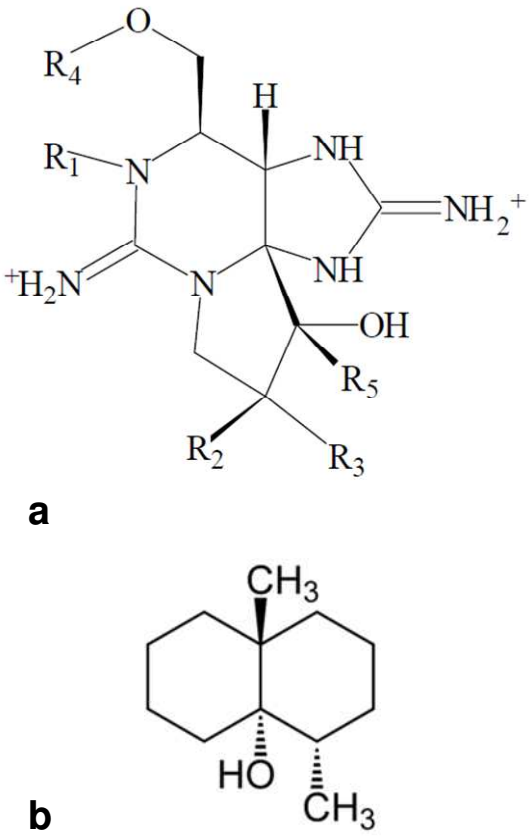
Release and oxidation of cell-bound saxitoxins during chlorination of *Anabaena circinalis* cells

Arash Zamyadi[1], Lionel Ho*[2], Gayle Newcombe[2], Robert I. Daly[2], Mike Burch[2], Peter Baker[2] and Michèle Prévost[1]*

[1] École Polytechnique Montreal, Civil, Mineral and Mining Engineering Department, P.O. Box 6079, Station Centre-ville, Montreal, Quebec, Canada, H3C 3A7
[2] Australian Water Quality Centre, SA Water Corporation, Australia
* Corresponding author Phone: 514-340-4711 local: 3711; Fax: 514-340-5918; E-mail: arash.zamyadi@polymtl.ca

Supporting Information (SI)

Number of pages: 7
Number of figures: 4
Number of table: 1



Toxin	R1	R2	R3	R5	Net Charge	Relative mouse toxicity
R4 = $CONH_2$ (carbamate toxins)						
STX	H	H	H	OH	+2	1.000
neoSTX	OH	H	H	OH	+2	0.924
GTX1	OH	H	OSO_3^-	OH	+1	0.994
GTX2	H	H	OSO_3^-	OH	+1	0.359
GTX3	H	OSO_3^-	H	OH	+1	0.638
GTX4	OH	OSO_3^-	H	OH	+1	0.726
R4 = $CONHSO_3^-$ (n-sulfocarbamoyl (sulfamate) toxins)						
GTX5 (B1)	H	H	H	OH	+1	0.064
GTX6 (B2)	OH	H	H	OH	+1	-
C1	H	H	OSO_3^-	OH	0	0.006
C2	H	OSO_3^-	H	OH	0	0.096
C3	OH	H	OSO_3^-	OH	0	0.013
C4	OH	OSO_3^-	H	OH	0	0.058
R4 = H (decarbamoyl toxins)						
dcSTX	H	H	H	OH	+2	0.513
dcneoSTX	OH	H	H	OH	+2	-
dcGTX1	OH	H	OSO_3^-	OH	+1	-
dcGTX2	H	H	OSO_3^-	OH	+1	0.651
dcGTX3	H	OSO_3^-	H	OH	+1	0.754
dcGTX4	OH	OSO_3^-	H	OH	+1	-
LWTX4	H	H	H	H	+2	<0.004
R4 = $COCH_3$ (Lyngbya wollei toxins)						
LWTX1	H	OSO_3^-	H	H	+1	<0.004
LWTX2	H	OSO_3^-	H	OH	+1	0.072
LWTX3	H	H	OSO_3^-	OH	+1	0.021
LWTX5	H	H	H	OH	+2	0.139
LWTX6	H	H	H	H	+2	<0.004
R4 = COC_6H_4OH (Gymnodinium catenatum toxins)						
GC1	H	H	OSO_3^-	OH	+1	-
GC2	H	OSO_3^-	H	OH	+1	-
GC3	H	H	H	OH	+2	-

Figure SI-*S1*. General structure of (a) Saxitoxins* (also known as paralytic shellfish poison) and its known analogues and (b) geosmin. These neurotoxins can be produced by cyanobacteria (CB)** species including, *Anabaena circinalis*, *Aphanizomenon ovalisporum*, *Cylindrospermopsis raciborskii* and *Lyngbya majuscula* (*1, 2*).

* The STXs molecules degree of ionization is a key factor in oxidation of these toxins. STXs are molecules with a purine alkaloid nucleus which contains nitrogen atoms. The general STXs structure has two guanidinium nitrogens of different pKa values (*3*). The degree of protonation of these nitrogen atoms depends on different factors including the water pH (*2*). Also there is a distinct difference in the net charge of these toxins (Figure SI-*S1*), STX has the highest (+2), followed by GTX2 and 3 (+1) and C1 and 2 (0) (*3*). This charge difference may also influence the degree of toxin molecule protonation in different chlorination conditions (e.g. different pH values). Also, STX has two free guanidinium ions whereas C-toxins have both and GTXs have one of these ions sulphated. This difference in degree of ions sulphatation may be the source of the difference in STXs reaction to chlorine (*3*).

** CB also known as blue-green algae are prokaryotic photosynthetic micro-organisms present in most ecosystems (*1, 4*). Some CB species are producers of a variety of potent toxins (*1, 5, 6, 7*). The increasing frequency and intensity of cyanobacterial proliferation leading to neurotoxin and hepatotoxin production is a universal problem (*5, 6, 8*). Freshwater CB including *A. circinalis* blooms have been found worldwide, including Australia and North American (*7*).

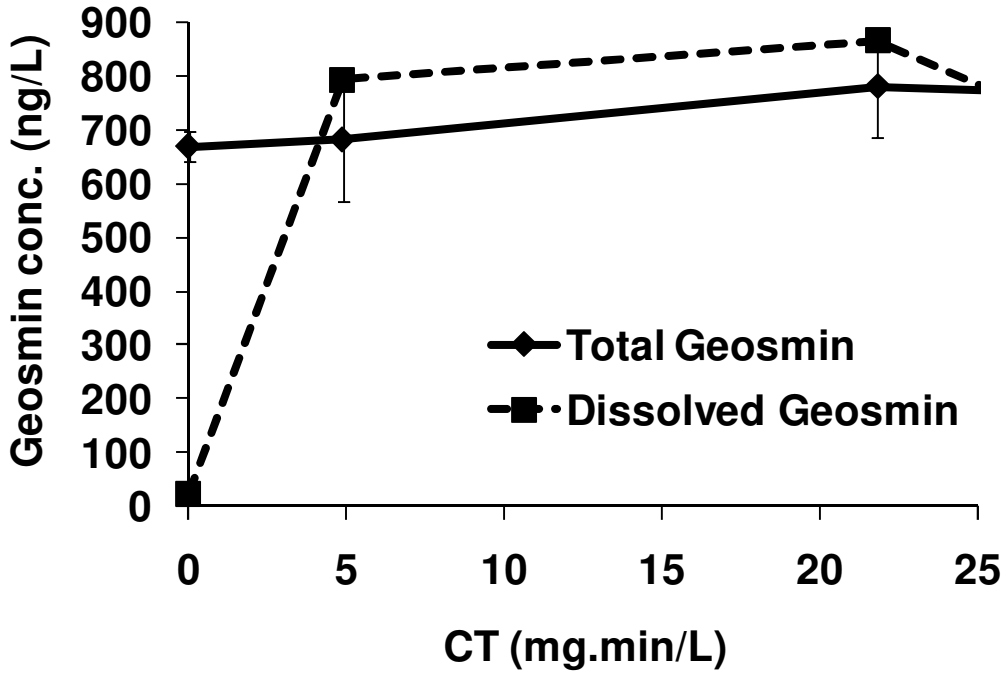


Figure SI-*S2*. Geosmin release in Murray River water (MR) water at pH 8 with 3 mg/L Cl_2.

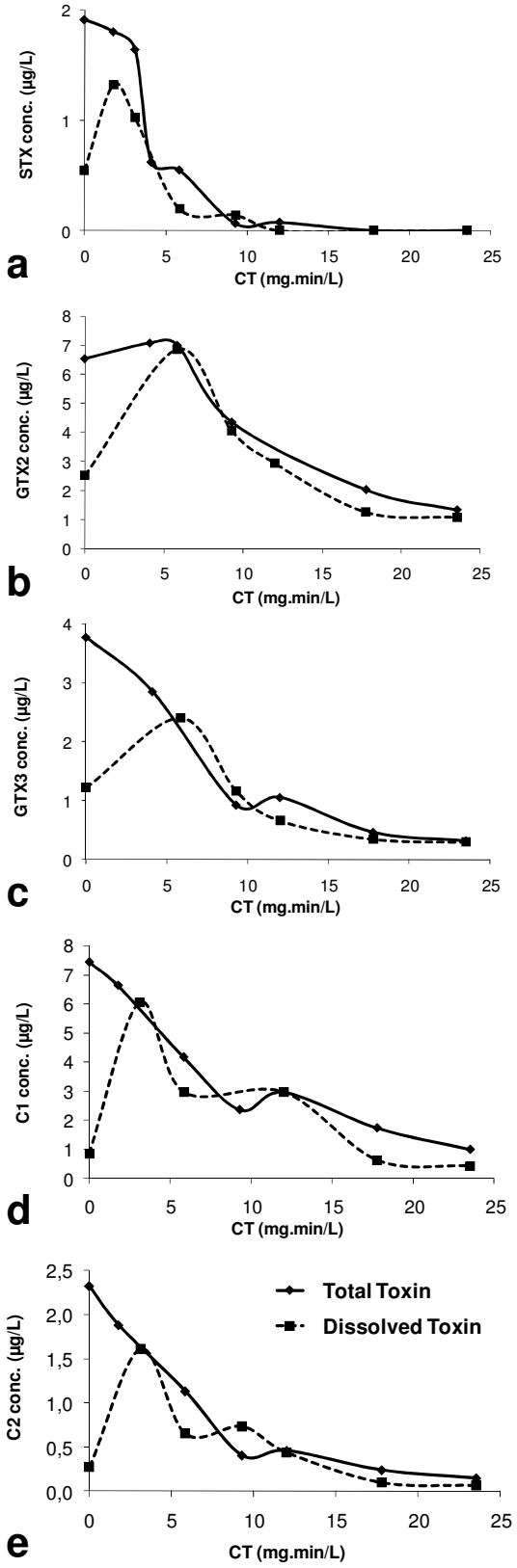


Figure Sl-*S3*. Toxin release and oxidation in MR water at pH 8 with 2 mg/L Cl_2 (a) STX, (b) GTX2, (c) GTX3, (d) C1, (e) C2.

Table Sl-*S4*. Measured concentrations of TTHM, HAA9 and NDMA as chlorination by-products with the highest CT value (50.3 mg.min/L) in MR water without and with *A. circinalis* cells.

Water type	TTHM (µg/L)	HAA9 (µg/L)	NDMA (ng/L)
MR without cells	40	39	6
MR with *A. circinalis* cells	49	42	11

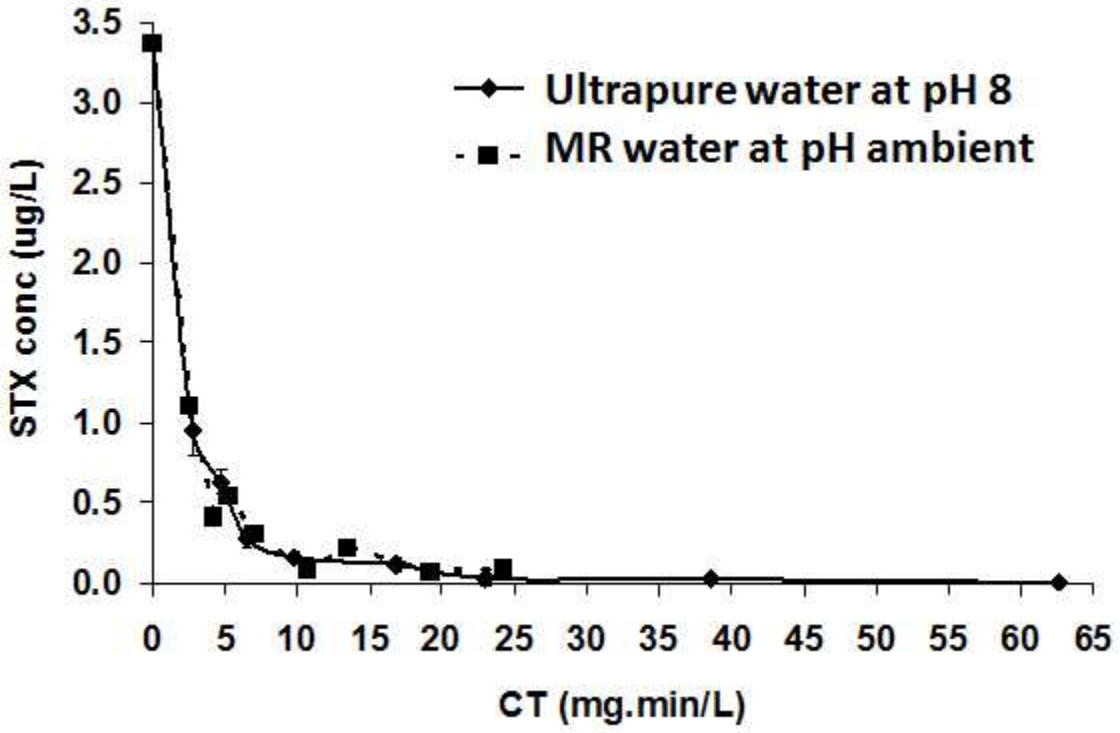


Figure Sl-*S5*. 122 µg/L STX extract oxidation with 3mg/L Cl_2 (a) in ultrapure water at pH 8, (b) in MR river water at pH ambient

Literature Cited

(1) Agence Française de Sécurité Sanitaire des Aliments (AFSSA) et l'Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET). *Avis de relatif à l'évaluation des risques liés à la présence de cyanobactéries dans les cours d'eau destinés à la baignade et/ou à d'autres usages*; AFSSA-AFSSET: France; 2006.

(2) Nicholson, B. C.; Shaw, G. R.; Morrall, J.; Senogles, P. J.; Woods, T. A.; Papageorgiou, J.; Kapralos, C.; Wickramasinghe, W.; Davis, B. C.; Eaglesham, G. K.; Moore. M. R. Chlorination for degrading saxitoxins (paralytic shellfish poisons) in water. *Environmental Technology 2003*, 24, 1341-1348.

(3) Cooperative Research Centre (CRC) for Water Quality and Treatment. *Chlorination of saxitoxins, research stages 1 to 3*; CRC for Water Quality and Treatment: Water Services Association of Australia; South Australia; Australia, 2005.

(4) Fristachi, A.; et al. Occurrence of Cyanobacterial Harmful Algal Blooms. In *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*; Hudnell, H. K., Ed; Springer Science+Business Media, LLC: New York 2008; pp 45-104.

(5) Chorus, I., Bartram J., Eds. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*, 1st, ed.; Taylor & Francis Group: New York, 1999.

(6) Carmichael, W. W.; Azevedo, S. M. F. O.; An, J. S.; Molica, R. J. R.; Jochimsen, E. M.; Lau, S.; Rinehart, K. L.; Shaw, G. R.; Eaglesham, G. K. Human fatalities from Cyanobacteria: chemical and biological evidence for Cyanotoxins. *Environmental Health Perspectives* **2001**, 109, 663-668.

(7) Merel, S.; Clément, M.; Thomas, O., Review State of the art on cyanotoxins in water and their behaviour towards chlorine. *Toxicon* **2010**, 55, 677–691.

(8) Svrcek, C.; Smith, D. W. Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *Environmental Engineering and Science* **2004**, 3, 155-185.