Physical, chemical, and mineralogical controls on retardation of anatoxin-a migration by sorption to natural soils with implications for groundwater protection

by Justin L. Hobart [1,2], Andrew M. O'Reilly [1,3,*] and Jennifer N. Gifford [1]

[1] Department of Geology & Geological Engineering, University of Mississippi, 120A Carrier Hall,

P.O. Box 1848, University, MS 38677-1848, USA

[2] Nyrstar East Tennessee Mines, New Market, TN 37820, USA

[3] National Sedimentation Laboratory, Agricultural Research Service, U.S. Department of Agriculture,

Oxford, MS, 38655 USA

* Correspondence: andrew.oreilly@usda.gov

Table of Contents

Methods used to perform batch experiments and analyze anatoxin-a concentrations

Equilibrium batch experiments were performed for each of the soil and sediment samples and generally follow procedures outlined in similar adsorption studies by Miller et al. (2001) and Klitzke et al. (2011). For each experiment, six batch tests were performed for each soil sample, and for simplicity, the six batch tests will henceforth be referred to as a "batch set." All ATX solutions and batch sets in this study were prepared at 25°C, and this temperature was kept constant throughout the batch test procedures.

The ATX-water solutions were prepared in glass flasks using 10 µg/mL Anatoxin-a (+) standards (PN 300620, Abraxis, Inc., Warminster, PA, USA). All flasks were covered in aluminum foil to prevent photodegradation. An initial stock solution of 15 µg/L was prepared from the standards and diluted with DIW to 0.3, 1, 3, and 7 µg/L. Approximately 3 mL were pipetted from each solution into 4 mL amber glass vials with polytetrafluoroethylene (PTFE) lined caps so that the concentration of ATX in each could be confirmed by enzyme-linked immunosorbent assay (ELISA) using a microtiter plate kit (PN 520060, Abraxis, Inc., Warminster, PA, USA).

In each batch set, the batch tests were conducted by placing 6 g of soil in six separate 50 mL centrifuge vials and then adding 30 mL of DIW in one vial and an ATX-water solution to each of the other vials to yield ATX at concentrations of 0, 0.3, 1, 3, 7, and 15 µg/L. After the soils and ATX-water solutions were combined in each batch set, the vials were capped and shaken in a covered box on an orbital shaker at 110 rpm for 24 hours. (Klitzke et al. (2011) noted that adsorption equilibrium between ATX and soil occurred after ~16 hours). After shaking, the pH and specific conductivity of the solutions were measured using a double junction pH meter (Orion 9102DJWP; Thermo Scientific, Waltham, MA, USA) and a conductivity meter (TraceableTM VWR International, Radnor, PA, USA). The solutions were then centrifuged at 400 rpm for 10 minutes and 5 mL of the resulting supernatant was withdrawn from each vial and temporarily placed in 50 mL graduated cylinders where 0.555 mL of a sample diluent concentrate (10x diluent supplied with ELISA kit) was added to prevent toxin degradation, and the mixture was then filtered using 0.2-micron polyethersulfone (PES) syringe filters (PN SF020E, Environmental Express, Charleston, SC, USA). Approximately 3 mL from each filtered sample was pipetted into 4 mL amber glass vials with PTFE-lined caps, covered in aluminum foil, and frozen at -17°C until ATX concentrations could be measured.

Within 18 hours after filtration, final toxin concentrations were measured by ELISA using an Abraxis Cyanotoxin Automated Assay System (PN 475200S, Abraxis, Inc., Warminster, PA, USA) at MSU. Toxin concentrations in each filtered sample were measured in duplicate on the microtiter plate following the standard instructions provided with the ELISA kit, which has minimum and maximum detection limits of 0.1 and 5.0 µg/L. For each of the two ELISA kits used, a 4-parameter logistic curve was used to calculate toxin concentrations from each sample via colorimetric absorbance methods (read at 450 nm). The calibration curves produced for each ELISA kit resulted in coefficient of determination (r^2) values greater than 0.999 and errors in the 0.75 µg/L positive control of less than 3%. Since the ELISA kit ATX maximum detection limit is 5.0 µg/L, the initial 7 and 15 µg/L stock solutions were diluted by factors of 2.8 and 6.0, respectively, such that an approximate concentration of 2.5 µg/L would be obtained. The sample diluent concentrate provided with the ELISA kit was mixed with DIW at a ratio of 1:10 and used for all sample dilutions. The batch test supernatant samples in which the 7 and 15 µg/L concentrates were used were diluted by factors of 3.111 and 6.666, respectively, accounting for the 0.555 mL of sample diluent concentrate previously added as a preservative. The initial stock solutions containing 0.3, 1, and 3 µg/L were not diluted, but the batch set samples containing ATX at these concentrations were diluted by a factor of 1.111 due to the addition of the 0.555 mL of sample diluent concentrate. The ATX quantification limit recommended by the ELISA kit manufacturer is that concentration equal to a relative absorbance of 90% (0.1 µg/L); for this work, data up to a relative absorbance of 98% were retained (0.02 µg/L). Percent recoveries for the stock solution nominal concentrations of 0.3, 1, 3, 7, and 15 µg/L ranged from 93 to 109%, with an average of 100%; the measured values—0.32, 1.09, 2.83, 6.77, and 13.99 µg/L—were used for all subsequent calculations.

Table S1. Pearson product-moment correlation coefficient matrix of physical, chemical, and mineralogical characteristics for the eight samples studied. Pearson r values < -0.7 or > 0.7 are shown in bold italics.

			Clay-fraction mineral (wt %)									Extractable concentration (mg/kg)							Total solid-phase concentration (mg/kg)													
	Kd (L/kg)	Rf (-)	Chlor-ite	Kaolin-ite	Mica	Smec-tite	Quartz	pH soil	pH sol'n	SC (µS/cm)	CEC (cmol+/kg)	P	K	Na	Mg	Ca	Zn	Silt + Clay (%)	Al	Si	K	S	P	Ca	Ti	Cr	Mn	Fe	Zn	Sr	Zr	SiO2
Kd (L/kg)	1.00																															
Rf (-)	0.47	1.00																														
Chlorite (wt %)	0.19	0.07	1.00																													
Kaolinite (wt %)	***0.76***	0.13	-0.18	1.00																												
Mica (wt %)	-0.26	0.12	0.45	-0.33	1.00																											
Smectite (wt %)	0.38	***0.88***	-0.22	0.13	-0.01	1.00																										
Quartz (wt %)	-0.42	-0.48	-0.25	-0.42	-0.67	-0.39	1.00																									
pH soil	0.10	-0.03	0.08	0.13	0.63	0.10	-0.67	1.00																								
pH sol'n	-0.11	-0.16	0.08	-0.08	0.30	-0.11	-0.18	0.68	1.00																							
Sp. Cond.(µS/cm)	-0.28	-0.39	-0.27	-0.02	0.38	-0.17	-0.25	***0.83***	***0.79***	1.00																						
CEC (cmol+/kg)	-0.18	0.67	0.03	-0.34	0.57	0.54	-0.45	0.25	0.31	0.13	1.00																					
Extract. P (mg/kg)	-0.05	0.52	-0.25	-0.01	0.36	0.32	-0.40	-0.06	-0.31	-0.16	0.62	1.00																				
Extract. K (mg/kg)	-0.19	0.53	0.26	-0.39	***0.70***	0.34	-0.47	0.37	0.47	0.22	***0.95***	0.48	1.00																			
Extract. Na (mg/kg)	-0.14	***0.79***	-0.13	-0.26	0.36	***0.75***	-0.38	-0.01	-0.13	-0.18	***0.86***	0.67	0.69	1.00																		
Extract. Mg (mg/kg)	-0.11	***0.76***	-0.02	-0.31	0.40	0.63	-0.34	0.09	0.20	-0.03	***0.98***	0.62	***0.89***	***0.89***	1.00																	
Extract. Ca (mg/kg)	-0.17	0.54	0.09	-0.32	0.69	0.51	-0.55	0.55	0.53	0.40	***0.93***	0.40	***0.94***	***0.73***	***0.86***	1.00																
Extract. Zn (mg/kg)	-0.01	0.09	0.03	-0.02	0.68	0.16	-0.63	***0.95***	***0.77***	***0.83***	0.49	0.10	0.60	0.16	0.34	***0.73***	1.00															
Silt + Clay (%)	-0.12	0.26	0.41	-0.24	***0.83***	-0.07	-0.56	0.39	0.30	0.19	0.68	0.59	***0.81***	0.36	0.57	0.65	0.55	1.00														
Total Al (mg/kg)	0.33	0.67	0.40	0.11	0.67	0.36	***-0.80***	0.37	0.14	-0.03	***0.71***	0.64	***0.76***	0.54	0.66	0.67	0.48	***0.85***	1.00													
Total Si (mg/kg)	-0.26	***-0.75***	-0.28	0.04	-0.63	-0.51	***0.70***	-0.40	-0.26	-0.06	***-0.85***	-0.62	***-0.87***	-0.65	***-0.82***	***-0.82***	-0.57	***-0.80***	***-0.95***	1.00												
Total K (mg/kg)	0.12	0.40	0.30	-0.02	0.66	0.06	-0.59	0.38	0.33	0.16	0.68	0.64	***0.78***	0.35	0.61	0.63	0.55	***0.95***	***0.90***	***-0.88***	1.00											
Total S (mg/kg)	-0.24	***-0.75***	-0.51	0.11	-0.44	-0.40	0.47	-0.04	-0.17	0.27	***-0.77***	-0.44	***-0.82***	-0.60	***-0.79***	-0.66	-0.23	-0.69	***-0.84***	***0.87***	***-0.74***	1.00										
Total P (mg/kg)	0.05	0.17	***0.82***	-0.18	0.45	0.00	-0.31	0.24	0.43	-0.01	0.30	-0.34	0.49	0.10	0.23	0.41	0.25	0.35	0.37	-0.34	0.25	-0.60	1.00									
Total Ca (mg/kg)	0.02	0.47	0.08	-0.14	0.67	0.42	-0.63	***0.71***	0.66	0.54	***0.83***	0.36	***0.88***	0.52	***0.74***	***0.94***	***0.88***	0.70	***0.72***	***-0.85***	***0.74***	-0.61	0.34	1.00								
Total Ti (mg/kg)	-0.19	0.11	0.59	-0.42	***0.89***	-0.17	-0.47	0.46	0.34	0.24	0.55	0.35	***0.74***	0.22	0.42	0.60	0.57	***0.94***	***0.72***	-0.69	***0.81***	-0.59	0.47	0.64	1.00							
Total Cr (mg/kg)	-0.43	-0.43	-0.19	-0.56	-0.46	-0.18	***0.85***	-0.42	-0.23	-0.13	-0.44	-0.49	-0.48	-0.27	-0.37	-0.43	-0.45	-0.62	***-0.81***	0.69	***-0.73***	0.58	-0.28	-0.54	-0.41	1.00						
Total Mn (mg/kg)	0.16	0.33	-0.04	0.05	0.39	0.09	-0.39	0.37	0.42	0.30	0.59	0.59	0.64	0.23	0.55	0.53	0.57	***0.76***	***0.71***	***-0.76***	***0.90***	-0.54	-0.06	***0.72***	0.59	-0.58	1.00					
Total Fe (mg/kg)	-0.16	0.45	0.26	***-0.73***	0.20	0.44	0.16	-0.09	0.00	-0.22	0.48	0.05	0.47	0.48	0.54	0.46	0.06	0.17	0.17	-0.37	0.10	-0.42	0.26	0.32	0.32	0.40	0.07	1.00				
Total Zn (mg/kg)	0.11	0.67	0.28	-0.23	0.64	0.48	-0.57	0.44	0.42	0.17	***0.89***	0.50	***0.94***	0.64	***0.85***	***0.89***	0.64	***0.79***	***0.86***	***-0.96***	***0.83***	***-0.84***	0.41	***0.91***	***0.72***	-0.53	***0.74***	0.51	1.00			
Total Sr (mg/kg)	0.08	0.42	0.19	-0.10	0.62	0.11	-0.52	0.34	0.28	0.16	***0.70***	0.70	***0.77***	0.39	0.65	0.63	0.54	***0.92***	***0.86***	***-0.87***	***0.98***	-0.70	0.12	***0.74***	***0.79***	-0.63	***0.93***	0.19	***0.84***	1.00		
Total Zr (mg/kg)	-0.08	***-0.82***	***0.02***	0.12	-0.14	***-0.88***	0.33	0.02	0.05	0.23	-0.69	-0.28	-0.53	***-0.88***	***-0.75***	-0.63	-0.09	-0.05	-0.37	0.51	-0.07	0.56	-0.31	-0.42	0.03	0.17	0.04	-0.52	-0.51	-0.08	1.00	
Total SiO2 (mg/kg)	-0.26	***-0.75***	***-0.28***	0.04	-0.63	-0.51	***0.70***	-0.40	-0.26	-0.06	***-0.85***	-0.62	***-0.87***	-0.65	***-0.82***	***-0.82***	-0.57	***-0.80***	***-0.95***	1.00	***-0.88***	***0.87***	-0.34	***-0.85***	-0.69	0.69	***-0.76***	-0.37	***-0.96***	***-0.87***	0.51	1.00

Table S2. Anatoxin-a stock water (C_w), equilibrium aqueous (C_e), and computed sorbed (C_s) concentrations and raw absorbance values for all samples and the standard curves.

Sample ID	Soil Series	C_w (µg/L)	C_e (µg/L)	C_s (µg/kg)
CS0.3	Cs	0.32	0.05	1.36
CS_1	Cs	1.10	0.11	4.93
CS_3	Cs	2.83	0.08	13.7
CS_7	Cs	6.77	0.29	32.4
CS_15	Cs	14.0	0.89	65.5
FF0.3	Ff	0.32	0.08	1.20
FF_1	Ff	1.10	0.08	5.09
FF_3	Ff	2.83	0.53	11.5
FF_7	Ff	6.77	0.73	30.2
FF_15	Ff	14.0	1.65	61.7
AT0.3	At	0.32	0.02	1.49
AT_1	At	1.10	0.09	5.06
AT_3	At	2.83	0.56	11.3
AT_7	At	6.77	0.57	31.0
AT_15	At	14.0	1.84	60.8
MEF1_0.3	MeF	0.32	0.05	1.35
MEF_1	MeF	1.10	0.11	4.96
MEF_3	MeF	2.83	0.2	13.1
MEF_7	MeF	6.77	0.46	31.6
MEF_15	MeF	14.0	1.29	63.5
CRF1_0.3	Ru1	0.32	0.06	1.30
CRF1_1	Ru1	1.10	0.06	5.18
CRF1_3	Ru1	2.83	0.21	13.1
CRF1_7	Ru1	6.77	0.62	30.8
CRF1_15	Ru1	140	1.72	61.4

Sample ID	Abs1	Abs2	Plate #	Comment
ATX1 Std 0	1.106	1.102	1	plates 1 & 2 standard 0.0 ug/L
ATX1 Std 1	0.957	0.954	1	plates 1 & 2 standard 0.15 ug/L
ATX1 Std 2	0.775	0.782	1	plates 1 & 2 standard 0.40 ug/L
ATX1 Std 3	0.549	0.537	1	plates 1 & 2 standard 1.0 ug/L
ATX1 Std 4	0.320	0.307	1	plates 1 & 2 standard 2.5 ug/L
ATX1 Std 5	0.164	0.157	1	plates 1 & 2 standard 5.0 ug/L
ATX1 Ctrl	0.613	0.615	1	plates 1 & 2 control 0.75 ± 0.185 ug/L
Stock0.3	0.835	0.826	1	stock solution (Cw) nominal 0.3 ug/L
Stock1	0.559	0.476	1	stock solution (Cw) nominal 1 ug/L
Stock3	0.281	0.272	1	stock solution (Cw) nominal 3 ug/L
Stock7	0.310	0.311	1	stock solution (Cw) nominal 7 ug/L
Stock15	0.312	0.326	1	stock solution (Cw) nominal 15 ug/L
CS0.3	1.058	1.056	2	environmental sample (Ce)
FF0.3	1.048	1.008	2	environmental sample (Ce)
AT0.3	1.082	1.080	2	environmental sample (Ce)
MEF1_0.3	1.058	1.050	2	environmental sample (Ce)
CRF1_0.3	1.070	1.022	2	environmental sample (Ce)
CRF2_0.3	1.033	1.073	2	environmental sample (Ce)
GT1_0.3	1.097	1.067	2	environmental sample (Ce)
GT2_0.3	1.034	1.010	2	environmental sample (Ce)
CS_1	1.008	0.994	2	environmental sample (Ce)
FF_1	1.029	1.028	2	environmental sample (Ce)
AT_1	1.030	1.015	2	environmental sample (Ce)
MEF_1	1.005	1.007	2	environmental sample (Ce)
CRF1_1	1.036	1.052	2	environmental sample (Ce)
CRF2_1	1.017	1.038	2	environmental sample (Ce)
GT1_1	0.996	1.045	2	environmental sample (Ce)
GT2_1	0.970	0.968	2	environmental sample (Ce)
CS_3	0.999	1.051	2	environmental sample (Ce)

Sample ID	Soil Series	C_w (µg/L)	C_e (µg/L)	C_s (µg/kg)
CRF2_0.3	Ru2	0.32	0.05	1.34
CRF2_1	Ru2	1.10	0.08	5.09
CRF2_3	Ru2	2.83	0.45	11.9
CRF2_7	Ru2	6.77	1.10	28.4
CRF2_15	Ru2	14.0	2.64	56.8
GT1_0.3	Ct1	0.32	0.02	1.50
GT1_1	Ct1	1.1.0	0.09	5.05
GT_3	Ct1	2.83	0.26	12.9
GT1_7	Ct1	6.77	0.78	30.0
GT1_15	Ct1	14.0	1.98	60.1
GT2_0.3	Ct2	0.32	0.09	1.17
GT2_1	Ct2	1.10	0.15	4.74
GT2_3	Ct2	2.83	0.32	12.5
GT2_7	Ct2	6.77	0.45	31.6
GT2_15	Ct2	14.0	1.28	63.6

Sample ID	Abs1	Abs2	Plate #	Comment
FF_3	0.752	0.729	2	environmental sample (Ce)
AT_3	0.724	0.727	2	environmental sample (Ce)
ATX3 Std 0	1.165	1.152	3	plate 3 standard
ATX3 Std 1	0.990	--	3	plate 3 standard 0.15 ug/L; Abs2 bad reading
ATX3 Std 2	0.799	0.804	3	plate 3 standard 0.40 ug/L
ATX3 Std 3	0.561	0.543	3	plate 3 standard 1.0 ug/L
ATX3 Std 4	--	--	3	plate 3 standard 2.5 ug/L; bad readings
ATX3 Std 5	0.162	0.155	3	plate 3 standard 5.0 ug/L
ATX3 Ctrl	0.636	0.623	3	plate 3 control 0.75 ± 0.185 ug/L
MEF_3	0.960	0.970	3	environmental sample (Ce)
CRF1_3	1.019	0.902	3	environmental sample (Ce)
CRF2_3	0.801	0.796	3	environmental sample (Ce)
GT_3	0.936	0.916	3	environmental sample (Ce)
GT2_3	0.885	0.870	3	environmental sample (Ce)
CS_7	1.057	1.048	3	environmental sample (Ce)
FF_7	0.929	0.917	3	environmental sample (Ce)
AT_7	0.967	0.962	3	environmental sample (Ce)
MEF_7	0.992	1.003	3	environmental sample (Ce)
CRF1_7	0.950	0.952	3	environmental sample (Ce)
CRF2_7	0.842	0.827	3	environmental sample (Ce)
GT1_7	0.893	0.927	3	environmental sample (Ce)
GT2_7	1.005	0.996	3	environmental sample (Ce)
CS_15	1.005	1.018	3	environmental sample (Ce)
FF_15	0.890	0.933	3	environmental sample (Ce)
AT_15	0.894	0.885	3	environmental sample (Ce)
MEF_15	0.978	0.937	3	environmental sample (Ce)
CRF1_15	0.920	0.886	3	environmental sample (Ce)
CRF2_15	0.803	0.809	3	environmental sample (Ce)
GT1_15	0.869	0.879	3	environmental sample (Ce)
GT2_15	0.941	0.975	3	environmental sample (Ce)

References

Klitzke, S.; Beusch, C.; Fastner, J. Sorption of the Cyanobacterial Toxins Cylindrospermopsin and Anatoxin-a to Sediments. Water Research 2011, 45, doi:10.1016/j.watres.2010.10.019.

Miller, M.J.; Critchley, M.M.; Hutson, J.; Fallowfield, H.J. The Adsorption of Cyanobacterial Hepatotoxins from Water onto Soil during Batch Experiments. Water Research 2001, 35, doi:10.1016/S0043-1354(00)00419-X.