Clays and Clay Minerals, Vol. 70, No. 2:155–164, 2022.

EFFECTS OF METAL-POLYCATION PILLARING AND EXCHANGEABLE CATIONS ON AFLATOXIN ADSORPTION BY SMECTITE

AHMAD KHAN[1,3], MOHAMMAD SALEEM AKHTAR[1], SABA AKBAR[1,3], KHALID SAIFULLAH KHAN[1],
MAZHAR IQBAL[3], ANA BARRIENTOS-VELAZQUEZ[3], AND YOUJUN DENG[3]*

[1]Institute of Soil Science, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan
[2]National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan
[3]Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77845, USA

Abstract—Natural smectites bind aflatoxins from water effectively, but the complex chemical environment in the guts of mammals and other animals can limit binding of aflatoxins. Many efforts have been made to enhance the adsorption capacity and affinity of smectites for aflatoxins in the presence of biological compounds. The main objective of the present study was to modify smectite structures by pillaring and cation exchange to enhance aflatoxin B1 adsorption capacity and selectivity. Smectite was pillared with Al and Al-Fe polycations or saturated with Ca, Mg, Zn, or Li. Structural changes in smectites with or without heat treatment were determined using X-ray diffraction and Fourier-transform infrared spectroscopy. Equilibrium aflatoxin B1 adsorption to the smectites was measured in aqueous solution and in simulated gastric fluid. Pillaring with the polycations expanded smectites in the *z*-direction to 18.6 Å and the expansion was stable after heating at 500°C. Changes in the Al–OH–Al infrared bands in the stretching region supported the formation of pillared clays. Migration of Mg, Zn, and Li into the octahedral sites of the smectite was observed as Mg and Zn saturation yielded a *d* spacing of 15 Å at 200°C which collapsed to 9.6 Å at 400°C. The 14.6 Å peak of the Li-saturated smectite collapsed to 9.6 Å at 200°C while the 15 Å Ca-saturated smectite peak was stable up to 400°C. The unheated Al- and AlFe-pillared smectites adsorbed significantly more aflatoxin B1 from an aqueous suspension than did unpillared clay. In both water and simulated gastric fluid, heat treatment decreased aflatoxin B1 adsorption to pillared smectites, but heat treatment increased aflatoxin B1 adsorption to unpillared smectites. Without heat treatment, smectites saturated with divalent cations (Ca, Mg, Zn) adsorbed more aflatoxin B1 from an aqueous suspension than the smectite saturated with a monovalent cation (Li). Ca-saturated smectite showed the greatest aflatoxin B1 adsorption, 114 g kg^{-1}, from aqueous suspension after 400°C heat treatment. The Zn-, Mg-, and Li-saturated smectites showed maximum aflatoxin adsorption of 107, 93, and 90 g kg^{-1}, respectively, after 200°C heat treatment. From simulated gastric fluid with pepsin, the 200°C heated, Zn-saturated smectite had maximum aflatoxin B1 adsorption of 68 g kg^{-1}. Pillared smectites effectively adsorbed aflatoxin B1 from aqueous suspension, but Ca- and Zn-saturated smectites after heat treatment might improve the selectivity of smectites for aflatoxin B1 over pepsin and enhance the efficacy of smectite as a feed additive.

Keywords—Adsorption · Aflatoxin · Cation exchange smectite · Pillaring · Selectivity

INTRODUCTION

Aflatoxins are toxic metabolites of *Aspergillus* that can occur in animal feed and human food (Akinmusire et al., 2019; Guerre, 2016; Morrison et al., 2017). More than 20 aflatoxins have been identified: aflatoxin B1, B2, G1, G2, and M1 are class 1 carcinogens according to the Agency of International Research on Cancer (Marchese et al., 2018). Among these five toxins, aflatoxin B1 (AfB1) is the most prevalent and toxic, and is the biosynthetic precursor of the other aflatoxins (Rejeb et al., 2019; Sadia et al., 2012). Many physical, chemical, and biological measures have been proposed and studied for aflatoxin detoxification; adding clay minerals into animal feed is recognized as one of the most practical approaches for aflatoxin control. The detoxification is achieved via adsorption of aflatoxin by argillaceous clays (Huwing et al., 2001; Kang et al., 2016; Pasha et al., 2007; Wang et al., 2018). Multiple adsorption experiments have indicated that smectites show significant adsorption capacity for aflatoxin and can adsorb up to 20% aflatoxin by mass (Deng et al., 2012).

Aflatoxin is adsorbed at hydrophobic sites in the interlayer of smectite through ion-dipole interaction and H bonding (Barrientos-Velazquez et al., 2016b; Deng et al., 2010). Natural smectites are effective binders for aflatoxin in aqueous solutions but have limitations in the in situ gut environment due to interference and competition of biomolecules. A large mismatch between laboratory-measured adsorption capacity and the control have been reported through feeding trials (Barrientos-Velázquez et al., 2016a; Jaynes et al., 2007). Full recovery from aflatoxicosis has not been achieved in animal-feeding trials (Barrientos-Velazquez & Deng, 2020). The underperformance of smectites in feeding trials is attributed partially to the complex compositions of the gastrointestinal fluids (Barrientos-Velazquez & Deng, 2020). The gut contains numerous biomolecules including proteins and vitamins with variable pH at different tract locations. Protein molecules can access the interlayer space of smectite and block the reaction sites for aflatoxin adsorption (Barrientos-Velázquez et al., 2016a). Pepsin is considered as the major competitor of AfB1 for adsorption as it can

---

This paper belongs to a thematic set on 'Biological toxins–Clay Interactions'

* E-mail address of corresponding author: yjd@tamu.edu
DOI: 10.1007/s42860-021-00159-0

replace the exchange cations and block hydrophobic sites in the interlayer (Barrientos-Velázquez et al., 2016a). To enhance smectite effectiveness in in situ gut conditions, the selectivity of smectites for AfB1 needs to be improved. The general goal of the present study was to enhance the AfB1 adsorption and selectivity of smectites in complex gastrointestinal fluid via structural or surface modifications.

Many clay-modification methods have been developed to enhance their effectiveness as catalysts or as adsorbents (Daković et al., 2010; Marković et al., 2017; Rejeb et al., 2019). Acid activation, specific cation saturation, intercalation of large organic molecules, and pillaring are examples of these modifications (Huang et al., 2013). Pillaring in smectite increases its basal spacing and surface area and induces molecular sieve-like properties (Kloprogge et al., 2005; Marković et al., 2017; Tomul & Balci, 2008). Pillared smectite with metal polycations were reported to increase adsorption of aflatoxin in water (Zeng et al., 2013) but the selectivity of the pillared clays for AfB1 in gut fluid has not been evaluated; whether heating the pillared clays can further enhance their efficiency in binding aflatoxins is also unknown.

Cation-exchange capacity, layer-charge density, and the types of cations at exchange sites have significant effects on the ability of smectite to adsorb AfB1 (Deng et al., 2012; Gan et al., 2019). Exchange cations in smectite play a determinative role in its adsorption ability for AfB1 (Daković et al., 2008; Tong et al., 2005). Divalent cations, namely Ca, Mg, and Ba, exhibit greater affinity for and adsorption of aflatoxin compared to the monovalent cations K, Na, and Li (Deng et al., 2014). Heating the clays after exchange of various cations alters the structure as some cations can migrate into the octahedral sites of the 2:1 layers (Nones et al., 2015; Toor et al., 2015; Zivica & Palou, 2015). The heat treatments led to variation in charge density, surface area, interlayer water, and pore volume (Gan et al., 2019; Heller-Kallai, 2013; Noyan et al., 2007) in smectites and, correspondingly, their aflatoxin adsorption (Nones et al., 2015, 2016). How heating smectite affects its adsorption of AfB1 in the presence of other biological compounds is unclear.

The specific objectives of the current study were: (1) to evaluate the stability of smectite after pillaring and cation saturation with and without heating; and (2) to evaluate the effectiveness of the modified smectites in improving AfB1 adsorption in simulated gastric fluid.

MATERIALS AND METHODS

Two approaches were used to modify the smectite: one by pillaring a smectite with Al or Al-Fe polycations, and the other by saturating the smectite with Ca, Mg, Zn, or Li. Aflatoxin B1 can access the interlayer space of smectite and, therefore, the present authors have hypothesized that expanding and stabilizing the smectite interlayer would accommodate more AfB1 molecules. To optimize the smectite structures to enhance AfB1 selectivity over pepsin, pillaring of smectites using polycations was proposed. The pillared smectites were heated at elevated temperatures to modify further their charge-density and surface properties. As saturation with various cations can change the interlayer environment and subsequent heating results in reduction of layer charge, it is proposed here that exchanging the smectite interlayer with a specific cation and heating at selected temperatures may increase the hydrophobic domains for AfB1 adsorption and thus improve adsorption capacity and selectivity for aflatoxin.

PILLARING IN SMECTITE

A crystalline smectite from Bhimber, Pakistan, was fractionated to obtain <2 μm particles (Deng et al., 2006). Montmorillonite was the only or dominant mineral in the clay fraction. The smectite was pillared with Al and Al-Fe polycations following methods described by Kaloidas et al. (1995), Gil et al. (2000), Vicente et al. (2004), and Mojović et al. (2009). In brief, the solutions of 0.18 mol L^{-1} $AlCl_3$, 0.02 mol L^{-1} $FeCl_3$, and 0.2 mol L^{-1} NaOH were prepared using chemicals purchased from Sigma-Aldrich, Inc. (St. Louis, Missouri, USA). For Al pillaring, only Al solution was used while for Al-Fe pillaring, the Al and Fe solutions were mixed in an appropriate ratio to reach a final atomic ratio of 5.0% of the active Fe metal (AMR_{Fe}) and total metal concentration of 0.06 mol L^{-1} in the Al-Fe solution. Both pillaring solutions were hydrolyzed by adding NaOH solution dropwise at 70°C followed by ageing with constant shaking for 2 h. The pillaring solutions having pH 3.8 were added to pre-weighed smectites, maintaining a metal/clay ratio of 3.8 mol kg^{-1} dry clay. The dispersions were shaken for 24 h, washed with distilled deionized water twice to remove interstitial cations, and dried at 30°C. A portion of the dried pillared clay was heated at 500°C for 2 h as a calcination treatment. A schematic representation of pillaring in smectite interlayers is shown in Fig. 1.

Modifying the Interlayer Environment of Smectite by Means of Various Exchange Cations

The freeze-dried Na-saturated clay was washed three times with solutions of 0.5 M $CaCl_2$, $MgCl_2$, or $ZnSO_4$, or 1 M $LiNO_3$, in order to achieve saturation with Ca, Mg, Zn, or Li, respectively. The clay was washed with distilled, deionized water to remove the interstitial solution and dried at 30°C (Barrientos-Velazquez et al., 2016b). A portion of each cation-exchanged clay was heat treated at 200°C, and a portion at 400°C, to determine the structure stability of various cation saturations under heat treatment (Chorom & Rengasamy, 1996).

Characterization of Pillared and Cation-exchanged Smectites

All the clays modified by pillaring and cation exchange including various heat treatments were analyzed by X-ray diffraction (XRD) and Fourier-transform infrared (FTIR) spectroscopy. For XRD, 2 mg of clay was dispersed in 1 mL of distilled deionized water by sonication, and the dispersion was transferred to a glass slide and air-dried overnight. The XRD was measured over the range 2–45°2θ with a step size of 0.05°2θ and a dwell time of 5 s at each step on a D8 Advance

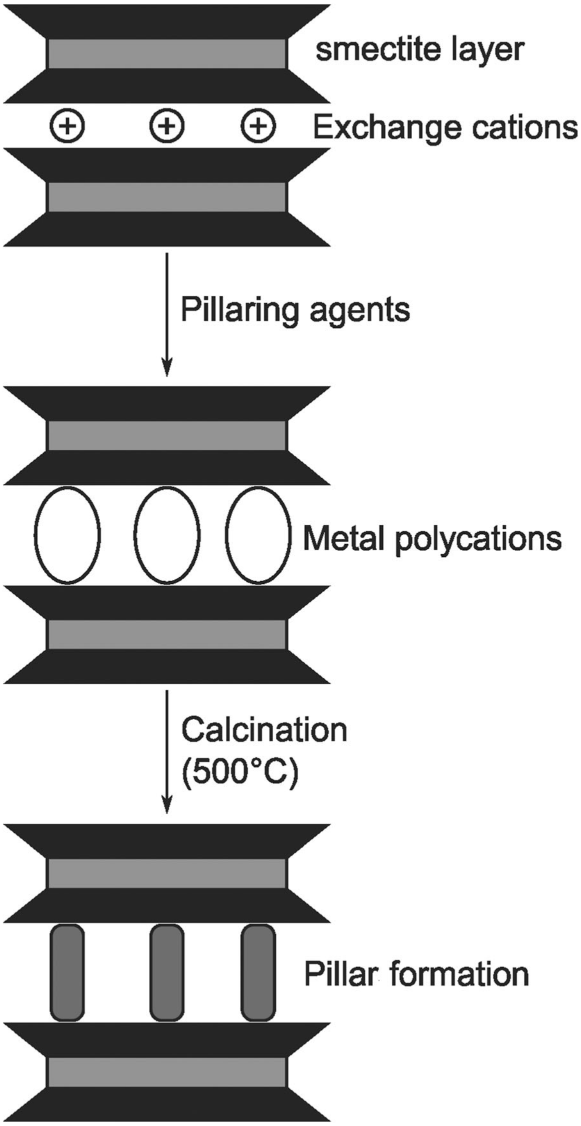

Fig. 1 Schematic representation of pillaring in smectite

Bruker diffractometer with CuKα radiation source and Sol-X detector (Bruker AXS GmbH, Karlsruhe, Germany).

For FTIR analysis, each clay dispersion was air-dried on a ZnS disc, positioned in a dewar accessory, and scanned under N_2 purging aimed at reducing CO_2 and moisture interference. The spectra were recorded in transmission mode on a Perkin-Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer, Waltham, Massachusetts, USA) with 2 cm^{-1} resolution and 32 scans for each spectrum.

Preparation of Simulating Gastric Fluid

The gastrointestinal fluid simulant was synthesized by adding 312 mg of pepsin, 125 mg of citric acid, 125 mg of malic acid, 125 µL of acetic acid, and 105 µL of lactic acid in 250 mL of distilled, deionized water, and mixed thoroughly by stirring (Barrientos-Velázquez et al., 2016a; Lemke et al., 2001). All the chemicals were purchased from Sigma-Aldrich. The solution was filtered through Whatman No. 42 filter paper and centrifuged at 4380×g for 30 min with an IEC PR-7000 centrifuge (International Equipment Company, Needham Heights, Massachusetts, USA) to remove any undissolved pepsin.

Aflatoxin Adsorption in Water and Simulated Gastric Fluid

A 1000 mg L^{-1} AfB1/acetonitrile stock solution was prepared by dissolving either 5 or 10 mg of AfB1 (Sigma-Aldrich) in a corresponding volume of acetonitrile. The stock solution was diluted with either distilled deionized water or the gastric fluid simulant to obtain solutions of 4 mg L^{-1} AfB1 in water and simulated gastric fluid, respectively. Aflatoxin B1 adsorption from a single concentration of 4 mg L^{-1} of AfB1 in water and simulated gastric fluid was quantified to select the promising treatments among the pillaring, cation exchange, and heating treatments of modified clays. 100 µg of clay was added to a 5 mL of 4 mg L^{-1} AfB1 solution in distilled deionized water or simulated gastric fluid and equilibrated overnight at 37°C with three replications. The suspension was centrifuged at 4380×g for 1 h and AfB1 in the supernatant was measured at 365 nm using a Beckman Coulter DU800 UV-spectrophotometer (Beckman Coulter Inc., Brea, California, USA) (Barrientos-Velázquez et al., 2016a; Kannewischer et al., 2006). The difference in the amount applied vs. in the supernatant was attributed to adsorption.

Aflatoxin Adsorption Isotherm

Based on the screening results of the single-concentration adsorption, AfB1 adsorption isotherms were conducted in duplicate for the pillared smectites and two selected cation-exchanged smectites before and after the heat treatment to evaluate further their maximum adsorption capacity and selectivity. Aflatoxin B1 concentrations of 0, 2, 4, 6, and 8 mg L^{-1} corresponding to 0, 6.4, 12.8, 19.2, and 25.6 micromoles L^{-1}, respectively, were prepared in water. From the 2 mg mL^{-1} clay dispersion in water, 50 µL was added in 5 mL of AfB1 standard solutions, allowed to equilibrate overnight at 37°C, and centrifuged at 6850×g for 1 h. The supernatant was decanted and analyzed for AfB1 (Kannewischer et al., 2006). The isotherms were fitted to the Langmuir equation (Eq. 1) to determine the adsorption parameters.

$$X = \frac{b\mathrm{K}C\mathrm{w}}{1 + \mathrm{K}C\mathrm{w}} \tag{1}$$

Equation 1 was rearranged in linear form:

$$\frac{C\mathrm{w}}{X} = \frac{1}{\mathrm{K}b} + \frac{C\mathrm{w}}{b} \tag{2}$$

where X is sorption of AfB1 by clay (g kg^{-1} clay), Cw is AfB1 concentration in the solution at equilibrium (mg L^{-1}), K is the constant related to binding strength (when $C\mathrm{w} = 1/\mathrm{K}$, $X = b/2$ or half of the sites on the clays are filled with AfB1), and b is the maximum adsorption capacity (Q_{max}) (g of aflatoxin kg^{-1} clay).

Statistical Analysis

Aflatoxin B1 adsorption in pillared smectite and/or cation-saturated smectite heated at different temperatures in two equilibrium fluids was analyzed by three-way analysis of variance and the treatment means were compared using Tukey's HSD test at $p < 0.05$.

RESULTS

Characteristics of Pillared Clays

The XRD and FTIR analyses indicated that pillaring of smectite was achieved with both Al and Al-Fe polycations and the pillared smectite was stable to the 500°C heat treatment. The intercalation of Al and Al-Fe polycations expanded the basal spacing of the smectite from 12.6 Å to similar values of 18.6 Å (Fig. 2a). The 500°C heat treatment shifted the basal spacing of both pillared smectite from 18.6 Å to 17.4 Å but broadened the peak width and reduced peak intensity. The decrease in intensity and increase in width of the peak may suggest a reduced crystallinity of the pillared clays that had gone through the heat treatments (Moreno et al., 1999; Taguchi & Schüth, 2005).

The Si–O stretching band at 987 cm^{-1} in unmodified smectite shifted to 991 cm^{-1} upon pillaring and to 1010 cm^{-1} upon heating pillared smectite (Fig. 2b). Similarly, the bands at 840, 913, and 1112 cm^{-1} shifted progressively to higher wavenumbers upon pillaring and subsequent heating. The IR bands associated with adsorbed water in the interlayers at 3410 cm^{-1} and 1634 cm^{-1} reduced in intensity suggesting water loss upon heating (Besson & Drits, 1997; Rejeb et al., 2019). The intensity of the IR band at 3616 cm^{-1} associated with Al–OH stretching was also reduced as a wider band at 3626 cm^{-1} formed in pillared clays and further shifted to 3637 cm^{-1} upon heat treatments of pillared clays (Farmer, 1974). The IR vibration at 1112 cm^{-1} moved to 1125 cm^{-1} in pillared clays with heating and is associated with in-plane vibrational movement in the Si–O stretch region. The new bands at 870 cm^{-1} formed in heated pillared clays suggested bonding between the tetrahedral sheet and intercalated pillaring ions.

Characteristics of Cation-exchanged Smectite before and after Heating

Saturating the smectite with Mg and Zn resulted in a 15 Å basal spacing and this basal spacing remained unchanged when the Mg- and Zn-saturated smectites were heated to 200°C and then cooled to room temperature. These smectites collapsed to 9.6 Å after heating at 400°C (Fig. 3) and did not return to their initial 15 Å basal spacing. For lithium-saturated smectite, the 14.6 Å peak collapsed to 9.6 Å after heating at 200°C while in the case of calcium saturation, the 15 Å peak was stable up to 400°C. The basal spacing responses to heating suggested that water molecules could still access the interlayer gallery in the Mg- and Zn-saturated smectite after 200°C heating and in the Ca-saturated smectite even after 400°C heating, but water cannot access the interlayer of the Li-saturated smectite after 200°C heating. The *d* spacing changes suggested that Mg, Zn, and Li could migrate to the empty octahedral sites in the smectite but Ca could not during the heating. Lithium, having a smaller cationic radius, could not

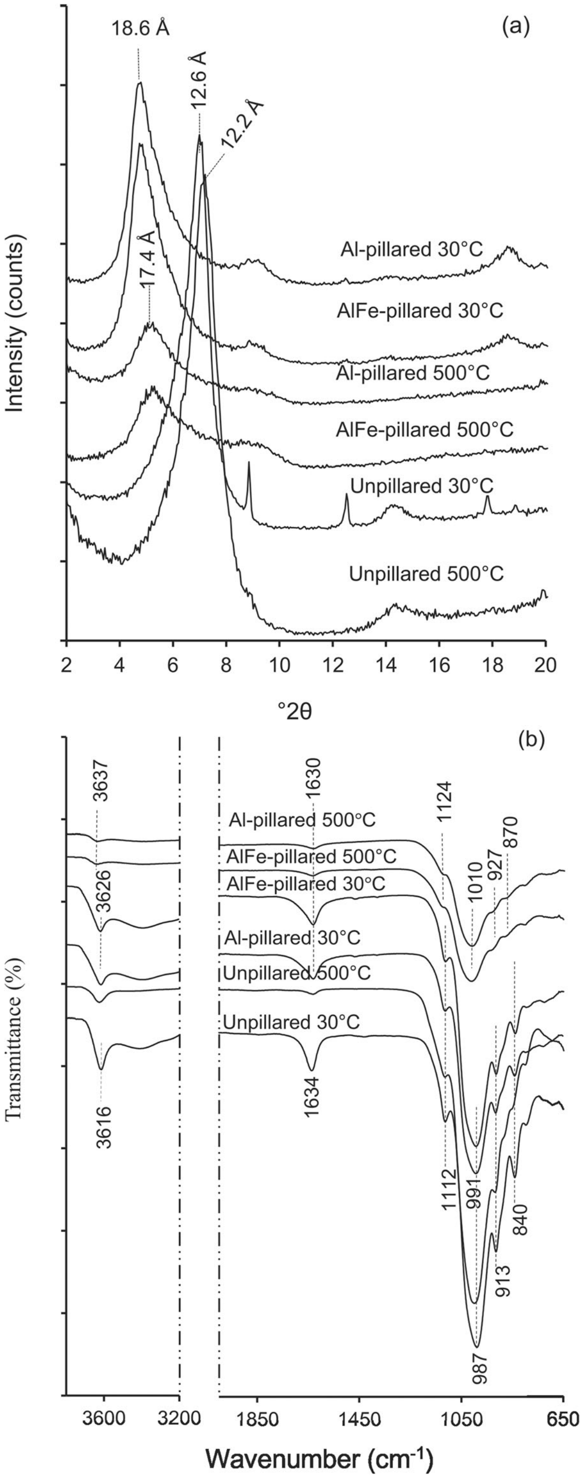

Fig. 2 **a** XRD patterns and **b** FTIR spectra of clays upon intercalation of metal polycations and subsequent heat treatments

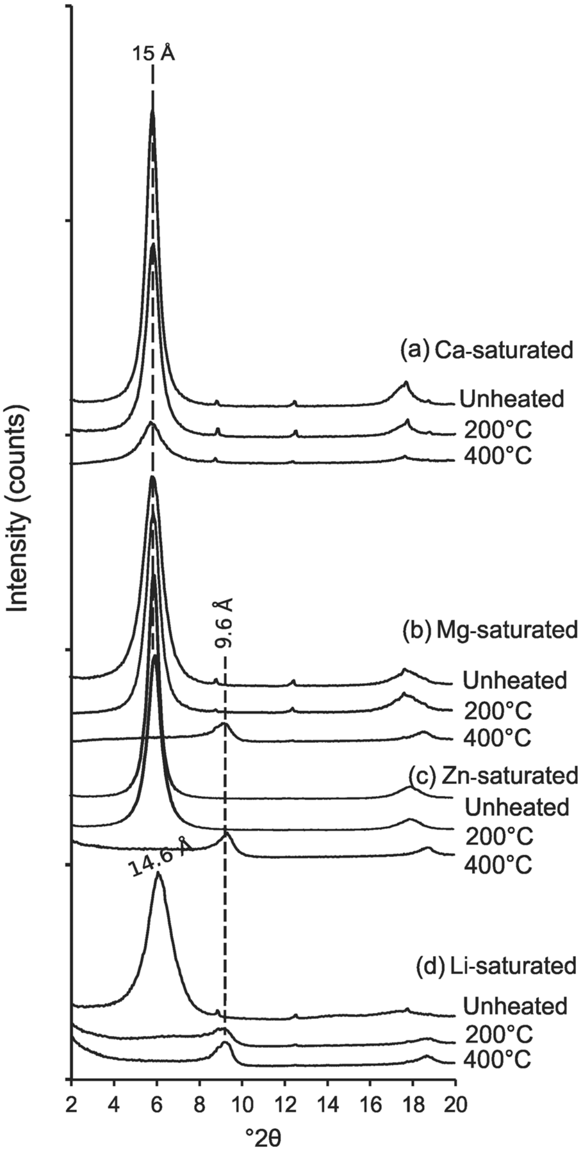

Fig. 3 XRD patterns of smectite saturated with Li, Mg, Zn, or Ca before and after heating for 2 h at 200 and 400°C

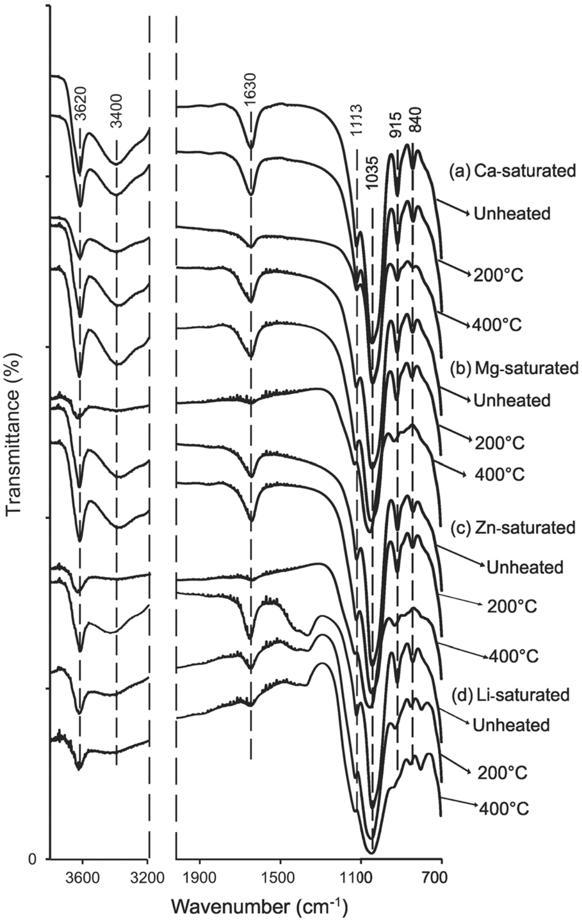

Fig. 4 IR spectra of smectite saturated with various cations and heated for 2 h

resist the heat effect, and the migration occurred at 200°C (Jaynes & Bigham, 1987) while the migration of Mg and Zn required a higher temperature. The XRD peak intensity decreased with the heat effect and subsequent broadening of peaks occurred in calcium-saturated smectite, which indicated loss of structure crystallinity upon heating (Calvet & Prost, 1971; Tlili et al., 2012).

The shift in IR band positions under saturation with different cations occurred only after heating at 200°C and 400°C and was constant irrespective of the cation on exchange sites. The shifts were associated with the heat effect rather than with the choice of cation (Fig. 4). The IR band position at 1035 cm^{-1} associated with out-of-plane Si–O stretching vibrations shifted to 1040 cm^{-1} upon heating at 400°C with Li and Ca saturation and to 1050 cm^{-1} in Mg- and Zn-saturated clays. In-plane stretching of Si–O resulted in a reflection at 1113 cm^{-1} which was shifted to 1121 cm^{-1} after heating at 400°C. The shifting of bands in the Si–O stretching region was due to the formation of new bonds between the tetrahedral-sheet and interlayer cations after loss of water molecules (Chorom & Rengasamy, 1996; Kloprogge et al., 1994).

Aflatoxin Adsorption by Pillared Smectites

A single-point (4 mg L^{-1}) aflatoxin adsorption experiment indicated that the interaction of three main factors, i.e. the type of pillaring, the heat treatment, and fluid type, was significant (F 330, $p < 0.0001$) (Fig. 5). Pillaring in smectite resulted in greater adsorption in aqueous solution but was on a par with unpillared smectite when the solution was simulated gastric fluid. Heat treatment increased AfB1 adsorption in unpillared smectite but not in the pillared smectites irrespective of the fluid type or the pillaring cation type. When aflatoxin adsorption was conducted in the absence of gastrointestinal compounds, the unpillared smectite heated at 500°C had the greatest mean adsorption of 120 g kg^{-1}, followed by the unheated Al-pillared of 90.4 g kg^{-1}, and the unpillared smectite heated at 500°C in simulated gastric fluid, 70.8 g kg^{-1}. Both the unheated Al and Al-Fe pillared smectites

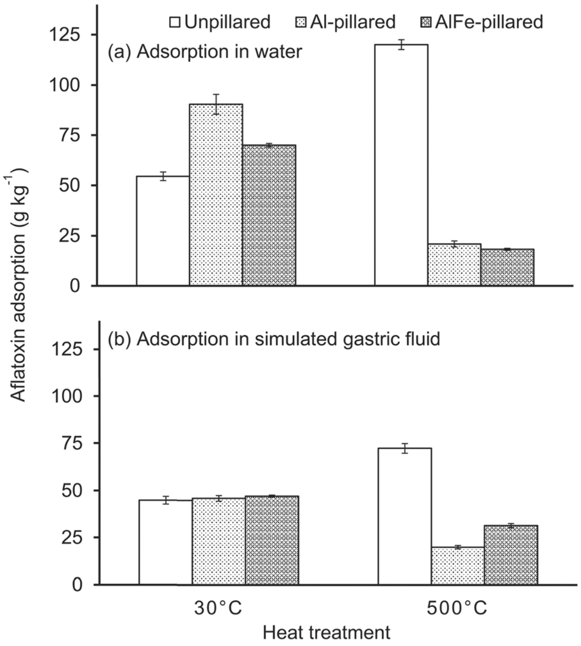

Fig. 5 Adsorption of aflatoxin B1 by pillared smectites from a single initial concentration of 4 mg L^{-1} aflatoxin B1 from aqueous solution and from simulated gastric fluids

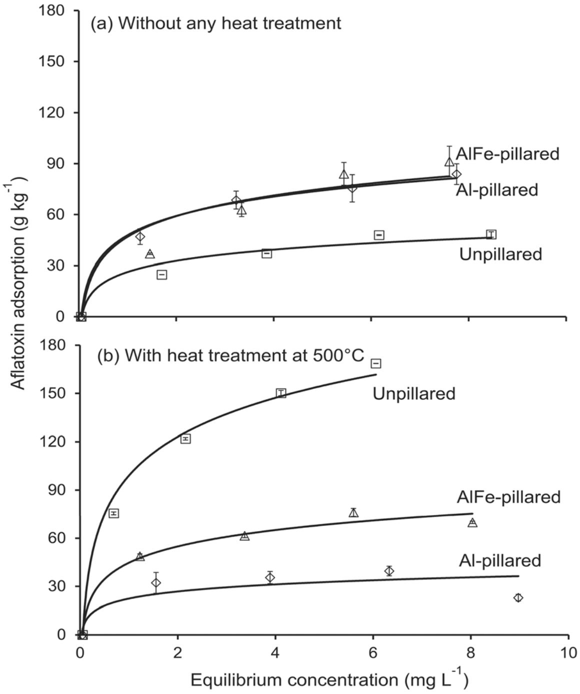

Fig. 6 Aflatoxin B1 adsorption isotherms on smectites before and after pillaring and heating treatments

had significantly greater adsorption in aqueous solution than unpillared smectite. Aflatoxin adsorption reduced in the pillared smectites and increased in unpillared smectites with heat treatment in each fluid type. A non-significant variation occurred among the two types of pillared and unpillared smectites without heat treatments when adsorption was carried out in simulated gastric fluid. The lesser adsorption in simulated gastric fluid over water may be attributed to the lower pH of 3.4 compared to 5.5 in aqueous suspensions and to pepsin interference. Apparently, pillaring with Al had a nominal advantage, while heating the natural smectite at 500°C had greater adsorption in aqueous suspension and the difference was maintained in gastric fluid also. When the same smectite had been heated at 500°C, the pillared smectite showed least adsorption in both the simple aqueous and in the simulated gastric fluids.

Aflatoxin adsorption isotherms on pillared smectites. The isothermal adsorption of AfB1 by the pillared and heat-treated smectite appeared to vary with treatments (Fig. 6). Each pillared smectite initially showed a significant increase and steeper slope (in terms of adsorption) than natural smectite where no heat treatment was carried out. In the case of heat treatment at 500°C, the natural smectite initially showed a steep increase in the isotherm and the steepest slope. Overall, the adsorption isotherms had an L2 shape as described by Grant and Phillips (1998). The Langmuir equation fitted the adsorption isotherms well (Table 1). The maximum AfB1 adsorption capacity (Q_{max}) varied, and was maximum in the case of natural smectite after heating at 500°C; and the value 0.64 mol kg^{-1} (20% w/w) matched the maximum adsorption reported so far (Deng et al., 2012). The Q_{max} reduced by heating in the case of pillared smectite and increased in the case of natural smectite. The adsorption strength increased nearly twofold by heating at 500°C in the case of the Al-Fe pillared smectite.

Aflatoxin Adsorption by Smectites Saturated with Various Cations

The interactive effect of the cation, heat treatment, and the equilibrium fluid type was significant (F 73.5, $p < 0.0001$) for adsorption of AfB1 at a single concentration of 4 mg L^{-1}. Mean AfB1 adsorption ranged from 12.4 to 114.1 g kg^{-1} among cation-exchanged smectites (Fig. 7). The Ca-saturated smectite showed the greatest adsorption followed by Zn- and Mg-exchanged smectites. Heating the smectites at 200°C resulted in greater AfB1 adsorption for Li-, Mg-, and Zn-saturated smectites but at 400°C reduced their efficacy in binding AfB1. Heating Ca-smectite at 400°C and Zn- and

Table 1 Langmuir model-fitted parameters for aflatoxin B1 adsorption in pillared smectites

Samples	Q_{max} (mol kg^{-1})	K	r^2
Unpillared 30°C	0.21	0.36	0.98
Al-pillared 30°C	0.31	0.72	0.99
AlFe-pillared 30°C	0.46	0.24	0.99
Unpillared 500°C	0.64	0.78	0.99
Al-pillared 500°C	0.07	0.86	0.88
AlFe-pillared 500°C	0.25	1.42	0.98

Q_{max} maximum adsorption capacity, K maximum binding strength, and r^2 correction coefficient

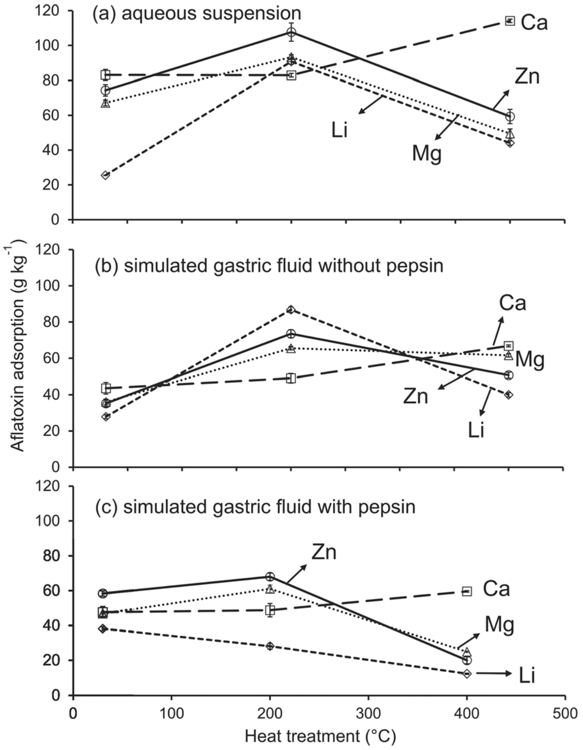

Fig. 7 Aflatoxin adsorption by smectites from 4 mg L^{-1} aflatoxin B1 after cation saturation and heating in three fluids: **a** aqueous solution; **b** simulated gastric fluid without pepsin; and **c** simulated gastric fluid with pepsin

Mg-smectites at 200°C resulted in greater AfB1 adsorption in aqueous suspension and also increased aflatoxin selectivity over pepsin in simulated gastric fluid. The greatest adsorption (114.3 g kg^{-1}) was obtained when Ca smectite was heated at 400°C, followed by Zn-smectite heated at 200°C. In the simulated gastric fluid with pepsin excluded, Li saturation and subsequent heat treatment at 200°C resulted in the maximum adsorption. A decline in AfB1 adsorption occurred after heating at 400°C for all cation saturations except Ca, which may be related to degeneration of the structure and charge reduction. In the simulated gastric fluid containing pepsin, the 200°C heated Zn-smectite had the maximal aflatoxin adsorption of 68.0 g kg^{-1}.

Table 2 Langmuir model-fitted parameters for aflatoxin B1 adsorption in selected cation exchanged smectites

Samples	$Q_{\max}$ (mol kg^{-1})	K	r^2
Zn smectite 30°C	0.44	0.42	0.95
Ca smectite 30°C	0.45	1.25	0.99
Zn smectite 200°C	0.42	2.03	0.99
Ca smectite 400°C	0.47	1.79	0.99

Q_{max} maximum adsorption capacity, K maximum binding strength, and r^2 correction coefficient

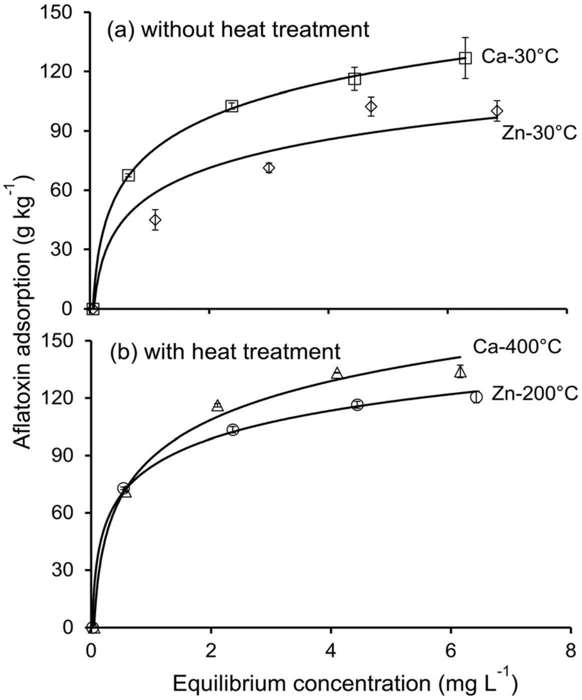

Fig. 8 Aflatoxin B1 adsorption isotherms for Ca- and Zn-saturated smectites after heating

Aflatoxin isotherms and adsorption capacity of cation-exchanged smectites. The exchange cation type or the heat treatment afterwards had little effect on maximum adsorption capacity (Table 2, Fig. 8). The heat treatment, however, caused the initial adsorption isotherm slope, an indication of binding strength, to increase. The natural smectite showed maximum AfB1 adsorption of 0.2 mol kg^{-1} which increased twofold with Ca and Zn saturation (Table 1). Though the additional heat treatment had no effect on $Q_{\max}$, it increased the maximum binding strength substantially. The increased adsorption by cation exchange is greater than the criterion (0.3 mol kg^{-1}) of an effective adsorbent set by Dixon et al. (2008).

DISCUSSION

Efficiency of Pillaring in Enhancing the Adsorption for Aflatoxin B1 by Smectite

Pillaring of smectite at room temperature increased adsorption of AfB1 in a simple aqueous solution, but did not increase the selectivity of smectite for AfB1 in the gastric fluid (Fig. 5). The relatively significant adsorption of AfB1 by the pillared smectite was associated with the expansion of the interlayer space as illustrated in Fig. 1 and confirmed by the XRD results (Fig. 2a). Several other studies also reported an increase in aflatoxin adsorption in pillared smectites over unmodified smectite (Lothenbach et al., 1997; Phillips et al., 1995; Vicente et al., 2004; Zeng et al., 2013). All of those results were obtained using simple aqueous solutions, however, not gastric fluids. The AfB1 adsorption enhancement effect of pillaring smectite was masked by gastric fluid (Fig.5, lower left). The simulated gastric fluid contained multiple biomolecules, and pepsin was a main biomolecule interfering with AfB1 adsorption by smectite (Barrientos-Velazquez & Deng, 2020; Jaynes et al., 2007). Pillaring of smectite was expected to limit access of the large protein molecules into the interlayer space. The small or negligible effects of pillaring on AfB1 adsorption in the simulated gastric fluid suggested that the small organic acids in the gastric fluid interacted with the pillaring polycations and made the interlayer environment less favorable for AfB1 adsorption.

Heating the pillared smectite was expected to might further enhance the stability of the porous interlayer structure of the pillared smectites and, therefore, enhance their adsorption of aflatoxin. The FTIR data (Fig. 3) suggested that the dehydration and dehydroxylation of interlayer water and pillars upon the 500°C heat treatment resulted in strong bonding of pillaring polycations to the tetrahedral sheet (Sergio et al., 2006; Yuan et al., 2008). These changes caused the reduction in the number of binding sites for AfB1 molecules in both the simple aqueous solution and the gastric fluid simulant. Reduced AfB1 adsorption after heating at 500°C and the lack of increment in selecting aflatoxin in gastric fluid suggested that heating at such high temperature was not optimal in terms of modifying smectite as aflatoxin binders. Further optimization of the pillaring is still needed.

Efficiency of Cation Exchange and Heating in Enhancing the Adsorption by Smectite of Aflatoxin B1

Both consistency and discrepancies were observed on the effects of exchange cations in the present study compared to those reported in the literature. Bonding between interlayer cations and two carbonyl oxygens is a major mechanism of aflatoxin binding with smectite (Deng & Szczerba, 2011), and thus the adsorption and affinity of aflatoxin depends on exchange cations (Daković et al., 2008; Deng et al., 2012). Smectite with Ca saturation adsorbed more AfB1 than those saturated with Zn and Mg. The greater adsorption by Ca-smectite could be related to a lower hydration energy of Ca than those of Mg and Zn. The increase in adsorption of cation-exchanged smectite upon heating was related to charge reduction (Chorom & Rengasamy, 1996; Madejová et al., 2006). Reduced aflatoxin adsorption by heating of natural bentonite was also reported (Nones et al., 2016). The maximum AfB1 adsorption was increased tenfold in Ca-saturated smectite and heated over the natural smectite (Gan et al., 2019). The decline in AfB1 adsorption after heating at 400°C in all cation saturations except with Ca was related to interlayer collapse as identified by XRD. Smectites have been reported to have an optimal charge density range for aflatoxin adsorption, the corresponding cation exchange capacity was between 80 and 110 cmol/kg. Reducing the charge density below the optimal range decreased AfB1 adsorption (Deng et al., 2012). The results observed in the current study suggested that saturating the smectite with Li, Mg, or Zn, and then heating Li-smectite at 200°C or Mg- or Zn-smectite at 400°C induced too much charge reduction of the smectite due to the migration of the cations into the octahedral sites. On the other hand, heating Mg- and Zn-smectite at 200°C caused only partial migration of the respective cation into the octahedral sites and induced greater AfB1 adsorption irrespective of fluid type over unheated cation-exchanged smectites. The greatest AfB1 adsorption of the 400°C heated Ca-smectite suggested that there were other structural or surface property changes on the smectite as Ca cannot occupy the octahedral sites.

Pepsin is a major interfering compound in aflatoxin adsorption as pepsin intercalated into the smectite interlayer at the expense of exchange cations according to Barrientos-Velázquez et al., (2016a) and Barrientos-Velazquez and Deng (2020). Those authors reported that the Ca- and Ba-smectite had greater maximum adsorption than Na-saturated smectite in water but the adsorption declined and the initial saturating cation effect diminished in simulated gastric fluid (Barrientos-Velázquez et al., 2016a). In the present study, smectites saturated with divalent cations Ca, Zn, or Mg adsorbed more aflatoxin than the Li-smectite in simulated gastric fluid. This could be associated with greater hydration energy and strong bonding of these cations to the clay surface (Deng et al., 2010; Deng & Szczerba, 2011). AfB1 adsorption by cation-exchanged smectites was observed to decline in simulated gastric fluid with and without pepsin. Though heating the Li-, Mg-, Zn-smectites at 200°C and Ca-smectite at 400°C enhanced their binding capacity for AfB1 in simple aqueous solutions, no improvement was observed in the presence of small organic acids or of pepsin in simulated gastric fluid. The increase in AfB1 adsorption in simple aqueous solution was related to charge reduction and favorable interlayer environment for aflatoxin binding. Likewise, heating also increased the affinity of AfB1 to smectite/clay that may have helped in greater adsorption of AfB1 over unheated cation-exchanged smectites in different fluid types.

CONCLUSIONS

Smectites modified by pillaring with Al and Al-Fe polycations enhanced maximum adsorption of AfB1 from aqueous suspensions through expansion in interlayer spaces, but the selective adsorption of aflatoxin over pepsin in simulated gastric fluid was not improved, which was probably due to the interference of simple organic acids that resulted in a less favorable interlayer environment for aflatoxin binding. Heating the pillared smectite reduced the binding ability of pillared clays in both simple aqueous solution and simulated gastric fluid due to strong bonding of pillars and exchange cations to clay upon dehydration and dehydroxylation leaving fewer binding sites for AfB1. Smectites saturated with Ca, Mg, or Zn cations had greater AfB1 adsorption compared to Li-exchanged smectite and was related to the divalent nature of the exchange cations. Heating increased selectivity for the adsorption of AfB1 over pepsin from simulated gastric fluid due to charge reduction and increase in hydrophobicity of the interlayer. Pillaring was effective without heating in aqueous suspensions only while smectites exchanged with Ca and Zn and heating effectively increased AfB1 adsorption from both aqueous suspensions and simulated gastric fluid.

ACKNOWLEDGMENTS

The present study was carried out during a student-exchange program funded by the Higher Education Commission of Pakistan.

FUNDING

Funding sources are as stated in the Acknowledgments.

Compliance with ethical statements

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

Akinmusire, O. O., El-Yuguda, A. D., Musa, J. A., Oyedele, O. A., Sulyok, M., Somorin, Y. M., Ezekiel, C. N., & Krska, R. (2019). Mycotoxins in poultry feed and feed ingredients in Nigeria. *Mycotoxin Research, 35*(2), 149–155. https://doi.org/10.1007/s12550-018-0337-y

Barrientos-Velazquez, A. L., & Deng, Y. (2020). Reducing competition of pepsin in aflatoxin adsorption by modifying a smectite with organic nutrients. *Toxins, 12*(1), 28. https://doi.org/10.3390/toxins12010028

Barrientos-Velázquez, A. L., Arteaga, S., Dixon, J. B., & Deng, Y. (2016a). The effects of pH, pepsin, exchange cation, and vitamins on aflatoxin adsorption on smectite in simulated gastric fluids. *Applied Clay Science, 120*, 17–23. https://doi.org/10.1016/j.clay.2015.11.014

Barrientos-Velazquez, A. L., Cardona, A. M., Liu, L., Phillips, T., & Deng, Y. (2016b). Influence of layer charge origin and layer charge density of smectites on their aflatoxin adsorption. *Applied Clay Science, 132*, 281–289. https://doi.org/10.1016/j.clay.2016.06.014

Besson, G., & Drits, V. (1997). Refined relationships between chemical composition of dioctahedral fine-grained micaceous minerals and their infrared spectra within the OH stretching region. Part II: The main factors affecting OH vibrations and quantitative analysis. *Clays and Clay Minerals, 45*(2), 170–183. https://doi.org/10.1346/CCMN.1997.0450205

Calvet, R., & Prost, R. (1971). Cation migration into empty octahedral sites and surface properties of clays. *Clays and Clay Minerals, 19*(3), 175–186. https://doi.org/10.1346/CCMN.1971.0190306

Chorom, M., & Rengasamy, P. (1996). Effect of heating on swelling and dispersion of different cationic forms of a smectite. *Clays and Clay Minerals, 44*(6), 783–790. https://doi.org/10.1346/CCMN.1996.0440609

Daković, A., Matijašević, S., Rottinghaus, G. E., Ledoux, D. R., Butkeraitis, P., & Sekulić, Ž. (2008). Aflatoxin B1 adsorption by natural and copper modified montmorillonite. *Colloids and Surfaces B: Biointerfaces, 66*(1), 20–25. https://doi.org/10.1016/j.colsurfb.2008.05.008

Daković, A., Kragović, M., Rottinghaus, G. E., Sekulić, Ž., Milićević, S., Milonjić, S. K., & Zarić, S. (2010). Influence of natural zeolitic tuff and organozeolites surface charge on sorption of ionizable fumonisin B1. *Colloids and Surfaces B: Biointerfaces, 76*(1), 272–278. https://doi.org/10.1016/j.colsurfb.2009.11.003

Deng, Y., & Szczerba, M. (2011). Computational evaluation of bonding between aflatoxin B1 and smectite. *Applied Clay Science, 54*(1), 26–33. https://doi.org/10.1016/j.clay.2011.07.007

Deng, Y., Dixon, J. B., & White, G. N. (2006). Adsorption of polyacrylamide on smectite, illite and kaolinite. *Soil Science Society of America Journal, 70*(1), 297–304. https://doi.org/10.2136/sssaj2005.0200

Deng, Y., Velazquez, A. L. B., Billes, F., & Dixon, J. B. (2010). Bonding mechanisms between aflatoxin B1 and smectite. *Applied Clay Science, 50*(1), 92–98. https://doi.org/10.1016/j.clay.2010.07.008

Deng, Y., Liu, L., Velazquez, A. L. B., & Dixon, J. B. (2012). The determinative role of the exchange cation and layer-charge density of smectite on aflatoxin adsorption. *Clays and Clay Minerals, 60*(4), 374–386. https://doi.org/10.1346/CCMN.2012.0600404

Deng, Y., Liu, L., Barrientos Velázquez, A., Szczerba, M., & Dixon, J. (2014). Interactions of aflatoxin B1 with smectites: Interlayer accessibility, bonding mechanisms, and size matching. *Aflatoxin Control: Safeguarding Animal Feed with Calcium Smectite*, 27–43. https://doi.org/10.2136/2014.aflatoxins.c4

Dixon, J. B., Kannewischer, I., Arvide, M. G. T., & Velazquez, A. L. B. (2008). Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan. *Applied Clay Science, 40*(1-4), 201–208. https://doi.org/10.1016/j.clay.2007.10.010

Farmer, V. C. (1974). *The Infrared Spectra of Minerals* (p. 331). Mineralogical Society. https://doi.org/10.1180/mono-4

Gan, F., Hang, X., Huang, Q., & Deng, Y. (2019). Assessing and modifying China bentonites for aflatoxin adsorption. *Applied Clay Science, 168*, 348–354. https://doi.org/10.1016/j.clay.2018.12.001

Gil, A., Gandia, L. M., & Vicente, M. A. (2000). Recent advances in the synthesis and catalytic applications of pillared clays. *Catalysis Reviews, 42*(1-2), 145–212. https://doi.org/10.1081/CR-100100261

Grant, P. G., & Phillips, T. D. (1998). Isothermal adsorption of aflatoxin B1 on HSCAS clay. *Journal of Agricultural and Food Chemistry, 46*(2), 599–605. https://doi.org/10.1021/jf970604v

Guerre, P. (2016). Worldwide mycotoxins exposure in pig and poultry feed formulations. *Toxins, 8*(12), 350. https://doi.org/10.3390/toxins8120350

Heller-Kallai, L. (2013). Thermally modified clay minerals. In *Developments in Clay Science* (Vol. 5, pp. 411–433). Elsevier Amsterdam. https://doi.org/10.1016/B978-0-08-098258-8.00014-6.

Huang, Y., Zhang, J., & Zhu, L. (2013). Evaluation of the application potential of bentonites in phenanthrene bioremediation by characterizing the biofilm community. *Bioresource Technology, 134*, 17–23. https://doi.org/10.1016/j.biortech.2013.02.009

Huwing, A. K., Freimurd, S., Kappeli, O., & Dulter, H. (2001). Mycotoxin detoxication of animal feed by different adsorbents. *Toxicology Letters, 122*(2), 179–188. https://doi.org/10.1016/S0378-4274(01)00360-5

Jaynes, W., & Bigham, J. (1987). Charge reduction, octahedral charge, and lithium retention in heated, Li-saturated smectites. *Clays and Clay Minerals, 35*(6), 440–448. https://doi.org/10.1346/CCMN.1987.0350604

Jaynes, W., Zartman, R., & Hudnall, W. (2007). Aflatoxin B1 adsorption by clays from water and corn meal. *Applied Clay Science, 36*(1-3), 197–205. https://doi.org/10.1016/j.clay.2006.06.012

Kaloidas, V., Koufopanos, C., Gangas, N., & Papayannakos, N. (1995). Scale-up studies for the preparation of pillared layered clays at 1 kg per batch level. *Microporous Materials, 5*(1-2), 97–106. https://doi.org/10.1016/0927-6513(95)00047-D

Kang, F., Ge, Y., Hu, X., Goikavi, C., Waigi, M. G., Gao, Y., & Ling, W. (2016). Understanding the sorption mechanisms of aflatoxin B1 to kaolinite, illite, and smectite clays via a comparative computational study. *Journal of Hazardous Materials, 320*, 80–87. https://doi.org/10.1016/j.jhazmat.2016.08.006

Kannewischer, I., Arvide, M. G. T., White, G. N., & Dixon, J. B. (2006). Smectite clays as adsorbents of aflatoxin B1: Initial steps. *Clay Science, 12*, 199–204. https://doi.org/10.11362/jcssjclayscience1960.12.Supplement2_199

Kloprogge, J., Booy, E., Jansen, J., & Geus, J. (1994). The effect of thermal treatment on the properties of hydroxy-Al and hydroxy-Ga pillared montmorillonite and beidellite. *Clay Minerals, 29*(2), 153–167. https://doi.org/10.1180/claymin.1994.029.2.02

Kloprogge, J. T., Duong, L. V., & Frost, R. L. (2005). A review of the synthesis and characterisation of pillared clays and related porous materials for cracking of vegetable oils to produce biofuels. *Environmental Geology, 47*(7), 967–981. https://doi.org/10.1007/s00254-005-1226-1

Lemke, S., Ottinger, S., Mayura, K., Ake, C., Pimpukdee, K., Wang, N., & Phillips, T. (2001). Development of a multi-tiered approach to the in vitro prescreening of clay-based enterosorbents. *Animal Feed Science and Technology, 93*(1–2), 17–29. https://doi.org/10.1016/S0377-8401(01)00272-3

Lothenbach, B., Furrer, G., & Schulin, R. (1997). Immobilization of heavy metals by polynuclear aluminium and montmorillonite compounds. *Environmental Science and Technology, 31*(5), 1452–1462. https://doi.org/10.1021/es960697h

Madejová, J., Pálková, H., & Komadel, P. (2006). Behaviour of Li^+ and Cu^{2+} in heated montmorillonite: Evidence from far-, mid-, and near-IR regions. *Vibrational Spectroscopy, 40*(1), 80–88. https://doi.org/10.1016/j.vibspec.2005.07.004

Marchese, S., Polo, A., Ariano, A., Velotto, S., Costantini, S., & Severino, L. (2018). Aflatoxin B1 and M1: biological properties and their involvement in cancer development. *Toxins, 10*(6), 214. https://doi.org/10.3390/toxins10060214

Marković, M., Daković, A., Rottinghaus, G. E., Kragović, M., Petković, A., Krajišnik, D., Milić, J., Mercurio, M., & de Gennaro, B. (2017). Adsorption of the mycotoxin zearalenone by clinoptilolite and phillipsite zeolites treated with cetylpyridinium surfactant. *Colloids and Surfaces B: Biointerfaces, 151*, 324–332. https://doi.org/10.1016/j.colsurfb.2016.12.033

Mojović, Z., Banković, P., Milutinović-Nikolić, A., Dostanić, J., Jović-Jovičić, N., & Jovanović, D. (2009). Al, Cu-pillared clays as catalysts in environmental protection. *Chemical Engineering Journal, 154*(1-3), 149–155. https://doi.org/10.1016/j.cej.2009.05.004

Moreno, S., Kou, R. S., Molina, R., & Poncelet, G. (1999). Al-, Al, Zr-, and Zr-pillared montmorillonites and saponites: Preparation, characterization, and catalytic activity in heptane hydroconversion. *Journal of Catalysis, 182*(1), 174–185. https://doi.org/10.1006/jcat.1998.2349

Morrison, D. M., Ledoux, D. R., Chester, L. F., & Samuels, C. A. (2017). A limited survey of aflatoxins in poultry feed and feed ingredients in Guyana. *Veterinary Sciences, 4*(4), 60. https://doi.org/10.3390/vetsci4040060

Nones, J., Nones, J., Riella, H. G., Poli, A., Trentin, A. G., & Kuhnen, N. C. (2015). Thermal treatment of bentonite reduces aflatoxin b1 adsorption and affects stem cell death. *Materials Science and Engineering: C, 55*, 530–537. https://doi.org/10.1016/j.msec.2015.05.069

Nones, J., Nones, J., Poli, A., Trentin, A. G., Riella, H. G., & Kuhnen, N. C. (2016). Organophilic treatments of bentonite increase the adsorption of aflatoxin B1 and protect stem cells against cellular damage. *Colloids and Surfaces B: Biointerfaces, 145*, 555–561. https://doi.org/10.1016/j.colsurfb.2016.05.061

Noyan, H., Önal, M., & Sarıkaya, Y. (2007). The effect of sulphuric acid activation on the crystallinity, surface area, porosity, surface acidity, and bleaching power of a bentonite. *Food Chemistry, 105*(1), 156–163. https://doi.org/10.1016/j.foodchem.2007.03.060

Pasha, T., Farooq, M., Khattak, F., Jabbar, M., & Khan, A. (2007). Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. *Animal Feed Science and Technology, 132*(1-2), 103–110. https://doi.org/10.1016/j.anifeedsci.2006.03.014

Phillips, T. D., Sarr, A. B., & Grant, P. G. (1995). Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. *Natural Toxins, 3*(4), 204–213. https://doi.org/10.1002/nt.2620030407

Rejeb, R., Antonissen, G., De Boevre, M., Detavernier, C. l., Van de Velde, M., De Saeger, S., Ducatelle, R., Hadj Ayed, M., & Ghorbal, A. (2019). Calcination enhances the aflatoxin and zearalenone binding efficiency of a Tunisian clay. *Toxins, 11*(10), 602. https://doi.org/10.3390/toxins11100602

Sadia, A., Jabbar, M. A., Deng, Y., Hussain, E. A., Riffat, S., Naveed, S., & Arif, M. (2012). A survey of aflatoxin M1 in milk and sweets of Punjab, Pakistan. *Food Control, 26*(2), 235–240. https://doi.org/10.1016/j.foodcont.2012.01.055

Sergio, M., Musso, M., Medina, J., & Diano, W. (2006). Aluminum-pillaring of a montmorillonitic clay: textural properties as a function of the starting mineral particle size. *Advances in Technology of Materials and Materials Processing Journal, 8*(1), 5. https://doi.org/10.2240/azojomo0179

Taguchi, A., & Schüth, F. (2005). Ordered mesoporous materials in catalysis. *Microporous and Mesoporous Materials, 77*(1), 1–45. https://doi.org/10.1016/j.micromeso.2004.06.030

Tlili, A., Saidi, R., Fourati, A., Ammar, N., & Jamoussi, F. (2012). Mineralogical study and properties of natural and flux calcined porcelanite from Gafsa-Metlaoui basin compared to diatomaceous filtration aids. *Applied Clay Science, 62*, 47–57. https://doi.org/10.1016/j.clay.2012.04.012

Tomul, F., & Balci, S. (2008). Synthesis and characterization of Al-pillared interlayered bentonites. *Gazi University Journal of Science, 21*(1), 21–31 https://dergipark.org.tr/tr/pub/gujs/issue/7395/96773

Tong, G., Yulong, M., Peng, G., & Zirong, X. J. (2005). Antibacterial effects of the Cu (II)-exchanged montmorillonite on *Escherichia coli* K88 and *Salmonella choleraesuis*. *Veterinary Microbiology, 105*(2), 113–122.

Toor, M., Jin, B., Dai, S., & Vimonses, V. (2015). Activating natural bentonite as a cost-effective adsorbent for removal of Congo-red in wastewater. *Journal of Industrial and Engineering Chemistry, 21*, 653–661. https://doi.org/10.1016/j.jiec.2014.03.033

Vicente, M., Belver, C., Trujillano, R., Rives, V., Álvarez, A., Lambert, J. F., Korili, S., Gandıa, L., & Gil, A. (2004). Preparation and characterisation of Mn-and Co-supported catalysts derived from Al-pillared clays and Mn-and Co-complexes. *Applied Catalysis A: General, 267*(1-2), 47–58. https://doi.org/10.1016/j.apcata.2004.02.023

Wang, G., Miao, Y., Sun, Z., & Zheng, S. (2018). Simultaneous adsorption of aflatoxin B1 and zearalenone by mono-and di-alkyl cationic surfactants modified montmorillonites. *Journal of Colloid and Interface Science, 511*, 67–76. https://doi.org/10.1016/j.jcis.2017.09.074

Yuan, P., Annabi-Bergaya, F., Tao, Q., Fan, M., Liu, Z., Zhu, J., He, H., & Chen, T. (2008). A combined study by XRD, FTIR, TG and HRTEM on the structure of delaminated Fe-intercalated/pillared clay. *Journal of Colloid and Interface Science, 324*(1-2), 142–149. https://doi.org/10.1016/j.jcis.2008.04.076

Zeng, L., Wang, S., Peng, X., Geng, J., Chen, C., & Li, M. (2013). Al-Fe PILC preparation, characterization and its potential adsorption capacity for aflatoxin B1. *Applied Clay Science, 83*, 231–237. https://doi.org/10.1016/j.clay.2013.08.040

Zivica, V., & Palou, M. T. (2015). Physico-chemical characterization of thermally treated bentonite. *Composites Part B: Engineering, 68*, 436–445. https://doi.org/10.1016/j.compositesb.2014.07.019

(Received 23 April 2021; revised 21 September 2021; AE: William F. Jaynes)