

SMECTITE CLAY ADSORPTION OF AFLATOXIN *vs.* OCTAHEDRAL COMPOSITION AS INDICATED BY FTIR

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Abstract—The fungus *Aspergillus flavus* Link ex Fries can infect grains and oil seeds and develop Aflatoxin B₁ (Afb₁) in the field or in storage. Aflatoxin contamination is a serious health hazard – it is extremely toxic and hepatocarcinogenic for animals and humans. A practical approach to solve this problem is to use smectite clay as an amendment to animal feed. The objective of this research is to investigate smectite clay–Afb₁ interactions by employing Fourier transform infrared (FTIR) spectroscopy to determine how clay composition influences Afb₁ adsorption by smectites. When Afb₁ was present in the clay, the spectral region from 1800 to 1300 cm⁻¹ was altered, and the regions between 4000 and 1800 and 1300 and 400 cm⁻¹ were unchanged except for the intensity in the broad region near 3400 cm⁻¹ related to the abundance of water. The 1300–400 cm⁻¹ region is attributed only to smectite clay properties, and it relates to the relative adsorption potential of the different smectites. Bonding between Afb₁ and smectite clay appears to be in the furan rings. Other possible bonding is with the two oxygens in the coumarin ring of Afb₁ and interlayer cations or their associated water molecules. The FTIR evidence of octahedral Fe in smectite and amorphous silica in the clays both indicate greater Afb₁ adsorption potential. Other smectites with spectral absorption indicating predominantly Al in the octahedral positions adsorbed less Afb₁.

Key Words—Aflatoxin, Adsorption Capacity, Cancer Prevention, FTIR, Smectite Clay.

INTRODUCTION

On British farms in 1960 a large number of ducklings and turkeys died; the cause was a toxin produced by a fungus identified as *Aspergillus flavus* Link ex Fries (Sargeant *et al.*, 1961). Nesbitt *et al.* (1962) observed that this material had a violet-blue fluorescence under ultraviolet light; it was named Aflatoxin B. Nesbitt *et al.* (1962) published the infrared (IR) spectrum of Aflatoxin B in chloroform indicating that OH groups were absent, unless chelated; two peaks were in the carbonyl region at 1755 cm⁻¹ and 1688 cm⁻¹. Hartley *et al.* (1963) established the molecular formula and the molecular weight of aflatoxin: C₁₇H₁₂O₆ at 312 g/mol. Finally, the structure was elucidated by Asao *et al.* (1963, 1965) and Büchi (1966). Van Soest and Peerdeman (1970a, 1970b) gave the absolute configuration of Afb₁. Pavão *et al.* (1995) used all the basic information previously established to explain the structure and activity of Afb₁ (Figure 1) and its optimized geometry.

The extreme toxicity of the Afb₁, its hepatocarcinogenic activity in animals, and its implications as a liver carcinogen in humans have been reported (Schoental, 1967; Busby and Wogan, 1984; Coulombe, 1991). The presence of Afb₁ has been confirmed worldwide in peanuts, corn, cottonseed, grain sorghum, millet, Brazil and pistachio nuts, almonds and other tree nuts, and dried fruits (Coulombe, 1991). The widespread occur-

rence of aflatoxin and its contribution to disease problems in humans in developing countries have led to regulation of grain imports in Europe (Official Journal of the European Union, 2006), and to development of health strategies to deal with concerns about aflatoxin in developing countries (Strosnider *et al.*, 2006). A strategy for reducing aflatoxins is its adsorption on smectite clay, *e.g.* Novasil, calcium montmorillonite clay (HSCAS) (Strosnider *et al.*, 2006). Phillips *et al.* (1995), using FTIR, explained the process as chemisorption by phyllosilicate clay. They found two new bands between 1600 and 1400 cm⁻¹, probably from metal chelates formed with the ions of the clay and the dicarbonyl system in Afb₁. Phillips (1999) showed that the dicarbonyl system of Afb₁ was essential for tight binding (chemisorption) and suggested that the adsorption process can be at edges, basal surfaces, and especially the interlayers of the smectite. The mineral clinoptilolite has also been used successfully to adsorb Afb₁ and showed 80% effectiveness (Tomašević-Čanović *et al.* 1994, 1996). However, Strosnider *et al.* (2006) recognized that “the efficacy, safety, and acceptability of enterosorption and chemoprotection require further study.”

Smectite has been used as an anticaking additive in animal feed and has been shown to protect animals from ingested aflatoxins. Smectite (*e.g.* Ca-montmorillonite or hydrated sodium calcium aluminosilicate) is needed as a practical control method for aflatoxin in animal feed; the smectite clay acts in the gastrointestinal tract decreasing Afb₁ bioavailability. Improvements in animal performance or survival where Afb₁ was present in the diet have been from 30 to 100% when smectite was

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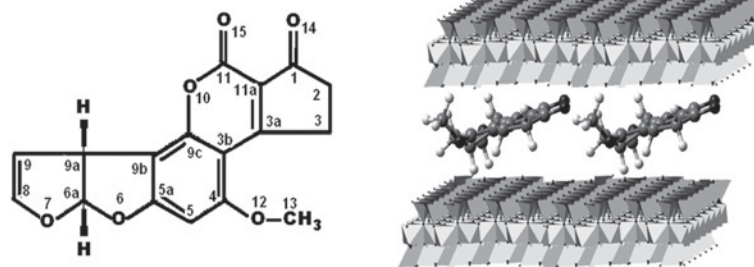


Figure 1. (Left) Aflatoxin B₁ molecule. The atom positions are in agreement with Pavão *et al.* (1995). (Right) Smectite clay with interlayer mycotoxin (water molecules and exchange cations omitted).

included in the feed (Desheng *et al.*, 2005; Dixon *et al.*, 2008; Kubena *et al.*, 1998; Phillips *et al.*, 2002).

One long-term goal of this AfB₁ research is to identify abundant bentonites of appropriate quality to sequester the mycotoxin in animal feeds. The specific objective of this research is to investigate smectite clay–AfB₁ interactions by employing FTIR to determine how clay composition influences AfB₁ sorption by smectites. The FTIR technique has been used successfully to study pesticides and the adsorption of other organic compounds by clay minerals (Homenauth and McBride, 1994; Gonzalez-Pradas *et al.*, 2003; Davies and Jabeen, 2003; Petit, 2006).

MATERIALS AND METHODS

Materials

The reagents and their sources are as follows: Aflatoxin B₁ from *Aspergillus flavus*, Sigma Chemical Co., CAS No. 1162-65-8, St. Louis, Missouri; Acetonitrile, Chromasolv[®] for HPLC, gradient grade, Sigma-Aldrich, CAS No. 75-05-8; KBr puriss, p.a. for IR spectroscopy, Fluka, CAS No. 7758-02-3.

Many samples used in this research are from the Office of the Texas State Chemist representing industrial products proposed as adsorbents of AfB₁ and their identity is withheld for proprietary reasons. Other samples were from the authors' own collection. Where the location of the natural source of the sample is known it is designated with the State abbreviation in the USA and three samples from Mexico are designated MX (Kannewisher, 2006).

Sample screening

Twenty samples of smectite were tested as adsorbents by screening their basic properties: pH, cation exchange capacity (CEC), organic carbon, and extractable bases, using standard methods (Soil Survey Staff, 1996).

Isotherm adsorption procedure

The procedure described by Grant and Phillips (1998) was used with some modifications (Kannewisher *et al.*, 2006). Aflatoxin was diluted with acetonitrile to make a stock solution. A working solution was prepared by

dilution of the stock solution with water to obtain the concentration required: 0.0, 0.4, 1.6, 3.2, 4.8, 6.4, and 8.0 ppm and then 0.1 mg of smectite was added. The samples were prepared in sterile FALCON[®] Blue Max Jr. 15 mL polypropylene conical test tubes and were shaken for 24 h at 200 motions/min on an orbital shaker (Cole-Parmer); the samples were then centrifuged (IEC PR-7000 Centrifuge) at 51,000 g for 57 min. The residual concentration of aflatoxin in the solution after the sorption process was measured using a UV/visible spectrophotometer at 365 nm. A blank and standards were also prepared. All samples were treated under the same conditions. Samples that had low (0.06 mol/kg) to high (0.68 mol/kg) sorption capacity were evaluated. The adsorption capacity was evaluated by plotting the adsorption data using the linear form of the Langmuir equation:

$$\frac{C_{eq}}{q} = \frac{1}{k_d Q_{max}} + \frac{C_{eq}}{Q_{max}} \quad (1)$$

First, Q_{max} and k_d were determined, where C_{eq} is the equilibrium concentration, q is the amount of AfB₁ adsorbed, k_d is a distribution coefficient, and Q_{max} the maximum sorption capacity.

Saturation of smectite samples with AfB₁ for FTIR analysis

Each 6 mg smectite sample was mixed in a test tube with 6 mL of working solution of 50 ppm AfB₁. In this case the working solution was adjusted with acetonitrile since the AfB₁ solubility is only 10–30 µg/ml in water (Busby and Wogan, 1984). The suspension was shaken at 175 motions/min overnight on an orbital shaker (Cole-Parmer); the samples were centrifuged (IEC PR-7000 Centrifuge) at 1000 g for 20 min and dried at room temperature on glass slides. The dry clay was scraped off and saved in labeled vials. Control samples were prepared without aflatoxin to check the possible influence of acetonitrile.

FTIR spectra acquisition

The FTIR spectra were obtained using a Perkin-Elmer System 2000 FTIR instrument by means of a diffuse

reflectance procedure (White and Dixon, 2003). Treatment and control samples were included. Sample preparation consisted of grinding the sample and mixing it with a known background material (KBr); in this case, 6 mg of each clay sample was ground and mixed with 1 g of KBr in a Wig-L-Bug shaker. The diffuse reflectance apparatus required 1 g of mixed sample.

RESULTS

Properties of the samples

The best performing adsorbents of aflatoxin (>0.4 mol/kg) had pH values from 7.1 to 8.8 and smectite clays with extreme pH values (4.7 or 9.6) were intermediate adsorbents. Calcium was the major exchangeable cation among the six best adsorbents, although the relative ion population was variable among the four exchange cations (Table 1). The first-order *d* spacings of the as-received smectites was largely the same, at 1.4 to 1.5 nm, except for three samples, 1.3 nm for 6WY and 1.6 nm for T8 and 16MX. The dominance of Na as an exchange ion may account for the small adsorption value for 6WY. Calcium ions have a greater hydration sphere and tend to be more abundant in the other samples. Potassium saturation reduced most spacings to 1.1 or 1.2 nm as expected, except for the very acid 17TX clay that may contain hydroxyl-Al polymers between the structural layers. Heating the K-saturated clays reduced the spacing to 1.0 nm except for two samples from Mississippi that had 1.1 and 1.2 nm spacings. The benzene-acetonitrile-treated samples without the mycotoxin (control) and the benzene-acetonitrile treatment with AflB₁ led to expanded *d* spacings of 1.5–1.6 nm, except for 6WY which persisted at 1.3 nm at room temperature.

The FTIR bands (Table 1) illustrate the consistency of Al as a structural ion in almost all of the smectite clays. The Fe absorbance at 880 cm⁻¹ and the non-crystalline silica absorbances at 793 to 790 cm⁻¹ suggest greater adsorption potential as illustrated by the top six smectites investigated. Other FTIR absorbances may be useful measures if their intensities can be increased to permit accurate measurement.

Adsorption capacity

The remarkable result of previous research was that the adsorption capacity range for the twenty samples was 10-fold (Table 1). The XRD patterns presented direct evidence that the AflB₁-molecules were adsorbed between the smectite layers and resisted collapse from heat treatment at 245°C (Kannewisher *et al.*, 2006). No smectite clay bands occurred in the region 1800–1300 cm⁻¹, thus it was possible to observe changes in the region when the samples were saturated with AflB₁.

FTIR spectra of smectite clay samples with/without AflB₁

The FTIR patterns of the smectite with and without AflB₁ saturation were compared and revealed that the

spectral bands in the regions 4000 to 1800 cm⁻¹ and 1300 to 400 cm⁻¹ originated from the smectite clays. All of the 20 smectite clays have OH-stretching bands at 3620 cm⁻¹ and ALOH bending bands at 915 cm⁻¹ (Gates, 2005) (Table 1, Figure 2). Consequently, all contain Al substituted in the octahedral sheet in moderate to abundant amounts.

The FeAl-OH absorbance at 885 cm⁻¹ was evident in five of the adsorbent smectite clays, questionable in sample 17TX, and absent from samples 7AZ and 5OK (Figure 2). Although AlMg-OH absorbance at 845–835 cm⁻¹ was evident for all 20 smectites, it was weak or very weak for many of them. Amorphous silica bands (~790 cm⁻¹) were present in the spectra of the four best adsorbents and in most others as weaker absorbance bands. They were the weakest for 7AZ and 5OK, two of the poorest adsorbents. At 616–624 cm⁻¹, Al_{oct}³⁺-O_{ap}-Si_{tet}⁴⁺ is a band diagnostic of Al³⁺-rich smectites in general (Madejová *et al.*, 1996).

The FTIR spectra were normalized using the bands at 915 cm⁻¹, 885 cm⁻¹, and at 845–835 cm⁻¹, yet no correlation was obtained with the adsorption capacity. The 885 cm⁻¹ absorbance is clearly stronger for the top three adsorbents and absent from clays 7AZ and 5OK; this indicates that they contain little or no structural Fe in the smectite. The poor adsorption by 6WY is attributed to the extremely high pH (9.6) and Na saturation that contribute to disorder in stacking the smectite layers. Sample 16MX differs from the other

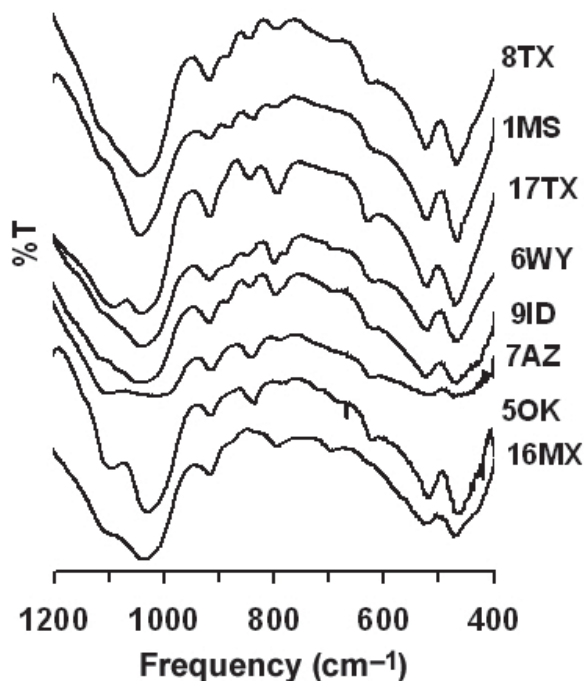


Figure 2. FTIR spectra for smectite clays 8TX, 1MS, 17TX, 6WY, 9ID, 7AZ, 5OK, and 16MX, sorted by adsorption capacity.

Table 1. Properties of the samples and FTIR absorbance bands ranked by adsorption capacity.

Sample*	pH	NH ₄ OAc extr. bases			CEC in NaOAc	Org. C (%)	Max. sorp. capacity (Q _{max}) (mol/kg)	As recd.	K-saturated 550°C	Blank (clay in benzene: acetonitrile 98:2)	<i>d</i> spacing from XRD (nm)		Saturated AfBI, 100 ppm in benzene: acetonitrile 98:2	AIOH	FTIR bands			
		Ca	Mg	Na							K	25°C			24.5°C	25°C	24.5°C	FeAl-OH
8TX	7.1	54.6	21.8	8.3	1.0	84.1	0.12	1.4	1.1	1.0	1.6	1.1	1.5	913	880	839	793	621
1MS	8.3	94.1	9.4	0.4	1.5	85.8	0.18	1.4	1.2	1.0	1.6	1.4	1.5	912	883	838	791	620
T7	8.8	51.7	15.3	43.5	0.5	87.6	0.27	1.5	1.2	1.0	1.5	1.0	1.5	912	881	840	793	623
11ID	7.4	56.6	15.6	9.2	0.7	76.4	0.18	1.5	1.1	1.0	1.5	1.2	1.5	914	881	839	792	621
T4	8.5	92.1	12.9	0.6	1.5	99.9	0.50	1.4	1.2	1.0	1.5	1.1	1.6	912	888	839	790	620
14MS	7.7	97.9	13.8	0.5	1.9	84.3	0.11	1.5	1.1	1.0	1.6	1.0	1.5	910	881	840	790	618
17TX	4.7	36.8	17.4	10.7	1.0	78.4	0.07	1.5	1.3	1.0	1.6	1.0	1.6	912	—	840	790	623
13ID	7.5	59.5	16.5	9.2	0.7	79.7	0.25	1.5	1.1	1.0	1.6	1.1	1.6	914	881	839	790	621
3MS	8.6	90.6	9.8	0.4	1.5	94.3	0.04	1.5	1.1	1.1	1.3	1.1	1.5	912	879	841	790	621
6WY	9.6	15.5	4.5	83.6	1.1	83.3	0.24	1.3	1.1	1.0	1.3	1.0	1.3	913	883	843	797	620
T8	7.8	43.1	14.5	12.1	0.9	74.2	0.16	1.6	1.2	1.0	1.5	1.0	1.6	910	881	839	793	620
15MX	8.7	39.0	8.9	59.7	1.3	93.7	0.23	1.5	1.2	1.0	1.4	1.0	1.5	914	—	838	795	621
4MS	8.5	115.0	14.7	0.4	1.6	93.3	0.04	1.4	1.1	1.2	1.5	1.0	1.6	912	878	838	791	620
10ID	7.8	44.7	18.5	11.9	0.8	75.1	0.13	1.4	1.1	1.0	1.5	1.1	1.6	914	882	840	793	618
2MS	8.7	93.3	9.2	0.4	1.7	85.4	0.17	1.5	1.2	1.0	1.6	1.0	1.5	910	881	838	788	621
12ID	7.4	58.3	15.3	8.9	0.7	75.9	0.17	1.5	1.1	1.0	1.5	1.2	1.6	913	882	839	793	621
9ID	7.5	50.0	18.3	9.4	0.7	77.8	0.17	1.4	1.1	1.0	1.5	1.0	1.6	912	882	840	793	621
7AZ	7.6	96.9	19.2	1.0	1.2	90.1	0.15	1.4	1.2	1.0	1.5	1.3	1.6	911	—	835	788	618
5OK	7.3	78.5	23.4	1.1	0.4	101.3	0.16	1.5	1.2	1.0	1.6	1.1	1.6	910	—	835	790	618
16MX	7.6	20.0	22.6	12.1	2.0	68.3	0.21	1.6	1.2	1.0	1.5	1.1	1.6	910	—	832	791	—

* Sample names include state-of-source abbreviations in the USA and Mexico (MX). Samples T4, T7, and T8 are commercial clay products.

samples, having a lower spectral absorbance and the peak at 880 cm⁻¹ apparently is not from Fe in the smectite structure. The particles failed to disperse well and thus were poor adsorbents. The average particle size was 50 μm, *i.e.* silt size (Mulder *et al.*, 2008).

FTIR spectra of smectite samples saturated with Aflatoxin B₁

The displacement of water by the Aflatoxin B₁ molecules is shown as the decrease in absorbance near the 1630 cm⁻¹ region and in the broad absorbance in the 3600–2800 cm⁻¹ range (Figure 3). Smectite clays saturated with Aflatoxin B₁ have absorbance bands for the mycotoxin molecules between 1800 and 1200 cm⁻¹ (Figure 3b). The strongest absorbance is between 1770 and 1725 cm⁻¹. Some of the weaker absorbance bands occur as sharp valleys and others as broad troughs. The prominence of the FTIR absorbance features and the amount of mycotoxin adsorbed decreased in the order 8TX > 17TX > 6WY > 16MX.

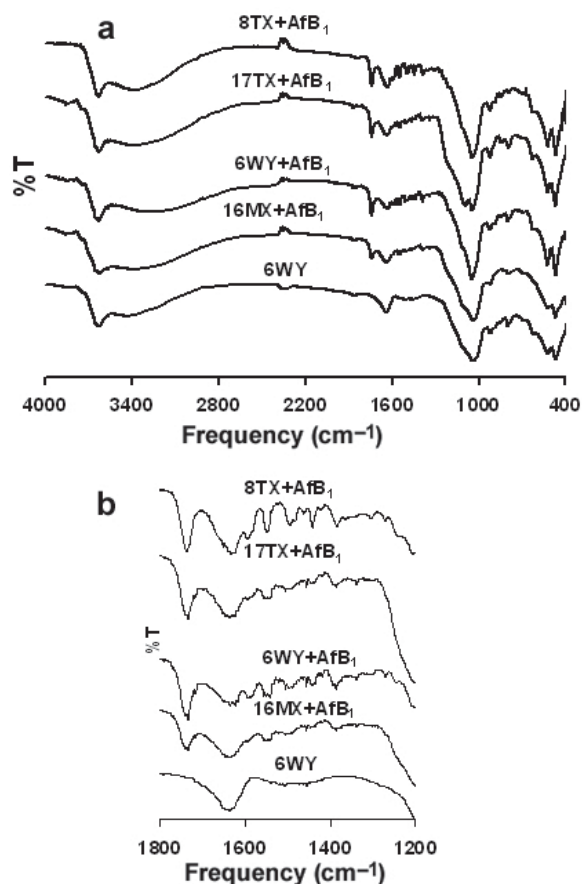


Figure 3. FTIR spectra of four smectites that represent the full range of Aflatoxin B₁ adsorption in (a) the spectra from 4000 to 400 cm⁻¹; and (b) spectra of four clays in order of decreasing adsorption of Aflatoxin B₁. Curve 6WY is a control sample.

DISCUSSION

Spectral changes during adsorption of Aflatoxin B₁

The major changes in the FTIR spectra of smectites when they adsorbed Aflatoxin B₁ were in the range 1800–1200 cm⁻¹, which is consistent with other studies, such as those involving smectite interactions with organic herbicides (Undabeytia *et al.*, 2000). Phillips *et al.* (1995) used FTIR to investigate the mechanism of Aflatoxin B₁ binding to HSCAS and showed that the interaction is in the range 1750–1400 cm⁻¹, including two new bands in the region 1600–1400 cm⁻¹. The intensities of principal bands in the IR spectra of the aflatoxins were obtained by Heathcote and Hibbert (1978). Also, the IR spectrum of the Aflatoxin B₁ was reported by Cole and Cox (1981) and Liu *et al.* (1998). In the present research the spectrum of Aflatoxin B₁ from the web site <http://www.chemexper.com/search/cas/1162658.html> (Figure 4) was used (with kind permission of the publishers).

FTIR comparison of adsorbed Aflatoxin B₁ and crystalline mycotoxin

Sample 8TX adsorbed the most mycotoxin in this study and produced sharper and stronger Aflatoxin B₁ bands than the other smectites (Figure 3; Table 2). Comparing the Aflatoxin B₁ bands in Figure 3b from the sample 8TX-mycotoxin complex with those in the published curve for mycotoxin (Figure 4) revealed 12 bands that correspond to those of mycotoxin alone. Seven of the bands indicate a red shift and five suggest a blue shift, although the differences are small. These features may imply a slight relaxation of the Aflatoxin B₁ bonds in the smectite clay compared to crystalline mycotoxin, a logical response for a molecule held between two silicate layers attracted by electrostatic forces.

Also, 12 bands for the mycotoxin were observed between 1133 and 463 cm⁻¹ which were not observed in the smectite-Aflatoxin B₁ complex. Four bands that were not shown in the adsorbed Aflatoxin B₁ data are prominent enough in Figure 4 to suggest that more investigation is needed of the properties of Aflatoxin B₁ adsorbed on smectite to better understand the relationships between the clay and the mycotoxin.

The reaction between smectite and Aflatoxin B₁ apparently involves carbonyl groups and oxygens O15 and O10 (Figure 1) of the coumarin moiety. The cyclopentanone ring and the interlayer metal cations such as Ca, Na, and Mg may interact *via* coordinate bonds formed in the adsorption process *i.e.* Mg-O-C(=O)-CH2 (Table 1). The stretching bands of C bonded to two oxygens (COO) of the coumarin ring appear at 1582 cm⁻¹ and 1425 cm⁻¹ (Nakamoto and McCarthy, 1968). This interpretation was also given by Phillips *et al.* (1995). The coumarin moiety absorbs at 1750, 1630, and 1590 cm⁻¹ (Ciegler and Peterson, 1968), and when the Aflatoxin B₁ was adsorbed on smectite, the coumarin moiety was modified in accordance with those bands. The bands at 1755 cm⁻¹

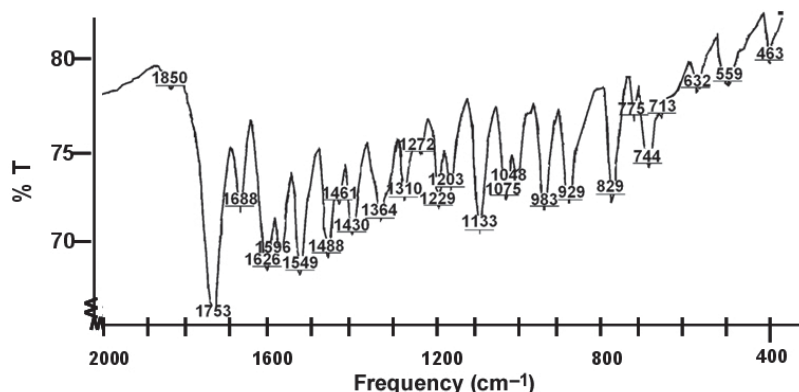




Figure 4. AfB₁ IR spectrum (source: <http://www.chemexper.com/search/cas/1162658.html>, (reproduced with permission of the publisher).

and 1688 cm⁻¹ are assigned to carbonyl groups from AfB₁ spectra (Nesbitt *et al.*, 1962), and the bands that confirm the C=C bond occur at 1670 cm⁻¹ and 1600 cm⁻¹, which disappear when the AfB₁ saturates the smectites.

The bands at 1410–1310 cm⁻¹ or 1350–1260 cm⁻¹ are assigned to OH-bending vibrations in the AfB₁ molecule (Coates, 2000); these bands are absent after AfB₁ was adsorbed by smectites. This observation is confirmed by the observations of Desheng *et al.* (2005). On the other hand, Pavão *et al.* (1995) indicated that AfB₁, an electrophilic reactant, is largely a planar molecule and the dihydrofuran ring protrudes from this plane (furan ring ) , so that C8 and C9 (Figure 1) are susceptible to reaction. Because the band at 1735 cm⁻¹ is in the region of C–O, a reaction between AfB₁ and smectite by epoxidation is suggested.

Displacement of much of the interlayer water by AfB₁ was evident from the FTIR spectra (Figure 3a), indicating that AfB₁ interacted with water molecules between smectite clay layers as the remaining water molecules are probably bonding intermediates in view of the large amount of aflatoxin adsorbed by smectite (Dixon *et al.*, 2008).

The strong band at 1735 cm⁻¹ apparently is associated with a six-membered lactone ring () (Coates, 2000). The prominence of this band in the present data raises the question that sequestration of AfB₁ may have influenced the lactone ring and possibly produced a second one, and thus modified AfB₁ to AfG₁. Another possible reaction is the epoxidation of carbons 8 and 9. This reaction enables the binding of AfB₁ to

cellular macromolecules such as DNA (Phillips *et al.*, 1995; Urbanek, 1997). The atoms C8 and C9 are also involved in hydroxylation reactions (Pavão *et al.*, 1995).

Structural basis for smectite selection criteria

The presence of amorphous silica and the presence of FeAl-OH (Jackson *et al.* 1948; Karathanasis, 2002) suggest that the bentonite was less weathered. These observations and the rounded and thicker particles of the 5OK smectite observed by transmission electron microscopy suggest that the better-adsorbing smectites are less weathered. They occur in thin particles that are frequently folded. They seem to be more accessible to the AfB₁ than the thick rounded platelets in the poorly sorbing 5OK sample (Mulder *et al.*, 2008).

The depletion of silica during weathering of soils and rocks is a generally recognized reaction. Thus, the removal of silicate glass indicates the extent of alteration of volcanic ash deposits such as those from which bentonites form. Also, aluminous, *i.e.* dioctahedral layer silicates, are more resistant to weathering than those containing Mg and Fe in the structure. The aluminous smectites, *e.g.* 7AZ and 5OK, are less effective as adsorbents of AfB₁. The 5OK sample had the largest CEC (101 cmol/kg) of the 20 bentonites, greater even than other bentonites which adsorbed larger amounts of AfB₁, suggesting some vermiculitic character. Preliminary tests indicated that vermiculite is a poor adsorbent of AfB₁ (Mulder *et al.*, 2008).

Na-smectites, *e.g.* 6WY, are modest adsorbents, yet their structural composition is like that of the better adsorbents. Sodium saturation tends to promote dis-

Table 2. Bands of FTIR absorption spectra of aflatoxin (Figure 4) and of adsorbed mycotoxin on smectite 8TX.

	cm ⁻¹											
AfB ₁	1753	1626	1596	1549	1488	1461	1430	1364	1310	1272	1229	1203
AfB ₁ adsorbed	1740	1594	1591	1552	1482	1462	1440	1381	1306	1264	1240	1200

orderly stacking of the layers and may reduce Aflatoxin B₁ adsorption.

The effectiveness of Aflatoxin B₁ adsorption in smectite suggests a bonding association on the clay surfaces where it replaces some of the interlayer water as shown by FTIR data. Both Aflatoxin B₁ and the divalent organic molecule, diquat, of similar configuration, can occupy most of the surface of smectite and neither fully saturates the interlayer surfaces of vermiculite (Dixon *et al.*, 1970). The vast surface area of smectite clays (800 m² g⁻¹) has proven effective at improving animal performance during *in vivo* research where the level of Aflatoxin B₁ added was much greater than can be legally marketed in feeds.

SUMMARY

Evidence for bonding between Aflatoxin B₁ and smectite particle surfaces is presented and a role for smectite in long-term practice for reduction of Aflatoxin B₁ as a threat to animal and human health is suggested. The relationships between Aflatoxin B₁ adsorption and octahedral ion population in smectites, evaluated by FTIR, provide useful information on their potential as adsorbents of Aflatoxin B₁. The method needs to be evaluated on a wider population of smectites to exploit the clay resources of the world, particularly in warm dry regions where Aflatoxin B₁ is a frequent threat. The octahedral cation population can be evaluated conveniently by FTIR analysis to predict the adsorption potential of smectite clays for Aflatoxin B₁. The FTIR absorbance features corresponded with 12 of the 24 most prominent absorbance features of crystalline Aflatoxin B₁ and several of them were red shifted, indicating interlayer bonding to the clay. The smectites that contain octahedral Fe (and Mg) are the most effective adsorbents of Aflatoxin B₁. Those smectites that have Al as the primary octahedral ion and contain little or no amorphous silica are among the poorest adsorbents. Several IR bands from the Aflatoxin B₁ molecule were modified by sorption to smectite. The molecular structure was apparently modified by adsorption in the interlayer region of the clays. The authors suggest that adsorption of Aflatoxin B₁ to smectite surface oxygens occurred *via* epoxidation involving the double bonded carbons on the furan ring of the Aflatoxin B₁. The data on the adsorption properties of Aflatoxin B₁ imply that smectites inherited from bentonites in soils may be a useful predictor of clay-organic reactions of those molecules that are attracted to interlayer regions of the smectite clays.

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