogrande II include hydroelectric generation and the water supply of a purification plant. In addition, farmers carry out intensive agricultural and livestock activities near the reservoir's area of influence.

The geographical coordinates in the current version of World Geodetic System 84 of the three points selected for sediment and water collection are shown in Fig. 1: S2, latitude (N) 6°32'26.912" and longitude (W) 75°30'23.648", at the end of the arm of the Las Animas ravine. S5, latitude (N) 6°30'4.997" and longitude (W) 75°27'41.9", catchment tower; S7, latitude (N) 6°31'42.523" and longitude (W) 75°27'47.303", the entrance of the Rio Grande to the reservoir.

Samples of *M. aeruginosa* blooms were taken in the Porce II reservoir, located in the jurisdiction of the municipalities of Gomez Plata, Yolombo, and Amalfi, between coordinates 75°09'14" − 75°04'59" W and 6°44'57" − 6°48'45" N. This reservoir became operational in 2001 and had a functional capacity of 96.2 Mm^3, with a total volume of 231.2 Mm^3. The maximum level of operation corresponds to 942.5 m above sea level. The reservoir is intended for electricity generation and fishing activity, which are economically important for the region. Therefore, we selected the point near the dam (6°48'18.88" N and 75°8'49.93" W) to collect bloom samples to determine the percentage of sediment adsorption.

The samples were taken at the water–sediment interface at the three selected stations using a Van Dorn-type bottle, stored in 10 L plastic bottles, and refrigerated until lab use.

2.2 Collection and analysis of sediment and water samples

The sediment samples were collected at the Riogrande II reservoir between June 2017 and March 2018; these were taken at an average depth of 15 m with an Eckman dredge. After collection, we transported the samples in 10 L plastic bottles while keeping them cool. Then, we conducted sedimentological analysis on the samples, which involved examining granulometry, the Biological Oxygen Demand (BOD), total phosphorus, total organic and material carbon, moisture, and organic matter losses on ignition (LOI) in muffle furnace (Terrigeno D8) and in a precision cultivation chamber (Memmert). In addition, Fe, Mn, and Cr of sediment content was also established [15].

2.3 Collection and analysis of phytoplankton samples

The bloom samples were collected directly from the surface in the Porce II reservoir and packaged in plastic bottles of 5 L. Using 10 mL of the sample, the cyanobacteria present under an optical microscope (Nikon YS2-H (Japan)) were determined, and using 20 mL of the sample, the MC HPLC/MS identified MC-LR. Taxonomic identification at the intragenic level was based on the specific literature for this group of cyanobacteria [16].

2.4 Detection and extraction of MC-LR by HPLC-MS and HPLC-DAD

The MC-LR standard was obtained from MP Biomedicals, LLC (Illkirch, France). From the sample collected

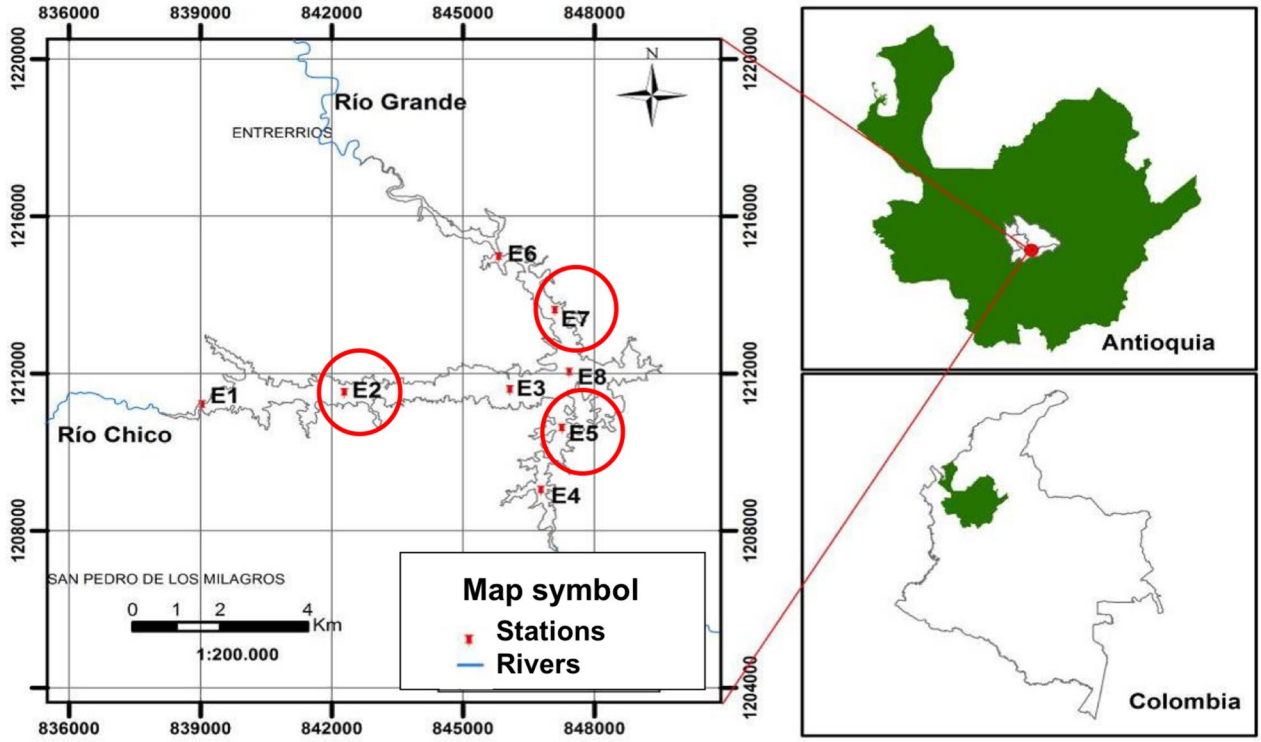

Fig. 1 Location of the three points selected for sediment collection in the Riogrande II reservoir [14]

during flowering, 15 L was freeze-dried using Labconco equipment (Freezone 12). The solid was sonicated using Branson equipment (2510 ultrasonic cleaner, USA) in a mixture of aqueous methanol-water (80:20, v/v) and centrifuged using Hermle equipment (Gosheim, Baden-Wurttemberg, Germany) at 3000 rpm for 5 min. The supernatant was dried in a rotator (Heidolph, Germany) at 45 °C and purified with C-18 cartridges (CNW Technologies CNWBOND, China), using solvents such as methanol, water, and aqueous methanol (methanol water 80:20, v/v). The sample was finally dried in a rotavator at the same temperature as before; 1 mL was used for detecting and quantifying the MC-LR toxin by HPLC/MS and HPLC/DAD according to the methodology previously described [14]. The collected sample, 350 mL, was taken for the MC-LR reduction tests.

2.5 Analysis of MC-LR reduction by adsorption in sediments

2.5.1 By simulating environmental conditions

For this analysis, 3 kg of sediment from stations S2, S5, and S7 of the Riogrande II reservoir were used. Each sediment sample was deposited in a plastic container of 50×50 cm; then, 5 L of the reservoir water was added, collected at the same points, and mixed with the extracted and quantified toxin, which had previously been by HPLC. The three sediments without the toxin mixture were treated under the same conditions for the control test, including mixing with a reservoir. All vessels were in agitation in a Shaker Actum HD-400 (Medellin, Colombia) at 90 rpm for 96 h under dark conditions and at 22 °C. Samples for MC-LR reduction were measured hourly for the first 4 h and then every 24 h until 72 h (Fig. 2a). According to the proposed methodology, 15 mL were taken to analyze MC-LR by HPLC (Chromaster, 5430 DAD, 5160 pump, Hitachi). The following formula was used to determine the percentage reduction of MC-LR: (Final MC-LR Concentration - Initial Concentration)/Final MC-LR Concentration)*100.

2.5.2 Sediment submitted to different treatments

This analysis was performed with sediments from S7. Each 50 g sample underwent a different treatment, yielding six treated sediments. The sterile sediment underwent autoclave sterilization for 20 min at 20 kPa while the sediment was sun-drying for 10 h or washed twice with 30% sodium hypochlorite and subsequently rinsed with sterile water five times with portions of 100 mL each. The sediment was also fractionated by different granulometric sieves, obtaining half silts (F1), fine silts (F2), and clays (F3). Sediment was used for the control without any treatment (Fig. 2b). Finally, the analysis of

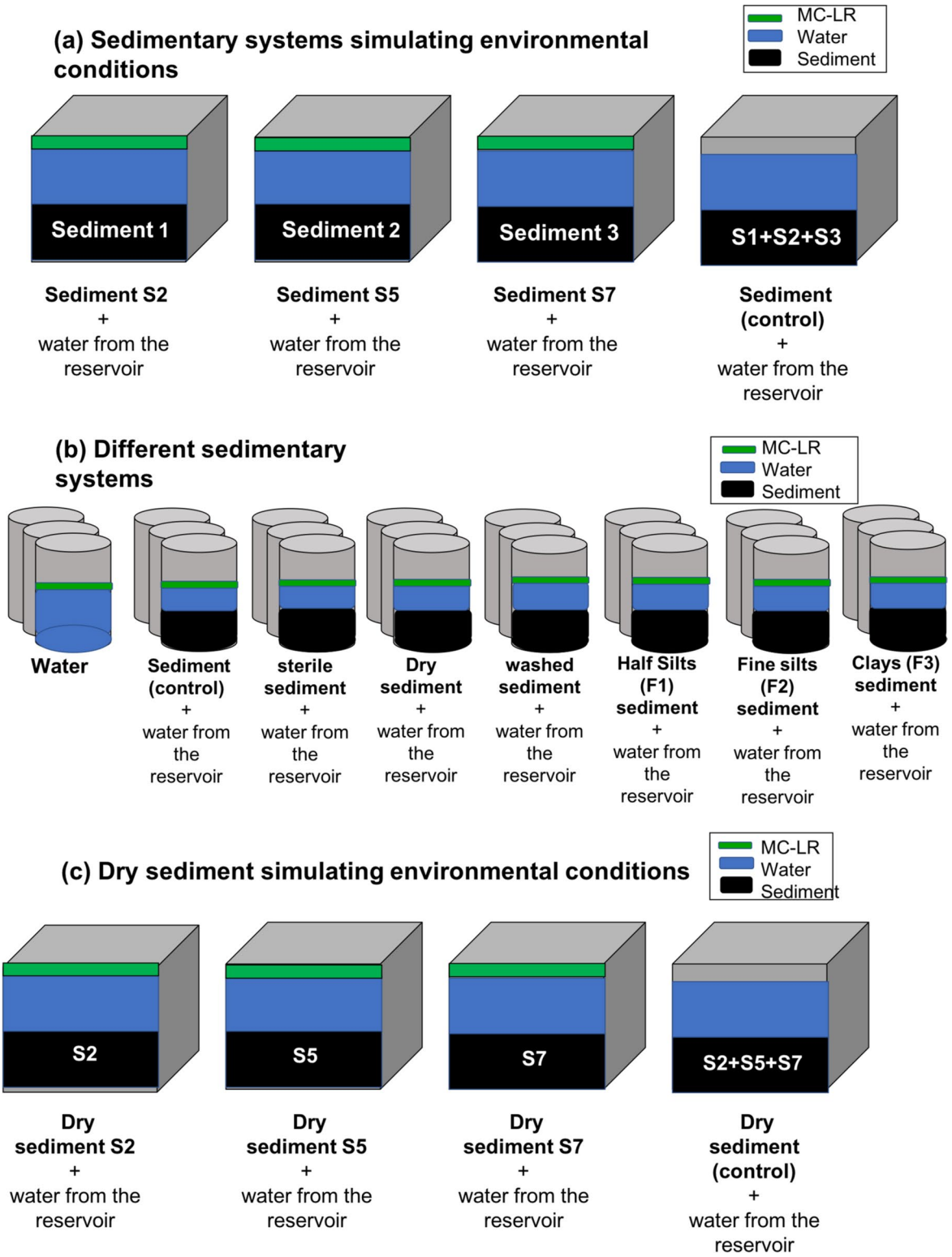

Fig. 2 General procedures for evaluating MC-LR reduction using different sedimentary systems. All assays were performed in triplicate

the MC-LR toxin was carried out with 20 g of sediment. For this, 5 mL of water samples were taken and analyzed by HPLC-DAD, according to the methodology described [14]. All trials were performed in triplicate.

2.5.3 MC-LR reduction analysis with the best sediment simulating environmental conditions

Subsequently, a dried sediment was chosen for the next test, with the simulation of certain environmental conditions. So, 3 kg of sediment was taken from the three stations of the Riogrande II reservoir (S2, S5, and S7), previously dried in the sun under the same conditions as in 2.5.1 (Fig. 2c). Then, after mixing water with the toxin, the sediment was added, and MC-LR was analyzed by HPLC-DAD. From these sediments, 15 mL of the sample were taken every hour, for 5 h and at 24 h.

2.5.4 Desorption analysis

For the desorption analysis, four tests were carried out with the sediment of S7. Initially, 50 g of sediment and a mixture of water with the toxin (MC-LR) were added. Then, after maintaining the adsorption process for 72 h, different treatments were carried out. In the first treatment, the mixture of sediment and water was sonicated, with a frequency between 50 and 60 Hz for 15 min in a Branson Sonicator 2510 (Branson Ultrasonics, Danbury, CT, USA). In the second treatment, sonication was maintained for 30 min, and in the third assay, it was kept under constant agitation for 24 h. Finally, the fourth treatment subjected the sediment to constant aeration using a pump AC 9904 (Xing Risheng Industrial Co, Shenzhen, China). As a control, sediment with added toxin was used without any treatment. Samples of free MC-LR were taken at the beginning of the experiment (time 0), at 72 h (time 1), and after performing the different processes for desorption (time 2). Subsequently, 1 mL of the sample was taken for the MC-LR analysis by HPLC/DAD.

2.6 Statistical analysis

ANOVA, followed by Tukey's test, evaluated the difference between treatments. The Shapiro-Wilk test and Levene's test were used to assess the normality and homoscedasticity of the data, respectively. If the data did not show normality, a logarithmic transformation of the values was applied. All tests were analyzed under a significance level of 0.05 and a confidence level of 95%. Statistical calculations were performed using the R Core Team Package 2018.

3 Results and discussion

3.1 Analysis of sediment samples

The reservoir's sediment was characterized by coarse granulometry, which varies between half silts, fine silts, and clays. The sediments were classified as inorganic, and their properties and composition can be seen in Table 1. The material from S7 had very different characteristics than the other two. Thus, BOD values of sediments were 1.3 g at station 2 (S2), 1.6 g at S5, and 5.1 g at S7. Thus, S7 exhibits a very high BOD, large amounts of phosphorus (0,40 mg P kg^{-1}, and a high LOI, 12%, compared to 7.2% and 4.6% of the other two sediments. However, S7 has the lowest N, organic carbon, and organic material (Table 2).

Accordingly, iron was slightly higher in S2 and S5, while sediment S7 showed the highest manganese concentration. The excess of an element concerning the natural abundance of iron (Fe), manganese (Mn), and chromium (Cr), allow sediments to be classified as contaminated, especially in S7.

3.2 Analysis of cyanobacteria samples and detection of MC-LR by HPLC/MS

Samples analyzed under the optical microscope revealed the presence of *M. aeruginosa* as the dominant species. This cyanobacterium was previously found as the main microorganism causing blooms in the Porce II reservoir [14]. In addition, HPLC/MS analyses of the bloom sample detected the presence of a majority peak with a molecular weight of 995 m z^{-1}, which corresponds to MC-LR as the main cyanotoxin [14]. However, the presence of other hepatotoxins has not been ruled out.

3.3 Analysis of MC-LR reduction by adsorption in sediments

3.3.1 Simulating environmental conditions

This assay was performed to select the sediment with the highest adsorption properties. Initially, when

Table 1 Parameters evaluated in the sediments of the three stations of the Riogrande II reservoir

Reservoir Station	Total phosphorus (mg P kg^{-1})	Total Organic Carbon (mg C kg^{-1})	Organic Material (mg MO kg^{-1})	Total Nitrogen (mg N kg^{-1})	Moisture (%)	LOI (%)	BOD (g)
º1S2	6.6	42.7	73.7	5.6	77.0	7.2	1.2
S5	1.0	44.4	76.5	4.2	80.2	4.6	1.6
S7	0.4	33.0	56.9	2.8	69.5	11.9	5.1

Table 2 Concentration of iron (Fe), manganese (Mn) and chromium (Cr) in reservoir sediments

Reservoir Station	Fe (mg cm^{-2} s^{-1})	Mn (mg cm^{-2} s^{-1})	Cr (mg cm^{-2} s^{-1})
S2	-3.62 E-06	7.15 E-09	-4.29 E-09
S5	-7.45 E-06	6.23 E-08	-2.50 E-09
S7	-8.22 E-06	-5.65 E-08	-4.29 E-09

comparing adsorption samples of MC-LR in the three stations of the reservoir S2, S5, and S7, it was found that their behavior was similar since there were no statistically significant differences ($P > 0.05$) in the concentrations of MC-LR (Fig. 3). However, from the second hour of exposure to the sediment, a decrease in the concentration of MC-LR was observed. After 24 h, MC-LR was no longer detected in the sediment of station 7 (S7), indicating the complete elimination of MC-LR, while for S2 and S5 concentrations, it was 0.22 µg L^{-1}, with reduction percentages of 96% and 91%, respectively, starting from an initial MC-LR concentration of 4.99 µg L^{-1}. Then, the sediment of S7 was used for the following assays because of its high capacity to adsorb the MC-LR toxin.

3.3.2 Exposing the sediment to different treatments

The adsorption of MC-LR in the six sediments subjected to different processes (drying, sterilizing, fractionating, and washing) showed statistically significant differences in concentration. According to Tukey's test, it was observed that there were significant differences with a 95% confidence level between the samples exposed to dry sediment, washing, half silts, fine silts, and those sterilized with the control sediment, clays, and the sample without sediment (Fig. 4).

The sterilized sediment samples showed statistically significant differences from the control, with a level of 95% confidence; the latter contained all the microflora involved in the biodegradation course and it was not subjected to a sterilization process. During the first three hours, reductions in the concentration of MC-LR of 53% were observed in the sterile sediment; meanwhile, in the unsterilized sediment, the value was only 18%. It has already been considered that biodegradation is usually one factor affecting this process [17]. Our results agree with what was previously found by Song et al. [18] regarding the ability of sediment to degrade the MC-LR. Previously, it has been proposed that the sediment absorption pathway is faster than biodegradation through the bacterial community. This complementary

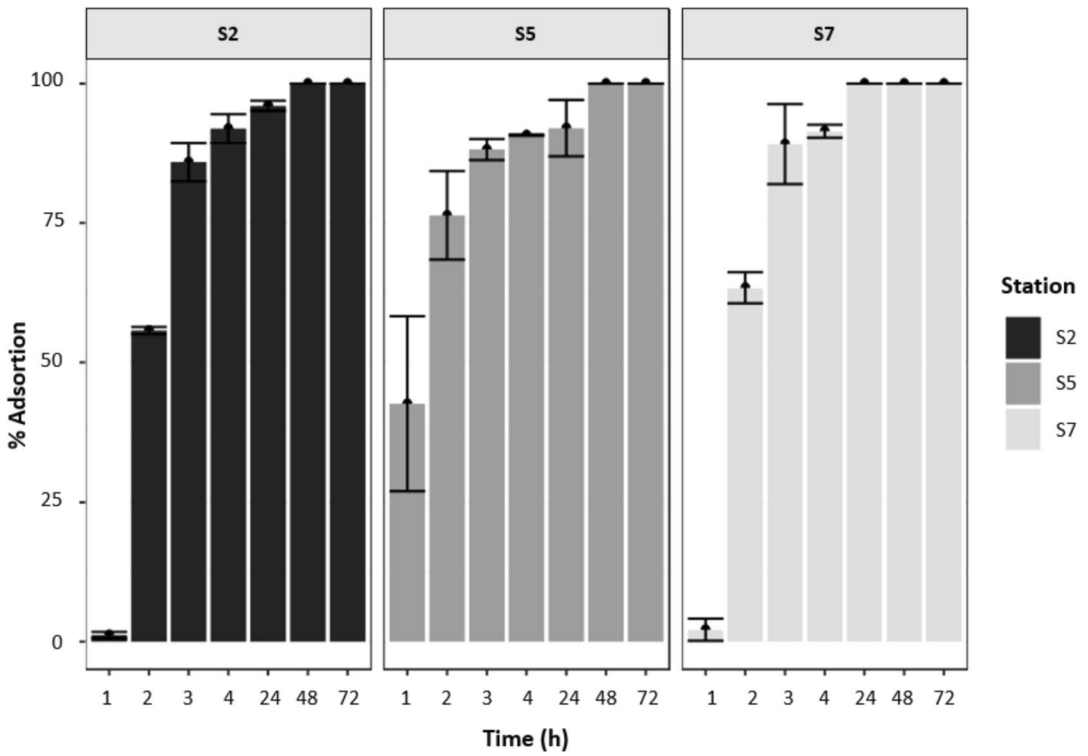

Fig. 3 Adsorption of MC-LR with sediments taken from the three points of reservoirs S2, S5, and S7

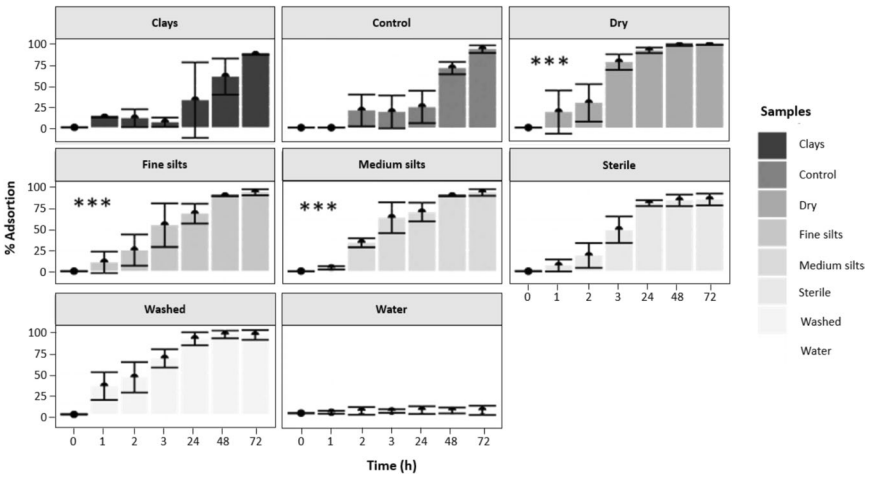

Fig. 4 Adsorption of MC-LR in different sediments subjected to various treatments. Whiskers are standard deviations. Different treatments are statistically indicated *** $p < 0.001$

biodegradation occurs due to aerobic and anaerobic microorganisms that can proliferate in this matrix and metabolize microcystins, which co-occurs with the degradation of organic matter in sediments [19]. Similarly, the hypochlorite-washed sediment had even higher percentages of toxin reduction than the sterile sediment (81 – 90%) because the oxidation process possibly cleans and activates more adsorption sites.

Otherwise, dry sediment caused a high reduction in the MC-LR toxin at 48 and 72 h, its presence in the sample was not detected. In contrast, for the sample of sediment sterilized during that same time, the toxin was detected at concentrations 0.75–0.69 μg L^{-1}, equivalent to a reduction of 86 and 87%, respectively.

After the sediment was fractionated into silt and clays, we found that the latter reduced the MC-LR toxin by 26% within 24 h, indicating that this type of sediment was the least effective, although removal efficiency may increase over time. Moreover, the fine and half silts were better adsorbents of the toxin, acting similarly over time with percentages of reduction of MC-LR 67–69% (Fig. 4). However, these results did not match those found in samples of Canada with sediment fractionated in fine silts, half silts, and clays [20]. It could be explained because these chemical and physical characteristics differences were inherent in sediments from different geographical areas, such as Colombia and Canada.

All these results indicate that fractionated sediments have an adsorption capacity higher than the entire sediment. The sediment texture, the surface area of its particles, pore size distribution, pH, dissolved organic content, and silt and clay content are important factors affecting the degree of adsorption capacity of MC-LR in sediment samples. In addition, sediments high in silt and clay have a greater adsorption capacity [21].

Also, the analysis of the MC-LR concentration in the control sample (only water toxin) indicated high stability over time, there was no degradation of toxin concentration 5.6 μg mL^{-1} during the 72 h of evaluation. Microcystins are usually stable in water under natural light conditions, mainly due to their cyclic structure [22].

3.3.3 MC-LR reduction with the best sediment, simulating environmental conditions

After establishing that the best material for reducing MC-LR was dry sediment, a new test was carried out, using sediments (3 kg) taken from the three points of the Riogrande II reservoir and simulating environmental conditions (Fig. 5). The sediment from S2 showed high adsorption after 2 h. Though, after a sudden decrease, several continuous increases were noticed. In this case, adsorption is a weak physicochemical process, and immediately after mixing the sediment with the toxin dissolved in water, it is quickly released. However, it is also possible that the toxin was not adsorbed in the entire sediment, and the remaining is released, after desorption. On the other hand, the sediment of S7 caused the highest toxin reduction in the fifth hour and up to 94%. Nonetheless, when the results were analyzed, no statistical differences were found in the adsorption capacity of the sediments among the stations at 24 h. Starting from the second hour for sediment S2, a sudden desorption process occurs, possibly caused by saturation or hydration or wetting processes in the dry sediment. Then, slow physical expansion induces the release of the previously adsorbed toxin. Though sediments can also be a risk issue since cyanobacteria have a natural habitat there, the adsorption of high concentrations of toxins is a dangerous reserve that can return to water bodies due to different physical factors [23], as discussed below.

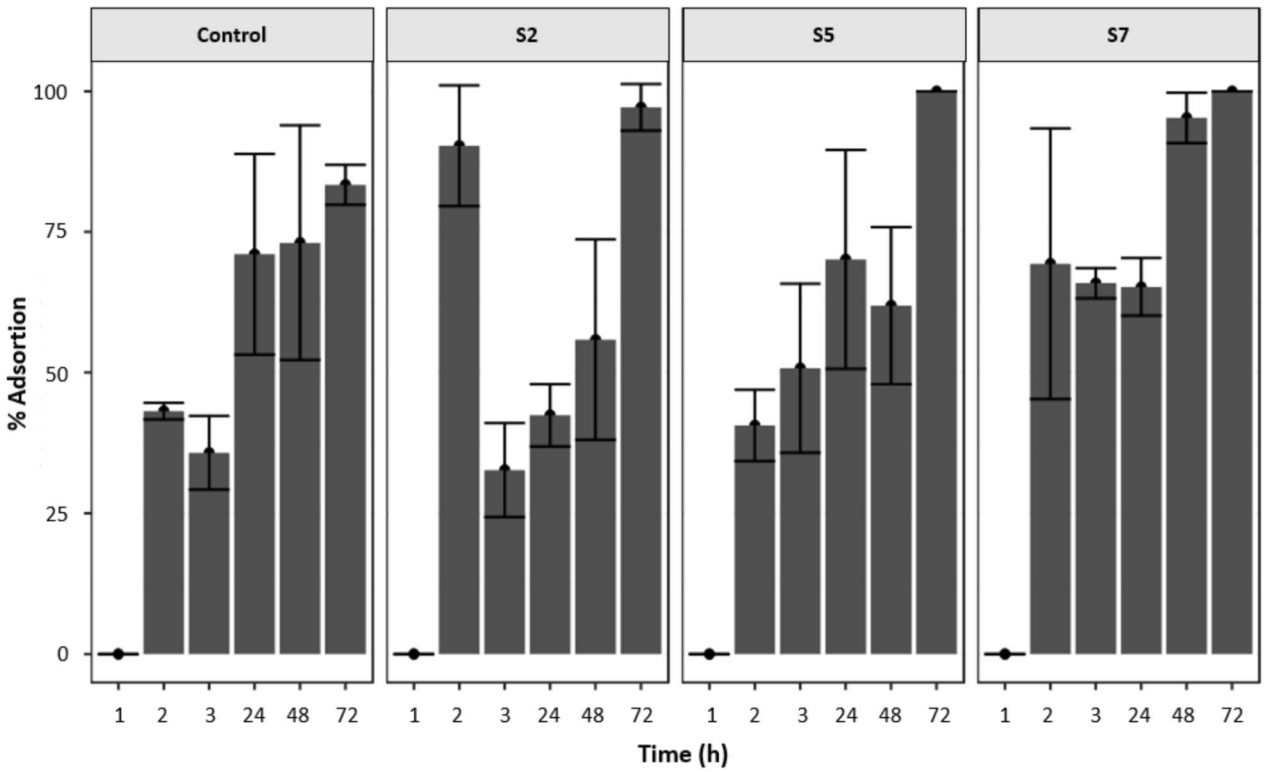

Fig. 5 Adsorption of the MC-LR toxin in dry sediment from the selected stations S2, S5, and S7

3.3.4 Analysis of the desorption process

Several assays were carried out with four treatments, aeration, agitation, and sonication, for 15 and 30 min to determine the stability of the adsorption process. These processes are like those used to remove toxins in some water purification plants. However, in toxins adsorbed in sediments, it is likely that such processes are affected by these treatments. After 72 h, no significant desorption occurred in the control experiment. However, the aeration treatment displayed a high ability to release the toxin, allowing the recovery of MC-LR at 96% (Fig. 6). Sonication for 15 and 30 min caused the desorption of the toxin at 68 and 24%, respectively, indicating a possible degradation of the toxin when was exposed to high frequencies for a long while. Finally, agitation did at 45%, like the control (49%).

The different treatments applied to the sediment for desorption of MC-LR allow for investigation of the natural processes of these toxins in the water. The ability of *M. aeruginosa to* migrate vertically in aquatic environments allows it to sequester biologically available nutrients, even at low concentrations [24]. This strategy is particularly effective in shallow, turbid, and eutrophic water systems. In addition, *M. aeruginosa* can quickly migrate between phosphorus-rich bottom sediments and take advantage of periodic sediment resuspension due to wind-caused agitation, [25]. This movement favors the desorption of the toxin.

Previous studies of toxin desorption have been carried out under in situ conditions without modifying the sediments. For example, Maghsoudi et al. [20] found that sediments quickly and immediately adsorbed microcystins. Thus, cylindrospermopsins MC-LW and MC-LF were adsorbed at 72, 56, and 55% at 2 h: Though, it was also shown that less than 9% of cyanotoxins are desorbed from the sediment within 96 h, indicating low desorption potential.

So far, few studies have analyzed different means of desorption of the toxin; however, some of them have found slight effectiveness with agitation and aeration. These studies also analyzed desorption and adsorption at the interface water sediment, under the influence of different factors such as temperature, pH, and aeration, reproducing natural conditions, but did not detect desorption of MC [26] even with times like those evaluated in this work.

All this seems to indicate that the high persistence of MC-LR in aquatic environments may be related to its high level of adsorption-desorption in sediments, thus maintaining relatively high levels of toxins, which escape microbial degradation, Due to its strong chemical stability, absence of specific degrading enzymes or possibly,

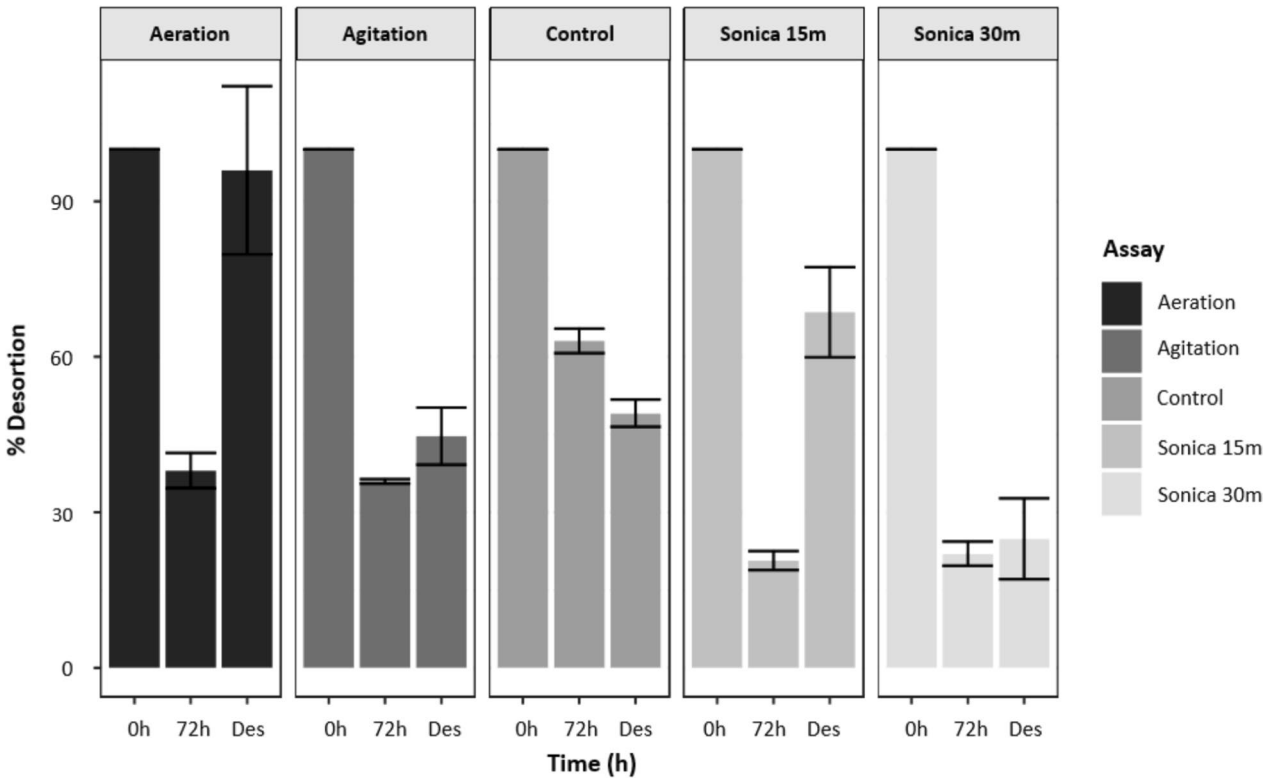

Fig. 6 Effect of several treatments on the desorption of MC-LR, from time 0 (free MC-LR, 100%) to 72 h. Des corresponds to the desorption (is a phenomenon by which a substance is released from or through a surface), equivalent to the final measurement taken after the process used. Whiskers are standard deviations. Different treatments are statistically indicated * $p < 0.001$

the presence of enzymatic inhibitors in water and other physicochemical influences such as pH and sunlight.

4 Conclusions

The result of this study suggests that the sediment at the bottom of the Riogrande II reservoir significantly contributes to the removal of MC-LR fast and effectively during the first 24 h. However, this adsorption is not an irreversible interaction since, after 48 h, a high percentage of the toxin was spontaneously desorbed from the sediment. So, it is possible to use the sediment only in cases of blooms, as an emergency measure to trap released toxins. Additionally, the applicability of using sediments in drinking water treatment is suggested, in which the sediment filter should be periodically removed once it becomes saturated. Moreover, some results of this research were different from several previous reports. For example, adsorption under normal conditions is an effective method for MC-LR uptake. Nonetheless, although it is a relatively fast process, it is also reversible since after 12 h, spontaneous desorption of more than 50% has occurred.

Surprisingly, some mechanical processes used in detoxifying water for human consumption, such as aeration, agitation, and sonication, need to be improved since they promote the desorption of the toxin from the sediments and its direct incorporation into the water. In addition, clays showed lower removal effectiveness than fine and half silts, but the latter materials were like dry sediment. The above indicates that Rio Grande II reservoir sediments are unsuitable for removing MC-LR toxin due to fast desorption. Definitive conclusions about the usefulness of sediments in the elimination of toxins it could be drawn after more intensive studies, which include a large variety of sediments with different silt and clay content, physicochemical properties, and a using a wide range of toxins and environmental conditions.

Acknowledgements
The authors thank Empresas Publicas de Medellin (EPM) for their collaboration in the sampling and the GAIA group of the University of Antioquia for their technical assistance with some processes.

Authors' contributions
NH and FE conceived the idea, planned the work, and wrote the manuscript; NH and MTF experimental work. The authors read and approved the final manuscript.

Funding
Funding was received from Minciencias-Colombia (Grant 844, Project 831–2019).

Availability of data and materials
All data generated during this work are available upon request.

Declarations

Competing interests
The authors declare that they have no competing interest.

Received: 14 September 2022 Accepted: 2 November 2023
Published online: 18 December 2023

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