

Article

Physical, Chemical, and Mineralogical Controls on Retardation of Anatoxin-a Migration by Sorption to Natural Soils with Implications for Groundwater Protection

Justin L. Hobart^{1,2}, Andrew M. O'Reilly^{1,3,*}  and Jennifer N. Gifford¹¹ Department of Geology & Geological Engineering, University of Mississippi, 120A Carrier Hall, P.O. Box 1848, University, MS 38677-1848, USA² Nyrstar East Tennessee Mines, New Market, TN 37820, USA³ National Sedimentation Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Oxford, MS 38655, USA

* Correspondence: andrew.oreilly@usda.gov

Abstract: Increasing prevalence of cyanotoxins in surface water bodies worldwide threatens groundwater quality when contaminated water recharges an aquifer through natural or artificial means. The subsurface fate of anatoxin-a (ATX) is not well studied. Laboratory batch experiments were performed to expand the current knowledge of ATX sorption affinities to geologic media, with a focus on natural soil (Vertisol, Ultisol, Alfisol, and Inceptisol) and physical, chemical, and mineralogical characteristics. For a range of aqueous ATX concentrations (0.3–14 µg/L), linear, Freundlich, and Langmuir isotherms fit observed data well ($r^2 = 0.92$ – 1.00 , RMSE = 0.4 – 6.3 µg/kg). Distribution coefficient (K_d) and retardation factor (R_f) values were computed for the linear isotherm, giving K_d of 22.3 – 77.1 L/kg and R_f of 62 – 256 . Average percent removals were 85.0 – 92.2% . The strongest predictors of K_d were kaolinite and smectite group mineral abundances and for R_f were smectite group and silt and clay abundances. Results indicate that loamy, silty, or clayey soils—particularly Vertisols—tend to substantially slow migration of ATX through natural soil systems. Where implemented as a functionalized amendment in an engineered pollution control media, such soils may enhance natural ATX attenuation processes, thereby supporting the protection of in situ and extracted groundwater during irrigation, natural and managed aquifer recharge, or riverbank filtration.

Keywords: anatoxin-a; cyanotoxin; emerging contaminants; sorption; soil; clay mineralogy; pollution control



Citation: Hobart, J.L.; O'Reilly, A.M.; Gifford, J.N. Physical, Chemical, and Mineralogical Controls on Retardation of Anatoxin-a Migration by Sorption to Natural Soils with Implications for Groundwater Protection. *Water* **2022**, *14*, 2869. <https://doi.org/10.3390/w14182869>

Academic Editors: Jiangshan Li, Xiao Yang and Fei Wang

Received: 31 July 2022

Accepted: 9 September 2022

Published: 14 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Groundwater shortages are increasing worldwide, leading to an increasing use of artificial recharge techniques to support sustainable aquifer management [1,2]. Using surface water for enhancement of aquifer recharge or irrigation carries with it the risk of contaminants impacting groundwater quality. One group of contaminants of emerging concern are toxins produced by cyanobacteria—also called cyanotoxins—which commonly proliferate in rivers, lakes, and reservoirs [3–6]. Cyanotoxins pose a risk to human and animal health and can bioaccumulate in food crops [7–10]. This risk is of particular concern for artificial recharge facilities where infiltrated water may subsequently be withdrawn for consumption or irrigation, such as riverbank filtration and stormwater harvesting [11,12]. Consequently, recognition is growing of the need for groundwater protection technologies [13].

Cyanotoxins comprise over 100 compounds with differing toxicological characteristics that generally can be classified into one of three groups based on targeted organ systems: hepatotoxins (causing liver damage), neurotoxins (causing neuromuscular disruption), or dermatotoxins (causing skin irritation) [4]. One particularly potent neurotoxin is anatoxin-a (ATX), which is a secondary bicyclic amine, related to alkaloids in structure, produced

by various species of cyanobacteria in freshwater ecosystems [14,15]. Once ingested by livestock or other animals, this cyanotoxin irreversibly disrupts nerve receptors in the muscular system, which results in paralysis, respiratory arrest, and death, usually within a few minutes to a few hours after ingestion [16], earning it the dubious distinction of being termed “Very Fast Death Factor” upon its discovery in 1961 [17]. ATX is described as an acutely toxic compound—having a median lethal dose (LD₅₀) of 20–250 µg/kg in mice—and has been observed in water bodies throughout North America, Europe, Asia, and Australia as well as in New Zealand and Kenya [18–22]. The toxin was found to be the likely culprit responsible for the rapid deaths of over 100 elk in rural New Mexico during the summer of 2013, and several cases of dog poisoning by ATX have been reported over the years [23–25]. The first known case of a human death attributed to possible poisoning by ATX occurred in Wisconsin in 2002 after a teenager ingested pond water containing the cyanotoxin; however, the cause of the fatality remained inconclusive [19]. Recently, the first cases of human food poisoning where ATX was definitively identified as the possible cause were reported by Biré et al. [26], where 26 people reported being sick after eating sea figs over three events in 2011, 2012, and 2018. Despite its acute toxicity, no water quality standards have been outlined for ATX by federal agencies in the United States or by governments in many other countries, and concerns regarding human injury by ATX and other cyanotoxins are becoming more prevalent [7,27]. Based on the limited data currently available for acute and subchronic (28 days) ATX toxicity, Testai [28] recommends provisional health-based reference values for short-term exposure via drinking water of 30 µg/L for adults and 6 µg/L for infants and small children; exposure to concentrations up to these values is expected to be safe.

ATX is highly soluble in water, thus environmental transport of the toxin is a concern. ATX has a low molecular weight of 165 Da and the chemical formula C₁₀H₁₅NO [21,29]. ATX occurs predominantly in the protonated form under typical environmental conditions (pH of 6–9) and has been shown to decompose rapidly in sunlight as well as under alkaline pH conditions [30–32]. Stevens and Krieger [31] found that ultraviolet (UV) radiation converts the toxin into harmless organic compounds within a few hours in natural hydrologic systems at a pH of 8–9. However, the rate of ATX degradation as a function of UV radiation is disputed among studies [32]. Although Stevens and Krieger [31] found ATX to have a half-life of only 1–2 h under natural sunlight conditions at pH of 8–9, a study by Smith and Sutton [33] showed a much slower degradation time. Smith and Sutton [33] reported, at pH 8 and 10 under laboratory experiments using reservoir water spiked with the toxin and natural photoperiod conditions, less than 5% of ATX was present after 14 days, implying a half-life of 3.2 days assuming an exponential decay. Kaminski et al. [34] reported only a 10% degradation of ATX after 1 h of irradiation under photosynthetically relevant UV conditions, but this may be largely attributable to acidic experimental conditions with a pH of 3.5. Furthermore, Stevens and Krieger [31] noted that the rate of ATX degradation in natural systems is dependent on the UV radiation intensity and radiation exposure in the water column. The New Zealand Ministry of Health [22] reports that the lifespan of ATX can range from days to months in the absence of sunlight.

Accounts of ATX in lakes and rivers have been extensively reported and investigated since the toxin was discovered decades ago. Owing to its photosensitivity, ATX persistence and transport in surface water may be limited; however, few researchers have investigated ATX occurrences in wells, aquifers, or vadose zone systems. The presence of ATX in an agricultural setting has been confirmed, with the toxin being found in agricultural soil, subsurface tile drainage water, and in groundwater from an artesian well at the farm [35]. Zhang et al. [35] also identified the prevalent cyanotoxin microcystin in agricultural soil and groundwater from municipal drinking water wells in the watershed. Microcystin-LR has also been measured at concentrations exceeding 1 µg/L (the World Health Organization has issued a provisional guideline of 1 µg/L in drinking water [27]) in groundwater samples from open wells in Saudi Arabia, and several studies have reported the accumulation of microcystin-LR as well as cylindrospermopsin in crops that were irrigated with

contaminated water [8,36,37]. Understanding the transport and fate of ATX in addition to other cyanotoxins in these environments could help identify potential groundwater sources vulnerable to cyanotoxin contamination.

Given the net positive charge of the ATX molecule under typical environmental conditions, its fate may be significantly affected by electrostatic attraction with negatively charged sites on soil particle surfaces or ion exchange with other soil cations. Klitzke et al. [38] were some of the first researchers that investigated ATX sorption affinities to different geologic media using equilibrium batch experiments. They quantified the abilities of ten hydromorphic sediments and three soils to remove ATX via adsorption to sediment or soil grains. The researchers produced two nonlinear adsorption isotherms from batch test results and reported adsorption coefficients for six of the sediments and two of the soils. Klitzke et al. [38] concluded that clay-rich and organic-rich materials removed ATX from water more efficiently than sandier materials; however, the study is limited with respect to ATX adsorption in natural soils since sorption coefficients for only two soils (a sandy soil and an organic mud) was reported. Bialczyk et al. [39] conducted kinetic batch experiments to measure the ATX adsorption and desorption characteristics of three clay minerals (palygorskite, kerolite, and sepiolite) and one clay sample (bentonite predominantly composed of montmorillonite) and found that sorption was related to clay mineralogy, particularly surface area. All samples had low organic carbon content (0.2–1.6 weight [wt.] %), thus the effects of organic materials were not apparent. The sorption properties of other cyanotoxins, such as nodularin, microcystin LR, and cylindrospermopsin, have been reported in the literature [12,36,38,40,41], but different charge characteristics of these molecules limits the relevance of these findings to ATX.

Even though batch experiments using geologic materials have been performed with respect to ATX to describe sorption characteristics of the cyanotoxin in geologic media, the current literature on this topic is limited and rarely focuses on the toxin adsorption as a function of soil type, individual soil horizons, or soil chemical or mineralogical properties. Therefore, the purpose of this study is to expand the current knowledge of ATX sorption affinities to geologic media, specifically with a focus on the physical, chemical, and mineralogical characteristics of natural soils (Vertisol, Ultisol, Alfisol, and Inceptisol). The results from this study will provide additional insight regarding the transport and fate of ATX in natural soil systems, including those involving bank filtration, agricultural irrigation, and artificial recharge. In closing, we discuss the potential to protect groundwater from impacts posed by the presence of ATX in the environment, and we comment on the role of functionalized amendments for targeting ATX removal via engineered media.

2. Materials and Methods

2.1. Study Area

Soil samples were collected at five sites along an east–west transect through Grenada County in north-central Mississippi (MS), USA, across a gradient of land resource areas and a variety of soil types overlying Eocene (56 Ma) to Quaternary (present) age sediments (Figure 1). Land resource areas are defined by the Natural Resources Conservation Service (NRCS) by geographic location, topography and landscape features, geology, soil group, hydrologic unit, and land use, and are typically used for agricultural planning and resource conservation programs [42]. The land resource areas in Grenada County (from west to east) are the Delta, Upper Thick Loess, Upper Thin Loess, and Upper Coastal Plain (Figure 1). Soils in these areas include Quaternary expansive clay deposits in the Delta [43], silty pedoglacial Pleistocene soils in the loess hills of western and central Grenada County, and soils from Eocene fluviomarine deposits in the hilly eastern half of the county. Grenada County is also home to Grenada Lake, which serves as a major flood-prevention and recreational reservoir in MS. Although ATX has not been reported in these waters, Dash et al. [44] revealed the presence of a cyanobacterial pigment, phycocyanin, and microcystin-LR in Grenada Lake. This suggests that the lake is prone to harmful algal blooms and, therefore, these surface waters could serve as potential sources of ATX in the study area.

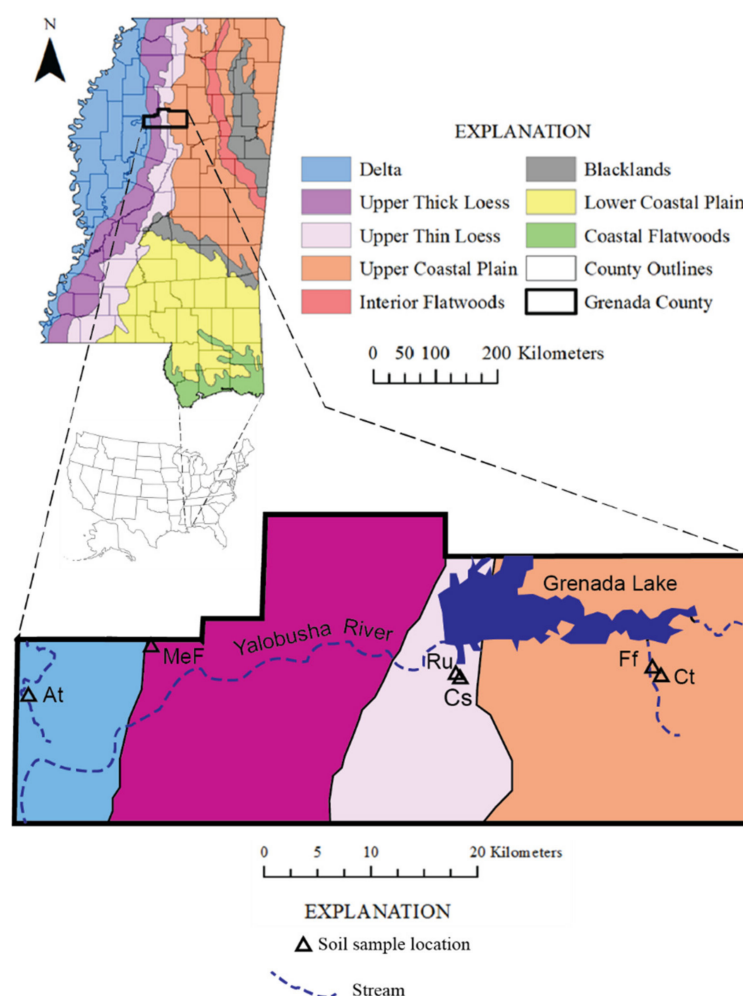


Figure 1. Locations of study area and land resources areas (modified from Vanderford [45]) in Mississippi, USA, and sample collection sites. Soil and sediment abbreviations are At, Alligator Association; Cs, channel sand; Ct, Cuthbert Series (Ct1, A horizon; Ct2, B horizon); Ff, Falaya Silt Loam Series; MeF, Memphis Silt Loam Series; Ru, Ruston Series (Ru1, A horizon; Ru2, E horizon).

The climate in the study area is humid and subtropical, with average temperatures ranging from -0.4 to 33 °C and an annual average rainfall of 156 cm (1991–2020 climate normals; [46]). These climatic factors affect soils in the county by leaching or removing soluble materials, encouraging moderately to highly acidic soil conditions, and by transporting colloids and organic substances deeper into soil profiles, resulting in the rapid formation of soils in younger sediments and well-developed horizons in older soils [43].

2.2. Soil Characteristics

2.2.1. Pedology

Eight samples were collected in Grenada County, MS, representing four soil orders: Vertisol, Ultisol, Alfisol, and Inceptisol. The soils chosen for this study were selected based on general prevalence in each of the four land resource areas in the county indicated by NRCS [47]. Samples consisted of soils from the Alligator Association and the Memphis, Falaya, Cuthbert, and Ruston Series, as well as sediment from gullied land that visual observation indicated is ephemerally inundated.

Because previous research has shown adsorption of cyanotoxins occurs in clays [38,39], it is assumed that clay-dominant soils will adsorb ATX more effectively than coarser-grained soils. Thus, sampling focused on soils with greater proportions of fines, containing more than ~25% silt and clay.

Alligator Association

Soils in the Alligator Association (At) are thermic Vertisols that occur in broad flat woodlands, backswamps, and meander scrolls within the Mississippi River alluvial plain in the Delta land resource area of Grenada County [43,48]. Alligator Association soils are moist for most of the year and, thus, belong to the Aquert soil suborder [48]. These soils generally consist of strongly acidic, poorly-drained, plastic, dark-grey to brown montmorillonitic clays and silty clay loams [43]. Alligator soils contain few organics, lack a B horizon, and have similar A and C horizons [43,47].

Memphis Silt Loam Series

The Memphis silt loam (MeF) is a thermic Alfisol that is commonly found along narrow ridges and steep slopes in forested areas in the western parts of the Upper Thick Loess as well as on terraces in the Upper Coastal Plain [43,48]. Alfisols are weathered soils that form in humid climates (often in forests) and show apparent signs of downward clay movement within the soil profile [49]. In Grenada County, the Memphis silt loam was derived from pedoglacial windblown silt and clay [50], and the loess deposits that underlie this soil generally range in thickness from ~3 to 16 m, with the maximum thickness occurring along the eastern margin of the Delta land resource area [51]. The A horizon of this well-drained soil is composed of a slightly-acidic brown silt loam underlain by a strongly-acidic dark-brown silty clay loam to silt loam in the B horizon [43].

Falaya Silt Loam Series

The Falaya silt loam (Ff) is derived from floodplain alluvium and has been described as an Inceptisol as well as an Entisol [43,48]. These soil orders are similar in that they describe immature soils that lack well-developed horizons; however, Inceptisols are considered better-developed than Entisols [49]. Since the Grenada County Soil Survey [43] characterizes the Falaya silt loam as an Inceptisol, the soil is classified as such in this study. The Falaya soil serves as a major agricultural soil in the county and is commonly found adjacent to streams in the Upper Thick Loess, Upper Thin Loess, and Upper Coastal Plain regions. This soil consists of strongly-acidic, friable, brown to grey silt loams containing only A and C horizons within a depth of ~1.5 m [43].

Ruston Series

Ruston Series soils (Ru) are siliceous Ultisols found in the middle to upper parts of steep slopes in the hilly uplands in eastern Grenada County [43]. Soils in this series are derived from highly weathered fluviomarine sediments in the Eocene Claiborne Group, which consist of sands, loamy sands, and sandy clays [43,51]. These soils are extremely well-developed. The A horizon in Ruston Series soils is composed of dark-brown to yellow-brown fine sandy loams underlain by a yellow-red sandy clay loam or loam in the B horizon [43,45]. The presence of an eluviated or leached E horizon has also been reported in Ruston soils [48]. These soils are well-drained, are strongly to very strongly acidic, and have thick soil profiles that extend to a fine sandy loam C horizon at a depth of ~1–1.5 m [43].

Cuthbert Series

Cuthbert soils (Ct) are Ultisols that occur with and are very similar to the Ruston soils, but generally contain more clay and have shallower soil profiles [43,48]. These soils contain distinct A, B, and C horizons that generally grade from friable grey-brown or brown fine sandy loams to yellowish-red and mottled brown clays to light-brown or light-grey clay shales and yellow-red sand lenses [39]. These soils are somewhat poorly-drained, strongly to very strongly acidic, and contain a strong fragipan near the ground surface [43,45].

Channel Sand

Although not a true soil, a fluvial channel sediment (Cs) was collected in an area mapped as sandy gullied land downslope of Ruston soils in the Upper Thin Loess region. The sample location is near the base of the gullied section in an ephemeral inundated area near a stream (Gibbs Creek). Sediment is composed of poorly-sorted, yellow-brown, angular to sub-rounded, fine to coarse sand grains (~70–75%) with notable silt or clay fractions (~25–30%) and little to no gravel. Sand lenses are common in the parent materials of the Cuthbert and Ruston soils; therefore, it was suspected that the channel sediment might serve as an analogue for soils in the deeper C horizons of soils from these series [43].

2.2.2. Sample Collection

A 91.4-cm chrome-plated soil probe with a 30.5-cm long by 1.9-cm diameter core extractor was used to collect core samples from each of the five soils and channel sediment. The collected cores ranged from 15.2 to 76.2 cm in total length, were first described in the field, and then subjected to a more thorough analysis at the University of Mississippi (UM) to identify the soil horizons, horizon depths, soil grain-size composition, and soil series at the sampling locations. The cores were stored in 76.2-cm split polyvinyl chloride (PVC) pipes. Loose soil samples from each horizon were collected using a small hand shovel and stored in labeled resealable bags.

The At and Ff soils contained only a single observable horizon, thus, only one sample was collected from each of these soils. The MeF soil contained two observable horizons, but only the upper horizon was sampled. The Ct soil contained apparent A and B horizons from which samples were collected. These horizons are referred to as “Ct1” and “Ct2”, respectively, in this study. The Ru soil contained three notable horizons in the upper 76.2 cm; however, only samples from the uppermost A and E horizons were sampled (labeled “Ru1” and “Ru2”, respectfully). Soil materials from a total of seven horizons and the channel sediment were sampled for ATX sorption analyses.

After collection and description, the soil core samples were oven-dried for 12 h at 37 °C. Once dry, the samples were disaggregated using a mortar and either a rubber or ceramic pestle so that the maximum surface area of the soil grains was exposed without crushing the grains.

2.2.3. Physical and Extractable Chemical Properties

Soil grain size analyses were performed for each sample to estimate the overall abundances of sands and combined silt and clay fractions. The grain size analyses were conducted by mechanical sieving using standard 1400, 500, 355, 250, 180, 125, 90, and 63 µm sieves.

The bagged samples were sent to the Mississippi State Extension Service Laboratory at Mississippi State University (MSU) to determine the following soil chemical properties: pH, cation exchange capacity (CEC), and abundances of extractable P, Na, K, Mg, Ca, and Zn. Soil pH values were measured using a soil:water slurry with a 1:2 ratio of soil to distilled, deionized water and mercury-free pH combination electrodes (AccuTupH Rugged Bulb; PN 13-620-183A, Fisher Scientific, Pittsburgh, PA, USA). Elemental concentrations were measured using an inductively coupled plasma-optical emission spectrometer (Spectroblue ICP-OES SOP; PN 760004562, Spectro Analytical Instruments, Inc., Mahwah, NJ, USA), and CEC values were calculated using the sum of measured K, Ca, Mg, and acidity as described by Sikora et al. [52].

2.2.4. Elemental Composition and Mineralogy

Samples analyzed for elemental composition and mineralogy were pulverized using a benchtop ring mill batch pulverizer (Rocklabs-Scott, Charlotte, NC, USA). The pulverizer was thoroughly cleaned prior to and between samples to minimize any potential contamination between samples. Samples were sent to the University of Florida (UF) Environmental Pedology Laboratory for X-ray fluorescence (XRF) analysis to quantify the

elemental composition of the soil samples. XRF was conducted in a He atmosphere using an X-ray fluorescence spectrophotometer (EDX 7000, Shimadzu Corporation, Kyoto, Japan). Each sample was loaded in a polypropylene cup with a mylar bottom and analyzed twice: once for 100 s with an X-ray voltage of 15 kV (for improved sensitivity of light elements) and once for 100 s with an X-ray voltage of 50 kV. Elemental concentrations (Al, Si, K, S, P, Ca, Ti, Cr, Mn, Fe, Zn, Sr, Zr) were estimated using linear calibration curves based on five standard reference materials analyzed alongside unknown samples. Coefficients of determination for the calibration curves ranged from 0.95 to 1.00 with a median of 0.98. Silica (SiO₂) concentration was computed from the measured Si concentration.

After XRF analysis, samples were analyzed by X-ray diffraction (XRD) at the UF Mineralogy and Soil Chemistry Laboratory. Due to the limited sample size, the clay-sized fraction of soil samples was collected using a modified micropipette method [53]. The sample was suspended in deionized water (DIW) at a 1:10 soil to DIW ratio, shaken overnight, and allowed to settle for 1 h and 50 min, after which the top 2.5 mL of the suspension was collected. The sample was then centrifuged briefly at 14,000 rpm, after which the DIW was decanted and replaced with 1 M MgCl₂. The sample was resuspended, shaken for 10 min, and centrifuged once again. The MgCl₂ was pipetted off and replaced with DIW, after which the clay particles were resuspended. This sample was dried down on a low-background silicon-based slide mount in a laminar flow hood and analyzed. If clay minerals were present, the slide was subjected to glycerol treatment and heating at 110 °C as needed to identify minerals present.

XRD analysis [54] was conducted using a computer-controlled X-ray diffractometer (Ultima IV, Rigaku Corporation, Tokyo, Japan) equipped with stepping motor and graphite crystal monochromator. Scans were conducted from 2–60° 2θ at a rate of 2 degrees 2θ per minute using Cu Kα radiation. Minerals were identified by referencing XRD data for minerals published by the Joint Committee on Powder Diffraction Standards (JCPDS). Semi-quantitative relative abundances of minerals were estimated using a modified version of the method reported by Poppe et al. [55] and reported as percentages of water-dispersible clay fraction.

2.3. Batch Experiments

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. [56] and Klitzke et al. [38]. For each experiment, six batch tests were performed for each soil sample at ATX concentrations ranging from 0 to 15 µg/L. These relatively low concentrations were selected based on environmental concentrations summarized by the New Zealand Ministry of Health [22] and the U.S. Environmental Protection Agency (USEPA; [57]), which indicate that ATX commonly occurs in natural waters at concentrations below about 15 µg/L and concentrations exceeding 500 µg/L are relatively rare. The ATX-water solutions were prepared using anatoxin-a (+) standards (PN 300620, Abraxis, Inc., Warminster, PA, USA) and diluted with DIW to yield nominal concentrations of 0.3, 1, 3, 7, and 15 µg/L. The concentration of ATX-water and batch test solutions were analyzed by enzyme-linked immunosorbent assay (ELISA) using a microtiter plate kit (PN 520060, Abraxis, Inc., Warminster, PA, USA) and an Abraxis Cyanotoxin Automated Assay System (PN 475200S, Abraxis, Inc., Warminster, PA, USA) at MSU. Batch tests were prepared by placing 6 g of soil in six separate 50 mL centrifuge vials and then adding 30 mL of DIW in one vial and the ATX-water solutions to each of the other vials to yield concentrations of 0, 0.3, 1, 3, 7, and 15 µg/L. The vials were capped, placed in a covered box to prevent photodegradation, and shaken on an orbital shaker at 110 rpm for 24 h. The solutions were then centrifuged at 400 rpm for 10 min; the resulting supernatant was withdrawn from each vial and filtered using 0.2-micron polyethersulfone syringe filters (PN SF020E, Environmental Express, Charleston, SC, USA). Toxin concentrations in each filtered sample were measured in duplicate on the microtiter plate following the standard instructions provided with the ELISA kit, and a 4-parameter logistic curve was used to calculate toxin concentrations from each

sample via colorimetric absorbance methods. 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 ATX-water nominal concentrations were 93–109% 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. A detailed description of the methods applied to perform the batch experiments and analyze ATX concentrations as well as all original absorbance data and computed aqueous ATX concentrations are provided in the Supplementary Materials.

2.4. Data Analysis

2.4.1. Sorption Isotherms

Linear, Freundlich, and Langmuir isotherm equations (Equations (1)–(3), respectively) have commonly been used to model cyanotoxin adsorption abilities in geologic materials. Therefore, these isotherms were selected for describing ATX sorption in the collected samples:

$$C_s = K_d C_e, \quad (1)$$

$$C_s = K_f C_e^n, \text{ and} \quad (2)$$

$$C_s = C_m \frac{K_l C_e}{1 + K_l C_e}, \quad (3)$$

where C_s is the sorbed concentration [M sorbate/M dry sorbent], K_d is the linear isotherm distribution coefficient [L^3 solution/M dry sorbent], C_e is the equilibrium aqueous sorbate concentration [M sorbate/ L^3 solution], n is an exponential decay term in the Freundlich equation, K_f is the Freundlich isotherm coefficient [L^3 solution/M dry sorbent], K_l is the Langmuir isotherm coefficient [L^3 solution/M sorbate], C_m is the maximum sorbed concentration [M sorbate/M dry sorbent] that can be held by the sorbent [58,59].

Linear, Freundlich, and Langmuir isotherm models were developed for each soil from the batch experiment results by plotting the average equilibrium concentrations (C_e) of ATX in the filtered solutions against the toxin mass sorbed on respective soils (C_s). The observed concentration of ATX sorbed on the soil particle surfaces was determined by:

$$C_s = (C_w - C_e) \frac{V_w}{M_t}, \quad (4)$$

where C_w is the aqueous toxin concentration in the initial stock solution, V_w is the volume of the aqueous solution, and M_t is the dry mass of sorbent (soil). In all experiments, V_w was 0.030 L and M_t was 0.006 kg. All isotherm equations were fit using the Microsoft Excel Solver function by minimizing the sum-of-squared errors between observed and predicted C_s values.

2.4.2. Retardation Factors

Retardation factors were calculated to provide an estimate of ATX transport in natural soil systems. Values of retardation factors (R_f) were calculated for the soils using the following equations [49,59]:

$$R_f = 1 + K_d \frac{\rho_b^d}{\phi} \quad (5)$$

$$\phi = 1 - \frac{\rho_b^d}{\rho_s}, \quad (6)$$

where, ρ_b^d represents dry soil bulk density [M/ L^3 bulk], ϕ is the soil porosity [L^3 voids/ L^3 bulk], and ρ_b^d is the soil solid fraction [M/ L^3 soil solid]. Values for ρ_b^d were estimated using bulk density measurements reported by Bruce et al. [46] for the Cuthbert Series samples (Ct1 and Ct2), Memphis Series sample (MeF), and Ruston Series samples (Ru1 and Ru2). Values for ρ_b^d were estimated using soil solid fraction data for the Alligator Association

sample (At) based on observations by Keenan et al. [60], and for the Falaya Silt Loam (Ff) sample based on observations by Longwell et al. [61]. Data were not available for Cs, and its bulk density was assumed to be equal to the average of Ru1 and Ru2 given their similar soil and clay percentages and considering that Cs may be composed of eroded sediments from upslope Ruston soils. Porosity values were estimated using a value of 2.65 g/cm^3 for ρ_s in Equation (6), assuming soil solids are predominantly composed of quartz or similar silicate minerals.

2.4.3. Statistics and Correlation

Error-based and correlation-based goodness-of-fit statistics were computed for the fitted isotherm models, consisting of the mean error (ME), root-mean-square error (RMSE), and r^2 . Associations among values for K_d and physical, chemical, and mineralogical properties were quantified using the Pearson product-moment correlation coefficient (r). Given the small sample size, r values are meant to provide only an approximate estimate of the degree of linear association, and are intended to be interpreted only in a more qualitative predictive fashion.

Multilinear regressions were performed for K_d and R_f against potential explanatory variables consisting of physical, mineralogical, extractable chemical, and total solid-phase chemical characteristics. All multilinear regression equations were fit using the Microsoft Excel Solver function by minimizing the sum-of-squared errors between observed and predicted K_d and R_f values. Candidate explanatory variables were selected based on r values computed in the correlation analysis, and final explanatory variables were selected in a stepwise fashion. Both forward and backward variable selection processes were applied, with variables added one-by-one or removed one-by-one, respectively, in an iterative process until reaching the lowest RMSE. Given the small sample size, the final equations were restricted to no more than three degrees of freedom.

3. Results

3.1. Soil Properties

3.1.1. Physical and Extractable Chemical Properties

Samples represent a range of soil horizons, with five samples from the A horizon and one each from the B and E horizons (Table 1). The non-soil sample (Cs) may be representative of eroded sediment from the C horizon of Cuthbert and Ruston soils. Under this assumption, the following samples represent a downward gradient in the soil profile from a zone of greater soil development/weathering to a zone of lesser weathering: Cuthbert Series, samples Ct1 (A horizon), Ct2 (B horizon), and Cs (C horizon analogue); and Ruston Series samples Ru1 (A horizon), Ru2 (E horizon), and Cs.

Table 1. Sample physical and extractable chemical characteristics (SC, specific conductance; CEC, cation exchange capacity).

Sample	Soil Type	Horizon	Depth	Silt + Clay (wt%)	pH		SC ^a ($\mu\text{S/cm}$)	CEC ^b (cmol/kg)	Extractable Concentrations (mg/kg)					
			(cm)		Soil ^c	Soil + ATX ^d			Na	Mg	P	K	Ca	Zn
Ru2	Ultisol	E	15	24.3	4.0	5.3	40.4	5.2	16	92	21	86	227	2.6
Ct1	Ultisol	A	~8	87.8	5.0	6.4	113.2	18.0	21	699	46	461	4062	14.3
At	Vertisol	A	~30	88.4	4.3	5.5	40.0	33.2	158	2191	123	660	5500	7.9
Ff	Inceptisol	A	15	83.0	4.4	5.0	48.6	10.5	48	256	120	195	1135	6.2
Ru1	Ultisol	A	8	29.9	4.1	5.5	43.8	7.1	25	138	33	114	444	3.0
Ct2	Ultisol	B	~15	96.7	4.4	5.6	31.2	14.5	30	596	40	430	2432	6.8
MeF	Alfisol	A	15	80.1	4.0	5.5	31.5	14.2	25	839	98	308	1010	4.2
Cs ^e	-	-	~30	28.1	4.5	5.4	44.4	7.5	33	293	42	106	1179	6.9

^a SC, specific conductance. ^b CEC, cation exchange capacity. ^c Measured on soil and distilled water slurry.

^d Measured on soil and anatoxin-a solution used in batch experiments. ^e Non-soil fluvial channel sediment, possibly representative of the C horizon of the Cuthbert and Ruston soils.

All samples had an appreciable fine-textured fraction. Values for the combined silt and clay percentage are based on the mass fraction passing a 63 μm sieve. Three samples (Ru1, Ru2, and Cs) consisted of 24–30% silt and clay, with the remaining five samples exceeding 80% up to nearly 97% (Table 1). The coarser grained samples had lower CEC values, ranging from 5.2 to 7.5 centimoles of positive charge per kilogram of dry soil (cmol^+/kg), whereas values for the finer grained samples ranged from 10.5 to 33.2 cmol^+/kg . Extractable concentrations of Na, Mg, P, K, Ca, and Zn varied among the samples (Table 1). Sample At had the highest concentrations for all extractable elements other than Zn, which contributed to it having the largest computed CEC. Organic carbon content was not measured. All samples were mineral dominated and no organic matter was visually apparent, although small fractions of organic matter are probable in all samples given their locations within or adjacent to forested areas and the shallow collection depths.

Silt and clay abundance and CEC have a moderate linear association, with an r value of 0.68, whereas CEC is more strongly correlated with extractable Na, with an r value of 0.86 (Supplemental Table S1).

3.1.2. Elemental and Mineralogical Composition

All samples had generally similar solid-phase elemental total concentrations as determined by XRF, with Si, Al, Fe, and K exceeding 1% (10,000 mg/kg) by weight (Table 2). The sum of SiO_2 and all other measured elements varied from ~60% for At to 116% for Ru2. These variations are attributable to one or more of the following factors: analytical error in XRF measurements (most likely where sum exceeds 100%), omission of elements especially those lighter than Al, or the assumption of SiO_2 for silicate minerals. For all eight samples on average, based on using SiO_2 in place of Si, the sum total of all elemental abundances is 99.7%.

Table 2. Sample solid-phase elemental total concentrations (mg/kg) from XRF analysis.

Sample	Al	Si	P	S	K	Ca	Ti	Cr	Mn	Fe	Zn	Sr	Zr	SiO_2
Ru2	29,757	543,071	4044	3239	5584	3433	3268	641	264	36,664	40	41	470	1,161,734
Ct1	67,519	378,292	4288	2484	18,937	5931	3922	81	611	22,602	87	98	487	809,241
At	86,600	279,652	4314	1099	19,187	5766	3751	75	537	39,178	109	103	217	598,230
Ff	72,471	415,679	3903	2776	16,860	4150	3762	68	467	17,195	57	90	499	889,218
Ru1	46,026	517,550	4308	2699	8330	3533	3016	122	259	10,765	34	39	427	1,107,141
Ct2	84,261	349,958	4764	1261	19,195	4737	4158	63	445	32,091	87	93	431	748,628
MeF	72,480	378,583	4002	1787	20,579	4422	3573	80	678	22,771	76	109	506	809,863
Cs	64,389	408,612	4096	2504	10,988	4475	3100	129	411	24,979	64	61	381	874,101

Clay minerals identified by XRD—kaolinite, chlorite minerals, mica minerals, and smectite minerals—dominated the water-dispersible clay fraction of all samples with the exceptions of Ru1, Ru2, and MeF, which had quartz (SiO_2) abundances exceeding 50% (Table 3). Kaolinite was the dominant clay mineral in the water-dispersible clay fraction for all samples, ranging from 9 to 44%.

Table 3. Sample water-dispersible clay-fraction mineralogical characteristics.

Sample	Clay Fraction Mineral Abundance (wt%)				
	Chlorite	Kaolinite	Mica	Smectite	Quartz
Ru2	0	9	0	0	91
Ct1	0	28	35	0	37
At	0	19	30	16	35
Ff	0	35	33	0	32
Ru1	0	44	0	0	56
Ct2	3	25	37	0	35
MeF	0	37	0	0	63
Cs	0	53	0	13	34

Smectites, micas, and chlorites each represent groups of clay mineral species, and hereafter are referred to as smectite, mica, and chlorite for simplicity. Smectite was found in samples Cs and At with abundances of 13% and 16%, respectively. Sample At has a CEC over four times that of Cs (Table 1). This difference may be due to the presence of mica in At (30% of the clay fraction) compared to its absence in Cs and a kaolinite abundance in Cs over double that of At (Table 3). Mica was present in four samples, ranging from 30 to 37% of the clay fraction. Chlorite was present in only one sample representing 3% of the clay fraction.

Varying linear correlations were identified among elemental and mineralogical abundances and physical and extractable chemical properties. Total Ca (from XRF analysis) was moderately correlated with soil pH ($r = 0.71$); CEC had moderate positive correlations with total Al, Ca, Zn, and Sr ($r > 0.7$) and moderate inverse correlations with total Si and S ($r < -0.7$); and silt and clay fraction was moderately to strongly positively correlated with Al, K, Ti, Mn, Zn, and Sr ($r \geq 0.85$) and inversely correlated with Si ($r = -0.80$). Mica abundance was moderately correlated with extractable K^+ and silt and clay fraction, with r values of 0.70 and 0.83, respectively (Supplemental Table S1). Quartz abundance was inversely correlated with total Al ($r = -0.80$) and positively correlated with total Si ($r = 0.70$).

3.2. Sorption Isotherms

Linear, Freundlich, and Langmuir sorption isotherms generally fit the measured ATX aqueous equilibrium and sorbed concentrations well (Table 4). As expected, the Freundlich and Langmuir isotherms fit the data better, but only slightly. Values of RMSE and r^2 for the linear isotherm were 2.60–6.31 $\mu\text{g}/\text{kg}$ and 0.92–0.99, respectively; and were slightly better for the Freundlich (0.40–6.10 $\mu\text{g}/\text{kg}$ and 0.92–1.00, respectively) and Langmuir isotherms (0.75–6.12 $\mu\text{g}/\text{kg}$ and 0.92–1.00, respectively). Values of ME, however, were substantially greater in magnitude for the linear isotherm compared to the Freundlich and Langmuir isotherms, with average values (all eight samples) of -5.11 , 1.95 , and 1.55 $\mu\text{g}/\text{kg}$, respectively. The fact that all fitted models are biased is attributable in part to the limited set of only five data points for each isotherm. Differences between duplicate ATX aqueous equilibrium concentrations were typically small, with a median standard deviation of 0.022 $\mu\text{g}/\text{L}$ ranging from 0.00 to 0.60 $\mu\text{g}/\text{L}$ (Figure 2). Owing to the relatively high sorption affinity of all samples, values of C_e were below the quantification limit (0.1 $\mu\text{g}/\text{L}$) recommended by the ELISA kit manufacturer for some batch experiments, specifically those with C_w of 0.3 $\mu\text{g}/\text{L}$ for all samples and C_w of 1 $\mu\text{g}/\text{L}$ for some samples (At, Ct1, Ff, Ru1, Ru2). All C_e values were retained (minimum 0.02 $\mu\text{g}/\text{L}$) and used in further analyses; exclusion of data where $C_e < 0.1$ $\mu\text{g}/\text{L}$ resulted in negligible change in best-fit isotherm parameter values (for example, percentage differences for K_d were $<0.3\%$).

Table 4. Linear, Freundlich, and Langmuir isotherm parameters and goodness-of-fit statistics.

Sample	Linear Isotherm				Freundlich Isotherm					Langmuir Isotherm				
	K_d (L/kg)	RMSE ^a ($\mu\text{g}/\text{kg}$)	ME ^b ($\mu\text{g}/\text{kg}$)	r^2 ^c	K_f (L/kg)	n	RMSE ($\mu\text{g}/\text{kg}$)	ME ($\mu\text{g}/\text{kg}$)	r^2	K_l (L/ μg)	C_m ($\mu\text{g}/\text{kg}$)	RMSE ($\mu\text{g}/\text{kg}$)	ME ($\mu\text{g}/\text{kg}$)	r^2
Ru2	22.31	2.60	-7.12	0.99	25.56	0.83	1.19	-0.25	1.00	0.15	202.6	1.27	-1.85	1.00
Ct1	31.83	3.56	-10.21	0.99	35.87	0.76	0.40	0.66	1.00	0.33	152.9	0.75	-1.55	1.00
At	33.83	6.31	-5.40	0.92	35.41	0.89	6.10	-0.78	0.92	0.12	335.1	6.12	-1.65	0.92
Ff	36.73	4.11	3.01	0.97	36.73	1.00	4.11	3.01	0.97	0.01	3141.0	4.16	2.92	0.97
Ru1	37.54	4.48	-10.93	0.98	41.16	0.75	1.89	2.19	0.99	0.46	138.4	1.28	0.66	1.00
Ct2	50.74	4.73	2.98	0.96	50.78	0.98	4.72	4.06	0.96	0.10	580.8	4.61	5.71	0.97
MeF	51.75	3.90	-5.29	0.97	52.29	0.84	2.72	3.50	0.99	0.39	191.9	1.97	4.08	1.00
Cs ^d	77.15	6.08	-7.92	0.94	73.16	0.82	5.06	3.24	0.96	0.68	175.4	4.57	4.06	0.96

^a RMSE, root-mean-square error. ^b ME, mean error. ^c r^2 , coefficient of determination. ^d Non-soil fluvial channel sediment, possibly representative of the C horizon of the Cuthbert and Ruston soils.

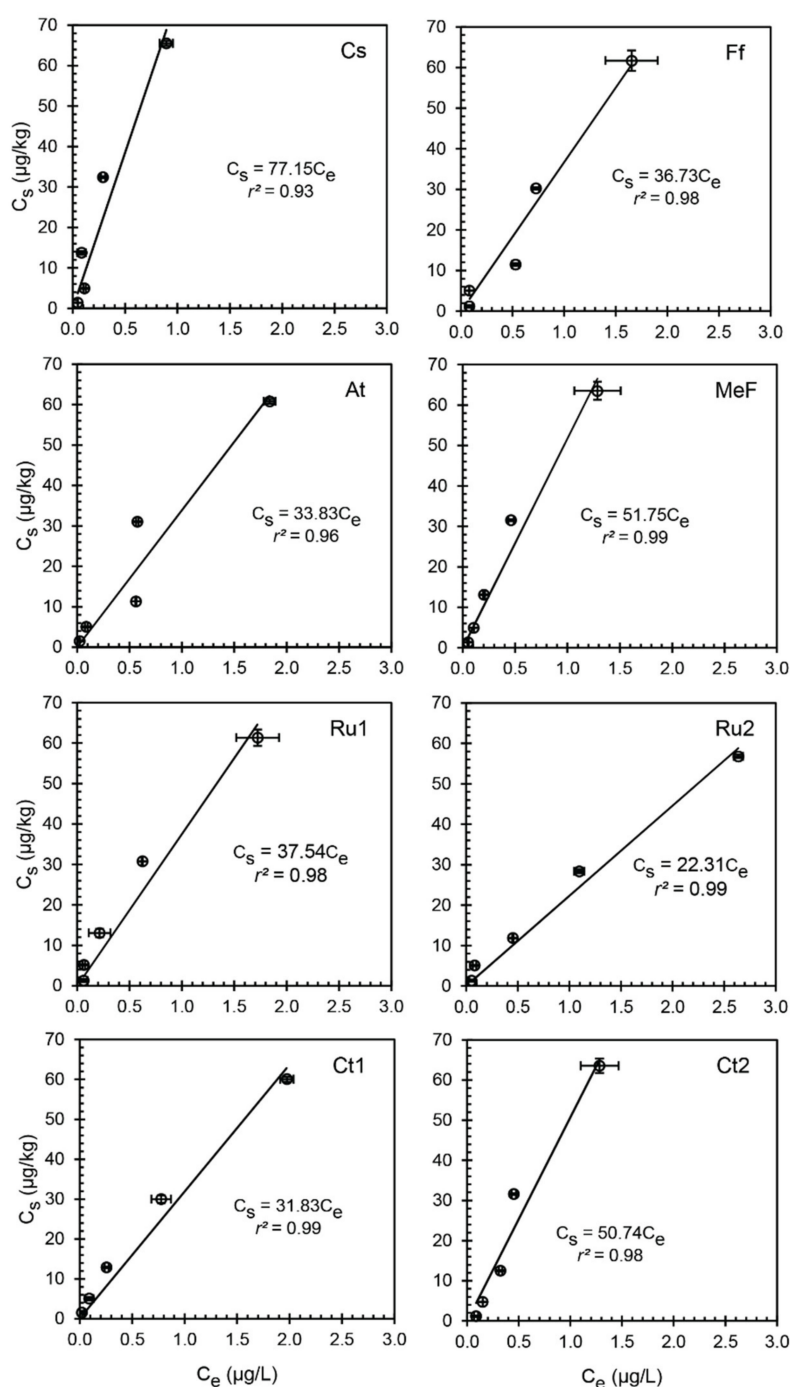


Figure 2. Sorbed (C_s) and aqueous equilibrium (C_e) concentrations for each sample (error bars represent ± 1 standard deviation; At, Alligator Association; Cs, channel sand; Ct1, Cuthbert Series A horizon; Ct2, Cuthbert Series B horizon; Ff, Falaya Silt Loam Series; MeF, Memphis Silt Loam Series; Ru1, Ruston Series A horizon; Ru2, Ruston Series E horizon).

All samples indicate substantial removal of ATX via sorption. Average percent removals ranged from 85.0% to 92.2% for all samples. Values for K_d varied by a factor of nearly 3.5, with the lowest value of 22.31 L/kg for Ru2 and highest value of 77.15 for Cs (Table 4), giving substantial differences in slope of the linear isotherm (Figure 3). Correlations between K_d and physical, mineralogical, extractable chemical, and total solid-phase chemical characteristics were limited. The strongest linear correlation is with kaolinite abundance with an r value of 0.76 (Supplemental Table S1). Other weakly correlated analytes are smectite abundance and total solid-phase Al concentration ($r = 0.38$ and 0.33 ,

respectively), and inverse correlations with total solid-phase Cr concentration and quartz abundance ($r = -0.43$ and -0.42 , respectively). All other analytes had correlations with magnitudes less than 0.30.

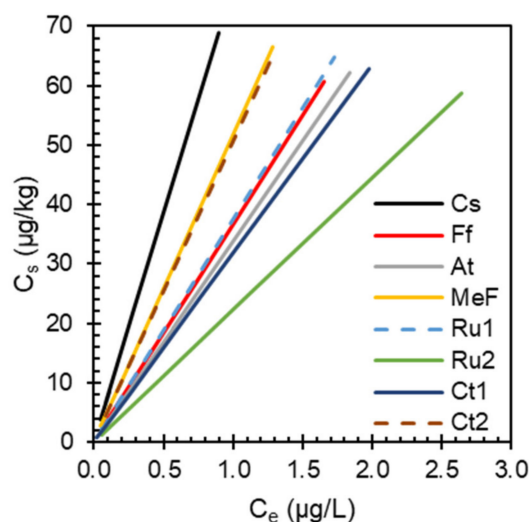


Figure 3. Best-fit linear isotherms for all samples (At, Alligator Association; Cs, channel sand; Ct1, Cuthbert Series A horizon; Ct2, Cuthbert Series B horizon; Ff, Falaya Silt Loam Series; MeF, Memphis Silt Loam Series; Ru1, Ruston Series A horizon; Ru2, Ruston Series E horizon).

3.3. Retardation Factors

A retardation factor describes the reduction in chemical mobility in porous media due to sorption processes and is only applicable when sorption equilibrium occurs quickly between adsorbate and adsorbent and a linear adsorption isotherm is applicable [59]. Under these conditions the computed R_f values can be used to estimate the propensity of ATX migration in soil given the good fit of the linear isotherms for all samples. Retardation factors generally were higher for larger K_d values, with two notable exceptions. Samples At and Ru1 had the third and fifth smallest K_d value, respectively (Table 4), but the highest and third lowest R_f value, respectively (Table 5). Larger and smaller values of ρ_b^d and ϕ , respectively, result in higher R_f for a given K_d in accordance with Equation (5). Thus, on a per unit mass basis the Cs sample can sorb the greatest amount of ATX as indicated by its large K_d of 77.15 L/kg, whereas under typical in situ conditions the At sample would sorb the most ATX. The heavy, swelling clay and silty clay loam nature of the Alligator Association soil is consistent with this finding.

Table 5. Sample bulk density (ρ_b^d), porosity (ϕ), and retardation factor (R_f).

Sample	ρ_b^d ^a (g/cm ³)	ϕ ^b (-)	R_f (-)
Ru2	1.35	0.49	62
Ct1	1.22	0.54	73
At	1.96	0.26	256
Ff	1.36	0.49	104
Ru1	1.22	0.54	86
Ct2	1.37	0.48	145
Mef	1.36	0.49	146
Cs	1.29	0.52	193

^a Values for ρ_b^d based on literature values typical for soil type. ^b Computed from ρ_b^d and soil mineral solid density of 2.65 g/cm³.

Correlations between R_f and physical, mineralogical, extractable chemical, and total solid-phase chemical characteristics tended to be stronger compared to those for K_d

(Supplemental Table S1). Moderate positive correlations with R_f were identified for smectite abundance ($r = 0.88$) and extractable Na and Mg ($r = 0.79$ and 0.76 , respectively), and moderate inverse correlations occurred with total solid-phase concentrations of Zr ($r = -0.82$) and Si and S ($r = -0.75$ for both). Positive correlations with magnitudes between 0.50 and 0.70 were indicated between R_f and CEC, all measured extractable element concentrations except Zn, and total solid-phase concentrations of Al and Zn. Correlations with K^+ , Mg^{2+} , and Ca^{2+} are expected, however, given that CEC is computed from these extractable cation concentrations.

3.4. Association between Sorption Affinity and Textural and Mineralogical Characteristics

When considered together rather than individually, soil properties representing texture and mineralogical composition have the strongest association with sorption affinity as represented by K_d and R_f values. A generally positive relation exists between K_d and the sum of kaolinite and smectite abundances (Figure 4A) with an r value of 0.81 . The relation with kaolinite does not hold for R_f , where the two samples containing smectite had the largest R_f value (Figure 4B).

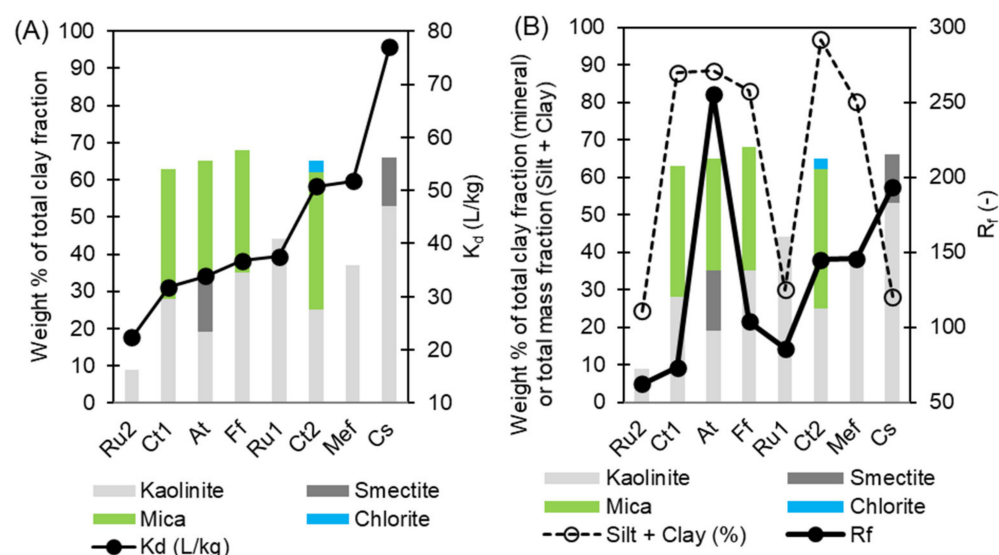


Figure 4. Water-dispersible clay-fraction mineral abundances and (A) linear isotherm coefficient (K_d), and (B) retardation factor (R_f) and silt and clay fraction (At, Alligator Association; Cs, channel sand; Ct1, Cuthbert Series A horizon; Ct2, Cuthbert Series B horizon; Ff, Falaya Silt Loam Series; MeF, Memphis Silt Loam Series; Ru1, Ruston Series A horizon; Ru2, Ruston Series E horizon).

Multilinear regressions were performed for K_d and R_f against potential explanatory variables representing clay mineralogy and texture (silt and clay abundances and mineralogical abundances), concluding that a bivariate regression showed reasonable relations for each. The strongest predictors of K_d were identified as kaolinite and smectite abundances and for R_f were smectite and silt and clay abundances (Figure 5).

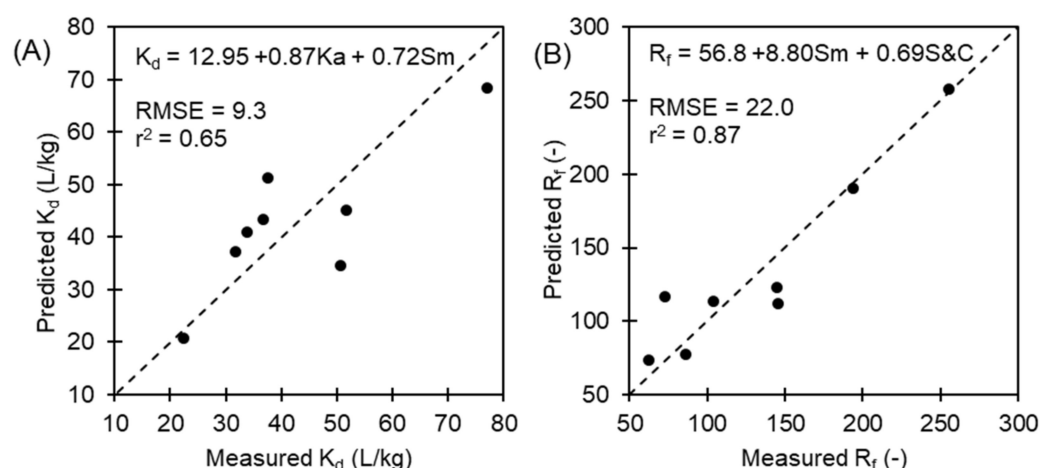


Figure 5. (A) Linear isotherm coefficient predicted using clay-fraction kaolinite (K_a) and smectite (S_m) weight percentages, and (B) retardation factor (R_f) predicted using smectite and silt and clay fraction ($S\&C$) (RMSE, root-mean-square error).

4. Discussion

4.1. Sorption Isotherm Characteristics

Linear isotherms closely fit observed data (Figure 2). Linear isotherms are commonly observed when adsorbate concentrations are relatively low or where the number of adsorption sites greatly exceeds the number of adsorbed molecules. For example, Homenauth and McBride [62] reported similar behavior for sorption of aniline on several clay minerals (montmorillonite, vermiculite, and kaolinite) and an organic muck. Freundlich isotherms are only slightly nonlinear, with best-fit n values of 0.75–1.00 and K_f values similar to K_d values. Very large values of C_m for Langmuir isotherms (Table 4) also indicate their nearly linear nature within the concentration range tested. For these reasons, the slight curvilinear nature indicated by the Freundlich and Langmuir isotherms should not be overinterpreted, and given their simplicity the linear isotherms are better suited for representing ATX sorption for aqueous concentrations (C_w) below 15 $\mu\text{g/L}$. Klitzke et al. [38] also reported linear behavior for isotherms at low equilibrium concentrations (C_e) of ATX (less than ~ 5 –10 $\mu\text{g/L}$).

The sorption capacities of many soils likely would not be met at the relatively low ATX concentrations tested. Considering a charge of +1 per ATX molecule, 1 $\mu\text{g/L}$ is equivalent to 0.60×10^{-6} cmol+/L or 3.03×10^{-6} cmol+/kg for the 30 mL ATX solution and 6 g of soil used in the batch experiments—many orders of magnitude smaller than the CEC of any of the samples. Thus, even considering competition with other cations for sorption sites, it is unlikely the maximum sorption capacity would be reached with the maximum ATX nominal concentration of 15 $\mu\text{g/L}$.

Separate batch experiments using sterilized samples were not performed, thus degradation due to microbial activity cannot be definitively excluded. Biological degradation likely was a minor effect, however, given the 24-h duration of the batch experiments combined with the fact that our samples were from locations not perennially submerged with potentially ATX-contaminated water. Rapala et al. [30] noted a 4-day lag in degradation in ATX water solutions inoculated separately with water and sediments from several lakes. Klitzke et al. [38] also reported little (8%) to negligible differences between batch experiments using unsterilized and sterilized samples. However, samples At and Cs were collected in areas that possibly had been occasionally inundated; although mixed results were found in terms of sorption affinity, with Cs having the largest K_d value, whereas At has the third smallest.

4.2. Influence of Clay Mineralogy on Anatoxin-a Sorption

Results suggest that clay mineralogy and texture are important factors for explaining ATX sorption in natural soils. Clay minerals have a permanent negative charge that is independent of pH owing to isomorphous substitution of lower charge metal ions (typically Al^{3+} , Mg^{2+} , Fe^{2+} , or Li^{+}) for the central cation in a tetrahedrally coordinated Si-O crystal and octahedrally coordinated Al-O (or Al-OH) crystal [63,64]. Formation of the crystals into tetrahedral and octahedral sheets distributes this negative charge imbalance across layers composing the clay platelet. The ATX cation thus would tend to be electrostatically attracted to the minerals' surfaces. Clay minerals also possess a variable charge owing to the coordinated O^{2-} or OH^{-} ions being exposed at the clay platelet surface as well as charges exposed by broken crystal bonds at platelet edges, all of which may be protonated and deprotonated. This pH-dependent charge can be characterized by the pH at which the net surface charge is zero, called the point of zero charge (pH_{pzc}). Values of pH_{pzc} vary by clay mineral type and impurities due to site-specific geologic conditions or processing of the sample. Some reported values of pH_{pzc} include <2–4.6 for kaolinite, <3.3 for mica (muscovite), <3 for chlorite, and <2–3 for bentonite/montmorillonite (smectite group mineral) [58,59,65]. The pH values of the ATX and soil solutions used in the batch experiments (5.0–6.4, Table 1) were higher than these pH_{pzc} values, indicating that the pH-dependent charge on the clay platelet surface would be negative. This variable charge would supplement the permanent charge, further attracting ATX cations, although perhaps less so for kaolinite given its potentially higher pH_{pzc} value.

Kaolinite, mica, and smectite were the most prevalent minerals identified by XRD in eight samples tested. Both Cs and At samples had similar smectite abundances (13% and 16%, respectively), which when combined with the high silt and clay abundance for At of 88.4% may explain the large R_f value of 256. Additionally, even though Cs has the lowest silt and clay abundance of 28.1%, the presence of smectite may contribute to it having the second largest R_f value of 193. Sample Ru2 has the smallest sorption affinity as indicated by the lowest K_d value and the lowest R_f value of 62. This may be related to the presence of kaolinite as the only identified clay mineral in Ru2, occurring at only 9% of the water-dispersible clay fraction, which represents the smallest percentage compared to other samples (Table 3).

The characteristics of individual clay minerals likely influence ATX sorption. Kaolinite is a non-expansive high Al clay mineral with a 1:1 layer structure that consists of one sheet of tetrahedrally coordinated Si-O shared with one sheet of octahedrally coordinated Al. Among the clay minerals identified, kaolinite typically has the lowest CEC (~1–5 cmol+/kg; [66]). Smectite, mica, and chlorite have 2:1 layer structure with one octahedral sheet sandwiched between two tetrahedral sheets.

Smectite has the highest shrink/swell potential of all 2:1 clays [67]. In wet soils, water is drawn into the interlayer space within the 2:1 structure and the clay swells [68]. This allows for an increased surface area to potentially be exposed for adsorption, substantially increasing the potential ability of smectite to adsorb cations compared to other clay minerals. Norrish [69] reported an interlayer distance of ~5 nm for a solution of distilled water and Na-montmorillonite (smectite group mineral) at a water content of 2 g H_2O /g clay, with an increasing linear trend at higher water contents. Our batch experiments were conducted at 5 g H_2O /g soil. The relatively small size of an ATX molecule, six times smaller in mass than microcystin LR which is reported to have an estimated size of 1.2–2.6 nm [70], would allow ample space for ATX molecules to migrate into the interlayer space. Among the clay minerals identified, the swelling potential of smectite contributes to it typically having the largest CEC (~80–150 cmol+/kg; [71]).

Micas have a separate cation (usually K^{+}) held in the interlayer space between two adjacent tetrahedral sheets which minimizes the expansion ability of the minerals [67], limiting the adsorption potential to that slightly greater than kaolinite.

Chlorite is similar to mica in its non-expansive nature, except it generally has complex cations (typically Al and Mg hydroxides) in the interlayer space between 2:1 layers, limiting the adsorption potential to that similar to kaolinite [72].

Larger values of R_f indicate slower chemical transport by advection or dispersion processes, thus migration of ATX would likely be most limited in soils with higher smectite or silt and clay abundances (At and Cs, Figures 4B and 5B). The strong positive effect of smectite is consistent with its high CEC compared to other clay minerals. Mica abundance was not correlated with either K_d or R_f ($r = -0.26$ and 0.12 , respectively; Supplemental Table S1), even though mica (muscovite) has been reported to readily sorb mono- and multivalent cations via ion exchange [73]. The apparent lack of sensitivity of mica on ATX sorption may be due to the small range of mica abundances of 30–37% for our samples. Also, sorption could be limited by other cations, as Shao and Czajkowski [74] described the inhibiting action of monovalent cations K^+ , Na^+ , and Li^+ on protein sorption on muscovite mica. We found that mica abundance was moderately correlated with extractable K^+ concentration ($r = 0.70$; Supplemental Table S1), consistent with the common presence of K^+ between mica layers that limits sorption. Kaolinite typically has the lowest CEC compared to smectite and mica, and its importance as a predictor for K_d may be related to its prevalence in all soil samples, ranging from an estimated 29 to 100% (average of ~68%) of the non-quartz water-dispersible clay fraction.

Studies specifically on ATX sorption in natural soil systems are limited, but our results are generally consistent with previous research in regard to the importance of particle size. Klitzke et al. [38] reported that sediment texture is a key parameter, with greater sorption for clayey sediments but also significant sorption to sandier sediments. Using batch experiments with ATX concentrations of 1–30 $\mu\text{g/L}$, their sample that experienced the greatest sorption ($K_f = 24.45 \text{ L/kg}$, $n = 0.93$) was 26% silt and clay, whereas a sand sample with 1% silt and clay had the least sorption ($K_f = 1.42 \text{ L/kg}$, $n = 0.75$). Organic carbon content was also an important predictor of sorption, with an organic mud with 66% silt and clay showing the second greatest sorption affinity ($K_f = 12.28 \text{ L/kg}$, $n = 0.87$). Overall, our samples showed greater ATX sorption than those of Klitzke et al. [38], with all K_f values exceeding 25 L/kg (Table 4). These differences may be due in part to particle size. Our samples were generally finer textured, with all eight having silt and clay contents exceeding 24%, whereas four of the eight samples analyzed by Klitzke et al. [38] had silt and clay contents below this level.

Bialczyk et al. [39], reported substantial removal of ATX on four clay minerals with maximum removals of 34.2%, 44.8%, 64.5%, and 92.1% for bentonite, sepiolite, palygorskite, and kerolite, respectively. The bentonite sample was composed primarily of montmorillonite, a smectite group mineral, yet had the lowest removals contrary to our findings. Our removal efficiencies of 85.0–92.2% fell within the upper end of this range. Differences may be attributable in part to the much higher ATX concentration of 5000 $\mu\text{g/L}$ used in the Bialczyk et al. [39] experiments. Additionally, the presence of other minerals could be an important factor. Bialczyk et al. [39] used relatively pure samples, but noted the presence of calcite, dolomite, quartz, and organics in their samples despite the use of commercially available clay minerals. Comparison of our results based on natural samples collected directly from a temperate environment with the mineral-specific results of Bialczyk et al. [39] is difficult. Any natural soil would contain a wide range of minerals as well as organic compounds, which likely were present to some degree in our samples but were not quantified. For example, Fe oxides are a group of minerals that may be present owing to the Fe-rich nature of our samples (Fe ranging from ~1 to nearly 4 wt%; Table 2), which commonly have substantial sorption capacity [75]. Nevertheless, our results illustrate the potential importance of clay mineralogy and silt and clay content on estimating the commonly applied transport parameters, K_d and R_f .

Rapala et al. [30] found removals of 25–48% attributable to sorption, which are substantially lower than our results but similar to the lower end of the range of removals measured by Bialczyk et al. [39]. Experiments were performed using ATX water solutions inoculated

separately with water and sediments from several lakes and then sterilized by autoclaving. Texture of the lake sediments was not reported, although they were characterized as either mud or sand. Differences with our removal efficiencies also may be partly attributed to the higher ATX concentrations of 2200–2600 µg/L used in the Rapala et al. [30] experiments.

Further elucidation of mechanisms driving the observed sorption behavior is limited by our experimental conditions, in part, due to the relatively small size of the ATX molecule and the low concentrations used, which were selected to be consistent with typical environmental concentrations. Conditions limiting sorption, such as saturation of sorption sites, that could provide insight into sorption mechanisms likely were not encountered under our experimental conditions. The potential number of sorption sites as implied by reported CEC values is at least four orders of magnitude greater than the total charge attributable to ATX sorption, considering sample Ru2 which has the smallest clay abundance composed of kaolinite with the lowest CEC combined with the highest ATX concentration of 14 µg/L. Likewise, given the small size of an ATX molecule (<1.2 nm), a monolayer of molecules at a concentration of 14 µg/L would cover an area at least three orders of magnitude smaller than the 5–39 m²/g specific surface area for kaolinite [66], which typically would have the smallest specific surface area of the clay minerals identified in our samples.

4.3. Influence of Soil Horizons on Anatoxin-a Migration

An examination of the possible influence of position in the soil profile suggests that sorption affinity may be related to the degree of soil leaching/weathering in Ultisols, based on the assumption that Cs can serve as an analogue for parent material of the Cuthbert and Ruston Series soils. Leaching of soluble minerals may deplete a horizon of potential adsorbents, transporting the solutes deeper in the soil profile where they may precipitate and accumulate as potential adsorbents; or deeper, less weathered soil horizons may preserve existing adsorbents. For the Cuthbert Series, samples Ct1, Ct2, and Cs represent the A, B, and C horizons, respectively, showing an increasing trend in K_d and R_f consistent with decreasing soil weathering with increasing depth. A similar increasing trend in K_d and R_f is indicated for the Ruston Series by samples Ru2, Ru1, and Cs that represent the E, A, and C horizons, respectively, but is not depth related. Sample Ru2, lying below Ru1, had the lowest K_d and R_f values of all samples. Even though the E horizon lies below the A horizon (Ru1), greater eluviation of soluble minerals from this horizon may limit the availability of adsorbents. Implications under these conditions is the migration of ATX may be faster in the shallow soil profile—for example, in the root zone of cultivated crops—and retarded with increasing depth as water percolates through the vadose zone. The greater partitioning of ATX to the soil solid phase is illustrated by the findings of Zhang et al. [35], where ATX in soil extracts (3.3–5.8 µg/L) were over an order of magnitude greater than those in subsurface tile drainage water (maximum of 0.2 µg/L) or groundwater (maximum of 0.3 µg/L) from a nearby well.

Soils tested here were not subject to prolonged inundation under natural field conditions, other than possibly Cs and At that could have been submerged occasionally, and sorption behavior might be different for sediments under saturated conditions. Klitzke et al. [38] analyzed samples collected from saturated sediments and unsaturated soils and found ATX sorption varied for both types, suggesting ATX sorption might not correlate strongly with saturation status. Therefore, our soil samples collected under unsaturated conditions may also be reasonable surrogates for saturated sediments with similar physical, chemical, and mineralogical characteristics.

4.4. Implications of Anatoxin-a Migration on Groundwater Protection

Results from this study illustrate the importance of considering the potential impact of ATX migration in the subsurface—and ultimately on groundwater—due to natural and artificial recharge with contaminated surface water. Given the appropriate soil conditions, physicochemical sorption processes likely can substantially retard the transport of ATX in natural soil systems. For soils containing more than ~25% silt and clay, migration of ATX in

the subsurface may be reduced by factors of ~60 to 250, based on the eight soils tested. Such behavior is relevant for irrigation conditions and managed aquifer recharge via infiltration. Under agricultural irrigation, for example, loamy, silty, or clayey soils would tend to slow migration of ATX to the water table, although limitation of retardation by sorption capacity and potential desorption may exist. Results may be beneficial for initial screening assessment of potential ATX migration under saturated flow conditions such as riverbank filtration. Given the substantial retardation of ATX in fine textured soils, consideration of sediment texture in or near the riverbed may be desirable with regard to ATX removal during selection of riverbank filtration sites. Under infiltration-based managed aquifer recharge, incorporation of loamy, silty, or clayey soils as an amendment may enhance natural ATX attenuation processes prior to recharge water reaching the water table.

Incorporation of functionalized amendments targeted at mitigating contaminant leaching is becoming more common in stormwater management in the context of green infrastructure for the protection of groundwater quality [13,76,77], but this technology has seen little application in managed aquifer recharge. A layer of native soil can be replaced with an engineered layer where one or more specific amendments, such as clay [76,78], iron filings [79], woodchips [80], or biochar [81], are added to achieve required functionality, such as meeting moisture content [76,80], sorption capacity [78,81], or water flux [76,80] specifications. Even though fine-grained materials such as loamy, silty, or clayey soils may reduce hydraulic conductivity, incorporation of an appropriate fraction can provide sufficient pollution control while not reducing water flux to unacceptable levels. For example, Shokri et al. [78] and O'Reilly et al. [76] incorporated silt/clay fractions of 5% and 27%, respectively, in engineered media to remove nutrients (nitrogen and phosphorus species) from stormwater runoff in roadway and suburban residential settings. In both applications, hydraulic performance of the media was sufficient to maintain adequate water fluxes in a roadway vegetated filter strip [78] and an infiltration basin [76] to satisfy stormwater volume control requirements. The effectiveness of clay minerals for sorption of environmental contaminants has been demonstrated frequently in the literature, with recently published work including metals [82–84], uranyl [85], aflatoxin [86,87], and polyelectrolytes [88]. In the water treatment field, potential sorbents, such as activated carbon [89], have been shown to remove ATX, although further research is recommended [32]. By combining successful pollutant control technologies from the fields of green infrastructure, surface and interfacial science, and water treatment with the improved understanding of ATX sorption affinity that our results provide, opportunity exists to develop engineered media incorporating functionalized amendments. Specific amendments can be targeted at limiting the environmental migration of ATX during irrigation, natural and managed aquifer recharge, or riverbank filtration, thereby advancing the protection of in situ and extracted groundwater.

Finally, it is important to note that conclusions drawn from these results are limited by the relatively small number of soil samples. Variations in the properties of natural soils extend well beyond those tested here. Thus, correlations and predictive relations reported herein should be interpreted only qualitatively and with caution when considered in relation to other soils based on similar physical, mineralogical, or chemical characteristics. Furthermore, results are applicable only for the range of aqueous ATX concentrations tested (0.3–14 µg/L); extrapolation of sorption isotherms for higher concentrations should be avoided as ATX removal would be expected to eventually decrease as sorption sites become filled.

5. Conclusions

The sorption of cyanotoxins, including microcystin-LR, cylindrospermopsin, and ATX, has been described in the literature, with solution and substrate properties of pH, ionic strength, particle size, and CEC generally found to be most influential. Little information, however, is available on the mineralogy of the substrate in previous studies, and the authors are unaware of other publications that relate ATX sorption to the mineralogy or extractable

chemical and solid-phase elemental composition of natural soils including Alfisols, Ultisols, Vertisols, and Inceptisols.

Mineralogy is expected to be particularly relevant given its fundamental control on sorption affinity. Results from this study are generally consistent with this hypothesis, showing the influence of kaolinite and smectite on ATX sorption capacity. Results suggest that potential retardation of ATX transport through in situ soil systems is most highly related to smectite abundance and particle size (silt and clay) fractions, and that soils with greater silt and clay fractions composed of minerals with moderate to higher sorption affinities would be more likely to retard movement of ATX through natural soil systems. No particular trend was found in regard to the four soil types tested, other than Vertisols likely have relatively large K_d and R_f values given the expansive clay-rich nature typically attributed to smectite group minerals common in these soils as identified in sample At. A potential effect of soil leaching/weathering is suggested by trends in sorption affinities showing smaller K_d and R_f values in the more highly leached A and E horizons of the two Ultisols tested.

Ultimately, results from this study indicate that ATX sorption in geologic materials may be more complex than previously thought, with soil mineralogy and particle size likely being key factors influencing sorption capacity. More investigations are needed to develop a mechanistic understanding of the effects of soil profile leaching/weathering and mineralogical and chemical soil properties on ATX removal as well as potential desorption in soil systems, focusing separately on contributions by the sand, silt, clay, and organic fractions. Fruitful future research may include transient variably saturated laboratory soil column tests run under realistic field water-flux conditions as well as field observations during irrigation, natural and artificial recharge, or riverbank filtration. Finally, further development of enhanced groundwater protection technologies is needed—such as engineered media incorporating functionalized amendments—to optimize in situ retardation of ATX migration based on an improved mechanistic understanding of the subsurface fate and transport of ATX.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/w14182869/s1>, as Supplementary Material: detailed description of methods used for the batch experiments and ELISA analysis; Table S1 Pearson product-moment correlation coefficient matrix of physical, chemical, and mineralogical characteristics for the eight samples studied; and 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.

Author Contributions: Conceptualization and writing—review and editing, J.L.H., A.M.O. and J.N.G.; methodology, formal analysis, data curation, funding acquisition, and visualization, J.L.H. and A.M.O.; investigation and writing—original draft preparation, J.L.H.; resources and supervision, A.M.O. and J.N.G.; project administration, A.M.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in part by the Douglas Geology Endowment, Department of Geology & Geological Engineering, UM.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are provided as Supplementary Materials as described above.

Acknowledgments: X-ray fluorescence data were collected by Allan R. Bacon and Kayci R. Anderson and X-ray diffraction data were collected by Jonathan Judy and Jennifer A. Sarchapone of the Soil and Water Sciences Department at UF. The assistance of A.R. Bacon and J. Judy in interpreting these data is gratefully acknowledged. The authors thank Padmanava Dash at MSU for the use of laboratory facilities where ATX concentrations were measured. The findings and conclusions in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation

or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Döll, P.; Schmied, H.M.; Schuh, C.; Portmann, F.T.; Eicker, A. Global-scale assessment of groundwater depletion and related groundwater abstractions: Combining hydrological modeling with information from well observations and GRACE satellites. *Water Resour. Res.* **2014**, *50*, 5698–5720. [CrossRef]
2. Stefan, C.; Ansems, N. Web-based global inventory of managed aquifer recharge applications. *Sustain. Water Resour. Manag.* **2018**, *4*, 153–162. [CrossRef]
3. Chorus, I.; Bartram, J. (Eds.) *Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring, and Management*; World Health Organization: Geneva, Switzerland, 1999.
4. Merel, S.; Walker, D.; Chicana, R.; Snyder, S.; Baurès, E.; Thomas, O. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* **2013**, *59*, 303–327. [CrossRef] [PubMed]
5. Loftin, K.A.; Graham, J.L.; Hilborn, E.D.; Lehmann, S.C.; Meyer, M.T.; Dietze, J.E.; Griffith, C.B. Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. *Harmful Algae* **2016**, *56*, 77–90. [CrossRef]
6. Snow, D.D.; Cassada, D.A.; Larsen, M.L.; Mware, N.A.; Li, X.; D'Alessio, M.; Zhang, Y.; Sallach, J.B. Detection, Occurrence and Fate of Emerging Contaminants in Agricultural Environments. *Water Environ. Res.* **2017**, *89*, 897–920. [CrossRef] [PubMed]
7. Codd, G.; Bell, S.; Kaya, K.; Ward, C.; Beattie, K.; Metcalf, J. Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* **1999**, *34*, 405–415. [CrossRef]
8. Corbel, S.; Mougin, C.; Bouaïcha, N. Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. *Chemosphere* **2014**, *96*, 1–15. [CrossRef]
9. Liang, C.; Wang, W.; Wang, Y. Effect of irrigation with microcystins-contaminated water on growth, yield and grain quality of rice (*Oryza sativa*). *Environ. Earth Sci.* **2016**, *75*, 505. [CrossRef]
10. Lee, S.; Jiang, X.; Manubolu, M.; Riedl, K.; Ludsin, S.A.; Martin, J.F.; Lee, J. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. *Food Res. Int.* **2017**, *102*, 234–245. [CrossRef]
11. Lahti, K.; Vaitomaa, J.; Kivimäki, A.-L.; Sivonen, K. Fate of cyanobacterial hepatotoxins in artificial recharge of groundwater and in bank filtration. In *Artificial Recharge of Groundwater*; Peters, J.H., Ed.; A.A. Balkema: Nieuwegein, The Netherlands, 1998; pp. 211–216.
12. O'Reilly, A.M.; Wanielista, M.P.; Loftin, K.A.; Chang, N. Bin Laboratory simulated transport of microcystin-LR and cylindrospermopsin in groundwater under the influence of stormwater ponds: Implications for harvesting of infiltrated stormwater. In *Proceedings of the IAHS-AISH Publication, Zurich, Switzerland, 13–18 June 2010; Volume 342*.
13. U.S. Environmental Protection Agency (USEPA). *Enhanced Aquifer Recharge of Stormwater in the United States: State of the Science Review*; Report # EPA/600/R-21/037F; USEPA: Washington, DC, USA. Available online: <http://www.epa.gov/research> (accessed on 7 February 2022).
14. Trainer, V.L.; Hardy, F.J. Integrative Monitoring of Marine and Freshwater Harmful Algae in Washington State for Public Health Protection. *Toxins* **2015**, *7*, 1206–1234. [CrossRef]
15. Colas, S.; Marie, B.; Lance, E.; Quiblier, C.; Tricoire-Leignel, H.; Mattei, C. Anatoxin-a: Overview on a harmful cyanobacterial neurotoxin from the environmental scale to the molecular target. *Environ. Res.* **2020**, *193*, 110590. [CrossRef] [PubMed]
16. Van der Merwe, D. Freshwater cyanotoxins. In *Biomarkers in Toxicology*; Academic Press: Cambridge, MA, USA, 2014.
17. Gorham, P.R. Laboratory Studies on the Toxins Produced by Waterblooms of Blue-Green Algae. *Am. J. Public Health Nations Health* **1962**, *52*, 2100–2105. [CrossRef] [PubMed]
18. Ferrão-Filho, A.D.S.; Kozłowsky-Suzuki, B. Cyanotoxins: Bioaccumulation and effects on aquatic animals. *Mar. Drugs* **2011**, *9*, 2729–2772. [CrossRef]
19. Krienitz, L.; Ballot, A.; Kotut, K.; Wiegand, C.; Pütz, S.; Metcalf, J.S.; Codd, G.A.; Stephan, P. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiol. Ecol.* **2003**, *43*, 141–148. [CrossRef] [PubMed]
20. Rantala-Ylinen, A.; Känä, S.; Wang, H.; Rouhiainen, L.; Wahlsten, M.; Rizzi, E.; Berg, K.; Gugger, M.; Sivonen, K. Anatoxin-a Synthetase Gene Cluster of the Cyanobacterium *Anabaena* sp. Strain 37 and Molecular Methods to Detect Potential Producers. *Appl. Environ. Microbiol.* **2011**, *77*, 7271–7278. [CrossRef] [PubMed]
21. Walker, H.W. *Harmful Algae Blooms in Drinking Water*; CRC Press: Boca Raton, FL, USA, 2015.
22. Ministry of Health. *Guidelines for Drinking-Water Quality Management for New Zealand*, 2nd ed.; Ministry of Health: Wellington, New Zealand, 2016; ISBN 9780947491789.
23. Cadel-Six, S.; Peyraud-Thomas, C.; Brient, L.; de Marsac, N.T.; Rippka, R.; Méjean, A. Different Genotypes of Anatoxin-Producing Cyanobacteria Coexist in the Tarn River, France. *Appl. Environ. Microbiol.* **2007**, *73*, 7605–7614. [CrossRef] [PubMed]
24. Matlock, S. Toxic algae blamed for elk deaths in northeastern New Mexico. *Santa Fe New Mexican*, 22 October 2013.

25. Puschner, B.; Hoff, B.; Tor, E.R. Diagnosis of Anatoxin-a Poisoning in Dogs from North America. *J. Vet. Diagn. Investig.* **2008**, *20*, 89–92. [\[CrossRef\]](#)
26. Biré, R.; Bertin, T.; Dom, I.; Hort, V.; Schmitt, C.; Diogène, J.; Lemée, R.; De Haro, L.; Nicolas, M. First Evidence of the Presence of Anatoxin-A in Sea Figs Associated with Human Food Poisonings in France. *Mar. Drugs* **2020**, *18*, 285. [\[CrossRef\]](#)
27. Ibelings, B.W.; Backer, L.C.; Kardinaal, W.E.A.; Chorus, I. Current approaches to cyanotoxin risk assessment and risk management around the globe. *Harmful Algae* **2014**, *40*, 63–74. [\[CrossRef\]](#)
28. Testai, E. Anatoxin-a and analogues. In *Toxic Cyanobacteria in Water*; Chorus, I., Welker, M., Eds.; World Health Organization: Geneva, Switzerland; Boca Raton, FL, USA, 2021; pp. 72–93.
29. Wood, S.A.; Holland, P.T.; MacKenzie, L. Development of solid phase adsorption toxin tracking (SPATT) for monitoring anatoxin-a and homoanatoxin-a in river water. *Chemosphere* **2011**, *82*, 888–894. [\[CrossRef\]](#)
30. Rapala, J.; Lahti, K.; Sivonen, K.; Niemelä, S. Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. *Lett. Appl. Microbiol.* **1994**, *19*, 423–428. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Stevens, D.; Krieger, R. Stability studies on the cyanobacterial nicotinic alkaloid snatoxin-A. *Toxicon* **1991**, *29*, 167–179. [\[CrossRef\]](#)
32. Vlad, S.; Anderson, W.B.; Peldszus, S.; Huck, P.M. Removal of the cyanotoxin anatoxin-a by drinking water treatment processes: A review. *J. Water Health* **2014**, *12*, 601–617. [\[CrossRef\]](#)
33. Smith, C.; Sutton, A. *The Persistence of Anatoxin-a in Reservoir Water*; Report No. FR0427; Foundation for Water Research: Marlow, Buckinghamshire, UK, 1993.
34. Kaminski, A.; Bober, B.; Lechowski, Z.; Bialczyk, J. Determination of anatoxin-a stability under certain abiotic factors. *Harmful Algae* **2013**, *28*, 83–87. [\[CrossRef\]](#)
35. Zhang, Y.; Husk, B.R.; Duy, S.V.; Dinh, Q.T.; Sanchez, J.S.; Sauvé, S.; Whalen, J.K. Quantitative screening for cyanotoxins in soil and groundwater of agricultural watersheds in Quebec, Canada. *Chemosphere* **2021**, *274*, 129781. [\[CrossRef\]](#)
36. Miller, A.; Russell, C. Food crops irrigated with cyanobacteria-contaminated water: An emerging public health issue in Canada. *Environ. Health Rev.* **2017**, *60*, 58–63. [\[CrossRef\]](#)
37. Mohamed, Z.A.; Al Shehri, A.M. Microcystins in groundwater wells and their accumulation in vegetable plants irrigated with contaminated waters in Saudi Arabia. *J. Hazard. Mater.* **2009**, *172*, 310–315. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Klitzke, S.; Beusch, C.; Fastner, J. Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. *Water Res.* **2011**, *45*, 1338–1346. [\[CrossRef\]](#)
39. Bialczyk, J.; Natkański, P.; Kuśtrowski, P.; Czaja-Prokop, U.; Bober, B.; Kaminski, A. Removal of cyanobacterial anatoxin-a from water by natural clay adsorbents. *Appl. Clay Sci.* **2017**, *148*, 17–24. [\[CrossRef\]](#)
40. Aba, R.; Mugani, R.; Hejjaj, A.; de Fraissinette, N.B.; Oudra, B.; Ouazzani, N.; Campos, A.; Vasconcelos, V.; Carvalho, P.; Mandi, L. First Report on Cyanotoxin (MC-LR) Removal from Surface Water by Multi-Soil-Layering (MSL) Eco-Technology: Preliminary Results. *Water* **2021**, *13*, 1403. [\[CrossRef\]](#)
41. Klitzke, S.; Apelt, S.; Weiler, C.; Fastner, J.; Chorus, I. Retention and degradation of the cyanobacterial toxin cylindrospermopsin in sediments—The role of sediment preconditioning and DOM composition. *Toxicon* **2010**, *55*, 999–1007. [\[CrossRef\]](#) [\[PubMed\]](#)
42. United States Dep. Agric. (USDA). *Land Resource Regions and Major Land Resource Areas of the United States, the Caribbean, and the Pacific Basin, Handbook 296*; USDA: Washington, DC, USA, 2006.
43. Thomas, A.E. *Soil Survey of Grenada County, Mississippi*; US Dep. Agric.: Washington, DC, USA, 1967; pp. 1–76.
44. Dash, P.; Silwal, S.; Ikenga, J.O.; Pinckney, J.L.; Arslan, Z.; Lizotte, R.E. Water Quality of Four Major Lakes in Mississippi, USA: Impacts on Human and Aquatic Ecosystem Health. *Water* **2015**, *7*, 4999–5030. [\[CrossRef\]](#)
45. Vanderford, H.B. *Soils of Mississippi*; Mississippi Agricultural Experiment Station: Starkville, MS, USA, 1962.
46. NOAA National Centers for Environmental Information, U.S. Climate Normals Quick Access. Available online: <https://www.ncei.noaa.gov/access/us-climate-normals/> (accessed on 8 October 2021).
47. Natural Resources Conservation Service. Web Soil Survey. Available online: <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm> (accessed on 23 May 2019).
48. Natural Resources Conservation Service. Official Soil Series Descriptions. Available online: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/scientists/?cid=nrcs142p2_053587 (accessed on 5 October 2021).
49. Brady, N.C.; Weil, R.R. *Nature and Properties of Soil*, 13th ed.; Prentice-Hall Inc.: Hoboken, NJ, USA, 1998; 960p.
50. Snowden, J.O.; Priddy, R.R. *Loess Investigations in Mississippi: Geology of Mississippi Loess*; Bulletin 111; Mississippi Geological, Economic and Topographical Survey: Jackson, MS, USA, 1968; pp. 13–204.
51. Bruce, R.; Raney, W.A.; Broadfoot, W.M.; Vanderford, H.B.; Lyle, C.Y. *Characteristics of Important Mississippi Soils*; Mississippi State University, Agricultural Experiment Station: Starkville, MS, USA, 1958.
52. Skikora, F.J.; Crouse, K.K.; Heckendorn, S.; Huluka, G.; Mitchell, C.C.; Moore, K.P.; Oldham, J.L. Cation Exchange Capacity. In *Soil Test Methods From the Southeastern United States*; Skikora, F.J., Moore, K.P., Eds.; South. Coop. Ser. Bull. No. 419; Southern Extension and Research Activity Information Exchange Group: Clemson, SC, USA, 2014; pp. 170–179.
53. Kaplan, D.I.; Bertsch, P.M.; Adriano, D.C.; Miller, W.P. Soil-borne mobile colloids as influenced by water flow and organic carbon. *Environ. Sci. Technol.* **1993**, *27*, 1193–1200. [\[CrossRef\]](#)
54. Harris, W.; White, G.N. X-ray diffraction techniques for soil mineral identification. In *Methods of Soil Analysis, Part 5: Mineralogical Methods*; Soil Science Society of America (SSSA) Book Series 5; SSSA: Madison, WI, USA, 2015.

55. Poppe, L.J.; Paskevich, V.F.; Hathaway, J.C.; Blackwood, D.S. *A Laboratory Manual for X-ray Powder Diffraction*; Open-File Report 2001-41; U.S. Geol. Survey: Woods Hole, MA, USA, 2001; 88p. [\[CrossRef\]](#)
56. Miller, M.; Critchley, M.; Hutson, J.; Fallowfield, H. The adsorption of cyanobacterial hepatotoxins from water onto soil during batch experiments. *Water Res.* **2001**, *35*, 1461–1468. [\[CrossRef\]](#)
57. United States Environmental Protection Agency (USEPA). *Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A*; USEPA: Washington, DC, USA, 2015.
58. Appelo, C.A.J.; Postma, D. *Geochemistry, Groundwater, and Pollution*; A.A. Balkema Publishers: Amsterdam, The Netherlands, 2005.
59. Hemond, H.F.; Fechner, E.J. *Chemical Fate and Transport in the Environment*, 3rd ed.; Academic Press: Cambridge, MA, USA, 2014.
60. Keenan, W.E.; McNutt, E.J.; Warren, R.B.; Morris, W.M.; Wynn, A.H. *Soil Survey of Leflore County, Mississippi*; US Dep. Agric.: Washington, DC, USA, 1959; pp. 1–62.
61. Longwell, T.J.; Parks, W.L.; Springer, M.E. *Moisture Characteristics of Tennessee Soils*; University of Tennessee Agricultural Experiment Station: Knoxville, TN, USA, 1963; p. 47. Available online: https://trace.tennessee.edu/utk_agbulletin/303/ (accessed on 8 September 2022).
62. Homenauth, O.P.; McBride, M.B. Adsorption of Aniline on Layer Silicate Clays and an Organic Soil. *Soil Sci. Soc. Am. J.* **1994**, *58*, 347–354. [\[CrossRef\]](#)
63. Barton, C.D.; Karathanasis, A.D. Clay Minerals. In *Encyclopedia of Soil Science*; Lal, R., Ed.; Marcel Dekker: New York, NY, USA, 2002; pp. 187–192.
64. Starkey, H.C. *The Role of Clays in Fixing Lithium*; Geological Survey Bulletin 1278-F; US Government Printing Office: Washington, DC, USA, 1982. [\[CrossRef\]](#)
65. Kosmulski, M. The pH-dependent surface charging and points of zero charge. *J. Colloid Interface Sci.* **2010**, *353*, 1–15. [\[CrossRef\]](#)
66. White, G.N.; Dixon, J.B. Kaolin-serpentine minerals. In *Soil Mineralogy with Environmental Applications*; Dixon, J.B., Schulze, D.G., Eds.; Soil Science Society of America (SSSA) Book Series 7; SSSA, Inc.: Madison, WI, USA, 2002; pp. 389–414.
67. McBride, M.B. *Environmental Chemistry of Soils*; Oxford University Press, Inc.: New York, NY, USA, 1994.
68. Kolstad, D.C.; Benson, C.H.; Edil, T.B. Hydraulic Conductivity and Swell of Nonprehydrated Geosynthetic Clay Liners Permeated with Multispecies Inorganic Solutions. *J. Geotech. Geoenviron. Eng.* **2004**, *130*, 1236–1249. [\[CrossRef\]](#)
69. Norrish, K. The swelling of montmorillonite. *Discuss. Faraday Soc.* **1954**, *18*, 120–134. [\[CrossRef\]](#)
70. Newcombe, G. Removal of natural organic material and algal metabolites using activated carbon. In *Interface Science and Technology*; Newcombe, G., Dixon, D., Eds.; Elsevier: Amsterdam, The Netherlands, 2006; Volume 10, pp. 133–153.
71. Reid-Soukup, D.A.; Ulery, A.L. Smectites. In *Soil Mineralogy with Environmental Applications*; Dixon, J.B., Schulze, D.G., Eds.; Soil Science Society of America (SSSA) Book Series 7; SSSA, Inc.: Madison, WI, USA, 2002; pp. 467–499.
72. Kahut, C.K.; Warren, C.J. Chlorites. In *Soil Mineralogy with Environmental Applications*; Dixon, J.B., Schulze, D.G., Eds.; Soil Science Society of America (SSSA) Book Series 7; SSSA, Inc.: Madison, WI, USA, 2002; pp. 531–553.
73. Osman, M.A.; Suter, U.W. Determination of the Cation-Exchange Capacity of Muscovite Mica. *J. Colloid Interface Sci.* **2000**, *224*, 112–115. [\[CrossRef\]](#)
74. Czajkowski, D.M.; Shao, Z. Inhibition of protein adsorption to muscovite mica by monovalent cations. *J. Microsc.* **2003**, *211*, 1–7. [\[CrossRef\]](#)
75. Bigham, J.M.; Fitzpatrick, R.W.; Schulze, D.G. Iron Oxides. In *Soil Mineralogy with Environmental Applications*; Dixon, J.B., Schulze, D.G., Eds.; Soil Science Society of America (SSSA) Book Series 7; SSSA, Inc.: Madison, WI, USA, 2002; pp. 323–366.
76. O'Reilly, A.M.; Wanielista, M.P.; Chang, N.-B.; Xuan, Z.; Harris, W.G. Nutrient removal using biosorption activated media: Preliminary biogeochemical assessment of an innovative stormwater infiltration basin. *Sci. Total Environ.* **2012**, *432*, 227–242. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Payne, E.G.; McCarthy, D.T.; Deletic, A.; Zhang, K. Biotreatment technologies for stormwater harvesting: Critical perspectives. *Curr. Opin. Biotechnol.* **2019**, *57*, 191–196. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Shokri, M.; Kibler, K.M.; Hagglund, C.; Corrado, A.; Wang, D.; Beazley, M.; Wanielista, M. Hydraulic and nutrient removal performance of vegetated filter strips with engineered infiltration media for treatment of roadway runoff. *J. Environ. Manag.* **2021**, *300*, 113747. [\[CrossRef\]](#)
79. Wen, D.; Chang, N.-B.; Wanielista, M.P. Assessing Nutrient Removal in Stormwater Runoff for Urban Farming with Iron filings-based Green Environmental Media. *Sci. Rep.* **2020**, *10*, 9379. [\[CrossRef\]](#)
80. Chang, N.-B.; Wen, D.; Colona, W.; Wanielista, M.P. Comparison of Biological Nutrient Removal via Two Biosorption-Activated Media Between Laboratory-Scale and Field-Scale Linear Ditch for Stormwater and Groundwater Co-treatment. *Water Air Soil Pollut.* **2019**, *230*, 151. [\[CrossRef\]](#)
81. Krasucka, P.; Pan, B.; Ok, Y.S.; Mohan, D.; Sarkar, B.; Oleszczuk, P. Engineered biochar—A sustainable solution for the removal of antibiotics from water. *Chem. Eng. J.* **2021**, *405*, 126926. [\[CrossRef\]](#)
82. Zhu, S.; Xia, M.; Chu, Y.; Khan, M.A.; Lei, W.; Wang, F.; Muhmood, T.; Wang, A. Adsorption and Desorption of Pb(II) on L-Lysine Modified Montmorillonite and the simulation of Interlayer Structure. *Appl. Clay Sci.* **2018**, *169*, 40–47. [\[CrossRef\]](#)
83. Zhu, S.; Chen, Y.; Khan, M.A.; Xu, H.; Wang, F.; Xia, M. In-Depth Study of Heavy Metal Removal by an Etidronic Acid-Functionalized Layered Double Hydroxide. *ACS Appl. Mater. Interfaces* **2022**, *14*, 7450–7463. [\[CrossRef\]](#)
84. Stuckey, J.W.; Livada, J.; Martínez, C.E. Structural charge location dictates speciation and lability of copper in swelling layer silicates. *Appl. Clay Sci.* **2022**, *216*, 106332. [\[CrossRef\]](#)

-
85. Zhang, J.; Mallants, D.; Brady, P.V. Molecular dynamics study of uranyl adsorption from aqueous solution to smectite. *Appl. Clay Sci.* **2022**, *218*, 106361. [[CrossRef](#)]
 86. Barrientos-Velázquez, A.L.; Cardona, A.M.; Liu, L.; Phillips, T.; Deng, Y. Influence of layer charge origin and layer charge density of smectites on their aflatoxin adsorption. *Appl. Clay Sci.* **2016**, *132–133*, 281–289. [[CrossRef](#)]
 87. D’Ascanio, V.; Greco, D.; Menicagli, E.; Santovito, E.; Catucci, L.; Logrieco, A.F.; Avantaggiato, G. The role of geological origin of smectites and of their physico-chemical properties on aflatoxin adsorption. *Appl. Clay Sci.* **2019**, *181*, 105209. [[CrossRef](#)]
 88. Shen, C.C.; Petit, S.; Li, C.J.; Li, C.S.; Khatoon, N.; Zhou, C.H. Interactions between smectites and polyelectrolytes. *Appl. Clay Sci.* **2020**, *198*, 105778. [[CrossRef](#)]
 89. Vlad, S.; Peldszus, S.; Anderson, W.B.; Huck, P.M. Aflatoxin-a adsorption by virgin and preloaded granular activated carbon. *AWWA Water Sci.* **2019**, *1*, e1116. [[CrossRef](#)]