Study of cyanotoxin degradation and evaluation of their transformation products in surface waters by LC-QTOF MS

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ABSTRACT

In the present work, the degradation of three cyanotoxins from the hepatotoxins group was investigated under laboratory-controlled experiments in water samples. Surface waters spiked with microcystin-LR (MC-LR), nodularin (NOD) and cylindrospermopsin (CYN) were subjected to hydrolysis, chlorination and photo-degradation, under both sunlight (SL) and ultraviolet (UV) radiation. A total of 12 transformation products (TPs) were detected and tentatively identified by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF MS). These comprised: 6 chlorination TPs (3 from CYN and 3 from MC-LR, 2 isomers); 4 UV TPs (all from CYN); and 2 sunlight TPs (one isomer from MC-LR and another from NOD). No TPs were observed under hydrolysis conditions. The chemical structures for all TPs were tentatively proposed based on the accurate-mass QTOF MS full-spectra. Analysis of real-world samples collected from the Peñol reservoir (Antioquia, Colombia) revealed the presence of MC-LR and CYN as well as a sunlight TP identified in the laboratory experiments. Data presented in this article will assist further research on TPs potentially formed in future tertiary degradation processes applied for the removal of organic micro-pollutants in water; as well as improving available knowledge on the toxic implications of cyanobacterial toxins TPs in surface waters.

Keywords

cyanotoxin degradation; photo-elimination; quadrupole time-of-flight mass spectrometry; transformation products; waters.

1. Introduction

Cyanobacteria have become the focus of extensive research due to increased blooms and the consequent presence of toxic compounds known as cyanotoxins (Chorus and Bartram, 2000; Codd et al., 2017; Corbel et al., 2014). Cyanotoxins are categorized into five groups depending on their toxicity and specific hazards: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (Carmichael, 1992; Jonas et al., 2015). The hepatotoxins group includes microcystins, cylindrospermopsins and nodularins. Cylindrospermopsin (CYN) is an alkaloid, while microcystins (MCs) and nodularins (NODs) are cyclic peptides that both contain the unusual amino acid Adda, ((2S,3S,8S,9S)- 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl deca-4,6-dienoic acid), which is responsible for their hepatotoxic activity (Meriluoto and Codd, 2005). These toxins have been detected in various aquatic environments for more than two decades (Zhang et al., 2015), and cases of gastroenteritis poisoning in humans and animals due to consumption or contact with water contaminated with hepatotoxins have been reported worldwide. Severe exposure to hepatotoxins has also been responsible for chronic liver diseases (Merel et al., 2010b; Z. Zhang et al., 2016). Of the hepatotoxins mentioned, the World Health Organization (WHO) has suggested a provisional limit in drinking water only to microcystin-LR (MC-LR), of 1 μg L^{-1} (WHO, 1998).

Some studies have reported elimination treatments for cyanotoxins in water (Pantelić et al., 2013; Westrick et al., 2010). For example, chlorination has been studied in several works (Merel et al., 2010a; Z. Zhang et al., 2016). From the perspective of drinking water safety, chlorination appears to be a valid degradation method for cyanotoxins, although the potential formation of microcystin-LR disinfection by-products merits further attention (Liao et al., 2015; Zamyadi et al., 2013), while partially chlorinated samples have been shown to retain a certain degree of toxicity (Zong et al., 2015). Other oxidants,

such as potassium permanganate and ozone, have also been studied with satisfactory results. However, factors such as water pH, contact time and oxidant concentration can present difficulties in the treatment plant (Fan et al., 2013; Sharma et al., 2012). Similarly, satisfactory elimination has been achieved using advanced oxidation processes (AOPs), such as photocatalytic UV/TiO_2 degradation on MC-LR, photocatalytic degradation on CYN, and UV/H_2O_2 on MC-LR. However, the use of AOPs commonly involves high treatment costs, this being a drawback to their application in conventional treatment plants (Chen et al., 2015; Fotiou et al., 2015; He et al., 2012; Jacobs et al., 2013; Pestana et al., 2015).

The application of UV radiation as a possible tertiary treatment for wastewater and drinking water is subject to some controversy. Some limitations of direct photolysis have been reported, because microcystins are resistant to UV treatments (He et al., 2015). Pinho et al. 2015, demonstrated that UV alone was unsatisfactory for MC-LR degradation (Pinho et al., 2015). However, Jacobs et al. showed that the substrate was quickly degraded by photolysis with UV-C radiation (Jacobs et al., 2013).

In most studies, degradation have been focused only on the elimination of the parent toxins. However, the potential formation of degradation/transformation products (TPs) also requires to be further evaluated. TPs may also be present in surface waters. This can be generated under natural conditions such as hydrolysis, solar radiation, biodegradation or after water treatments such as chlorination (Li et al., 2017; Y. Zhang et al., 2016). Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the preferred technique for quantification of several analytes. UHPLC tandem MS spectrometry is a powerful analytical tool in a wide variety of matrices due to its high sensitivity and selectivity in very low concentrations of cyanotoxins. It is particularly useful for the analysis of very specific congeners of cyanotoxins, of which the molecular

95 weight and fragmentation pattern are known (Beltrán et al., 2012; Codd et al., 2017;
96 Fotiou et al., 2015; Jacobs et al., 2013; Zervou et al., 2017). Additionally, high resolution
97 mass spectrometry (HRMS) instruments such as Orbitrap (Boix et al., 2016a; Merel et
98 al., 2009) or time-of-flight (QTOF), allow the detection and identification of
99 transformation products from different organic pollutants including cyanotoxins (Boix et
100 al., 2016b; Hernández et al., 2015a; Hernández et al., 2015b; Ibáñez et al., 2017; Sanz et
101 al., 2015). Hybrid analyzers, such as QTOF MS, allow data acquisition under different
102 collision-induced dissociation conditions within the same single injection, i.e. working
103 under MS^E mode. MS^E acquisitions are very useful for the investigation of TPs due to the
104 relevant information obtained on the fragmentation of compounds without precursor ion
105 selection. MS^E mode involves the sequential acquisition of accurate mass data at low and
106 high collision energy within one single injection. At low energy (LE), fragmentation is
107 minimized and the most abundant ion normally corresponds to the parent molecule
108 (adducts in some cases). However, at high collision energy (HE), fragmentation of the
109 molecule is favored. Therefore, both the (de)protonated molecule and fragment ion data
110 provide useful information on accurate masses, which is essential for elucidation of the
111 TPs potentially formed from cyanotoxins (Ibáñez et al., 2017).

112 Several TPs of MCs have been detected by various authors in UV and H_2O_2 experiments.
113 Some of these proposed a rupture in the double bond of the Adda group of *m/z* 795 and
114 835 (He et al., 2015); 759, 783 and 811 (Liu et al., 2003); 303 and 570 (Andersen et al.,
115 2014); and 825 (Jacobs et al., 2013). Others proposed demethylation of the Adda group
116 with *m/z* 965 by (He et al., 2015). Other research detected mono and polyhydroxylations
117 in the aromatic ring, or in the conjugated double bond of the Adda group with *m/z* 1011,
118 1013, 1027 and 1045 (He et al., 2015); 1029 (Pinho et al., 2015); or others. Other TPs
119 have been detected via chlorination processes, for example carboxylation of the same

120 methoxy with *m/z* 1025 and addition of 1 to 4 chlorine atoms to the aromatic ring of the
121 Adda group with *m/z* 1029, 1064, 1098 and 1133, respectively (Y. Zhang et al., 2016).
122 Hydroxylation and chlorination have also been proposed for *m/z* 1047, 1063, 1097, 1115,
123 993 and 1011 by (Merel et al., 2009); and 795, 1029, 1047, 1081 and 1099 by (Zong et
124 al., 2013).

125 TPs of CYN detected via UV/H_2O_2 or UV/TiO_2 processes have involved losses of the
126 sulphate group with *m/z* 334, 336, 350, 382, 384 and 398 (He et al., 2014); opening of the
127 uracil group or addition of the hydroxyl group with *m/z* 316, 375, 448, 446, 464, 432 and
128 434 (Fotiou et al., 2015); opening or oxidation in the tricyclic guanidine alkaloid with *m/z*
129 200, 210, 214, 226 and 242 (He et al., 2014); dihydroxylation with *m/z* 450 (Chen et al.,
130 2015). Meanwhile, via a chlorination process, CYN has generated TP of *m/z* 274, 375
131 and 450 due to the loss of the uracil group and the addition of a chlorine atom (Merel et
132 al., 2010a).

133 The aim of this work was to evaluate the degradation of three relevant cyanotoxins from
134 the hepatotoxins group in water subjected to hydrolysis, UV radiation, sunlight simulation
135 and chlorination. The experiments were performed under laboratory-controlled
136 conditions, and the detection and tentative identification of transformation products
137 formed during the processes applied were carried out by LC-QTOF-MS. Finally, the
138 presence of these cyanotoxins (MC-LR, CYN and NOD) and the elucidated TPs were
139 searched in surface water collected from the Peñol reservoir in Colombia.

140

2. **Material and methods**

2.1. Reagents and chemicals

Microcystin LR (>95%) isolated from *Microcystis aeruginosa* and cylindrospermopsin (>95%) isolated from *Cylindrospermopsis* sp. analytical standards were purchased from Cyano Biotech GmbH (Berlin, Germany). Nodularin (99%) analytical standard isolated from *Cylindrospermopsis* sp., was obtained from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany). The three toxins employed for elimination experiments were bioreagent grade (bioreagent grade, >90%) and were obtained from CyanoBiotech GmbH (Berlin, Germany).

2.2. Liquid Chromatography

Analyses were carried out using an Acquity Waters ultra-performance liquid chromatography (UHPLC) system (Waters, Milford, MA. USA) equipped with a binary solvent manager and sample manager. The chromatographic separation was performed using an Acquity UPLC BEH C18 1.7 μm particle size analytical column of 100×2.1 mm (Waters) at a flow rate of 300 μL min^{-1}. The mobile phase used were A = H_2O with 0.01% HCOOH and B = MeOH with 0.01% HCOOH in gradient mode. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 10%; 14 min, 90%; 14.1 min, 10%; 8 min, 10%. The injection volume was 20 μL.

2.3. Mass Spectrometry

2.3.1. QTOF

A hybrid quadrupole-orthogonal acceleration - QTOF mass spectrometer (Q-TOF XEVO G2), Waters Micromass (Manchester, UK), with an orthogonal Z-spray-ESI interface operating in positive ionization mode, was used (for further details, see SI and (Boix et al., 2016b; Decision, 2002)).

165 *2.3.2. QqQ*

166 A triple quadrupole (TQD, Waters) with an orthogonal Z-spray ESI interface was
167 interfaced with a Waters Acquity UPLC system (for further details, see SI).

168 *2.4. Data processing*

169 QTOF MS data were processed using MetaboLynx XS and TargetLynx application
170 managers (Micromass v 4.1). The strategy followed for identification of cyanotoxin TPs
171 can be found in SI and (Boix et al., 2013).

172 *2.5. Degradation experiments*

173 A total of four degradation experiments were performed (hydrolysis, sunlight photo-
174 elimination, UV photo-elimination, and chlorination). Experiments under hydrolysis
175 conditions was control experiment. It will help determine the causes of formation of
176 transformation products when sunlight, UV radiation, and chlorination are applied. Milli-
177 Q water and surface water from three different sources was evaluated, to inquire possible
178 interactions with natural organic matter present in the water. Surface water (SW) from
179 the Mijares river, Sitjar reservoir and María Cristina reservoir (Castellon, Spain) were
180 collected in March 2016. After analyzing these samples to confirm the absence of the
181 selected hepatotoxins (i.e., to use them as blanks for degradation experiments), samples
182 were individually spiked with each hepatotoxin (MC-LR and CYN at 500 μg L^{-1} and
183 NOD at 200 μg L^{-1}). These high concentrations were required to detect possible TPs or
184 intermediates that commonly appear at very low levels. Non-spiked Milli-Q water and
185 surface water were used as the control samples. These samples were subjected to the same
186 conditions as the spiked samples. Aliquots of 1 mL or 200 μL were taken for each
187 experiment at different time intervals and stored at -20ºC until analysis.

188

2.5.1. Control experiment

Hydrolysis experiments were carried out at room temperature in darkness for 18 days. A 1 mL aliquot was taken daily. The first measurement was on day 0.

2.5.2. Sunlight degradation conditions

Sunlight photo-elimination was performed using a solar simulation system (Suntest XLS+. Atlas MTT, Linsengericht, Germany), equipped with a xenon arc lamp as radiation source and a solar light filter allowing wavelengths in the range of 300–800 nm. The radiation intensity was set to 500 W m^{-2} and the light dose per hour of irradiation to 1.8 MJ h^{-1}. At these settings, 144 irradiation hours corresponded to 20 days of natural sunlight (dose: 288 MJ m^{-2}). The degradation was performed using 15 mL closed quartz glass tubes and the sample temperature was set to 25°C to minimize evaporation of the sample and possible thermal transformation. During irradiation, 1 mL water samples were taken at different time intervals (0, 30 min, 2 h, 8 h, 18 h, 28 h, 48 h, 75 h, 100 h and 144 h) and immediately stored at -20°C.

2.5.3. UV radiation conditions

A mercury lamp (250 W m^{-2}) with main output at 254 nm was used for the UV photo-degradation experiments. The UV radiation lamp was placed at a distance of 15 cm from the samples. Before each experiment, the UV lamp was warmed up for 15 min to ensure stable output. The surface water samples and Milli-Q water were kept in 15 mL closed quartz glass tubes with a lid to prevent evaporation. Over an irradiation period of 72 h, 200 µL aliquot water samples were taken at different time intervals (0, 5 min, 20 min, 40 min, 90 min, 4 h, 24 h, 48 h and 72 h).

212 *2.5.4. Chlorination conditions*

213 Chlorination experiments were carried out by adding 30 μL of commercial NaOCl
214 solution 1% w/v to 10 mL of spiked sample; giving a final concentration of NaOCl 3.0
215 mg L^{-1}. This oxidant concentration value coincides with the value normally used in
216 treatment plants in Colombia. In the case of the surface water samples, these were
217 previously centrifuged to minimize interference with natural organic matter (NOM),
218 simulating the conventional treatment process in which coagulation and sedimentation
219 stages are applied. Afterwards, the working solution was placed in darkness at 20 °C to
220 avoid the photo-degradation phenomenon. To assess the formation of transformation
221 products, 1 mL aliquots were sampled at different time intervals (0 min, 5 min, 20 min,
222 40 min, 90 min, 4 h, 24 h and 48 h) and kept at -20ºC. In each aliquot, 50 μL of 200 mg
223 L^{-1} ascorbic acid (final concentration 10 mg L^{-1}) was added to stop the reaction.

224 *2.6. Sample analysis*

225 Three surface water samples suspected to be contaminated by cyanotoxins were sampled
226 from the Peñol reservoir (Antioquia, Colombia) at three different sampling points in June
227 2017. Samples were refrigerated at 4ºC. Sample treatment consisted of three
228 freezing/thawing cycles, aiming to cause cell lyses and determine the total amount of
229 cyanotoxins (extracellular and intracellular toxins). The samples were then filtered
230 through a 0.22 μm nylon filter and shipped under refrigerated conditions to Spain. As
231 soon as the samples were received in the laboratory, they were stored at -18ºC until
232 analysis. The samples were defrosted on the day of the analysis, and an aliquot was
233 directly injected into the LC-QqQ and LC-QTOF chromatographic system without any
234 additional treatment.
235

3. Results and discussion

3.1. Elimination rates

Surface and Milli-Q waters were subjected to 4 different degradation experiments (hydrolysis, sunlight, UV-light, and chlorination). The decrease in parent toxins and formation of transformation products were monitored in the four degradation experiments, obtaining the corresponding elimination curves.

3.1.1. Control experiment

Study of hydrolysis in Milli-Q water and surface water samples spiked with the three hepatotoxins over 18 days did not show significant elimination. Therefore, the possible reduction in the concentration of these toxins and potential formation of TPs observed in subsequent experiments (sunlight, UV and chlorination) should be associated exclusively to the specific processes applied and not to hydrolysis.

3.1.2. Sunlight photo-elimination

After 144 h of sunlight experiments, equivalent to approximately 20 days of natural sunlight, a decrease in the concentration of the three studied toxins was observed (**Figure 1a**). The elimination of the NOD and CYN toxins in the surface waters was higher than in Milli-Q water. Meanwhile the MC-LR toxin showed an opposite result, where in Milli-Q water the elimination was greater. Therefore, the average degradation of cyanotoxins in the surface waters, calculated as the toxin concentration percentages after irradiation divided by the initial spiked concentration of the toxin (C/C_0 x 100%), were 24%, 47% and 53%, for MC-LR, NOD and CYN, respectively. In contrast, the elimination rates observed in Milli-Q water were 43%, 25% and 16%, respectively. This difference could be explained by the influence that natural organic matter (NOM) and/or biocenosis present in the aquatic biota can have on the elimination of these cyanotoxins (Edwards et

al., 2008; Meriluoto et al., 2017). Despite not obtaining a positive result, and in accordance with the literature regarding the elimination of MC-LR, similar results were obtained for the CYN and NOD toxins (Buratti et al., 2017; Ho et al., 2012).

3.1.3. UV photo-elimination

In photo-elimination experiments under UV radiation, no substantial differences were found between Milli-Q water and the surface waters for the three hepatotoxins. In both cases, a rapid elimination of MC-LR and NOD occurred compared to CYN (**Figure 1b**). Under the conditions of this study, five minutes of UV radiation were enough for complete elimination (99%) of MC-LR and NOD, while the complete elimination of CYN by UV radiation required longer exposure (250 min). Direct photolysis has been reported as an important removal pathway, which is achieved via the formation of photoproducts after the generation of a photoexcited state (Porras et al., 2016). Other studies have reported the photolysis of MC-LR at different wavelengths (VIS, UV-A and UV-C) (Antoniou et al., 2008; Song et al., 2007). Some differences in UV elimination have been observed compared with other results reported in the literature. Thus, some authors have found that elimination of CYN was not appreciable under UV radiation (Chen et al., 2015; He et al., 2012), or that UV alone was unsatisfactory for the MC-LR degradation (He et al., 2015; Pinho et al., 2015). This was not the case in our work.

3.1.4. Chlorination-elimination

Similar degradation behavior was observed for cyanotoxins in Milli-Q and surface waters in both the chlorination and UV experiments. However, CYN showed higher reactivity with NaOCl than NOD and MC-LR. **Figure 1c** shows nearly complete elimination (96%) of this toxin, after barely two minutes of contact with the oxidant. In contrast, concentration levels decreased 67% for MC-LR and 55% for NOD after the same time,

with complete elimination of these two toxins observed after 30 min. The results for MC-LR were in accordance with the study conducted by Zhang (Y. Zhang et al., 2016). The different elimination rates observed for the three toxins could be explained by the differences in their chemical structure, i.e. the position of amino groups in microcystins could modify the removal rate. This was demonstrated in a degradation study of eleven MCs which, although a study of UV/TiO_2 degradation, allows the structural differences of the MC variants to be understood, independently of the elimination process applied. (Pestana et al., 2015). Thus, the MC-LR elimination observed in this work could not be extrapolated to other cyanotoxins unless they had great structural similarities. Chlorination, as a stage after coagulation and sedimentation of conventional treatment, can be an essential barrier against dissolved and intracellular cyanotoxins. According to Zamyadi, the release of intracellular cyanotoxins during cell lysis can occur during stages prior to chlorination (Zamyadi et al., 2013). Thus, secondary toxicity from the transformation products and/or disinfection products cannot be ignored (Y. Zhang et al., 2016; Zong et al., 2015).

3.2. Detection and elucidation of transformation products by QTOF MS

Regarding the four degradation conditions evaluated, no TPs were observed under hydrolysis conditions, which was expected in accordance with the chemical stability observed for all cyanotoxins (see section 3.1.1).

3.2.1. Transformation products by sunlight

In the photo-elimination experiments on the three evaluated cyanotoxins, CYN showed no TPs, but a different situation was observed for NOD and MC-LR toxins. NOD, in Milli-Q and the surface waters, showed only a single TP at *m/z* of 244.2260 ($C_{14}H_{20}NO_2^+$; mass error 1.1 mDa; Rt 10.26 min) named TPNOD_SL_244. The proposed structure

implies the total rupture of the pentapeptide ring and the loss of the aromatic ring of the Adda moiety.

A MC-LR TP at *m/z* of 498.2840 was detected at 10.69 min retention time (TPLR_SL_498). The detected TP was considered a doubly protonated $[M + 2H]^{2+}$ isomer TP for MC-LR, assigning an elemental composition of $C_{49}H_{75}N_{10}O_{12}^{2+}$. The peak area of the TPLR_SL_498 and MC-LR shows an inverse behavior in the transformation curve (see **Figure 2**), which could be explained by a transformation from *trans* to *cis* configuration present in the C6-C7 bond of the Adda moiety. The proposed structures are in agreement with Jacobs et al., where it is mentioned that solar energy induces structural changes in the MC-LR molecules (Jacobs et al., 2013). Furthermore, the removal, or isomerization in the hydrophobic Adda (C6-C7 bond) from *trans* to *cis*, will eliminate the associated toxicity of MCs (He et al., 2015). However, toxicity studies should be carried out in future works.

3.2.2. Transformation products by UV

The absence of detected TPs for MC-LR and NOD toxins could be associated with a rapid degradation rate of the cyclopeptides exposed to the UV radiation. Therefore, the rapid mineralization of the compounds makes detection of the corresponding intermediate TPs difficult (Song et al., 2007). Whereas the CYN toxin shows a slower removal of water, it suggests a lower degradation rate. In this way, it was possible to detect 4 TP when samples of water enriched with CYN were analyzed. (see **Table 1).**

Thus, for TPCYN_UV_327 at *m/z* 327.2017 (Rt 4.06 min) for example, an elemental composition of $C_{15}H_{27}N_4O_4^+$ was assigned. This involves a sulphate group loss as well as an opening of the uracil ring from the parent CYN. Transformation curves for TPCYN_UV_327, showing a rapid transformation in the first 240 min, and the proposed

chemical structure, can be seen in **Figure 3**. **Figure 1_SI** illustrates the spectrum of TPCYN_UV_327. This TP shows an important sodium adduct [M+Na] at *m/z* 349.1838. However, the presence of several fragment ions at *m/z* 275.1510; 187.1332 and 157.1242 facilitate corroboration of its possible chemical structure.

Two further TPs were detected following a different transformation route to that proposed above. TPs at *m/z* 288.0673 ($C_{10}H_{14}N_3O_5S^+$; Rt 2.64 min) and *m/z* 274.0866 ($C_{10}H_{16}N_3O_4S^+$; Rt 1.42 min) maintain the sulfate but lose the uracil group on their chemical structures. Proposed chemical structures for both TPs and fragment ions found in the MS spectra are shown in **Figure 2_SI**. One of these TPs (TPCYN_UV_274) was previously detected by Merel *et al* (Merel et al., 2010a).

Finally, another TP was found at *m/z* 215.1647, TPCYN_UV_215 ($C_{12}H_{23}O_3^+$; Rt 6.44 min) (**Figure 3)**. This TP presents complete rupture of the uracil group, rupture of the fraction of the tricyclic alkaloid, and, similarly to TPCYN_UV_327, loss of the sulfate group. The proposed structure is characterized mainly by an aliphatic chain of 11 carbons with a hydroxyl group and ketone group as substituents, including double conjugated bonds in most cases. **Figure 3_SI** shows the MSMS spectra and fragment ions found for TPCYN_UV_215.

3.2.3. Transformation products by chlorination

In the chlorination experiments, of the three evaluated cyanotoxins only NOD showed no TPs. From CYN, 3 TPs were detected. Two of these TPs, TPCYN_Cl_434 ($C_{14}H_{17}ClN_5O_7S^+$; Rt 2.97 min) at *m/z* 434.0558, and TPCYN_Cl_422 ($C_{15}H_{24}N_3O_9S^+$; Rt 3.34 min) at *m/z* 422.1232, have not been previously reported to our knowledge. TPCYN_Cl_434 has a chlorine atom in the proposed structure. The presence of the peak at *m/z* of 436.0741 (32% abundance of *m/z* 434.0558) confirms the presence of the isotope

356 ^{37}Cl (see **Figure 4**). **Figure 5** shows the QTOF MS^E spectrum (LE and HE) of
357 TPCYN_CL_422 and their fragments after chlorination experiments in surface water
358 spiked with CYN, as well as the transformation curve. The third TP detected was *m/z*
359 350.1028, TPCYN_Cl_350 TPs ($C_{12}H_{20}N_3O_7S^+$; Rt 1.28 min). **Figure 4_SI** shows the
360 QTOF MS^E spectra for TPCYN_Cl_350 in surface water spiked with CYN and its
361 transformation curve. This TP has been detected by Merel et al. (Merel et al., 2010a), and
362 was identified as cylindrospermopsic acid. The transformation path of the original
363 molecule could be a hydrolysis in the amide group (opening of the uracil group), followed
364 by subsequent loss of nitrogen from the same group. The proposed mechanism is in
365 agreement with the review by Pantelic et al., where it is mentioned that the uracil group
366 is the most susceptible to chemical oxidation (Pantelić et al., 2013).

367 The transformation of MC-LR to TPs in the chlorination experiments did not show
368 abundant TPs. **Figure 5_SI** shows the transformation curves for the detected MC-LR TPs
369 after chlorination experiments**.** Two isomeric TPs at *m/z* 1029.5610 ($C_{49}H_{77}N_{10}O_{14}^+$),
370 TPLR_Cl_1029_1 (Rt 8.82 min) and TPLR_Cl_1029_2 (Rt 9.08 min), with a mass error
371 around 1 ppm, were detected. In this case, a double hydroxylation should result from the
372 additive reaction of two hydroxyl groups on the conjugated diene in Adda residue. This
373 TP is consistent with the data found in the literature, where 4 isomers with a *m/z* 1029
374 have been observed. The 34 units of *m/z* difference with MC-LR (*m/z* 995.555) is
375 consistent with the fixation of 2 hydroxyl groups on the toxin with a chemical formula
376 $C_{49}H_{76}N_{10}O_{14}$, (Merel et al., 2010b; Y. Zhang et al., 2016; Zong et al., 2013). The
377 hydroxylation on the conjugated double bonds leads to the formation of isomers. This
378 proposal matches with the fragmentation observed for these TPs in comparison with MC-
379 LR, (see **Table 1**). **Figure 6_SI** shows QTOF MS^E spectra for both MC-LR TP isomers
380 after chlorination experiments in MQ and surface water. Another TP with *m/z* 895.4910,

TPLR_Cl_895 ($C_{45}H_{67}N_8O_{11}^+$; Rt 8.13 min), was also detected. For this TP the opening of the peptide ring and conservation of the Adda group is proposed, because the *m/z* 135 fragment is still present in the HE spectrum (see **Figure 7_IS**).

In summary, a total of 12 cyanotoxin TPs were tentatively identified by LC-QTOF MS: 6 chlorination TPs (3 from CYN and 3 from MC-LR, including two isomers), 4 UV TPs (all from CYN) and 2 sunlight TPs (one isomer from MC-LR and another from NOD).

The biological toxicity of the TPs found was not evaluated. However, the information reported in this work on the identity of the TPs is relevant for future investigations, as previous studies have confirmed a certain degree of toxicity for MC-LR by-products (He et al., 2015).

3.3. Summary of transformation products

The largest number of TPs detected in this study were from the transformation of the CYN toxin. Of the 7 TPs found, 3 result from the chlorination process and 4 from the UV radiation process. These two processes have in common the attack to the uracil group of the CYN structure, this being the point most susceptible to the attack both of photochemical and oxidative processes. However, under chlorination conditions, formation is proposed of Cl_350 and Cl_422 TPs by total elimination of the uracil group and oxidation to one and two carboxylic acids, respectively. Additionally, both retain the sulfate group. On the other hand, the TPCYN_Cl_434 conserved not only this sulphate group in the structure, but also the uracil group with the addition of a chlorine atom.

Similarly, the total elimination of the uracil group is proposed for TPs obtained under UV radiation. However, a double bond is instead formed in TPCYN_UV_274, and an

aldehyde group is added in TPCYN_UV_288. Additionally, both retain the sulfate group. Another product is TPCYN_UV_327, in which the opening of the uracil group generates the alcohol group on one side and the aldehyde group on the other, while additional loss of the sulfate group is also proposed. A final product is TPCYN_UV_215, for which the total opening of the uracil group and the tricyclic alkaloid with loss of the sulphate group is proposed. This is the TP from the original CYN molecule that is most degraded.

On the other hand, few TPs were detected during the transformation of the MC-LR toxin by oxidative methods in the chlorination experiment. In this case, only hydroxylation of the conjugated double bond of the Adda fraction was proposed for *m/z* 1029, which was present at two different retention times, confirming isomerization around the conjugated double bond. This compound was termed dihydroxy-microcystin by (Merel et al., 2009). Another slightly different TP was produced by the opening of the seventh amino acid polypeptide ring of the MC-LR with *m/z* 895. These are the double bond of the Adda fraction and the amino acid Mdha (Methyl-dehydro-alanine) of the peptide cycle, the most vulnerable sites to degradation processes. Other works have also shown these sites to be susceptible in different elimination processes (Y. Zhang et al., 2016).

3.4.Analysis of surface water samples

After the detection and identification of TPs for the selected cyanotoxins, three surface water samples collected from the Peñol reservoir. This reservoir is suspected to be contaminated by cyanotoxins, were analyzed by LC-MS/MS (QqQ) and LC-QTOF MS.

The first analysis was performed by LC-MS/MS with triple quadrupole at its highest sensitivity to facilitate the detection of TPs at low concentrations. Chromatographic separation was carried out under the same LC conditions used in the LC-QTOF

degradation experiments, in order to obtain the same retention times. The three parent cyanotoxins and their corresponding TPs, identified in the degradation experiments, were included in the target analysis. The availability of reference standards for the three parent compounds allowed us to perform quantification of these compounds in the samples, while for TPs only a qualitative analysis could be made. For each compound, at least two transitions were selected. For the TPs, selection was made based on the fragmentation observed by QTOF in the degradation experiments (**Table 2_SI**). A positive finding was considered to occur when the Q/q intensity ratio (between the two most sensitive transitions) and retention time were, for the parent cyanotoxins, consistent with those of the reference compound and, for the TPs, with those of the spiked samples subjected to the degradation experiments (Decision, 2002; Ibáñez et al., 2017).

MC-LR and CYN were found in the three analyzed samples at concentrations between 80 and 280 μg L^{-1} (MC-LR), and 30 and 120 μg L^{-1} (CYN). In other studies, higher values of cyanotoxins have been reported, for example, MC-LR concentrations of 48 μg L^{-1} (Argentina), 115 μg L^{-1} (Chile), 300 μg L^{-1} (Canada) and 100 μg L^{-1} (United States), among others (Pham and Dang, 2018). With respect to TPs, only sunlight TPs were expected as no UV or chlorination treatments were applied to the samples. The results showed the presence of TPLR_SL_498, an isomer of MC-LR, in the three sampling points. However, no TPs for CYN were detected, probably due to its lower degradation as observed in the laboratory degradation experiments. **Figure 6** shows the positive findings found by LC-MS/MS QqQ for the water collected in sampling point 1. The ion ratio agreement is also shown in the figure.

In order to confirm the identity of the cyanotoxins and the TP detected by LC-MS/MS in the reservoir waters, the samples were re-analyzed by LC-QTOF using the same LC conditions. Evaluation of QTOF analysis was performed by obtaining the extracted Ion

Chromatograms (XICs) with a mass window (±20 mDa) at the targeted cyanotoxins and TP exact m/z-values (nwXICs). Both MC-LR and CYN were confirmed in the three water samples by the presence of the corresponding chromatographic peak and by accurate-mass measurements with mass errors below 5 ppm. However, TPLR_SL_498 could be confirmed only in one sample, as the lower concentrations in the other two samples together with the lower sensitivity of LC-QTOF MS in comparison to LC-MS/MS QqQ, prevented its detection and identification.

4. Conclusions

This paper reports the study performed on the degradation/elimination and transformation of three cyanotoxins belonging to the hepatotoxins family, in surface water subjected to sunlight simulation, UV radiation and chlorination. This research has allowed the identification of TPs not reported until now, as well as the confirmation of others previously reported.

CYN was more persistent to sunlight radiation than MC-LR and NOD, with a lower degree of transformation to by-products. In natural environments, sunlight plays an important role in favoring cyanobacteria bloom formation. However, in the light of the results of this study, sunlight could also be considered a mitigating factor for some cyanotoxins, eliminating and/or transforming selected toxins. Similar behavior may occur for other toxins, a possibility that should be investigated in future works. Laboratory experiments performed with UV radiation and chlorination demonstrated that these were more efficient methodologies than sunlight radiation for toxin elimination.

LC-QTOF MS analysis with acquisitions at low and high energy, allowed confident detection and identification of up to 12 cyanotoxin TPs. Of these, 6 corresponded to chlorination TPs (3 from CYN and 3 from MC-LR, including 2 isomers); 4 were UV TPs (all from CYN); and 2 were sunlight TPs (one isomer from MC-LR and another from NOD). Given the generally high toxicity of cyanotoxins, other compounds from this family and other cyanotoxin groups need to be studied in relation to their persistence and potential degradation/transformation under different conditions. An evaluation of the toxicity of the TPs discovered would be also necessary after appropriate identification and detection in the real-world samples.

485 The analysis of surface waters collected from a reservoir in Colombia allowed
486 confirmation of the presence of CYN, MC-LR and the transformation product
487 TPLR_SL_498, an isomer of MC-LR. The fact that these cyanotoxins were found at
488 relatively high concentrations in real-world surface waters (between 30 and 280 $\mu g\ L^{-1}$)
489 supports the results obtained in laboratory experiments, which revealed low degradation
490 under sunlight conditions.

491

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Figure caption

Figure 1. Elimination curves for MC-LR, CYN and NOD in Milli-Q water (MQ) and surface water (SW) after sunlight experiments (a), UV experiments (b), and chlorination (c).

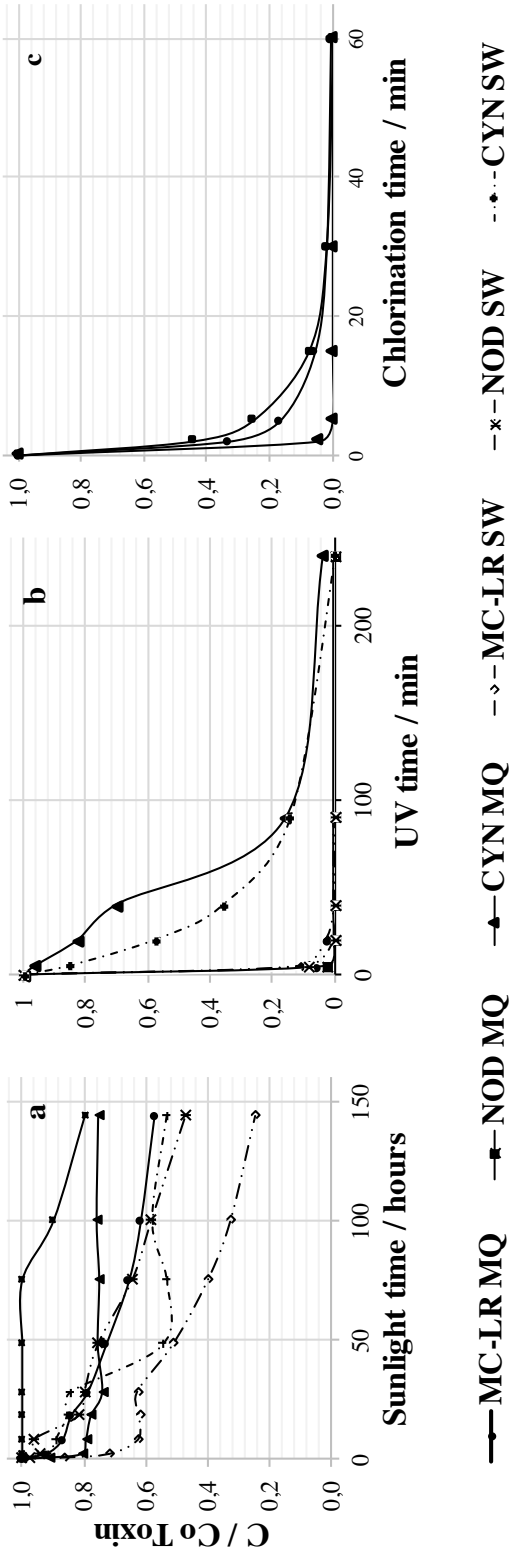
Figure 2. QTOF MS^E spectrum for TPLR_SL_498 *trans* (a) and *cis* (b), and transformation curves for MC-LR $[M+2H]^{2+}$ after sunlight experiments in Milli-Q water and surface water.

Figure 3. Transformation curves and proposed structures for TPCYN_UV_327 and TPCYN_UV_215 after UV experiments in Milli-Q water.

Figure 4. QTOF MS^E spectrum for TPCYN_CL_434 and fragments after chlorination experiments in surface water spiked with CYN.

Figure 5. Top: QTOF MS^E spectrum for TPCYN_CL_422 and fragments after chlorination experiments in surface water spiked with CYN. Bottom: transformation curve.

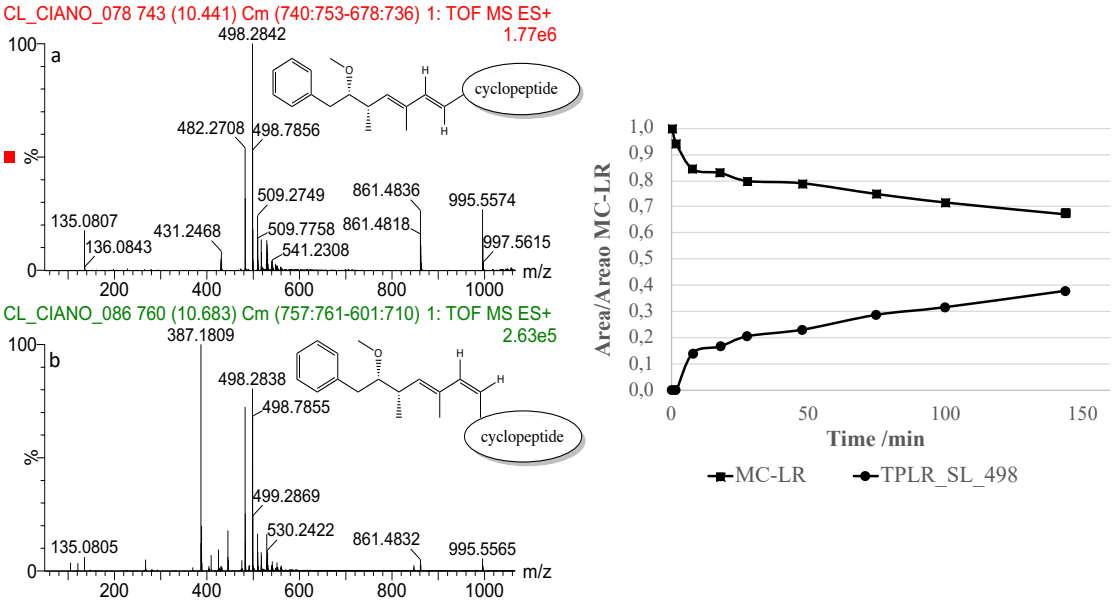
Figure 6. LC-MS/MS chromatograms corresponding to surface water from Peñol reservoir. CYN, MC-LR and TPLR_SL_498 were found. Q/q is the ratio of precursor and product ion



732

733 **Figure 1.** Elimination curves for MC-LR, CYN and NOD in Milli-Q water (MQ) and
734 surface water (SW) after sunlight experiments (a), UV experiments (b), and chlorination
735 (c).

736



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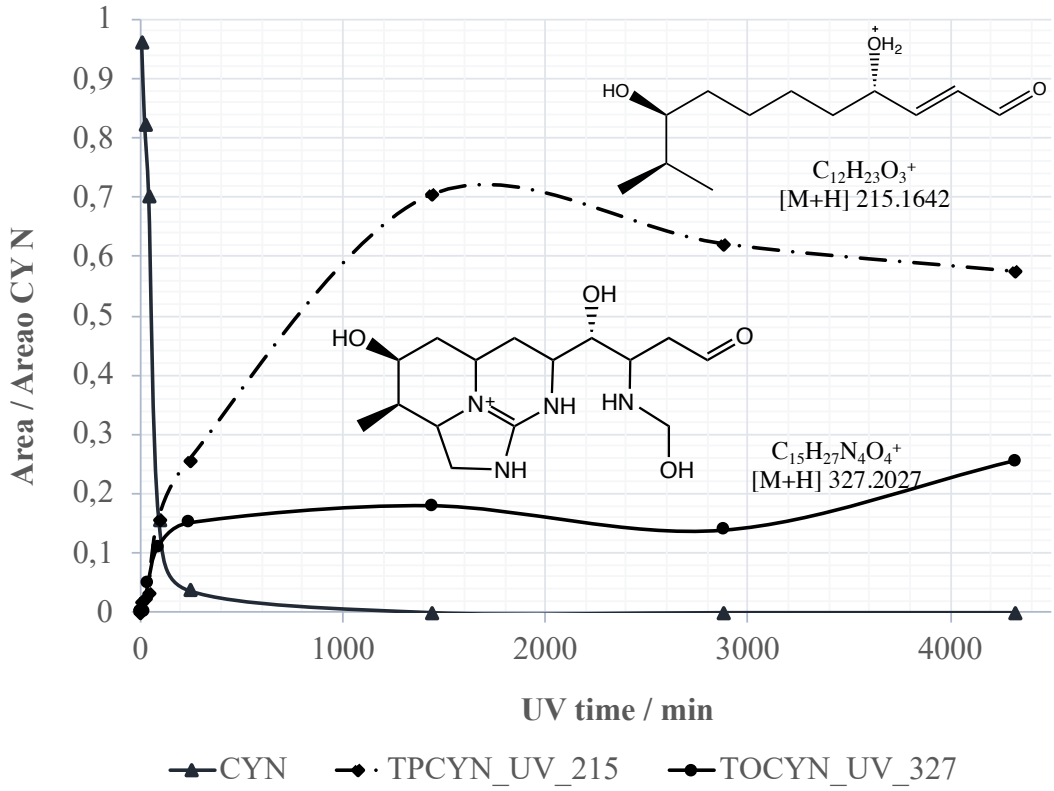

Figure 3. Transformation curves and proposed structures for TPCYN_UV_327 and TPCYN_UV_215 after UV experiments in Milli-Q water.

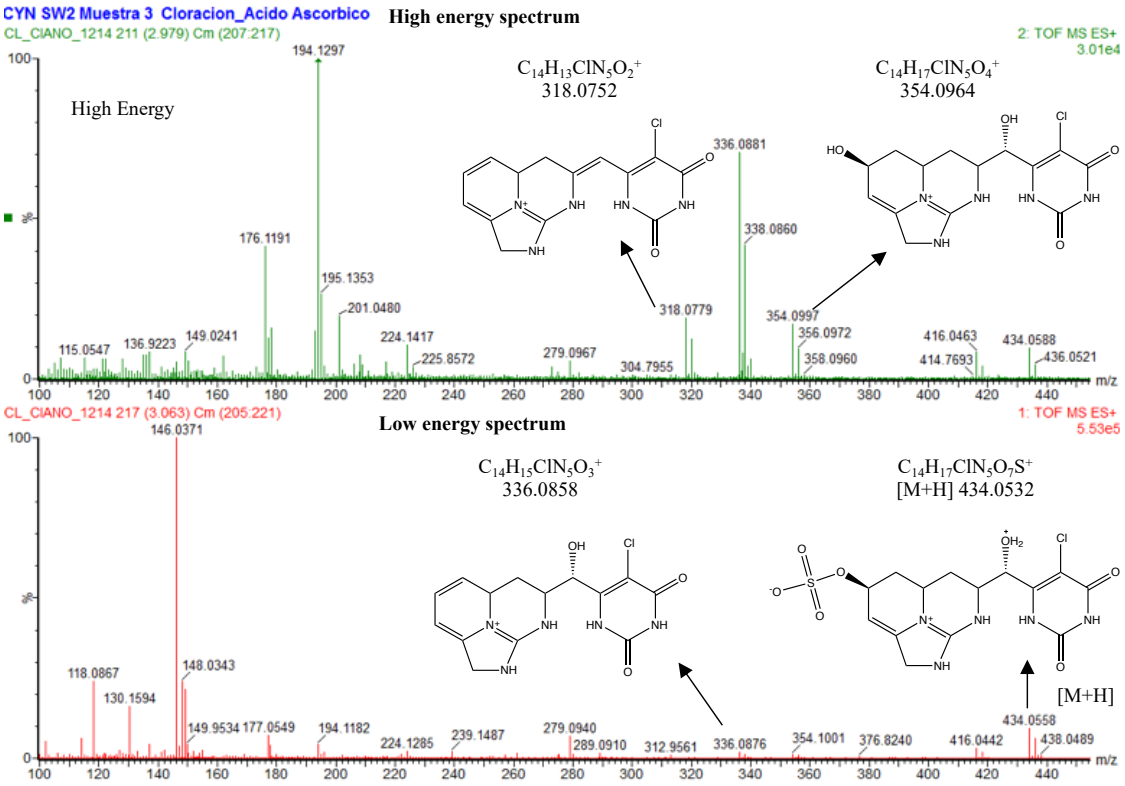

Figure 4. QTOF MS^E spectrum for TPCYN_CL_434 and fragments after chlorination experiments in surface water spiked with CYN.

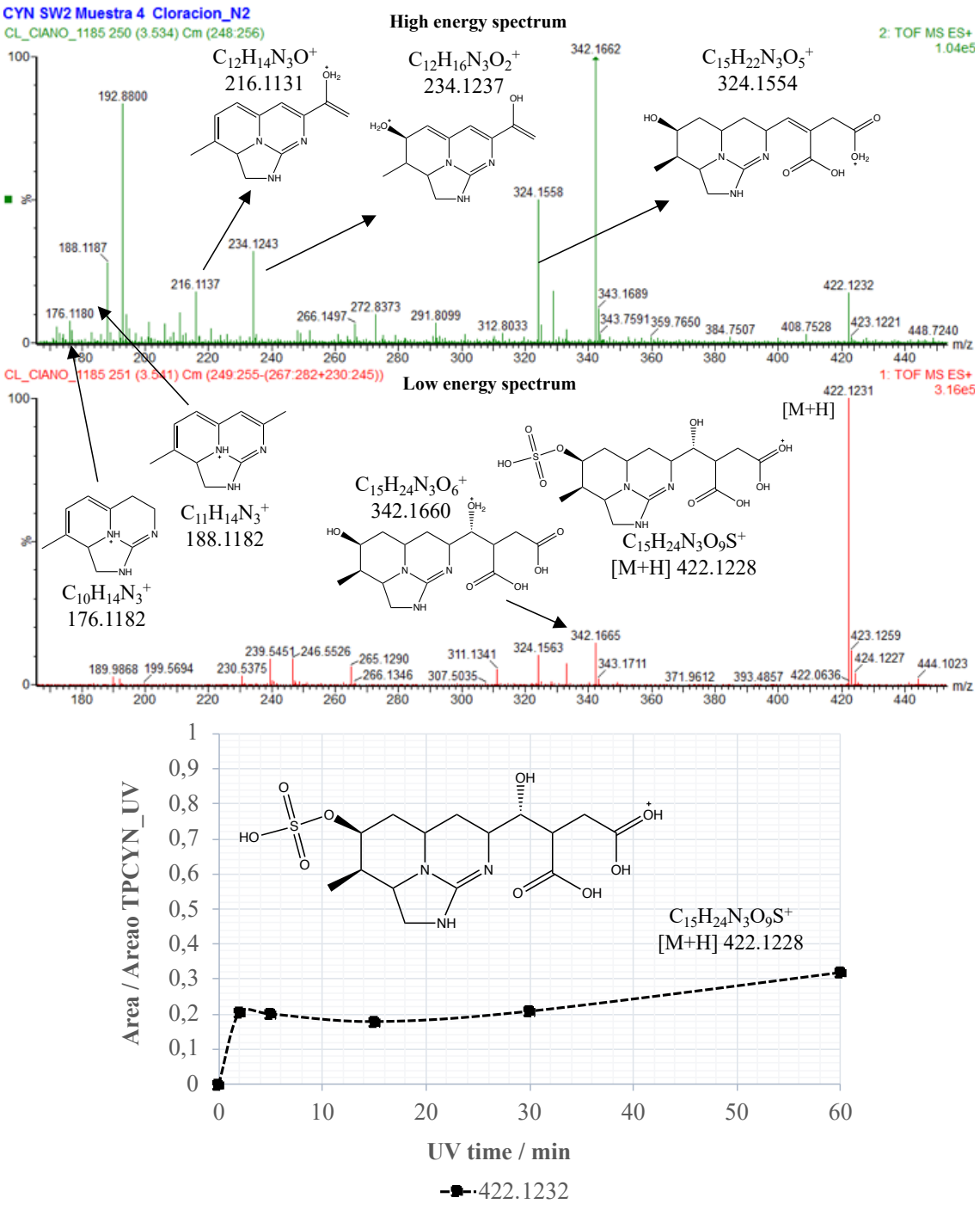

Figure 5. Top: QTOF MS^E spectrum for TPCYN_CL_422 and fragments after chlorination experiments in surface water spiked with CYN. **Bottom:** transformation curve.

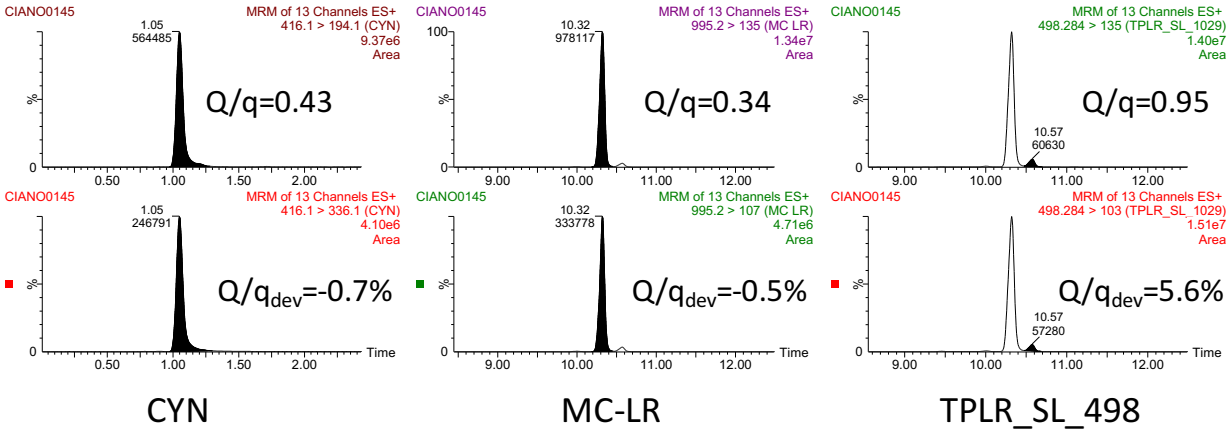

Figure 6. LC-MS/MS chromatograms corresponding to surface water from Peñol reservoir. CYN, MC-LR and TPLR_SL_498 were found. Q/q is the ratio of precursor and product ion.

Table 1. UV and chlorination TPs and fragments of CYN, MC-LR and NOD obtained by LC-ESI-QTOF MS

	UV radiation								Chlorination							
Toxin	**Experimental mass *m/z***	**ppm**	**mDa**	**DBE**	**Retention time / min**	**Elemental composition**	**Experimental fragments *m/z***	**Elemental composition**	**Experimental mass *m/z***	**ppm**	**mDa**	**DBE**	**Retention time / min**	**Elemental composition**	**Experimental fragments *m/z***	**Elemental composition**
CYN	327.2017 TPCYN_UV_327	4.7	1.3	4.5	4.06	$C_{15}H_{27}N_4O_4^+$	275.1510 187.1332 157.1242	$C_{14}H_{19}N_4O_2^+$ $C_{10}H_{19}O_3^+$ $C_9H_{17}O_2^+$	434.0558 TPCYN_Cl_434	4.8	2.1	8.5	2.97	$C_{14}H_{17}ClN_5O_7S^+$	354.0997 336.0881 318.0779	$C_{14}H_{17}ClN_5O_4^+$ $C_{14}H_{15}ClN_5O_3^+$ $C_{14}H_{13}ClN_5O_2^+$
	215.1647 TPCYN_UV_215	0.09	0.02	1.5	6.44	$C_{12}H_{23}O_3^+$	197.1542 185.1541 167.1436	$C_{12}H_{21}O_2^+$ $C_{11}H_{21}O_2^+$ $C_{11}H_{19}O^+$	422.1232 TPCYN_Cl_422	0.3	0.1	5.5	3.34	$C_{15}H_{24}N_3O_9S^+$	342.1662 324.1558 234.1243 216.1137	$C_{15}H_{24}N_3O_6^+$ $C_{15}H_{22}N_3O_5^+$ $C_{12}H_{16}N_3O_2^+$ $C_{12}H_{14}N_3O^+$
	317.1444[a]	5.4	1.7	4.5	8.22	$C_{12}H_{21}N_4O_6^+$	N.D.								188.1187 176.1180	$C_{11}H_{14}N_3^+$ $C_{10}H_{14}N_3^+$
	288.0673 TPCYN_UV_288	6.5	1.9	5.5	2.64	$C_{10}H_{14}N_3O_5S^+$	230.0248 215.0603 166.0761 137.0548	$C_7H_8N_3O_4S^+$ $C_7H_7N_2O_4S^+$ $C_9H_{12}NO_2^+$ $C_7H_9N_2O^+$	350.1028 TPCYN_Cl_350	1.7	0.6	4.5	1.28	$C_{12}H_{20}N_3O_7S^+$	270.1454 252.1355 224.1407	$C_{12}H_{20}N_3O_4^+$ $C_{12}H_{18}N_3O_3^+$ $C_{11}H_{18}N_3O_2^+$
	274.0866 TPCYN_UV_274	1.6	0.45	4.5	1.42	$C_{10}H_{16}N_3O_4S^+$	194.1293 176.1200	$C_{10}H_{16}N_3O^+$ $C_{10}H_{14}N_3^+$								
MC-LR	ND								1029.5610 TPLR_Cl_1029_1	1.0	1.1	16.5	8.82	$C_{49}H_{77}N_{10}O_{14}^+$	877.4774 534.2546	$C_{40}H_{65}N_{10}O_{12}^+$ $C_{20}H_{32}N_5O_{12}^+$
									1029.5608 TPLR_Cl_1029_2	1.2	1.3	16.5	9.08	$C_{49}H_{77}N_{10}O_{14}^+$	877.4745 534.2588 430.2379	$C_{40}H_{65}N_{10}O_{12}^+$ $C_{20}H_{32}N_5O_{12}^+$ $C_{16}H_{24}N_5O_9^+$
									895.4910 TPLR_Cl_895	2.1	1.9	16.5	8.13	$C_{45}H_{67}N_8O_{11}^+$	700.3622 607.3245 571.3212 515.2862 135.0810	$C_{29}H_{50}N_9O_{11}^+$ $C_{25}H_{47}N_6O_{11}^+$ $C_{25}H_{43}N_6O_9^+$ $C_{23}H_{43}N_6O_7^+$ $C_9H_{11}O^+$
NOD	ND								ND							

[a] Is not considered a TP.

782