SMECTITE CLAY ADSORBENTS OF AFLATOXIN B_1 TO AMEND

ANIMAL FEED

A Thesis

by

INES KANNEWISCHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Soil Science

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	Richard H. Loeppert
Committee Member,	Kevin J. McInnes
Head of Department,	C. Wayne Smith

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ABSTRACT

Smectite Clay Adsorbents of Aflatoxin B_1 to Amend Animal Feed. (December 2006)

Ines Kannewischer, Dip., University of Hannover, Germany

Co-Chairs of Advisory Committee: Dr. Joe B. Dixon
Dr. Richard H. Loeppert

Smectite clay has been shown in studies over the past 20 years to sorb aflatoxin B_1 (AfB_1) in animal feed and thereby reduce its toxic influence on animals.

In this study, 20 smectite samples were selected from industrial products or reference minerals. In the initial steps, it was shown that AfB_1 entered the interlayer galleries of smectites and a 10-fold range in sorption ability was observed in a set of 20 smectite samples. Yet, it was not clear which clay properties (CEC, pH, base saturation) influenced this variation.

In an effort to further explore properties that might influence the sorption of AfB_1, three good sorbent samples were chosen from our set of 20 samples along with one sample of low sorption capacity. Those samples were fractionated into sand, silt, coarse clay (CC), and fine clay (FC) fractions. From all sample fractions, sorption isotherms and X-ray diffraction patterns were obtained. Additionally, a vermiculite and a palygorskite were examined with regard to sorption capacity. Concentration of smectite and their adsorption test suggest that differences in smectite composition are responsible for difference in sorption, not so much their relative abundance or other mineral phases. Initial infrared analysis indicates that weathered aluminous smectites, which have no octahedral iron or magnesium, belong to the poor AfB_1 sorbents.

Palygorskite and vermiculite are not effective sorbents.

Based on the findings in this study, tentative quality criteria of sorbent selection for their use in animal feed were established. These criteria are: pH between 6.5 and 8.5, CEC >

$75 \text{cmol}_c/\text{kg}$, organic carbon < 2.5 g/kg, expression of XRD smectite peak and AlFeOH-bending in FTIR and Langmuir adsorption capacity for AfB_1 > 0.40 mol/kg.

DEDICATION

To my parents, who gave me roots and wings.

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First of all, I would like to thank Maria Guadalupe Tenorio Arvide and Joe B. and Martha J. Dixon for sharing almost two years of their study, work, travel and excellent catfish dinners with me. "Marilu" has often helped and supported me when working in the laboratory and I profited from her experience and friendliness. I would like to thank Dr. Dixon for being a wonderful mentor, and for generously sharing his knowledge with me.

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During my research, the following people provided support and I acknowledge and thank them for their contributions to our work: Stefan Dultz, G. Norman White, Richard Dress, C. T. Hallmark, Dave Zuberer, Annette Fincher, Donna Prochaska, Lea D. Morris, D.A. Zuberer, Kyung-Min Lee, T. J. Herrman, George Latimer.

It has been a great pleasure to learn from them and an honour to be able to work with all of them.

I want to thank all my fellow graduate students, who contributed immensely to the fact that I had a great time studying in the Soil and Crop Sciences Department.

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1. INTRODUCTION

Aflatoxins are a group of highly carcinogenic mycotoxins produced primarily by the fungus *Aspergillus flavus*. Within the group of aflatoxins, Aflatoxin B_1 (AfB_1) is the most toxic, and it is known for its harmful effects on humans and animals. Aflatoxin contamination is a worldwide problem, especially in warmer climates, and the toxin can enter the food chain through contaminated food and feed products. They are considered an unavoidable food and feed contaminant (Coulombe, 1991).

Smectite clay has been used for many years as an ingredient in animal feed to form better pellets and to improve animal growth and health (Grim, 1962), but recently its importance as an aflatoxin sequestration agent has become clear. Not only has it been investigated as an adsorbent when included in small amounts in aflatoxin-contaminated animal diets and shown effective, but in addition smectite clays offer a simple and economically feasible way to manage and remediate the aflatoxin-problem and protect farm animals and humans from aflatoxicosis.

Over the past 20 years scientists have demonstrated the effectiveness of smectites against aflatoxin inside the animals' gastrointestinal tract. Possible binding mechanisms of the toxin to the smectites were discussed (Phillips et al., 2002), yet, which physical and chemical properties of the smectites are crucial for them to qualify as good adsorbents is not clear. Another problem in the progress of the research on smectites as aflatoxin adsorbents is that two scientific areas, clay mineralogy and animal science, meet here and sometimes one scientific community is not aware of the progress made in another discipline. Clay often seems to be outside the viewing area of the biologically oriented scientific community (Barug et al., 2004).

If certain characteristics of smectites prove as reliable sorbent indicators and sorption mechanisms are well-understood, this understanding would allow state authorities to give seals of approval to industrial feed additives as good AfB_1-adsorbents.

This thesis follows the style of Soil Science Society of America Journal.

To facilitate interdisciplinary understanding, this thesis provides an overview of aflatoxins and smectite clays before focussing on the subject matter.

The research carried out in the course of my studies can be assigned to three different stages: (a) the screening of 39 samples of potential AfB_1 sorbents to characterize their chemical and physical properties, (b) the characterization of 20 smectite samples out of the larger set as sorbents of AfB_1, and (c) the fractionation of 4 smectite samples and the investigation of mineralogy and particle size on AfB_1 sorption of those samples.

Further objectives of this study are (i) to describe the range of properties of commercial sorbents offered to suppress aflatoxin toxicities in animal feeds, (ii) to present the chemical and physical properties of a set of 20 smectites of these commercial sorbents, and (iii) to discuss the relationships among clay-sorbent properties and the amounts of aflatoxin they adsorb, as well as (iv) test the hypothesis if differences in mineralogy and particle size of four selected smectites might influence aflatoxin sorption.

Based on the findings, a practical goal for the near future is to establish quality criteria for commercial sorbents. Controlled use of commercial sorbent smectites that fulfill these quality criteria could be labelled as approved dietary amendments to protect farm animals from aflatoxicosis and the economical interests of farmers.

2. LITERATURE REVIEW

2.1. Aflatoxin B$_1$

Mycotoxins are a group of chemically diverse fungal metabolites that may pose health risks to animals and humans when consumed though feed and food products. More than 100 mycotoxins have been structurally characterized, but few have been implicated in serious toxic syndromes. One of these few, aflatoxin, a group of highly carcinogenic, hepatotoxic, teratogenic, and mutagenic secondary metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* that infect many crops (Palgrem and Ciegler, 1983) have caused lethal episodic outbreaks of poisoning (Busby and Wogan, 1984). Means to limit infection of crops or to treat aflatoxin contaminated foods and feeds are of great economic importance to agriculture production.

The discovery of aflatoxin was triggered by the "Turkey X disease", which struck Great Britain in 1960, and led to the loss of near 100,000 turkey poults from liver damage after they consumed aflatoxin-contaminated groundnut *(Arachis hypogaea)* meal (Cullen and Newberne, 1994; Pereira Schuler and Bernal Gómez, 2001). The scale of the loss and the serious economic damages caused by this incident stimulated intensive research. Sargeant et al. (1961) provided evidence that the mold *Aspergillus flavus* produced the toxic substances that lead to the disease in the turkeys (the name aflatoxin is derived from *Aspergillus flavus*). Similar results were soon discovered by researchers in other countries, and it was realized that many previous, less well-documented losses of turkey, ducklings, chickens, pigs, and other animals (Schoental, 1967) could be attributed to aflatoxin poisoning. A review of pre-1960 incidences is given by Eaton and Groopmanns (1994).

The first aflatoxins to be recognized as the causative agent for toxicosis were the aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$, and were characterized independently by Van der Zidjen et al. (1962) and De Iongh et al (1962). Asao et al. (1963) then elucidated their chemical

structures. The AfB$_1$ molecule consists of five chemical ring structures (Phillips. 1999), namely a dihydrofuran and a furan ring, a methoxyphenyl ring, a δ-lactone ring, and a cyclopentenone ring (Figure 1, left to right). In addition to toxicity from direct
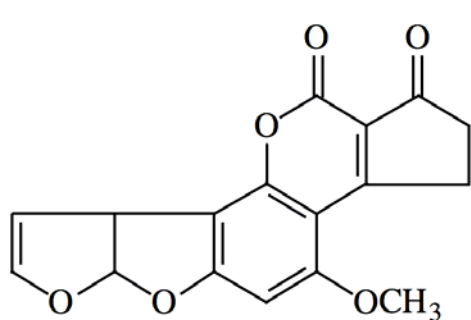
Fig. 1: Structure of the aflatoxin B$_1$ molecule.

consumption, aflatoxins were shown to move through the food chain as ducklings fed milk from cows that ate contaminated feed showed symptoms of aflatoxin toxicosis (Allcroft and Carnaghan, 1963). This observation lead to the identification of aflatoxin M$_1$ and M$_2$ (Holzapfel et al., 1966). Besides economic and health pressures, some characteristics of the aflatoxins contributed to their fast and exact identification: they are extractable into organic solvents, exhibit intense fluorescence, have high melting points and low solubilities in water (Schoental, 1967). Although there are more structural forms of aflatoxin known today than the ones mentioned above, the major aflatoxins are B$_1$, B$_2$, G$_1$, G$_2$, and M$_1$, with B$_1$ being by far the most toxic. AfB$_1$ is also the most prevalent in nature (Cole and Cox, 1981). An overview of the chemical and physical properties of AfB$_1$ is given in Table 1.

Within seven years after the discovery of the aflatoxins, more than 400 scientific papers on the topic were published (Schoental, 1967). Today there are about 8000 research articles, three books in English language (Goldblatt, 1969; Eaton and Groopman, 1994; Heathcote and Hibbert, 1978) and various book chapters dedicated to the numerous aspects associated with the aflatoxins. Much knowledge has been

assembled so far; nevertheless, solutions to the problem of aflatoxins as food and feed contaminants are still urgent.

Table 1: Selected chemical and physical properties of aflatoxin B$_1$.

Common name	Aflatoxin B$_1$
Molecular formula [1]	C$_{17}$H$_{12}$O$_6$
Molecular weight [1]	312.06
Melting point [1]	268° - 269°C
Vertical cross sectional area [2]	52.8 Å
Horizontal cross sectional area [2]	88.3 Å
Solubility [2]	11 to 33 µg/mL
Log K$_{ow}$ [2]	1.46 and 1.98
UV data (EtOH) [1]	λ_{max} nm (ε): 223(25,600), 265(13,400), and 362(21,800)
Flourescence emission [1]	425 nm

[1] Cole and Cox, 1981
[2] Phillips et al., 2002

Aflatoxins appear in many potential foods and feedstuff, such as cotton seed, peanut, corn, milo, rice, dried fish, shrimp and meat meals (Ellis et al., 2000). If the environmental conditions are favourable, toxin-producing strains of *A. flavus* may grow on a suitable substrate. Since the *Aspergillus* group of molds is ubiquitous and uses substrates of high carbohydrate content, agricultural commodities and their products are very vulnerable to aflatoxin contamination (Heathcote and Hibbert, 1978). Ideal environmental factors for the molds to grow are warm and humid climates in combination with high soil air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid dispersal of fungal conidia (CAST, 1989, Robens, 1990). Subsequent aflatoxin production in storage is also favoured at high humidity, high

temperature and insect or rodent activity (CAST, 1989). Also, long intervals between harvest and drying, and deficient aeration of stored commodities foster fungus growth (Lindner, 1990). The conditions favourable for natural aflatoxin contamination predominantly occur at latitudes between 40°N and 40°S of the equator (Williams et al., 2004), but in the times of globalisation it is a worldwide problem. Although aflatoxins are ubiquitous contaminants of several classes of commodities, contamination of corn likely poses the greatest risk to humans worldwide (Coulombe, 1991).

Once the toxin is present, there are currently few efficient and affordable ways available to degrade or remove the toxin.

Recent studies estimated that mycotoxins contaminate 25% of the world's food crops and account for more than $1.4 billion in economic loss in the United States alone (Bingham et al., 2004). Due to warm humid climate in the Southern United States aflatoxin contamination is widespread. According to an article in the Wall Street Journal, drought stress triggered an outbreak of aflatoxin in the Midwest of the United States in October 2005 (Kilman, 10/21/2005). In Iowa, as much as 20% of the corn brought to an elevator after harvest was found to have worrisome levels of aflatoxin. Heavily contaminated feeds are usually destroyed or are sold at a steep discount for non-feed uses. In the 90s, farmers in Texas suffered several consecutive years of economic hardship from contaminated corn crops.

In farm animals, even low levels of mycotoxicosis induced by aflatoxins are correlated with feed refusal, reduced feed conversion ratios, anaemia, reproductive failure, impaired immune response and renal damage (Hamilton, 1990). Similar symptoms can be found in hatchery-reared fish. Higher doses of aflatoxin are often lethal. One of the numerous more recent examples is the contamination of dog food that repeatedly lead to the deaths of dogs in Texas as reported by Bingham et al. (2004).

Aflatoxins are well recognized as a cause in liver cancer and toxic effects, not only in animals but in humans as well. In June 2004 the BBC reported in a news article on their website about an incidence in Kenya with more than 180 local people who had to be hospitalised due to the consumptions of aflatoxin with contaminated corn. They were

suffering symptoms of liver failure, yellow eyes, vomiting and bleeding from their nose. Eighty of those people died from this aflatoxin poisoning. Directly correlating certain disease in humans, such as the occurrence of liver cancer, to the consumption of aflatoxin contaminated food as the causative agent is difficult. However, there are an estimated 4.5 billion persons living in developing countries who are chronically exposed to uncontrolled amounts of the toxin (Williams et al., 2004). Williams et al. (2004) give a thorough overview over different aspects of aflatoxicosis in humans in developing countries. For the United States, Wood (1989) found "no direct evidence that implicated aflatoxins as the causal agents for human cancer". Stoloff (1983) published a probability study on aflatoxin as a cause for primary liver-cell cancer in men in the United States concluding that at the present time it is not possible to correlate the chronic carcinogenic symptoms in men as opposed to acute toxicosis from high levels of aflatoxin such as mentioned above. However, correlations of aflatoxins with carcinogenity have been found when tested in laboratory animals. Therefore, presence of aflatoxins should be restricted to the lowest practical level.

The US Food and Drug administration (FDA) considers aflatoxin to be an unavoidable food and feed contaminant, and it is the declared goal to minimize contamination by implementing regulations that require the survey and management of the problem. They set action levels that consider agricultural imported or domestic shipments adulterated at aflatoxin levels exceeding 20 ppb. The FDA action levels are listed in Table 2.

To reduce or solve the problem of aflatoxin contamination, there have been several different approaches ranging from physical separation of contaminated kernels to chemical treatment to degrade the toxin (Goldblatt, 1969; Bubsby and Wogan, 1984). Management practices can prevent or minimize the degree of aflatoxin occurrence of the produce in the field or storage (Riley and Norred, 1999). Once contaminated, aflatoxin detoxification measures of post-harvest treatment to remove or reduce the toxic effects need to be undertaken.

Table 2: Maximum levels of aflatoxin in agriculture depending on intended use.

aflatoxin contamination [ug/kg]	products	intended use
300	corn and peanut products	finishing (i.e., feedlot) beef cattle
300	cottonseed meal	beef cattle, swine, or poultry (regardless of age or breeding status)
200	corn or peanut	finishing swine of 45 kg or greater
100	corn and peanut	breeding beef cattle, breeding swine, or mature poultry
20	corn, peanut products, and other animal feeds and feed ingredients	immature animals
20	corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients	dairy animals, for animal species or uses not specified above, or when the intended use is not known

Possible strategies to destroy aflatoxin during food and feed processing are discussed by Phillips (1994) and include thermal inactivation, irradiation, solvent extraction and mechanical separation, density segregation, bio-control and microbial inactivation, ammoniation, treatment with bisulfide, heterogeneous catalytic degradation and several other chemical treatments.

Despite improved handling, processing and storage, aflatoxin remains a problem in the food and feed producing industries. Besides degradation of aflatoxin by ozonation (Proctor, 2004), one of the more promising approaches is the attempt to prevent the adsorption of aflatoxins in feeds in the gastrointestinal tract of animals. Hydrated sodium calcium aluminosilicate (HSCAS) has been shown to be an effective sorbent of AfB_1 and reduced the negative effects of that toxin when the clay is included as a non-nutritive supplement in the animals' diet (Phillips et al, 1987; Schell, 1993; Grant and Phillips,

1998). Since we are searching for an efficient and economically feasible way to suppress AfB$_1$ this is the subject of further discussion.

2.2. Smectite

When in 1847 the first montmorillonite (a mineral belonging to the smectite group) was discovered in France, the chemical analysis revealed an alumina hydrosilicate containing calcium and magnesium. In the research article published the same year in "Annales of Chemistry and Physics" by Damour and Salvétat the description of the mineral material discovered read the following (according to Arnoux et al., 2006):

> The Montmorillon mineral, very soft and soapy to the touch, is completely amorphous, and drops off easily between the fingers; its colour is light pink. Without having the clay plastic properties, it mixes easily with water; it is infusible to the blowtorch flame. It is also infusible in the high temperature of the porcelain kilns and takes on the whiteness and appearance of a biscuit; it becomes hard enough to scratch glass. Heated in a tube, it gives off a lot of water, loses its pink color and becomes grayish-white.

Although it is amazing how much scientists of those days knewalready found out about that smectite mineral, subsequent discoveries, especially the discovery of x-ray diffraction, led to much more detailed information on these materials. Most remarkably perhaps, the discovery in the early 1920s that clays are crystalline and by no means amorphous (Schulze, 1989). Their crystal structure leads to distinct properties and with the right tool at hand it is possible to distinguish between different types of smectites and understand differences in their behavior.

Many of the publications and papers that have been mentioned in the previous section refer frequently to a hydrated sodium calcium aluminium silicate (HSCAS) as a specific sorbent of AfB$_1$. In some cases, bentonite is used to identify the mineral (e.g. Galvano et al., 2001; Kubena et al., 1988, Grant and Phillips, 1998). It is not always clear if the authors are aware of knowledge available regarding those materials, information that would help to understand observations made in their experiments.

The title of this work now uses the term "smectite clays as adsorbents of aflatoxin B_1", and this section intends to clarify the relationship between these terms in use and to identify the most important characteristics of smectites, the sorbent material of AfB_1 in our study. It is a special concern of this section, to also provide an introduction to smectites and clay minerals to those readers, who have not familiarized themselves with this special field.

To begin with, I am going to define clay and clay minerals to provide a better general understanding of the subject area.

Minerals are defined as "naturally occurring homogeneous solids with a highly ordered atomic arrangement and a definite (but not fixed) chemical composition", which is usually formed by an inorganic process (Klein, 2002). In soil sciences, the most reactive minerals tend to be of microscopic scale, and they can be separated into primary and secondary minerals. Primary minerals found in soils are those that are inherited from decomposition of igneous, metamorphic or sedimentary rocks and that formed under elevated temperatures. Secondary minerals form mostly from low-temperature reactions in the soil occurring during weathering of primary weathering. They can also be inherited from sedimentary rocks (Schulze, 1989).

Since there is not yet a uniform nomenclature for clay and clay material, the definitions of clays and clay minerals are a little bit more difficult.

Clay, according to Guggenheim and Martin (1995), is "a naturally occurring material composed primarily of fine grained minerals, which is generally plastic at appropriate water contents and will harden" when dried or fired. Clay can also be used as a term for a particle size group but the exact size limit is not strictly set and varies between different disciplines. In soil sciences the clay fraction refers to a class of material whose particles are smaller than 2 μm in diameter.

Clay minerals tend to be of the same length scale as clays but a particle size is not associated with the definition. The term is used for a class of hydrated phyllosilicates making up the fine-grained fraction of soils, rocks and sediments (Bergaya, 2006).

Being a phyllosilicate, synonymous with layer- or sheet-silicate, the structure of smectites is closely related to that of the minerals mica and vermiculite. They belong to the group of 2:1 minerals, meaning that one octahedral sheet is sandwiched in between two opposing tetrahedral sheets, together making up one layer.

The basic units of the tetrahedral sheets are tetrahedrons of four oxygen atoms that bear one Si^{4+} in their center. Si^{4+} is thus tetrahedrally coordinated and has a coordination number of four. The octahedral sheets are a little bit more complex in that they can be either di- or trioctahedral, depending of the valence of the cation occupying the octahedral sites. In either case, the cation is octahedrally coordinated, thus surrounded by six OH^- anions. In a trioctahedral arrangement three out of every three octahedral sites are occupied by a divalent cation such as Mg^{2+}, resulting in a structural formula of $Mg_3(OH)_6$ or $Mg(OH)_2$. In a dioctahedral sheet two out of every three octahedral sites are occupied by a trivalent cation, most commonly Al^{3+}. This gives a structural formula of $Al_2(OH)_6$ or $Al(OH)_3$ (Schulze, 2002). Visualisations, such as those presented in Figure 2 and in the references cited, are very helpful, if not essential, in understanding the structural makeup of phyllosilicates and smectites.

In an ideal tetrahedral or octahedral sheet as well as in an ideal 2:1 layer, all cationic charges are balanced by the surrounding anions that are also shared between adjacent tetrahedrons or octahedrons. Figure 3 gives a model of an ideal dioctahedral layer. There are four planes of anions in the 2:1 layer structure. The outer two planes consist of the basal oxygens of the tetrahedral sheet (Fig 3. A and D) while the inner two planes consist of the hydroxyls (OHs) from the octahedral sheet and the oxygens that are common to both octahedral and tetrahedral (apical oxygen) sheets (Fig. 3 B and C).

A) Tetrahedral sheet. B) Trioctahedral sheet C) Dioctahedral sheet.

apical oxygens

basal oxygens

A) Tetrahedral sheet. B) Trioctahedral sheet. C) Dioctahedral sheet.

O^{2-}, OH^- Si^{4+} Mg^{2+} Al^{3+}
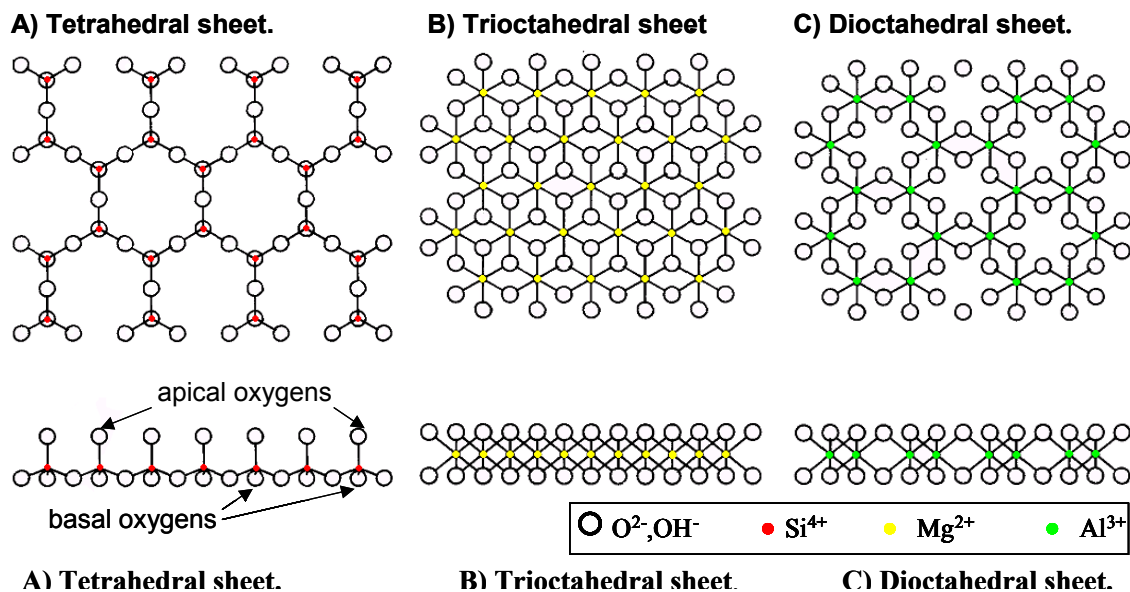
Fig. 2: Structural model of a) a tetrahedral sheet, b) a trioctahedral sheet and c) a dioctahedral sheet viewed from above (top) and from the side (bottom). Adapted from Schulze (1989, 2002).

A →
B →
C →
D →

O^{2-} OH^- Si^{4+} Al^{3+}
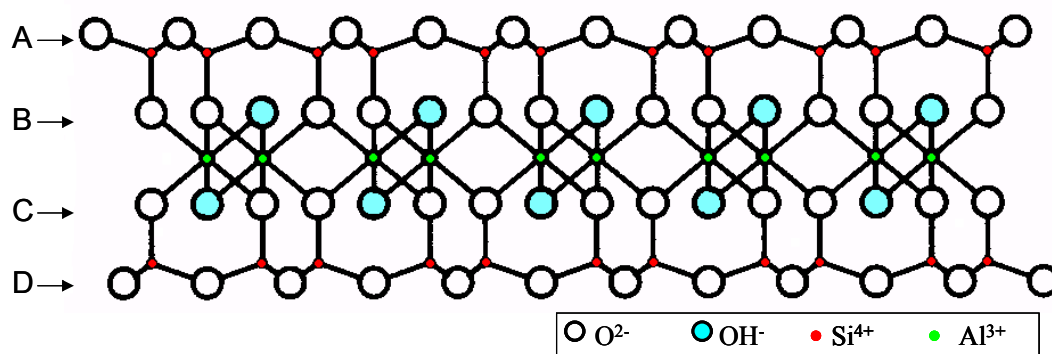
Fig. 3: Model of an ideal 2:1 layer. Note that sites located at the edges even under ideal conditions can develop variable charges. Adapted from Schulze (1989, 2002). In reality, almost all 2:1 clays have a permanent negative charge.

The cause for this charge deficit is the occurrence of isomorphic substitution where the tetrahedral Si^{4+} and octahedral Al^{3+} in the structure are replaced by ions with similar ionic radii and charge. Determining for possible substitution in tetrahedral and octahedral sites is the coordination number and the radius ratio. The radius ratio is defined as:

$$\text{Radius ratio} = \frac{\text{radius of the cation}}{\text{radius of the anion}} = \frac{r_c}{r_a}$$

From calculations it is known that the tetrahedral site can hold a sphere up to 0.414 and the octahedral site up to 0.732 times the radius of the O^{2-} ion (Klein, 2002). For example, the radius ratio of Si^{4+} is 0.278 and thus much smaller than the largest cation that would fit into the tetrahedral site. It therefore occupies almost always the tetrahedral site. Mg^{2+} has a radius ratio of 0.471, which is too large to fit in the tetrahedral site but it fits in the octahedral site. Al^{3+} with a radius ratio near the limit (0.364) can occur in both tetrahedral and octahedral coordination (Schulze, 1989). Common tetrahedral cations are Si^{4+}, Al^{3+}, and Fe^{3+} and octahedral cations are usually Al^{3+}, Fe^{3+}, Mg^{2+}, and Fe^{2+}. Other cations, such as Li^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, V^{3+}, Cr^{3+}, and Ti^{4+} were identified in octahedral positions (Brigatti et al., 2006).

As isomorphic substitution leads to the replacement of a higher charge with a lower charge cation, the charge in the structure decreases and can lead to permanent negative charge. A very common example is the replacement of one out of four tetrahedral Si^{4+} with Al^{3+} in mica. Because the structure was charge balanced before the isomorphic substitution it is obvious that a negative charge of -1 is created. In the case of mica, this charge is balanced by K^+ or other anhydrous cations that are fixed between the layers, in the interlayers.

With the preceding background information in mind, it is now easier to have a look at the smectite structure (Figure 4) and its characteristics. In smectites, different isomorphic substitutions can occur in both tetrahedral and octahedral sheet and the octahedral sheets can also be either di- or trioctahedral. Their permanent negative layer charge ranges from 0.2 to 0.6 per formula unit depending on degree of isomorphic substitution (Bailey, 1980). Those charges are balanced by cations in the interlayer that are hydrated to

varying degrees, depending on the hydration energy of the interlayer cation, the polarization of the water molecules by interlayer cations, variation of electrostatic surface potential (differences in charge location), the activity of water and the size and morphology of the smectite particles (Brigatti et al., 2006). A smectite saturated with Ca^{2+} and Mg^{2+} can stepwise take up up to four ~ 0.28 nm thick layers of water leading to and increase in the basal spacing up to ~ 2nm. In contrast a completely dehydrated layer would have a basal spacing of only ~ 1nm. Is the interlayer of a smectite mainly filled with strongly hydrated Na^+ ions instead of Ca^{2+} this leads to a less strong attraction between the layers and their disorderliness is promoted (Scheffer, 2002). At appropriate water levels Na-smectites can become gel-like. When smectites are heated so that the water in the interlayer is driven off the structure collapses around the remaining interlayer cations. In nature this happens for example during burial metamorphism and a montmorillonite structure can thereby be transformed into an illite-like structure (Klein, 2002).
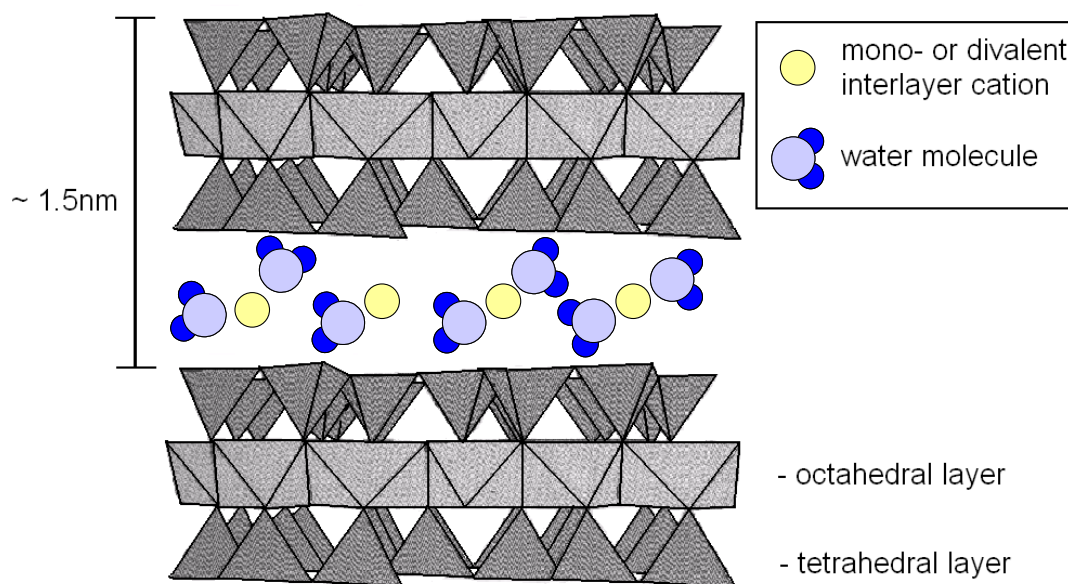
Fig. 4: Model of the smectite structure. One 2:1 layer has a thickness of about 1nm. Thickness including the interlayer depends on interlayer cations and their degree of hydration. Modified after Brigatti et al. (2002).

The unique property of smectites to swell is most probably due to their small layer charge. Expansion takes place as water or some polar organic compound enters the interlayer. The electrostatic attraction to hold the layers together is not strong enough, as opposed to the more highly charged vermiculites (layer charge 0.6 to 0.9), which do not swell as extensively (Moore and Reynolds, 1997).

The interlayer cations and their ability to exchange with other cationic species that the smectites sorb from a surrounding solution is given as the cation exchange capacity (CEC). The CEC is generally understood to be equivalent with the layer charge. The CEC is considered a material constant and lies between 47 and 162 $cmol_c$ kg^{-1} for smectites (Borchart, 1989). Because of their high CECs smectites are major contributors to the nutrient status of the soil such as K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} (Reid-Soukup & Ulery, 2002). Ca^{2+}, Mg^{2+}, Ba^{2+} and Na^+ are the most frequently encountered hydrated cations in the interlayer. Many of the properties of smectites are dependent on the adsorbed cations (Grim und Güven, 1978). The exchange between cations balancing the negative charges is a diffusion controlled, reversible process and in most cases there is selectivity of one cation over another (Brigatti et al., 2006). Cationic organic molecules, such as aliphatic and aromatic amines, pyridines or methylene blue, may replace the inorganic exchangeable cations while non-ionic polar molecules may replace water that was adsorbed to the siloxane surfaces of the clay (Brigatti et al, 2002, Deng and Dixon, 2002).

Due to their short-range order and small particle size smectites have an extraordinary high total surface area of $6 \cdot 10^5$ to $86 \cdot 10^5$ m^2/kg. The other surfaces alone (Fig. 5b) have values between $3 \cdot 10^4$ and $1 \cdot 10^5$ m^2/kg. The orderliness of the stacking of layers of smectites also is of importance. Both surface area and particle thickness (stacking order) can especially have influence the clays adsorption properties. Cations, to a much lesser anions and many types of organic compounds can find different sites on the surface of the smectite to bind or adsorb to, mainly the inner and outer surfaces and sites at the

edge of the smectite particles. The edges have broken O and OH bonds, which contribute to a small extent to the CEC, depending on the fineness of the particles (Borchart, 1989).
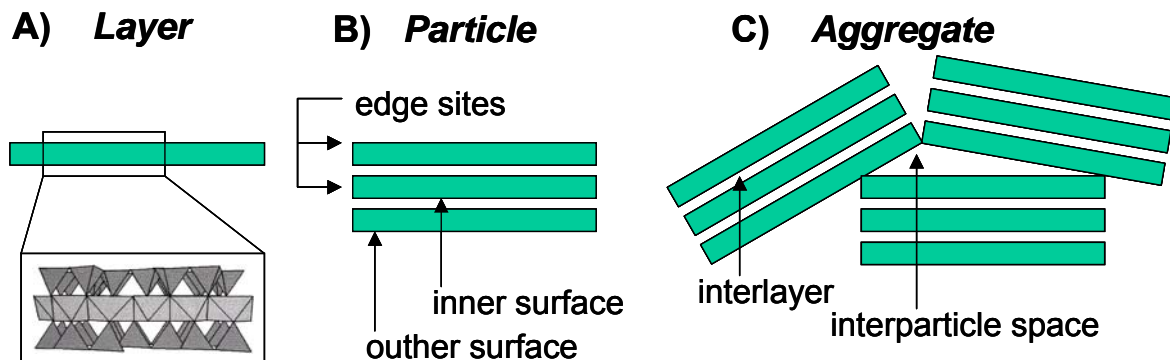
Fig. 5: Layer, particle and aggregate of layer silicates. Layer thickness or basal spacing d(001) and particle thickness of smectites can be determined using X-ray diffraction (XRD).

The charges arising from these broken bonds are pH dependent, thus variable charges; in contrast to the permanent charge that arises from isomorphous substitution. Edge charge at pH 7 typically would contribute about 5 $cmol_c$/kg to the measured CEC. Below pH 7 the many of the broken bonds attract H^+ ions and at pH above neutrality the charge becomes more negative and thus increase the CEC (Borchart, 1989).

To identify smectite and differentiate between smectite and other (clay) minerals x-ray diffraction (XRD) is the standard technique and is usually accompanied by supplementary analytical techniques. Transmission electron microscopy (TEM) is also a useful tool for clay mineral identification and plays an increasingly important role (Środoń, 2006). Since smectites are expandable depending on interlayer cation and humidity/solvent molecules XRD conditions must be carefully controlled (Borchart, 1989). Typical for smectite is a first order basal diffraction peak or d-spacing (001) at 1.4nm when magnesium saturated and at room temperature, which swells to 1.8nm when glycerol is added and x-rayed again. When potassium saturated, the d-spacing of smectites at room temperature lies between 1.0 and 1.4nm and collapses upon heating to 300ºC to 1.0 nm (White and Dixon, 2003). Moore and Reynold's (1997) book is very

user-friendly for clay mineral identification with XRD, and Brindley and Brown's (1980) book is probably the most comprehensive work. Thermogravimetry, differential thermal analysis, infrared spectroscopy, elemental and selective dissolution technique and Mössbauer spectroscopy can be used for smectite identification and are revised in Borchart (1989) and Bergaya et al. (2006). A good reference for many chemical methods used in clay mineral identification is the book by Jackson (2005, reprint from 1985).

To summarize, smectites can differ in chemical composition, the origin of charge from tetrahedral and/or octahedral sites and the amount of charge. Table 3 gives an overview over the minerals in the smectite group. Mg^{2+}- rich forms are called montmorillonites and they have predominantly octahedral charges. Mainly tetrahedrally charged Al^{3+}- rich smectites are beidellites and they are is Fe^{3+} rich they are called nontronite. Montmorillonites are the main components in bentonites and other smectite deposits.

Bentonite is a soft, plastic, light-colored rock that also contains some colloidal silica. It forms as a result of devitrification and chemical alteration of glassy igneous material, such as tuff or volcanic ash (Klein, 2002). An example are volcanic ash-fall layers that are subsequently exposed to mildly alkaline conditions such as they occur in a marine environment. Under those conditions the ash falls alter to smectites and form bentonite (Moore and Reynolds, 1997).

Table 3: Endmember formulas for minerals in the smectite group. Combined octahedral and tetrahedral isomorphic substitution is common in real smectites.

Site of charge	Dioctahedral	Trioctahedral
Octahedral	Montmorillonite $(M \times nH_2O) (Al_{2-y}Mg_y) Si_4 O_{10}(OH)_2$	Hectorite $(M \times nH_2O) (Mg_{3-y}Li_y) Si_4 O_{10}(OH)_2$
Tetrahedral	Beidellite $(M \times nH_2O) Al_2 (Si_{4-x}Al_x) O_{10}(OH)_2$	Saponite $(M \times nH_2O) Mg_3 (Si_{4-x}Al_x) O_{10}(OH)_2$
	Nontronite $(M \times nH_2O) Fe_2 (Si_{4-x}Al_x) O_{10}(OH)_2$	Sauconite $(M \times nH_2O) Zn_3 (Si_{4-x}Al_x) O_{10}(OH)_2$
	Volkonskoite $(M \times nH_2O) Cr_2 (Si_{4-x}Al_x) O_{10}(OH)_2$	

M: one or more mono- or divalent exchangeble cations that may have a value ranging from 0.2 to 0.6.
structural formula: (interlayer occupation) (octahedral cations) (tetrahedral cations) (structural anions)

In soils, the Fe- content of smectites is usually higher than that in geological deposits and the Mg content accordingly tends to be lower (Scheffer, 2002). Relating to this, the soil smectites are also predominantly dioctahedral (Borchart, 1989).

In summary, the properties of smectites discussed above are (see also Bergaya and Lagaly, 2006):

- particles of colloidal size $< 2\mu m$
- a high specific surface area $6 \cdot 10^5$ to $86 \cdot 10^5$ m^2/kg
- in general a high degree of stacking order
- moderate negative layer charge of 0.2 to 0.6 per formula unit
- a large CEC, that is only slightly dependent on ambient pH
- a low pH dependent anion exchange capacity
- swelling and shrinking due to variable water content in the interlayer
- basal spacing mostly between 1 to 2 nm
- extensive interlayer swelling of some members of the smectite group (Li^+- and Na^+ exchanged forms) that may lead under appropriate conditions to the complete dissociation of the mineral layers
- "propensity for intercalating extraneous substances, including organic compounds and macromolecules"

These properties provide the basis for the use of bentonites and smectites in many commercial applications. Those include their use in drilling muds, as catalysts, bonding clays in foundries, backfill to seal the space around high-level radioactive waste canisters and as an adsorbent for different purposes (Klein, 2002). Large volumes of bentonites today are needed for filtering, decolorizing, pelletizing animal feed, as pet litter adsorbents, pesticide carrier and oil and grease adsorbent. Smaller amounts are also needed for paints, pharmaceuticals, cosmetical use, additives for cement and mortar, water purification, fertilizers, ceramics and many more miscellaneous applications. The number of environmental application of bentonites is growing fast. The world's

production of bentonites today is an estimated 13 million tons/year (Harvey and Lagaly, 2006).

Smectites, and other clay minerals, can be found in soil ingested by humans for therapeutic effects (Mahaney et al., 2000). The phenomenon called geophagy, the ingestion of soil or clay, is also widespread in certain animal communities (Brightsmith et al., 2004) and one of the implications of this behaviour include the ability of clay minerals to adsorb and retain toxic environmental substances (Johns, 1986).

Smectites occur in soils sediments and hydrothermal deposits and can be found around the world (Borchart, 1989). Their properties, availability and inexpensiveness make them so attractive also for the fight against aflatoxicosis.

2.3. Smectites as Adsorbents of Aflatoxin B_1

Smectites, which are used in many applications, are used as well in industrially produced pharmaceuticals, where they are used as antidiarrhoeaics, dermatological protectors, as emulsions and creams, and in several other applications (Carretero et al., 2006). Interesting in this context is the inhibition of the harmful enzyme trypsin by smectites in patients with ulcerative colitis, which has been ascribed to an interaction of smectite with the gastrointestinal mucus layer and the binding of trypsin to the mineral (Droy-Lefaix and Tateo, 2006). Smectites interact closely with the mucus glycoproteins.

Whether this sort of interaction also plays a role for the adsorption of aflatoxin B_1 by smectites is not known. The effectiveness of smectite clays as aflatoxin B_1 sorbents is well documented. Originally, smectite clays were used in animal feed as pelletizing or anti-caking agents. The reduction in feedborne aflatoxin and its deleterious effects by using binding agents like smectites that can be added to the animals feed represents one of the more recent strategies in the struggle for aflatoxin detoxification.

Masimango et al. reported as early as 1978 that aflatoxin is bound with differing effectiveness by different sorbents. They performed *in vitro* experiments in which they

adsorbed AfB_1 from various media including beer, water and milk to different sorbent materials.

In initial studies in 1987 and 1988, hydrated sodium calcium aluminosilicate (HSCAS) was tested in animals' diets by Davidson et al. and Phillips et al., respectively. Phillips evaluated the binding capacities of alumina, silicates, and aluminosilicates in vitro before selecting HSCAS as the most suitable sorbent in *in vivo* trials with chickens. These early studies demonstrated that the inclusion of the clay reduced some of the adverse effects of aflatoxin in the animals. Phillips et al. (1988) found that HSCAS has a high binding affinity for AfB_1. The adsorption complex was stable at temperatures of 25 and 37˚C, over a pH range from 2 to 10 and against desorption by an eluotropic series of solvents. Chemisorption, was the proposed mechanism of adsorption.

The term HSCAS seems to be preferred in the literature. Unfortunately, this term lacks some precision, since it is a generic description and thus does not uniquely define the material of use. Based on some more detailed descriptions of sorbent materials in some studies and our own experimental findings, it can in general be assumed that HSCAS and smectite clays are the same, and I am going to use these terms as synonymous.

In the two decades following the initial studies, many animal scientists repeated and extended the initial experimental data and concluded that smectite clays almost without exception seem to effectively sequester aflatoxin when ingested with the animal feed. Symptoms of aflatoxicosis were reduced. Due to the vastness of the literature available and the important information in many of the papers, I have summarized 29 of them in Table 4. The table shows that in a variety of animals, such as chickens, turkeys, pigs, cows, rats, mink, dogs and even trout, HSCAS has been confirmed as an effective protection against the negative health effects of aflatoxin. HSCAS also has been reported to reduce AfM_1 residues in milk, which is a carry-over contamination resulting from the consumption of contaminated feed. HSCAS was an effective protection against AfM_1 in milk when included in the diets of dairy cows (Ellis, 1991) and goats (Harvey et al,

1993). Blüthgen and Schwertfeger (2000) studied the reduction of AfM_1 in the milk of cows and also demonstrated good adsorption qualities of smectites *in vitro*.

The protective effects in the animal studies were dependent on which parameters were observed, the animal studied, the levels of aflatoxin and the amount of smectite included in the animal's diet. For chickens, Kubena et al. found 100% protection when 5g/kg HSCAS was included at aflatoxin levels of 5.0 mg/kg (1988) and 3.5 mg/kg (1993a), while Doerr (1989) only found full protection at levels as low as 2 mg/kg – which still represents a rather extreme contamination. Overall, 5 g/kg of smectite adsorbent added to the animals feed appears to be appropriate and should be effective for most naturally occurring aflatoxin B_1 contamination levels.

Although smectite clays are generally recognized as safe feed additives, several authors were concerned about their nutritional inertness. Without exception in the cited literature, *in vivo* studies did not show any harmful effects by smectite clays alone on the animal health. Corresponding remarks can be found in Table 4 in the column "comments", whenever the researchers examined the effects of clay alone. The scientific groups of Phillips and Kubena represent more than one third of the papers summarized in Table 4.

In vitro studies of potential sorbents of mycotoxins include single concentration sorption studies (Phillips et al., 1988, and others), isothermal adsorption analysis (Ramos and Hernandez, 1996; Grant and Phillips, 1998) and chemisorption index determination (Phillips et al., 1995; Tomašević-Čanović et al., 2001). The major problem with most *in vitro* studies is that results in general do not allow a conclusion about sorption behaviour *in vivo*. Even as some studies try to imitate the conditions of the gastrointestinal tract, in *in vitro* studies oversimplification is not avoidable. For example, Scheideler (1993) performed adsorption experiments in the presence of real intestinal contents from chickens. But it remains a challenge to duplicate the complex natural systems, such as the pH gradient from acid in the stomach to basic in the small intestines depending also on animal species.

Table 4: Literature overview: reduction of adverse effects of aflatoxin on birds, rodents, mammals and fish by dietary inclusion of smectite clays. Listed by group of animal and year of study.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af[1] in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Davidson et al., 1987	Chickens	HSCAS[2]	1 and 5	0.02 and 0.04	Bioavailability of aflatoxin in liver and blood reduced in a dose dependent manner.	"Similar aluminosilicates were not as effective."
Kubena et al., 1988	Chickens	HSCAS	5	7.5 and 5.0	55 and 100% significant reduction of the growth inhibitory effects of AfB_1.	
Doerr, J.A., 1989	Chickens	HSCAS (NovaSil, Engelhard Corp.)	5 and 10 1.25 2.5 5 and 10	4 2 2 2	50% improvement. 50% improvement. 75% improvement. Fully protected.	Protection referring to body weight. HSCAS did not fully protect against liver or spleen weight changes caused by aflatoxin.
Araba and Wyatt, 1991	Chickens	HSCAS and sodium bentonite	5 and 10	5	Toxic effects on feed intake, body weight gains, liver weight and liver lipids reduced. Sodium bentonite better agent at 5g/kg dietary inclusion.	Clays alone did not alter the animals performance.
Huff et al., 1992	Chickens	HSCAS (Engelhard Corp.)	5	3.5	Reduced toxicity of evaluated parameters.	HSCAS alone did not alter examined parameters.

Table 4: Continued.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af[1] in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Jaraprakash et al., 1992	Chickens	HSCAS	2	1	Significantly reduced adverse effects of aflatoxin, like clinical manifestations, biochemical alterations and increase in cumulative weight of liver and kidneys.	
Kubena et al., 1993a	Chickens	HSCAS	5	3.5	Almost total protection against the effects caused by aflatoxin.	
Kubena et al., 1993b	Chickens	HSCAS	5	5.0	Significantly diminished by 39-68% in duplicate experiment by 46-88%.	
				2.5	Significantly diminished by 38-90%.	
Scheideler, 1993	Chickens	Ethacal®, Novasil, Perlite, Zeobrite	10	2.5	All tended to decrease negative effects. See paper.	
Madden and Stahr, 1995	Chickens	Silty clay loam	100-250	0.7	Effectively reduced.	
Kubena et al., 1998	Chickens	HSCAS (T-Bind™)	2.5	5.0	Toxic effects reduced by 43%.	Sorbent alone did not alter the performance of the chickens.
Ledoux et al., 1999	Chickens	HSCAS (Improved Milbond-TX®, Milwhite Inc.,TX)	10	4	Reduced incidence and severity of hepatic histopathology changes and completely prevented renal lesions.	*In vitro* studies showed 100% efficiency of Af-adsorption. No effect of HSCAS alone on animals.

Table 4: Continued.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af^1 in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Madden et al., 1999	Chickens	Silty clay coam	100 and 250	0.7	Reduced the detrimental effects of AfB_1 on the performance and biochemical parameters.	
Pimpukdee et al., 2004	Chickens	NovaSil Plus	1.25 – 5	5	Significant protection from the effects of high levels of aflatoxins. Preserved hepatic vitamin A levels, even at lower dietary intake of clay.	
Desheng et al., 2005	Chickens	Ca-Montmorillonite (from Ca-bentonite)	5	0.2	Significantly diminished.	
Kubena et al., 1990	Turkey poults	HSCAS	5	0.5	Diminished adverse effects on body weight gains, most relative organ weights, hematological values, serum biochemical values and enzyme.	
				1	68% decrease in mortality.	
Edrington et al.,1996	Turkey poults	HSCAS and acidic HSCAS	5	0.75	71% AfM_1 decrease in urine compared by HSCAS and 51% decrease by acidic HSCAS. Toxicosis alleviated in both cases.	Activated charcoal in this experiment reduced AfM_1 output in urine but did not alleviate aflatoxicosis.

Table 4: Continued.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af^1 in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Voss et al., 1993	Rats	Bentonite (Volcaly)	1	1.5	Body weight and food consumption significantly increased. Liver lesions.	Selected hematological and serum chemical values not influenced by bentonite consumption.
			10		Comparable performance to control, no signs of aflatoxicosis. Liver lesions less extensive.	
Sarr et al., 1995	Rats	HSCAS (Novasil^{TM})	5	0.125 – 1.0	AfM_1 output significantly decreased. Less AfM_1 with increasing dose of HSCAS.	
Mayura et al., 1998	Rats (pregnant)	HSCAS (Novasil^{TM})	5	2	Maternal, developmental and histological parameters comparable to controls.	HSCAS alone does not have negative effects on the performance of pregnant rats. Clinoptilolite in this study did not protect from aflatoxicosis.
Abdel-Wahab et al., 1999	Rats (pregnant)	HSCAS (Engelhard Corp.) Bentonite (Ain-Shams University, Cairo)	5	2.5	Prevents maternal and developmental effects of AfB_1	HSCAS or bentonite alone had no adverse effects on nutrient utilization.
Bingham et al., 2004	Rats dogs (Labrador retrievers)	HSCAS	5	0.1	Both rats and dogs showed reduction in urinary metabolites of aflatoxin B_1 when fed a diet with HSCAS clay.	

Table 4: Continued.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af[1] in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Bonna et al., 1991	Mink	HSCAS	5	0.034 0.102	Prevented mortality, eliminated histopathological lesions in the liver.	34 ppb and 102 ppb AfB$_1$ without HSCAS were 20 and 100% lethal.
Lindemann et al., 1993	Pigs	HSCAS (Novasil^{TM}, Engelhard Corp.), 2 sodium montmorillonites: Volclay, FD-181 (American Colloid Company, IL)	5	0.84	Both, HSCAS and Na-bentonites improved growth rate and restored serum clinical chemistry indicators.	There were no further benefits when more than 0.5% clay were included in these experiments.
Schell et al., 1993	Pigs	Ca bentonite (HSCAS)	2.5 to 20	0.8	Weight gain linearly improved. Growth inhibitory effects diminished by 64 to 82%.	
Harvey et al., 1994	Pigs	HSCAS-1 and HSCAS-3, two formulations of HSCAS	5	3	Body weight gain significantly improved, prevented most Af-induced biochemical values.	
Lindemann et al., 1997	Pigs	Sodium bentonite FD-181 (American Colloid Corp., IL)	5	0.5	Total growth recovery.	Kaolin and other adsorbents were also tested in this study.

Table 4: Continued.

Reference	Animal	Type of clay	Clay in diet (g/kg)	Af[1] in diet (mg/kg)	Effects of clay on adverse effects of aflatoxin	Comments
Blüthgen and Schwertfeger, 2000	Lactating cows	Na-bentonites and Ca-bentonite (Agromont-Ca®)	20	See paper	Tendency to reduce AfM_1 in milk but no significant results (small sample size).	Supplementary experiments with lactation cows. Na-bentonite adsorbs more than Ca-bentonite. Only vague conclusions in paper.
Ellis et al., 2000	Trout	Na-bentonite (Volclay™)	20	0.02	Intestinal absorption of dietary aflatoxin was blocked, reducing liver and kidney aflatoxin loads by at least 80±10%	

[1] Aflatoxin
[2] Hydrated calcium sodium aluminosilicat

Methods to detect aflatoxin concentration usually involve liquid chromatography or HPLC. Radiolabelling of AfB_1 molecules and subsequent determination by liquid scintillation has been used (e.g. Scheideler, 1993). UV-visible spectrophotometry is used in other cases (Grant and Phillips, 1998).

Several review articles were published on the sorption of mycotoxins and aflatoxin in specific and by different adsorbents, and I give a small overview in the following. Harvey et al. (1993) published a comprehensive review in which they report the development of their research and the evolution of new questions on the *in vivo* evaluation of aluminosilicate compounds to reduce aflatoxin toxicity. They found HSCAS able to protect chickens, swine and lambs from aflatoxicosis and that aflatoxins were also suppressed in milk of cows and dairy goats. They see a good prospect for the preventive management of contaminated feedstuff and the reduction of aflatoxin in the foodchain when used in conjunction with other management practices.

Ramos and Hernández (1997) discussed the prevention of aflatoxicosis by inclusion of HSCAS in feedstuffs and concluded that this is a promising field but that still more research is required, especially concerning long-term health effects of HSCAS on animals.

Huwing et al. (2001) reviewed the efficiency of different materials, such as activated charcoal, zeolites, HSCAS, other clays, polymers, yeast and yeast products, as adsorbents for different mycotoxins. They found that HSCAS showed almost total protection against the adverse effects of aflatoxins but were very limited in counteracting the mycotoxin zearalenone.

Phillips et al. (2002) presented a thorough and comprehensive overview of clay-based enterosorbents for the prevention of aflatoxicosis. Structural information on various adsorbent minerals is included and the specificity of HSCAS for AfB_1-adsorption is pointed out. Mechanisms of adsorption to the HSCAS surface are discussed, and their evidence suggests that aflatoxins react tightly at multiple sites at the clay surface, especially within the interlayer. They postulated conceivable risk arising from the

dietary inclusion of smectites on the animals health and stated the need for special attention to mineral-nutrient interactions in sensitive animals.

Trckova et al. (2004) compared kaolin, bentonite and zeolites as feed supplements and their health effects and risks. They stated that bentonites in animal diets act as gut protectants (enterosorbents) and that bentonites have been shown to be the most effective in binding toxins, especially aflatoxin.

Taylor (2001) reported that out of 21 commercially available sorbents about two-thirds are classified as montmorillonites and stated that if the sorbent material in commercial mycotoxin-binding products are clay minerals other than montmorillonites or zeolites research rarely confirms their quality as aflatoxin adsorbents.

Dixon et al. (2006) proposed quality labeling of smectite clays and presented an introductory plan regarding which parameters should be used as quality measures implemented by state authorities to regulate sorbent materials for the purpose of aflatoxin-binding in animal feed.

In most studies where both zeolites and smectites were studied, smectites seemed to be more effective, with an exception of the study by Tomašević-Čanović (2001) in which is was concluded that clinoptilolite had a higher chemisorption index, less desorption after adsorption of AfB_1, than montmorillonite.

Over all, over the past two decades previous studies have indicated that literature smectite clays are effective adsorbents of aflatoxin and successfully protected many farm animals from aflatoxicosis.

3. SMECTITE CLAYS AS ADSORBENTS OF AFLATOXIN B_1: INITIAL STEPS[*]

3.1. Overview

Smectite clay has been shown to sorb aflatoxin B_1 (AfB_1) in animal feed (Phillips et al., 1995 and 2002) and thereby reduce its toxic influence on animals and its entrance to the human food chain. In an effort to find effective adsorbents, 39 samples proposed to adsorb aflatoxin were analyzed and classified into four groups based on their properties: coefficient of linear extensibility (COLE), pH, cation exchange capacity (CEC), organic carbon, microbial content and x-ray diffraction (XRD) spacings. A subset of 20 bentonite samples from commercial sources and reference minerals from 6 US-states and 2 sites in Mexico was selected for sorption determinations. A 10-fold difference in sorption based on the Langmuir equation was observed. Yet clay properties were mostly clustered and it is not clear which properties influence this variation. The basal spacing of AfB_1 saturated smectites exhibited greater resistance to collapse on heating than untreated smectites indicating that AfB_1 entered the interlayer galleries of the smectites. After heating the mycotoxin-clay complex the desorbed mycotoxin was altered indicating a reaction of the molecules with the clay surface. The most effective sorbent smectite samples were from three US-states (MS, ID, TX).

3.2. Introduction

Aflatoxins are highly carcinogenic, hepatotoxic, teratogenic and mutagenetic secondary metabolites produced primarily by the fungi A*spergillus flavus* and A*spergillus parasiticus* (Palmgren and Ciegler, 1983). These fungi are widespread and especially a problem in warm climates.

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[*] Reprinted with permission of the publisher from: Kannewischer, I., M.G. Tenorio Arvide, G.N. White, and J.B. Dixon. 2006. Smectite Clays as Adsorbents of Aflatoxin B_1: Initial Steps. Clay Science, Japan 12 (Supplement 2).

In the US aflatoxins were found to contaminate peanuts, corn, cottonseed, grain sorghum, millet and a variety of nuts. Although aflatoxins are ubiquitous contaminants of several classes of commodities, contamination of corn likely poses the greatest risk to humans worldwide and they are considered unavoidable food and feed contaminants (Coulombe, 1991; Phillips et al., 1995).

Within the group of aflatoxins, AfB_1 is by far the most toxic and known for its deleterious effects on humans, poultry, livestock and other animals (Bilgrami and Sinha, 1992). It not only does harm to animals when ingested with their feed but also poses risks to human, e.g. when the animals' products are consumed. In addition to this, economic costs can be high when aflatoxin occurs at high concentrations in crops and renders the crop unusable.

To reduce or solve the problem of AfB_1 contamination, there have been several different approaches ranging from physical separation of contaminated kernels to chemical treatments to degrade the toxin. One of the more recently developed strategies is the attempt to prevent the adsorption of aflatoxins in feeds in the gastrointestinal tract of animals. Hydrated sodium calcium aluminosilicate (HSCAS) has been shown to be an effective sorbent of AfB_1 and reduces the negative effects of that toxin when the clay is included as a supplement in the animals' diet (Schell et al., 1993; Grant and Phillips, 1998). The inclusion of small amounts of clay in animal feed offers an economically feasible and relatively simple way to suppress AfB_1 influences. Several companies now propose their clay products as feed additives for sorption of AfB_1 sorbents. Despite the effectiveness in AfB_1-sorption by some clays demonstrated mainly in feeding studies, there is a lack of understanding the parameters influencing the effectiveness of smectites as AfB_1 sorbents from a scientific point of view. If certain characteristics of smectites prove as reliable sorbents due to well understood mechanisms, this would allow state authorities to give seals of approval to industrial feed additives as good AfB_1-sorbents without the companies being required to carry out expensive feeding studies.

The research carried out in this study can be assigned to two different stages: (a) the screening of 39 samples to characterize their chemical and physical properties and (b) the characterization of 20 smectite samples out of the larger set as sorbents of AfB_1.

Accordingly, the first objective of this study is to describe the range of properties of commercial sorbents offered to suppress aflatoxin in animal feed and to present the chemical and physical properties of these commercial sorbents for comparison.

The second objective of this work is to discuss the relationships among clay-sorbents' properties and the amounts of aflatoxin they adsorb.

3.3. Materials and Methods

3.3.1. Materials

Aflatoxin B_1 from Aspergillus flavus was purchased from Sigma Chemical Co. (St. Luis, MO 63118); CAS No. 1162-65-8. Acetonitrile, Chromasolv® for HPLC, gradient grade was purchased from Sigma-Aldrich; CAS No. 75-05-8. Benzene, GR, CAS 71-56-1, and Methanol, HPLC Grade, CAS 67-56-1, were from EM Sciences; CAS 71-56-1.

Many samples were submitted by the Office of Texas State Chemist representing industrial products proposed as sorbents of AfB_1 and others were reference minerals from our own collection. All 39 samples were analyzed and treated as received. The smectites included in this set were from 6 different states in the United States as well as from 2 sites in Mexico. Smectites from a third site in Mexico, Laguna del Carmen, a saline lagoon in a high desert environment, and their sorption potential will be discussed in a separate paper (Tenorio Arvide et al., 2005).

3.3.2. Sample Screening

The original set of 39 samples was screened for their chemical and physical properties using standard methods. Parameters determined for each sample were the coefficient of linear extensibility (COLE-value; Borchart, 1989), pH, CEC (Soil Survey Laboratory Staff, 1996), organic carbon and carbonates (Dremanis, 1962) and XRD. If a smectite

peak was present, the particle thickness L, was calculated using the Scherrer equation. XRD & TEM were used according to White and Dixon (2003).

If signs of fermentation were observed during the process of the COLE-value determination, a microbiological culture on Agar was prepared using the spread plate technique to test the sample for yeast or other living organisms (Zuberer, 1994).

After this screening process 20 samples with the characteristics of smectite were examined using the AfB_1-sorption procedures described below.

3.3.3. Isothermal Adsorption Procedure

We used the procedure as described by Grant and Phillips (1998) with some small changes.

Stock solution. To prepare the stock solution acetonitrile was injected directly into the bottle as received from Sigma Chemical Co. using disposable syringe and needle. Dissolved AfB_1 was taken out using the syringe and transferred to a glass flask. To wash the AfB_1 container acetonitrile was injected several times and each time liquid was then transferred to the flask. Acetonitrile was added to the stock solution to obtain the correct volume. The flask was wrapped tightly in Al foil and stored at 0-2 °C.

Working solution. An aliquot of the stock solution was transferred into a glass flask using an Eppendorf Research Pipette and diluted with distilled water to 8 ppm. The concentration of the working solution was verified by measuring the absorbance of the 365 nm AfB_1-peak in a scan (200-800 nm wavelength) with a Beckman Coulter DU800 UV-Visible–Spectrophotometer.

Isotherms. To each 5 mL of AfB_1-solution with the concentrations 0.0, 0.4, 1.6, 3.2, 4.8, 6.4 and 8.0 ppm were added 0.1 mg smectite-sample. We used sterile FALCON$^{®}$ Blue Max Jr. 15 mL 17 x 120 mm polypropylene conical tubes. The concentrations were obtained by dilution of the working solution with distilled water. In order to add the small amount of clay sample to each concetration first a suspension of 10 mg clay sample per 5 mL distilled water was prepared. Then 50 μL of this 2 mg/mL suspension was transferred to the test tubes using an Eppendorf Research Pipette. The samples were

prepared in duplicate. Along with the samples there were two controls consisting of 5 mL of stock solution (8 ppm) without adsorbent and 5 mL of the lowest concentration without adsorbent (0.4 ppm). After 24 hours of shaking at 200 motions/min on an orbital shaker (Cole-Parmer); the samples were centrifuged (IEC PR-7000 Centrifuge) at 51000 g for 57 min and the amount of adsorbed AfB_1 was determined, measuring the AfB_1-absorbance of the supernatant at 365 nm (in water-acetonitrile solvent) with UV/visible spectrophotometry.

For the standard curve AfB_1-solutions concentration at 0, 0.4, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2 and 8.0 were measured. Molar absorptivity (ε) was then derived from the standard curve. The supernatant concentrations of the toxin were calculated using this experimentally derived ε-value, which was equal to 18822.

Using the linear expression of the Langmuir equation

$$C_{eq}/q = (1/k_d Q_{max}) + (C_{eq}/Q_{max})$$

first Q_{max} and k_d were determined, where C_{eq} is the concentration in equilibrium, q is the amount AfB_1 adsorbed, k_d a distribution coefficient and Q_{max} the maximum sorption capacity. The data were then fitted to the Langmuir equation.

3.3.4. XRD of AfB_1 Saturated Smectites

Saturation of smectite with AfB_1. To 10 mg of sample 10 mL of a 100 ppm AfB_1-solution (benzene: acetonitrile, 98: 2) was added and shaken 24 hours at 200 motions/min on an orbital shaker in 50 mL PYREX® glass test tubes with screw caps. AfB_1-concentration left in solution was determined using UV/visible spectrophotometry at $\lambda = 348$ nm and a molar absorptivity of AfB_1 in benzene: acetonitrile $\varepsilon = 19800$ (Nesheim et al., 1999).

The benzene: acetonitrile solution was directly obtained by dissolving solid AfB_1 in it, parallel to the dissolution of AfB_1 in acetonitrile as described in detail above. The saturated smectite was mounted on a quartz plate in an aluminum frame for XRD analysis.

Along with each saturated sample control samples were prepared. The amount of sample used was 150 mg, which was first shaken 24 h in benzene: acetonitrile and then dried onto VICOR-glass slides.

Heat treatment. The heat stability of the smectite-aflatoxin complex was determined producing XRD-patterns of the saturated samples and their controls at room temperature and after heating to 100, 150, 200 and 245ºC for at least 2 h.

XRD-pattern. The XRD-patterns were obtained directly after the samples were taken out of the oven or a desiccator. XRD-patterns were obtained using a Philips X-ray-diffractometer with CuK_α-radiation, a graphite monochromator and a theta-compensation slit. The pattern was measured in 0.05 º2θ intervals from 2 to 32 º2θ for room temperature and 2 to 22 º2θ for all heat treatments of 100 ºC and above.

3.3.5. Desorption Experiment

AfB_1 was extracted with methanol (Dollear, 1969) after the heat treatments. After the last step of heating and XRD was completed, the samples were scraped off their slides. The amount of smectite was weighted and transferred into PYREX®-glass-test tubes. Five mL of methanol as extractor was added. The amount of methanol for desorption was only half of the 10 mL 100 ppm AfB_1 solution used for saturation to ensure measurable amounts of desorbed material. After 24 hours of shaking at 200 motion/min the samples were centrifuged to separate smectite from liquid. Molar absorptivity ε in methanol was 21500, according to AOAC standard method (1995). Instead of measuring at a single position, wavelength scans were performed of the supernatant with the UV/Visible-Spectrophotometer to screen for aflatoxin desorbed from the smectite sample.

3.4. Results and Discussion

3.4.1. Sample Screening

Table 1 and 2 summarize the results of the sample screening. The samples range in CEC from 21.7 to 101.3 $cmol_c$/kg. Organic carbon contents are found to range from 0.04 up to 47.99 wt%. The pH ranges from 4.0 to 10.3. In 11 samples organic activity was detected in the form of living yeast or other microorganisms. The division into 4 groups of sorbent samples was made according to their diverse characteristics. One group had about 13 % organic carbon and also exhibited smectite peaks as well as minor amount of quartz in XRD pattern. The other group was higher in organic carbon, which had a negative influence on the quality of XRD patterns. However, quartz peaks were found in the OSC-samples. The eight sample-group exhibited neither a high organic content nor a smectite XRD peak, although the CEC in this group ranges between 21.7 and 74.2 $cmol_c$/kg. Those samples need further study in order to determine the origin of the CEC and are found in the group "other mineral sorbents" (Table 5).

The 20 samples behaving like smectites were chosen to focus on characterization as AfB_1 sorbents (Table 6). All have a high CEC between 68.3 and 101.3 $cmol_c$/kg and the XRD-characteristics of smectite. The mean crystallite dimension, L, determined using the Scherrer equation (Moore and Reynolds, 1997) ranged from 10.3 to 25.7 nm (for untreated samples air-dried from water at room temperature on XRD-slides). Values for pH in this group were between 7.1 and 10.3 except one sample had an uncommonly low value of 4.7. The last one was included for its uniqueness in this set. Subsequent field study and observation confirmed a presumption that this low pH probably is a result of acidification caused by sulfide oxidation.

A consistency of characteristics can be observed in certain sub-groups, e.g. Mississippi (1MS, 2MS, 3MS, 4MS), Idaho (samples ending with ID) or Laguna del Carmen, Mexico (LC). When pH was plotted against CEC, L or COLE -value clustering of each sub-group can be observed which indicates the same origin of these sample sets and is important for quality assurance.

Table 5: Three of four groups of proposed sorbents listed by increasing pH.

Sample no.	pH	COLE value	CEC [cmol/kg]	Orgn. C %	Organic activity[1]	L [Å]
ORGANIC AND SMECTITIC SORBENTS						
2 VA	5.6	1.8	52.3	12.91	✓	98
1 VA	5.6	1.6	55.3	12.90	✓	137
3 VA	5.7	2.6	52.7	13.23	✓	99
4 VA	5.8	2.7	53.5	13.42	✓	73
SORBENTS HIGH IN ORGANIC CARBON						
T1	5.0	1.1	49.1	47.99	✓	NP
T2	5.5	2.0	54.1	14.05	✓	NP
5 OSC	5.7	1.1	57.3	25.01	✓	NP
6 OSC	5.7	0.6	37.1	22.98	✓	NP
7 OSC	5.7	0.8	32.6	23.91	✓	NP
8 OSC	5.7	0.9	36.5	24.01	✓	NP
T5	6.2	2.1	52.4	18.98	✓	NP
OTHER MINERAL SORBENTS						
T6	4.0	0.4	49.1	0.74	x	NP
18 VA	7.1	0.1	21.7	1.65	x	NP
T3	7.9	0.1	53.8	0.27	x	NP
LC1	10.1	0.9	62.1	0.24	x	NP
LC3	10.2	2.0	69.0	0.13	x	NP
LC4	10.2	1.3	69.6	0.17	x	NP
LC5	10.2	1.8	–	–	x	NP
LC2	10.3	1.4	–	–	x	NP

[1] X No activity; ✓ Activity
NP: No XRD smectite peak

Table 6: Smectite properties listed by increasing sorption.

Sample no.	pH	COLE value	CEC in NaOAC [cmol/kg]	Crystallite dimesion L			Maximum AfB$_1$-sorption capacity Q$_{max}$ [mol/kg]
				In Water	K Saturated	AfB$_1$ Saturated [nm]	
16 MX	7.6	1.1	68.3	10.3	7.0	18.6	0.060
5 OK	7.3	0.3	101.3	23.4	13.5	29.4	0.130
7 AZ	7.6	0.7	90.1	22.1	13.5	22.8	0.151
9 ID	7.5	1.3	77.8	22.7	15.6	18.6	0.208
12 ID	7.4	1.1	75.9	18.1	12.6	22.8	0.240
2 MS	8.7	1.0	85.4	17.3	10.6	22.8	0.282
10 ID	7.8	1.8	75.1	13.7	11.9	15.7	0.283
4 MS	8.5	0.9	93.3	15.9	9.7	18.6	0.284
15 MX	8.7	~3	93.7	23.4	18.5	15.8	0.288
T8	7.8	1.0	74.2	12.8	13.7	15.7	0.290
6 WY	9.6	1.8	83.3	22.7	14.5	29.4	0.294
3 MS	8.6	0.8	94.3	22.1	9.7	22.8	0.318
13 ID	7.5	0.7	79.7	22.7	13.5	15.7	0.355
17 TX	4.7	2.2	78.4	22.1	18.5	25.7	0.375
14 MS	7.7	0.6	84.3	17.7	13.5	18.6	0.403
T4	8.5	0.9	99.9	11.9	12.6	22.8	0.404
11 ID	7.4	1.3	76.4	25.7	10.8	15.8	0.481
T7	8.8	1.2	87.6	22.1	13.5	13.6	0.515
1 MS	8.3	0.6	85.8	17.3	9.7	18.6	0.526
8 TX	7.1	1.3	84.1	12.1	12.1	15.8	0.677

3.4.2. Sorption

The Langmuir equation yields an overall good fit of the different sorption values (Fig. 6). The maximum adsorption capacities obtained from this data treatment range from 0.060 (16MX) to 0.677 (8TX) mol/kg, representing a 10-fold increase in sorption capacities, as listed in table 6. Values for kd from the Langmuir equation did not show a meaningful relationship to the AfB_1 sorption and therefore are not included.
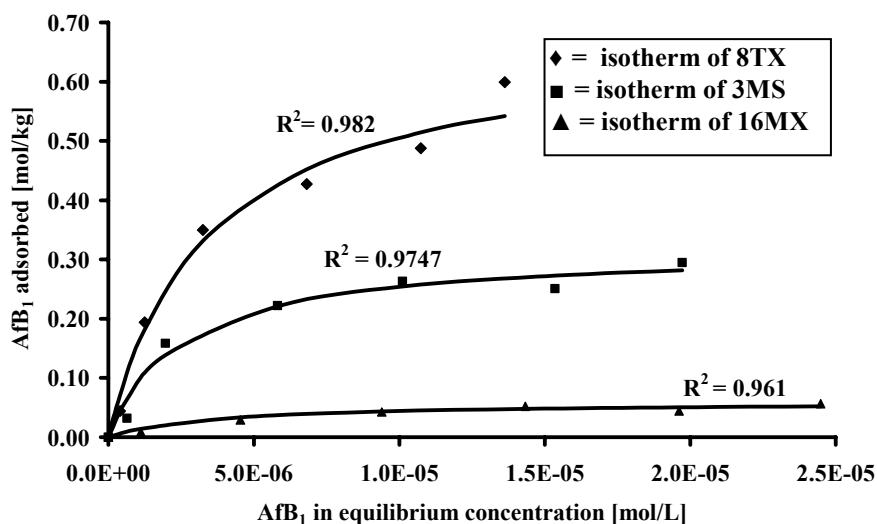
Fig. 6: Langmuir adsorption isotherms encompassing the range of observed sample adsorption capacities.

Amounts of sorption in relation to sample properties show no clear trends but are clusterd instead. In contrast to a previous hypothesis by Grant and Phillips (1998) also the Ca-ion content of samples does not have a distinct influence on sorption in our study (Fig. 7). Other cations present, K^+, Na^+ and Mg^{2+}, also seem to have no effect on the sorption ability of the untreated (as received) smectites. Preliminary data however show a reduction in sorption when smectites were K^+ saturated, which implies that the more highly charged sites are excluded from AfB_1-sorption.
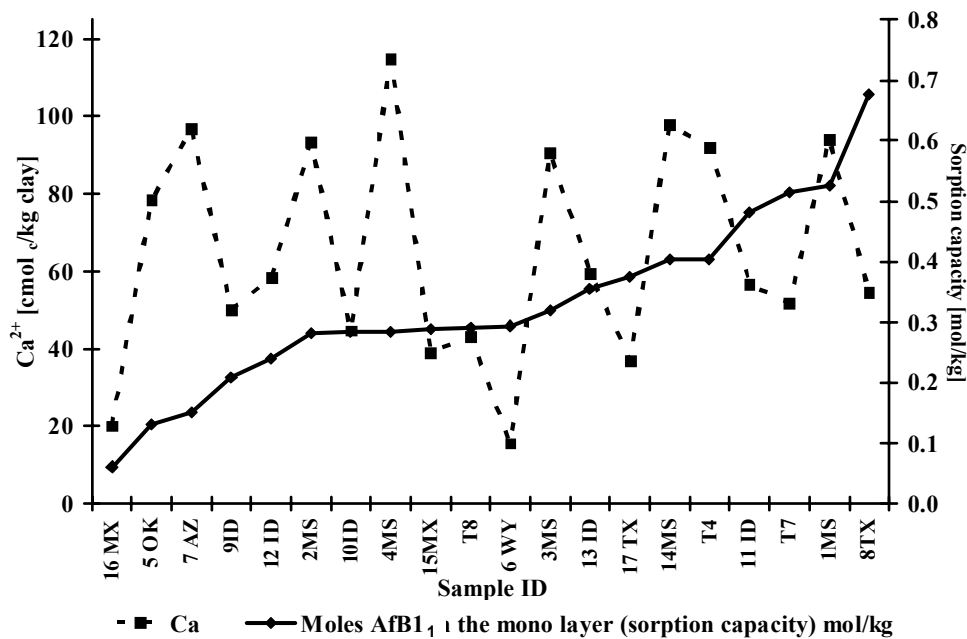
Fig. 7: Comparison of Ca²⁺-Ion content with sorption capacity.

The sorption data points at the highest concentration (8 ppm) using unmodified concentration values show that 6 to 47.7 % of the AfB_1 originally in solution was adsorbed by the 100 µg clay sample present. Examination of the low concentration data points shows that up to 99.8 % of the AfB_1 originally present (0.4 ppm) was adsorbed. An average of 22.2 % sorption at the 8 ppm point versus a much higher average of 47.7 % at 0.4 ppm. These observations indicate also that even if the maximum sorption capacity as calculated by Langmuir is low, the smectite still might be an effective adsorbent at low concentrations. Sorption behavior at concentration below the saturation level will be part of a further study. Chemisorption is indicated by XRD-results and by the overall good fitting of the data to the Langmuir equation concentrations support a more complex mechanism supporting previous suggestions that different sites and mechanisms of action are involved in sorbing aflatoxin to HSCAS (Phillip et al., 2002) and montmorillonite (Ramos and Hernandez, 1996).

Possible sites for sorption of AfB$_1$ to the smectites include its interlayers, external surfaces and edge sites. Phillips (1999) stated that the dicarbonyl system of aflatoxin was found essential for binding. The effects of this group on the binding of AfB$_1$ on smectite requires more research.

To our knowledge, this research represents the most extensive survey of smectite as AfB$_1$ sorbents. Besides smectites, zeolites, other clays, activated charcoal, polymers and yeast products have been tested for their use in mycotoxin detoxification, as reviewed by Huwig et al. (2001). Montmorillonites (smectites) have been shown to be the most effective. As shown in this research, AfB$_1$ sorption within this group of smectites differes substantially.

3.4.3. XRD of AfB$_1$ Saturated Smectites

The ratio of AfB$_1$ to clay was from 7.4 to 9.8 wt%. The subsequent XRD-pattern after heat treatment showed uniform resistance to collapse of the basal spacing whereas unsaturated control samples, dried from the same matrix solution, did collapse (Fig. 8). The d-spacing of AfB$_1$-treated samples did not collapse below 1.3 nm indicating that the AfB$_1$-molecules entered between the layers and prevented further collapse. The difference between collapsed and uncollapsed samples at 245ºC is about 0.3 nm, sufficient to allow AfB$_1$-molecules to fit in.

Phillips et al. (2002) found that the sorption capacities of collapsed HSCAS compared to untreated HSCAS derived from isotherm experiments was decreased by more than 85%, which also indicates interlayer sorption of AfB$_1$.

XRD-patterns also show the dissappearance of the third order peak of smectite samples saturated with AfB$_1$; this might have been caused by a different hydration state of ions in the interlayers caused by the interlayer AfB$_1$ sorption (Fig.9).
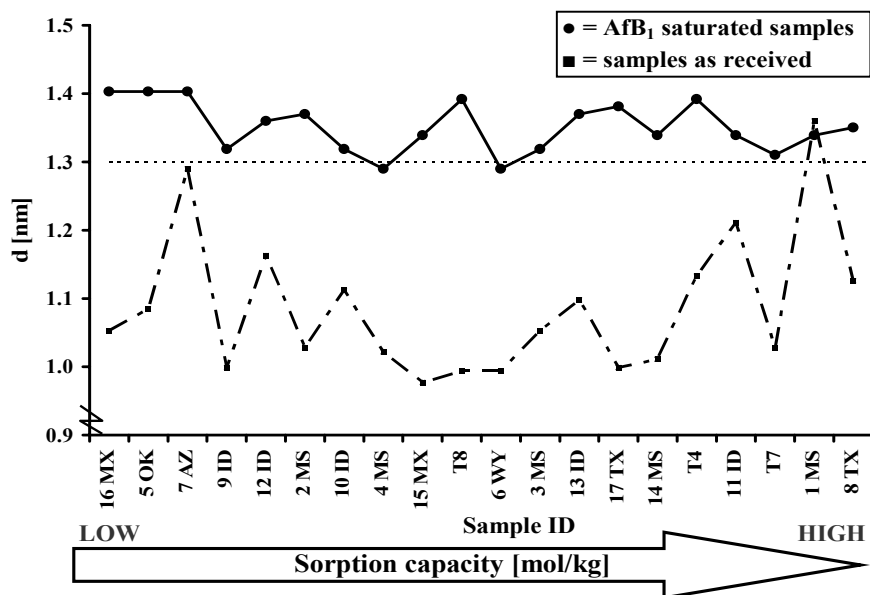
Fig. 8: Basal spacing of AfB$_1$ -saturated and −unsaturated samples.
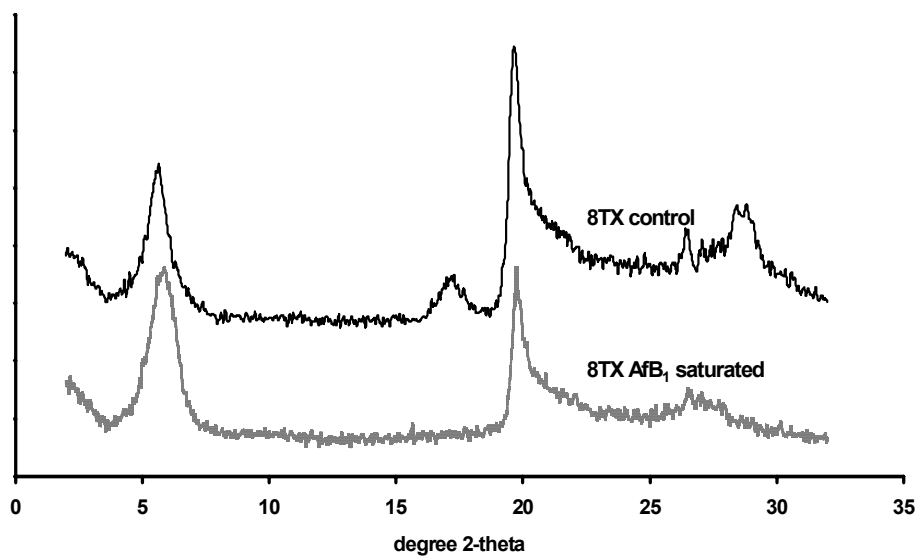
Fig. 9: Peak intensity and disappearance of 3rd order peak with AfB$_1$-saturation for sample 8TX.

3.4.4. Desorption Experiment

In the spectra from the wavelength scans an alteration of the AfB_1 absorbance peaks was observed. Calculated concentrations of desorbed compounds therefore refer to alteration products of AfB_1. In most cases the altered peaks were identified as aflatoxicol but few cases showed peaks that are still unidentified AfB_1-related compounds. The amount desorbed was estimated to be between 17 and 76% of the amount originally adsorbed. Regarding these differences in retention-ability of the smectites, the simplified system we used must be considered and was probably stronger than would be found in an animal's stomach, where the solvent is aqueous. Numerous studies have been carried out by mainly animal scientists, which have shown that toxic effects were effectively inhibited in cows (Harvey et al., 1991b), mink (Bonna et al, 1991), pigs (Schell et al., 1993), broiler chickens (Kubena et al., 1993a) turkey poults (Kubena et al., 1991) and other animals by the inclusion of clays, especially HSCAS. Another factor that might limit the reliablitiy of results obtained for retention-ability of the different smectites is the preceding heat treatment. Yet, the fact that heating induced alteration of sorbed AfB_1 to less toxic molecules like aflatoxicol suggests that AfB_1 reacts with the smectite surface. Doyle et al. (1982) reported that aflatoxin has been found to be quite stable to heat and was not degraded until 250˚C. Thus, what we found might be of interest for future research dealing with degradation of that toxin.

3.5. Conclusions

The commercial offerings of sorbents for aflatoxin include a wide array of clays, organics and mixtures. The smectite clays selected for further investigation had mostly clustered chemical properties with CEC between 68 and 101 $cmol_c$/kg and pH ranging from 7.1 to 8.8. An exceptional clay sample was pH 4.7 and field study indicated that it probably was acidified by sulfide oxidation.

Our sorption data suggest that smectites are generally suitable for AfB_1-sorption. However, a 10-fold difference was observed in the sorption capacity. The cause of these

differences is not well understood although many smectite parameters were measured. Also there are too few first quality sorbents for the vast need on a world scale.

The clustering of sample properties made it difficult to establish their relationships to the sorption capacity. Thus samples need to be investigated with more intensive investigation of individual properties. On the 20 chosen samples more basic data are needed e.g. octahedral composition. Also more unique mineral examples are needed to test functional data.

Consistency of sample sub-groups from sorbent-providing industries with regard to sorption and other characteristic properties can be seen as reliable sources for potential sorbent additives to animal feed. NovaSil® clays that have been used in various in vivo studies and which are named 1 to 4MS in our study were reliable sorbents in vitro, too. Also samples from Idaho and Texas have been shown to be reliable smectites for AfB_1-sorption.

Sorption data generally fit the Langmuir-equation and theoretical maximum adsorption capacities for AfB_1 can be inferred.

The AfB_1-molecule was documented the first time directly to enter into the interlayers of the smectites by means of XRD which is in agreement with previous indirect observations. New types of data are needed on bonding between AfB_1 and smectites.

Upon heating of AfB_1 adsorbed to the smectites it was indicated, that a degradation-reaction took place. This might be of interest for future research dealing with degradation of that toxin.

4. SMECTITE CLAYS AS ADSORBENTS OF AFLATOXIN B$_1$: MINERALOGY AND PARTICLE SIZE

4.1. Overview

Smectite clay has been shown to sorb aflatoxin B$_1$ (AfB$_1$) in animal feed and thereby reduce its toxic influence on animals and its entrance into the human food chain. In a previous study it was shown that AfB$_1$ entered the interlayer galleries of smectites and a 10-fold difference in sorption ability was observed in a set of 20 smectite samples. Yet, it was not clear which clay properties (CEC, pH, base saturation) influenced this variation (section 3).

In an effort to further explore properties that might influence the sorption of AfB$_1$, three effective sorbent samples (8TX, 1MS, 11ID) were chosen from our set of 20 samples along with one sample of low sorption capacity (5OK). These samples were fractionated into sand, silt, coarse clay (CC) and fine clay (FC) fractions. From all sample fractions, sorption isotherms and X-ray diffraction patterns were obtained. Additionally, a vermiculite and a palygorskite were examined with regard to AfB$_1$ sorption capacity and XRD-pattern.

Mineralogy of sand and silt fractions of the sorbent samples consisted mainly of quartz and feldspars, and in one case (8TX) dolomite, whereas coarse and fine clay fractions in all cases were almost exclusively smectite.

As expected, the sorbent samples showed a trend of increased sorption with decreasing particle size from the sand towards the clay fractions. Contrary to expectation, there were still large differences in adsorption capacities between samples in the clay fractions.

Coarse clay showed higher sorption capacity values than fine clay. Palygorskite and vermiculite, both had very low AfB$_1$ sorption capacities when compared to the better smectites.

4.2. Introduction

Aflatoxins are secondary fungal metabolites that contaminate crops worldwide and are a risk to the economical performance of farmers and to humans and animals who consume contaminated food and feed. To remediate the harmful effects of aflatoxin on farm animals, animal scientists have found an effective strategy: upon the addition of smectites in the animals diets at levels as low as 5g/kg almost total protection from aflatoxicosis could be achieved. The adsorption of aflatoxin takes place inside the animal gastrointestinal tracts (Phillips et al., 2002).

The questions as to how and why aflatoxin B_1 molecules are adsorbed to the smectites is of pivotal importance in predicting good adsorbent material to protect animal health in the future and to assign quality labels to industrial aflatoxin sorbent products.

In our previous study we provided strong evidence, by means of x-ray diffraction, that AfB_1 was adsorbed into the interlayer galleries of smectites. Desheng et al. (2005) came to the opposite conclusion of no interlayer adsorption in smectites, but their samples were tested at low AfB_1 saturation. Phillips et al. (2002) supported the hypothesis of interlayer adsorption of AfB_1 with an independent study.

Several theories for selective chemisorption of the AfB_1 by smectite can be found in the literature. Sarr et al. (1991) suggested, based on fourier transform infrared spectroscopy (FTIR) findings, that the β-dicarbonyl of the AfB_1 molecule complexes with incompletely coordinated metal ions of a hydrated sodium calcium aluminosilicate (HSCAS), which they used in their study. Phillips et al. (1995) agreed with this hypothesis for a binding mechanism in that the β-dicarbonyl system is an electron rich system that should readily form complexes with unfilled d-orbitals of transition metals. Computer modeling was used to show that AfB_1 may react at surfaces within the interlayer of HSCAS particles. Phillips (1999) suggests that AfB_1 may react as well on edge and basal surface sites of the HSCAS particles as on the interlayer sites. In 2002, Phillips et al. proposed that a potential chemical binding reaction between smectite surface and AfB_1 molecule may be an electron donor/acceptor (EDA) mechanism.

In addition to the β-dicarbonyl group, Pavão et al., (1995) indicated that the furan ring of AfB_1 is likely to participate in thermodynamically favoured epoxidation and hydroxylation reactions. These reactions are in agreement with Urbanek's findings (1997) when AfB_1 is bonding with DNA. Since they were working in a different adsorption system, the relevance of this type of reaction in the AfB_1-smectite system has yet to be determined.

Although, these adsorption mechanisms have been proposed, there is still a lack of understanding the main characteristics of clay minerals that influence sorption capacity. The 20 smectites that we tested for their adsorption of AfB_1 exhibited a ten-fold difference in adsorption capacity (section 3).

To find possible correlations of sorptive capacity with clay properties, multivariate analysis was performed on a set of characteristics (Lee et al., 2005). The results did not show clear correlations for any individual or combination of properties.

Although sorption mechanisms of AfB_1 on a molecular scale are being discussed and modeled, the smectite properties that make them effective or less effective sorbents are not known. Fröschel et al. (2000) tested 27 bentonites for their AfB_1 sorption and sample properties. They found low correlations between exchangeable cations and cation exchange capacity (CEC) and the AfB_1 sorption capacity, as was observed in our study (section 3).

In an effort to further explore properties that might influence the sorption of AfB_1, three effective sorbent samples (8TX, 1MS, 11ID) and one sample with low sorption capacity (5OK) were chosen from our set of 20 samples (section 3). It was the goal to characterize the effective sorbent smectites (8TX, 1MX, 11ID) and possibly to observe differences (in morphology using transmission electron microscopy (TEM); in their surface binding sites using FTIR, in their particle size distribution using laser diffraction particle size analysis), and to learn more about why these differences in sorption capacity arise.

Another objective of this study was to evaluate the possibility of minor mineral phases present in the smectite sorbent samples that could affect sorption capacity and to characterize the mineralogy of the sorbent samples by particle size fraction.

4.3. Materials and Methods

4.3.1. Materials

Samples 8TX (0.68 mol AfB_1/kg adsorption maximum), 1MS (0.53 mol AfB_1/kg), and 11ID (0.48 mol AfB_1/kg) were selected for fractionation procedure because they qualified previously as good AfB_1 adsorbents. Sample 5OK (0.13 mol AfB_1/kg) represented poor AfB_1 adsorption as determined by sorption isotherm analysis.

In addition, a freeze-dried palygorskite from Florida and a vermiculite from Llano,TX were used for comparative analysis. The vermiculite was machine-ground for 2 min with a mechanical grinder by Ångstrom, Inc., Chicago, IL.

Aflatoxin B_1 from Aspergillus flavus was purchased from Sigma Chemical Co. (St. Luis, MO 63118); CAS No. 1162-65-8. Acetonitrile, Chromasolv® for HPLC, gradient grade was purchased from Sigma-Aldrich; CAS No. 75-05-8. Benzene, GR, CAS 71-56-1, and Methanol, HPLC Grade, CAS 67-56-1, were from EM Sciences. For FTIR analysis KBr for IR-spectroscopy (Fluka) CAS No. 7758-02-3 was used.

Table 7: Some properties of the unfractionated smectite samples. The d-spacings are a result of x-ray-diffraction analysis after different treatments.

Sample	pH	NH₄OAc extractable bases				CEC in NaOAc	Langmuir equation			d-spacing (nm)		
		Ca	Mg	Na	K		Q_{max} (mol/kg)	K_d	R^2	as received	K saturated	K saturated at 550° C
		(cmol$_c$/kg)										
5 OK	7.3	78.5	23.4	1.1	0.4	101.3	0.13	38432	0.89	1.524	1.187	0.999
11 ID	7.4	56.6	15.6	9.2	0.7	76.4	0.48	415454	0.97	1.511	1.112	0.994
1 MS	8.3	94.1	9.4	0.4	1.5	85.8	0.53	633352	0.95	1.381	1.150	1.022
8 TX	7.1	54.6	21.8	8.3	1.0	84.1	0.68	295551	0.98	1.392	1.126	0.988

4.3.2. Fractionation and Laser Diffraction Particle Size Analysis

Before the samples were fractionated they were pretreated to remove carbonates, organic matter (OM) and MnO_2. For each of the 4 samples, 20 g subsample of the bulk material was used. Carbonate was removed by treating with 1 M NaOAc (pH 5) buffer solution while maintaining the temperature at 70˚C in a waterbath. For OM and MnO_2 removal, samples were treated with H_2O_2. To disperse samples, they were saturated with sodium using pH 10 Na_2CO_3 (Kunze and Dixon, 1986).

The sand fraction was obtained by sieving the samples through a 53 mm sieve. The silt and clay fractions were seperated gravimetrically via centrifugation according to Stokes law (White and Dixon, 2003). Silt and coarse and fine clay fractions were oven died from suspension in water at 105°C.

In addition to the fractionation procedures, bulk samples were analyzed for particle size with a laser diffraction particle-size analyser (LS13320, Beckman Coulter). This instrument uses laser diffraction technology and multi-wavelength light scattering to determine particle size distribution in a single analysis by virtue of binocular optics. To prepare the samples for analysis 1 g of bulk material was added to 100 ml of pH 9.5 $NaCO_3$ solution to obtain a suspension with 1% bentonite. Each sample was washed twice with $NaCO_3$ solution to enhance dispersion. The samples were shaken overnight, and finally dispersed in an ultrasonic bath for 30 minutes.

4.3.3. X-Ray Diffraction (XRD)

Slides with oriented particles were prepared for x-ray analysis of the silt, coarse, and fine clay fractions by drying a sample from suspension in water on VICOR® glass slides. The sand fractions were x-rayed on sample holders with quartz windows. XRD patterns were obtained using a Philips x-ray-diffractometer with CuK_{α}-radiation, a graphite monochromator and a theta-compensation slit. Each pattern was measured in 0.05 º2θ intervals from 2 to 64 º2θ to observe the first and higher order smectite peaks and possibly other mineral phases (White and Dixon, 2003).

The d-spacings were calculated according to Braggs law:

(1) $\qquad n\lambda = 2d\sin\theta$

with n being an integer, λ the wavelength of the CuK$_\alpha$-radiation, d the repeated distances of the crystal planes (of the smectite) and θ the angle between the incident x-ray beam and the scattering planes (Moore and Reynolds, 1997).

4.3.4. Isothermal Adsorption Procedure

To 5 mL of AfB$_1$-solution with the concentrations 0.0, 0.4, 1.6, 3.2, 4.8, 6.4 and 8.0 mg/L, 0.1 mg of smectite was added. The amount of adsorbed AfB$_1$ was determined after 24 hours of shaking, using a UV/visible spectrophotometer. Data were fitted to the Langmuir equation. The detailed desciption of the method used can be found in section 3.

4.3.5. Use of the Langmuir Equation

To fit experimental data to the Langmuir equation,

(2) $\qquad q = (Q_{max}k_dC_{eq})/ (1+k_dC_{eq})$

it was transformed into its linear form,

(3) $\qquad (C_{eq}/q) = (1/k_dQ_{max}) + (C_{eq}/Q_{max})$

where C_{eq} is the concentration in equilibrium (mol/L), q is the amount AfB$_1$ adsorbed (mol/kg), k_d a Langmuir coefficient (L/kg), and Q_{max} the maximum sorption capacity (mol/kg). In the Langmuir equation (2) the k_d influences the initial slope of the curve and the Q_{max} the height at which the curve converges to its plateau.

To determine Q_{max} and k_d, first q (mol/kg) was plotted against C_{eq} (mol/L) to observe if the adsorption data obtained for the sample resembles the shape of a Langmuir isotherm. For equation (3), a plot of C_{eq}/q (g/L) on the y-axis and q (mol/L) on the x-axis will yield a straight line. The slope of the line is equal to $1/Q_{max}$. When the value for the y-intercept is multiplied by Q_{max} and the reciprocal of the product value is taken, k_d is determined (Essington, 2004).

4.3.6. Transmission Electron Microscopy (TEM)

For TEM analysis, the coarse clay fractions of samples 8TX, 1MS, 11ID and 5OK were used. Before drying the clay fractions in the fractionation step (4.3.2.1.) one drop of the clay water suspension was saved in a small glass container and kept in the refrigerator at 2ºC. To mount the clay on a Formvar® carbon-coated copper grid, first water was added to the sample in the glass vessel until the suspension appeared almost completely transparent. Then, one drop was taken out and transferred onto the grid and dried under a heat lamp.

The TEM used was a JEOL 2010 (JEOL Ltd., Tokyo, Japan). For each sample pictures were taken from 4 locations on the grid. Selected area electron diffraction (SAED) and energy dispersive x-ray spectra (EDS) of coarse and fine clay fractions were obtained whenever possible. Locations that were too close to the copper grid of the sample holder gave disturbed results thus precluding SAED and EDS data acquisition. To obtain quantitative estimates values of the obtained 3 to 4 EDS pattern were averaged per sample.

4.3.7. Fourier Transform Infrared Analysis (FTIR)

Six mg of each of the bulk unfractionated samples 8TX, 1MS, 11ID and 5OK were shaken with 6 ml water-acetonitrile over night (for another experiment 50 mg/kg AfB_1 solution in water-acetonitrile was used). The suspension was centrifuged for 20 min at 9800 m/s^2 and samples were dried onto a glass slide at room temperature. After being dried they were removed by scraping.

This resulting sample was mixed with 1 g of KBr using a Wig-L-Bug (Crescent Dental Mfg. Co., Chicago, Illinois), and the mixture was transferred to a glass vial and stored in a desiccator. This material was then used for the FTIR-analysis.

The FTIR method used was by diffuse reflectance (White and Dixon, 2003).

4.4. Results

4.4.1. Conventional Fractionation and Laser Diffraction Particle Size Analysis

Table 8a summarizes the distribution of sample fractions. On average, more than 20% of the starting material was lost during the fractionation process, ranging from 16% for sample 5OK to 31% loss of starting material for 11ID. Except for sample 1MS, all samples consisted of 60% or more coarse and fine clay based on normalized values. Sample 1MS had 40% silt.

Table 8a: Results of gravimetric fractionation: normalized percent weight.

Fraction / Sample	8TX	1MS	11ID	5OK
		wt%		
sand	3.6	18.6	2.4	13.0
silt	13.7	40.5	18.8	26.4
coarse clay	38.7	27.8	47.2	40.2
fine clay	44.0	13.1	31.6	20.4

Table 8b: Percent volume for fractions according to LDPSA. In brackets: particle size range chosen arbitrarily according to geometry of distribution peaks (Fig. 11).

Fraction / Sample	8TX	(8TX)	1MS	(1MS)	11ID	(11ID)	5OK	(5OK)
				vol%				
sand (>50µm)	0.0	0.0	8.2	8.2	5.3	5.2	0.0	0.0
silt (14-50 µm)	96.2	13.0	90.3	16.2	92.8	35.7	98.1	30.0
coarse clay (4-14 µm)	3.8	51.5	1.5	40.9	1.9	36.9	1.9	48.7
fine clay (<4 µm)	0.0	35.5	0.0	34.7	0.0	22.2	0.0	21.3

The results obtained with the laser diffraction particle size analyzer (LDPSA) deviated from the results from the conventional fractionation method (Table 8b). Particles that fall in the sand fraction (50-2000µm) were only detected for sample 1MS and 11ID. Surprisingly, CC and FC clay fractions <2µm as detected with the instrument make up only minor parts of the samples. According to the data, samples consist mainly of silt size particles (2-50µm). The mean particle diameters range from 7.2 (8TX) to 15.6µm (11ID).

For quantitative comparison percentage of particles <10μm were determined. With increasing percentage of particles below that limit sorption tends to be higher (Figure 10).

The shapes of the particle size distributions are not unimodal but show different numbers of distinct peaks (Fig. 11). No sample has one clear maximum. Instead, all samples have distribution maxima around 2.5, 6 and 18 μm. Samples 8TX and 1MS are right-skewed while distributions of 11ID and 5OK are more symmetric. When setting arbitrary particle size limits to correspond to sand, silt and clay fractions orienting at the geometry of the distribution maxima, these values resembled more closely to the percentage of fractions that were observed in the conventional fractionation procedure (Table 8b).
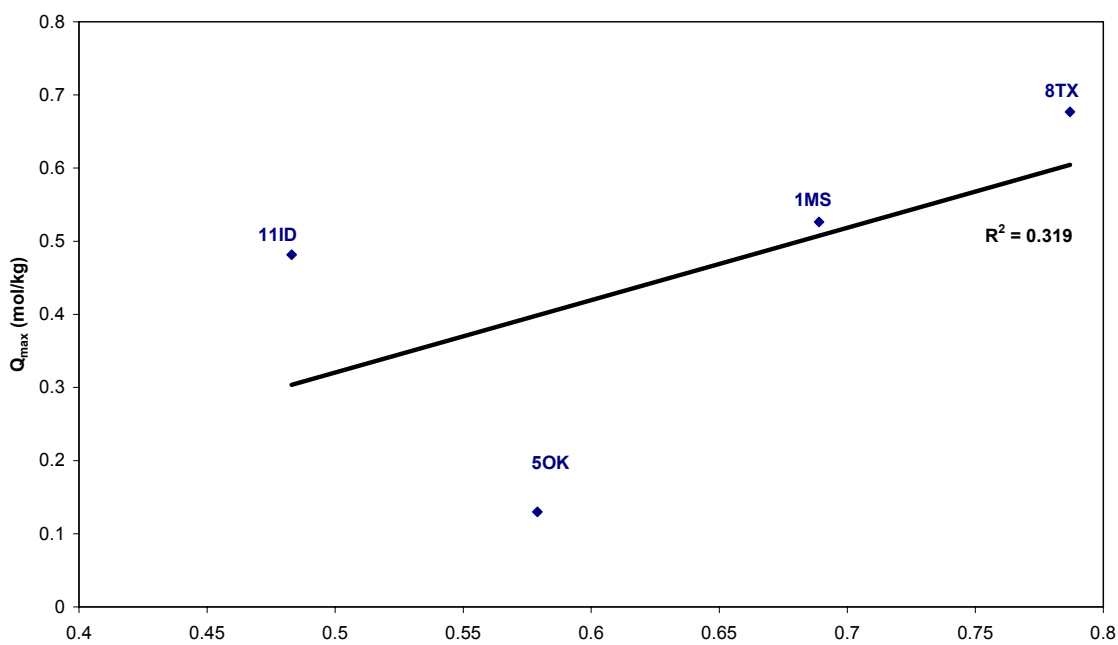
Fig. 10: Fraction of particles <10μm versus Langmuir maximum adsorption capacity (Q_{max}).
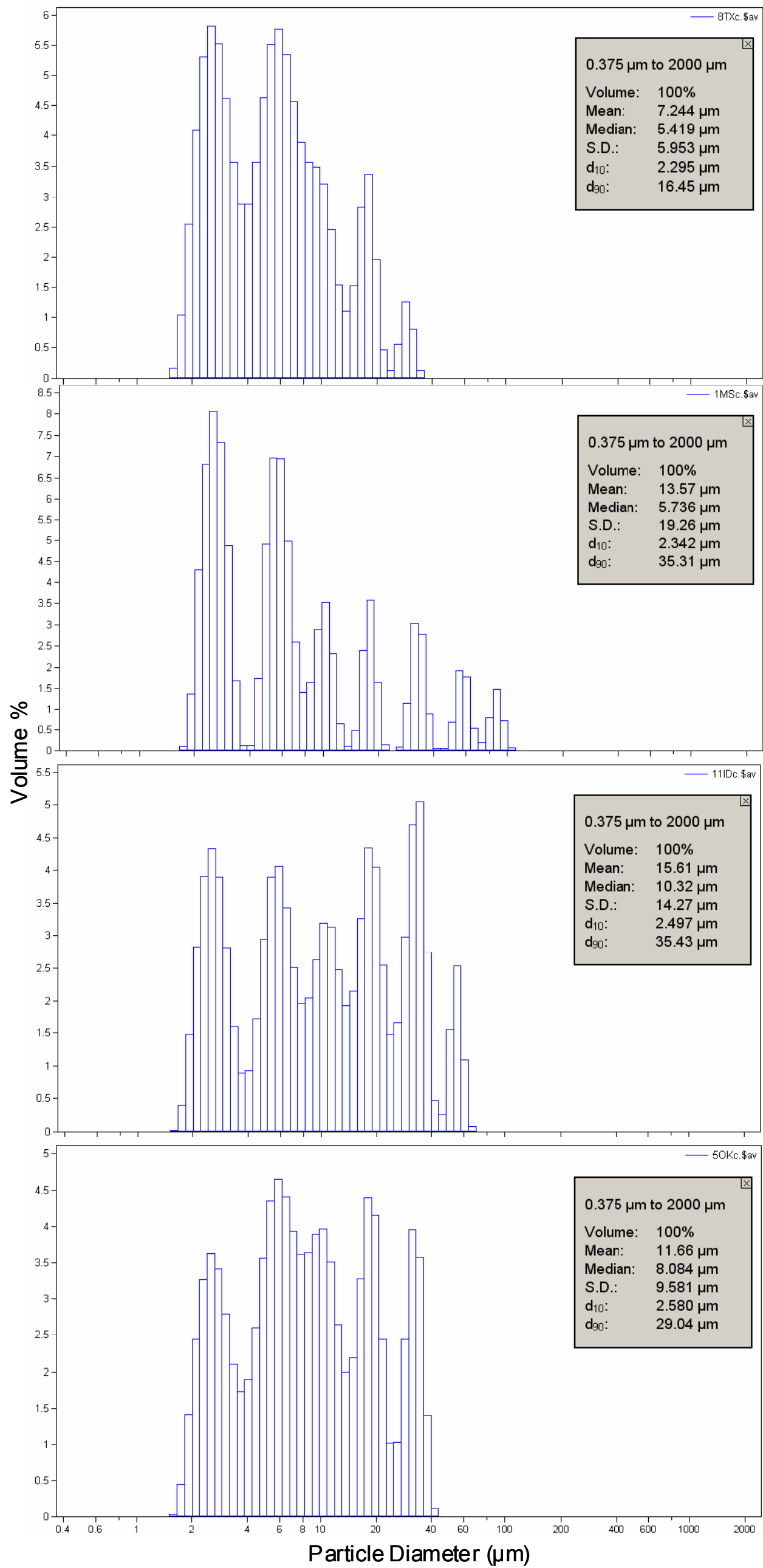
Fig. 11: Particle size distribution histograms of samples 8TX, 1MS, 11ID and 5OK (top to bottom) from laser diffraction particle size analyzer

4.4.2. XRD

In the XRD patterns the major diversity in mineralogy existed in the sand fraction of the samples. Minerals in the sand fraction were in all samples mainly quartz and feldspars (plagioclase, anorthite). Sample 8TX contains dolomite and probably quartz. The quartz peak indicated for 8TX in Figure 12 shares the same d-spacing (0.334 nm) as dolomite and seems to lack the intensity typical for quartz. In sample 1MS, small amounts of goethite were present, which is expressed also in the yellowish color of the sample material. In the sand fraction no smectite peaks were present or they were only weakly expressed as for example in sample 8TX.

Silt XRD patterns showed smectite and quartz peaks. Sample 11ID had a smectite peak of same relative intensity as the quartz peak, and for 1MS and 5OK samples quartz was the strongest XRD peak.

All CC and FC fractions almost exclusively consist of first and fourth order smectite peaks. Second and third order peaks of smectites are only weakly expressed. In those fractions no other mineral peaks were visible (Figure 12).

4.4.3. Isotherms

Isotherms consistently showed highest AfB_1-adsorption per unit mass for the clay fractions and lower adsorption for silt and sand fractions (Fig. 13 and 14). For sample 11ID, coarse and fine clay fractions show almost identical isotherms, while for the rest of the samples the coarse clay fraction appears to be a more effective adsorbent (Table 9). The adsorption observed for sample 5OK was exceedingly low and below 0.05 mol/kg for its strongest sorbing fraction, the CC. For sample 11ID clay fractions adsorbed well (both Q_{max}: 0.23 mol/kg), but adsorbed equally low amounts of AfB_1 adsorption in sand and silt fractions. Sample 8TX showed good adsorbent in all fractions (Q_{max} of CC: 0.36 mol/kg). Sample 1MS behaved similarly but with high adsorption in clay fractions (FC: 0.27 mol/kg, CC: 0.33 mol/kg) and lower adsorption in silt and sand. Comparison shows that samples 1MS and 8TX are the most effective AfB_1-adsorbents,
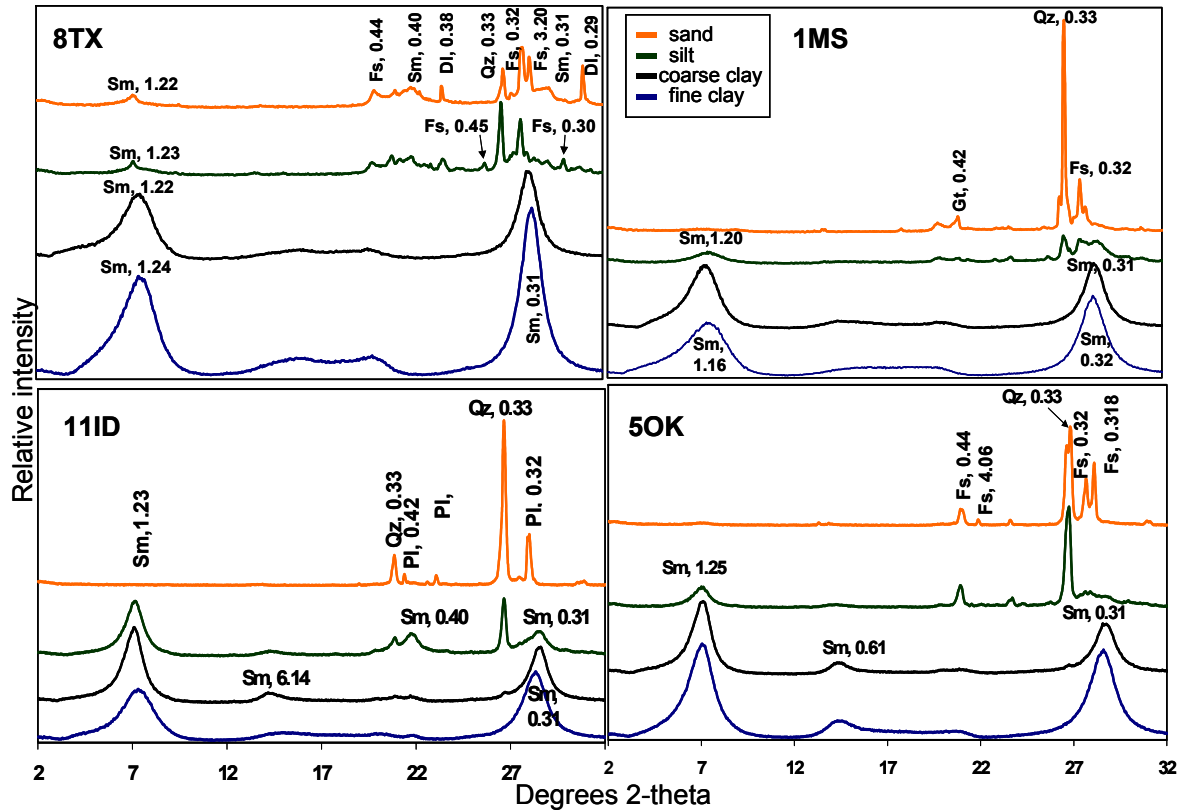
Fig. 12: XRD pattern of sand, silt, coarse clay and fine clay fractions of samples 8TX, 1MS, 11ID and 5OK in the range from 2 to 32 degree 2-theta. Sm = Smectite, Qz = Quartz, Fs = Feldspar, Dl = Dolomite, Gt = Goethite, Pl = Plagioclase. d-spacings in nm. Goethite in 1MS causes the yellow color of that sample.

followed by sample 11ID. This observation resembles observations made in the bulk samples (section 3) with the difference, that the adsorption values after fractionation were lower.

Good fit to the Langmuir equation was not consistently observed for all fractions, but was relatively good in the clay fractions. Figure 14 gives an example of the fitting of experimental data to the Langmuir curve. Adsorption capacities, Q_{max}, for the effective sorbent samples 8TX, 1MS and 11ID were slightly (11ID) to clearly (8TX) higher in the CC than in FC (Table 9). The Q_{max} and k_d values were decreased in the fractions compared to the untreated samples (Table 9).

The palygorskite and vermiculite that were examined along with the samples, had almost zero adsorption capacity.
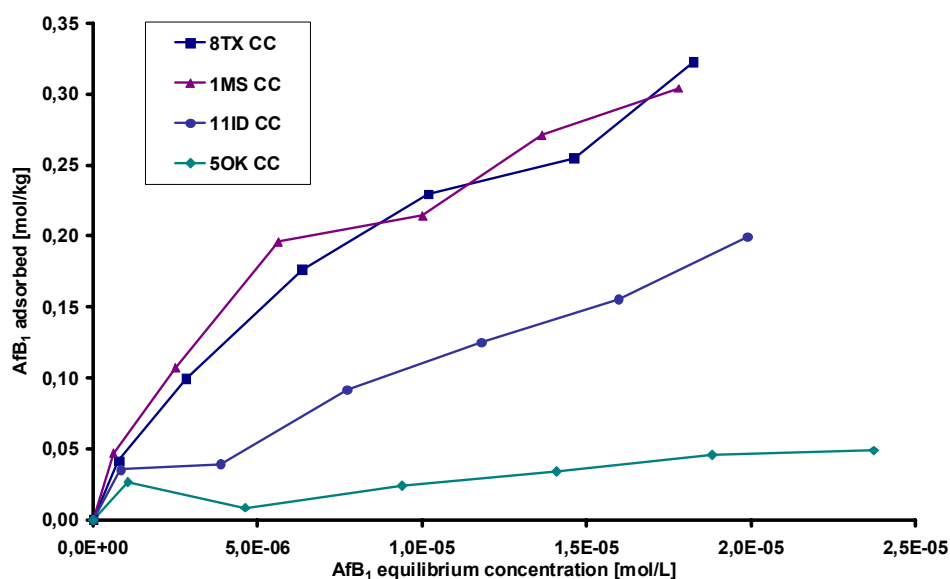
Fig. 13: Adsorption isotherms of coarse clay fractions. Langmuir fitting was not consistently applicable. Fit to straight line yielded better fit than Langmuir model.
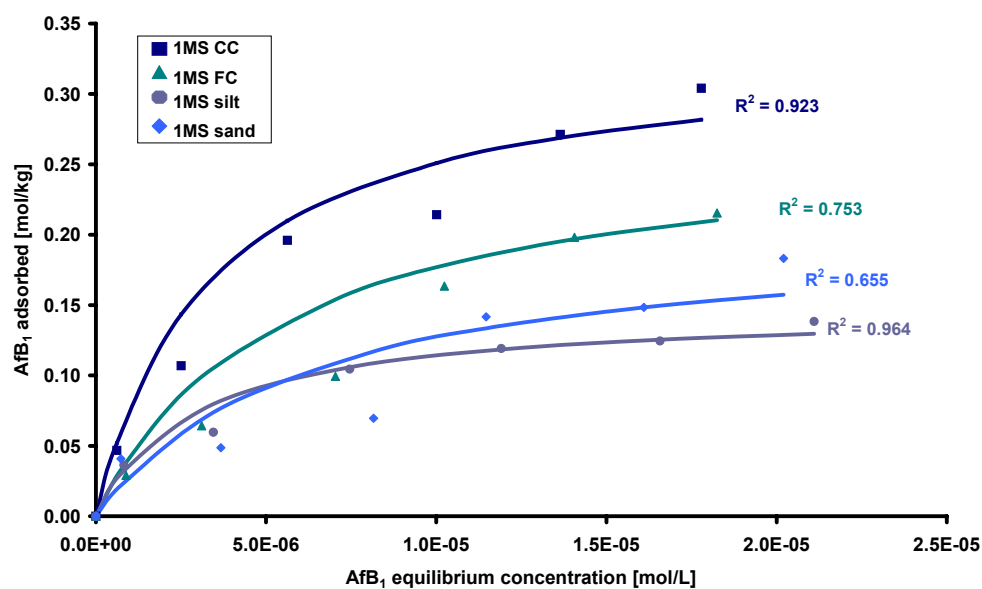
Fig. 14: Example of fitting of Langmuir curves to adsorption data of all fractions for sample 1MS.

Table 9: Langmuir values Q_{max} and k_d and the R^2 values of fitting to Langmuir equation and line. Langmuir fitting was not appropriate for sample 5OK.

Sample	Q_{max} (mol/kg)	k_d (L/kg)	Langmuir R^2	Linear R^2
8TX FC	0.235	1.42E+05	0.810	0.894
8TX CC	0.364	2.74E+05	0.868	0.902
8TX bulk	0.677	2.96E+05	0.898	0.776
1MS FC	0.273	1.83E+05	0.753	0.981
1MS CC	0.335	2.99E+05	0.923	0.909
1MS bulk	0.526	6.33E+05	0.943	0.705
11ID FC	0.227	1.47E+05	0.674	0.947
11ID CC	0.238	1.05E+05	0.580	0.757
11ID bulk	0.481	4.15E+05	0.916	0.498

4.4.4. TEM

TEM and SAED were used to observe the morphology and structure of the samples in the fine and coarse clay fractions. In Figure 15a and b, selected TEM and SAED patterns are displayed. Overall, morphologies appeared to be that of typical smectites for all samples, with many small thin particles in the viewing field that were often not very distinct from the surrounding. In the SAED pattern, diffuse rings were observed for all samples, which is caused by the turbostratic structure of smectites (Mering and Oberlin, 1971). This means that the layers of the smectites are disordered in the (a,b) plane with respect to the a and b crystallographic axis but not to the c axis (Elsass, 2006). Depending on degree of stacking disorder, the particles of the samples observed had more or less diffuse rings, with a tendency for 1MS and 11ID to have sharper rings and of 8TX and 5OK to have more diffuse rings.

The micrographs for 8TX and 1MS in Figure 15a are characterized by overall complex shapes, appearing like glass shards, fibers or cloudy diffuse material. Rolling of particles edges is predominant in sample 8TX. The corresponding SAED shows diffuse rings, and no sharp rings or spot pattern were observed.

The images of sample 1MS show mainly thin particles that were frequently folded. In the SAED pattern, sharp rings indicating higher order within a-b crystal plane is shown, which was common for this sample. In addition to the crisp edges as shown here, the specimen also contained some rather diffuse particles. Such amorphous features indicated evolution from residual glass as occurring in volcanic ash deposits (Grim and Güven, 1978).

The micrographs of 11ID showed several different features. The SAED pattern of a thick particle (15b E) shows a spots pattern suggesting mica with also diffuse rings present, possibly indicating illitic material. Smaller particles than the one shown in Figure 15b E prevail in the sample. Diffuse edges in material (Fig. 15b E and b F) is mixed with more cloudy smectites (Fig. 15b F). The SAED pattern of Figure 15b F taken from the small hexagonal particle in the center of the image showed several rings indicating smectite. Another location in the sample, not displayed here, showed sharper

rings than typical for smectite, and may indicate the presence of more highly ordered phyllosilicate phases. In both images of 11ID, some rolling of edges can be observed. The mixture of materials indicates a natural origin of the sample.

The images of sample 5OK show typical smectite with small and thin particles (Fig. 15b G and b H). Rounded edges and the absence of crisp detail may be a sign of weathering. The SAED pattern has weak diffuse rings suggesting poor crystallinity. The EDS data on this sample indicate that all four observed locations on the sample contain no or only very little iron (Table 10).

The chemistry of the coarse clay fraction of each sample was averaged over the 3-4 EDS analysis obtained and were mostly typical for smectite with about 24 atomic% of the structural cations being aluminium ions and 67 atomic% being silicon ions. Alkali or

Table 10: EDS data corresponding to images in Figures 15a and b. No EDS data could be obtained where the specimen was located too close to the sample holder grid.

Element	A) 8TX	B) 8TX	C) 1MS	D) 1MS	E) 11ID	F) 11ID	G) 5OK	H) 5OK
				atomic %				
Si		61.96	68.89	63.68	58.4	65.41	62.92	77.84
Al		24.79	26.68	24.69	25.37	18.41	25.25	22.16
Mg	no EDS data	3.14	/	4.97	/	/	11.48	/
Ca		/	/	1.2	/	/	/	/
Na		3.83	/	1.73	/	/	/	/
Fe		6.28	4.44	3.72	8.05	16.23	/	/
K		/	/	/	8.18	/	/	/
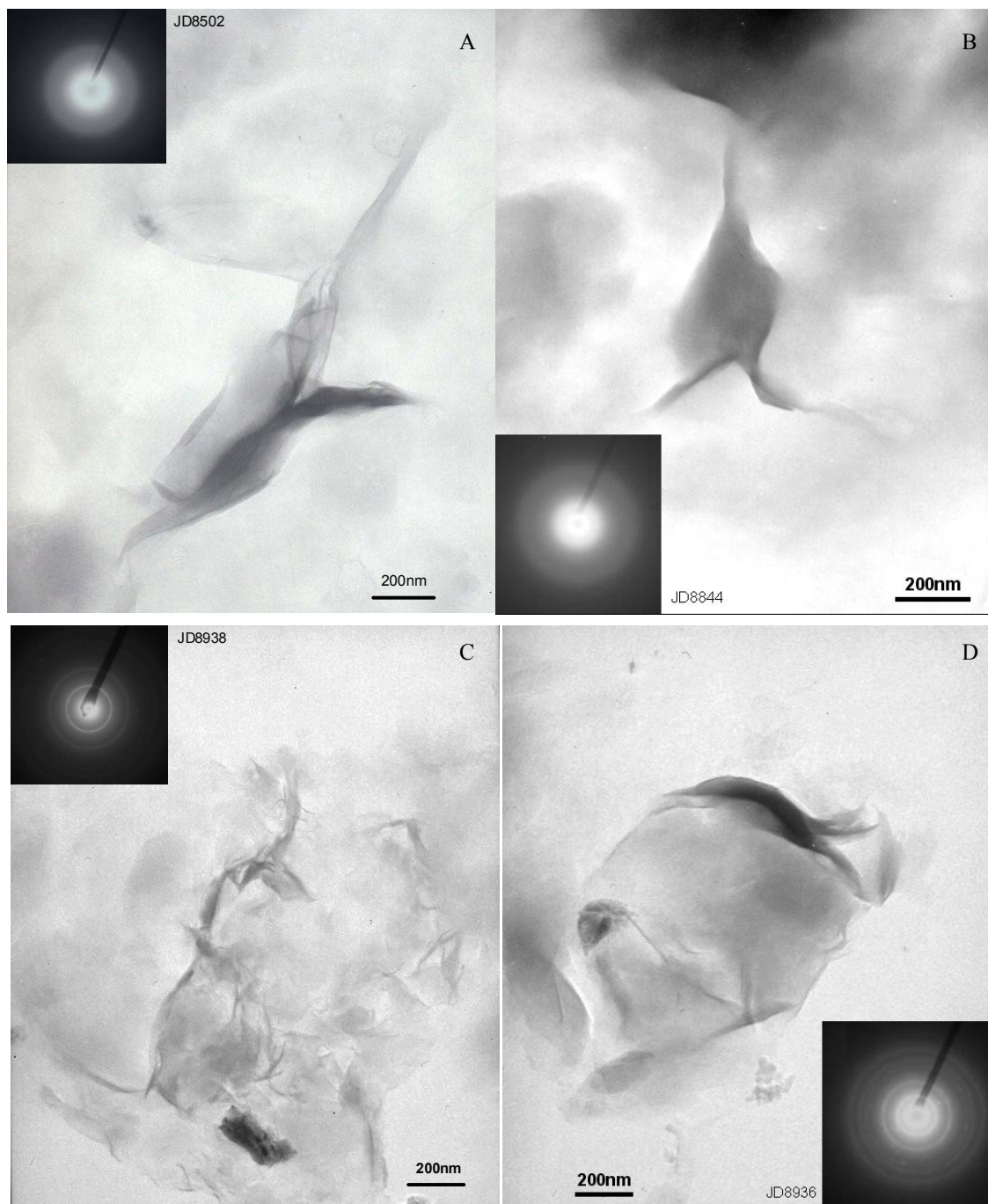
Fig. 15a: TEM micrographs and SAED pattern of samples 8TX (A, B) and 1MS (C, D).
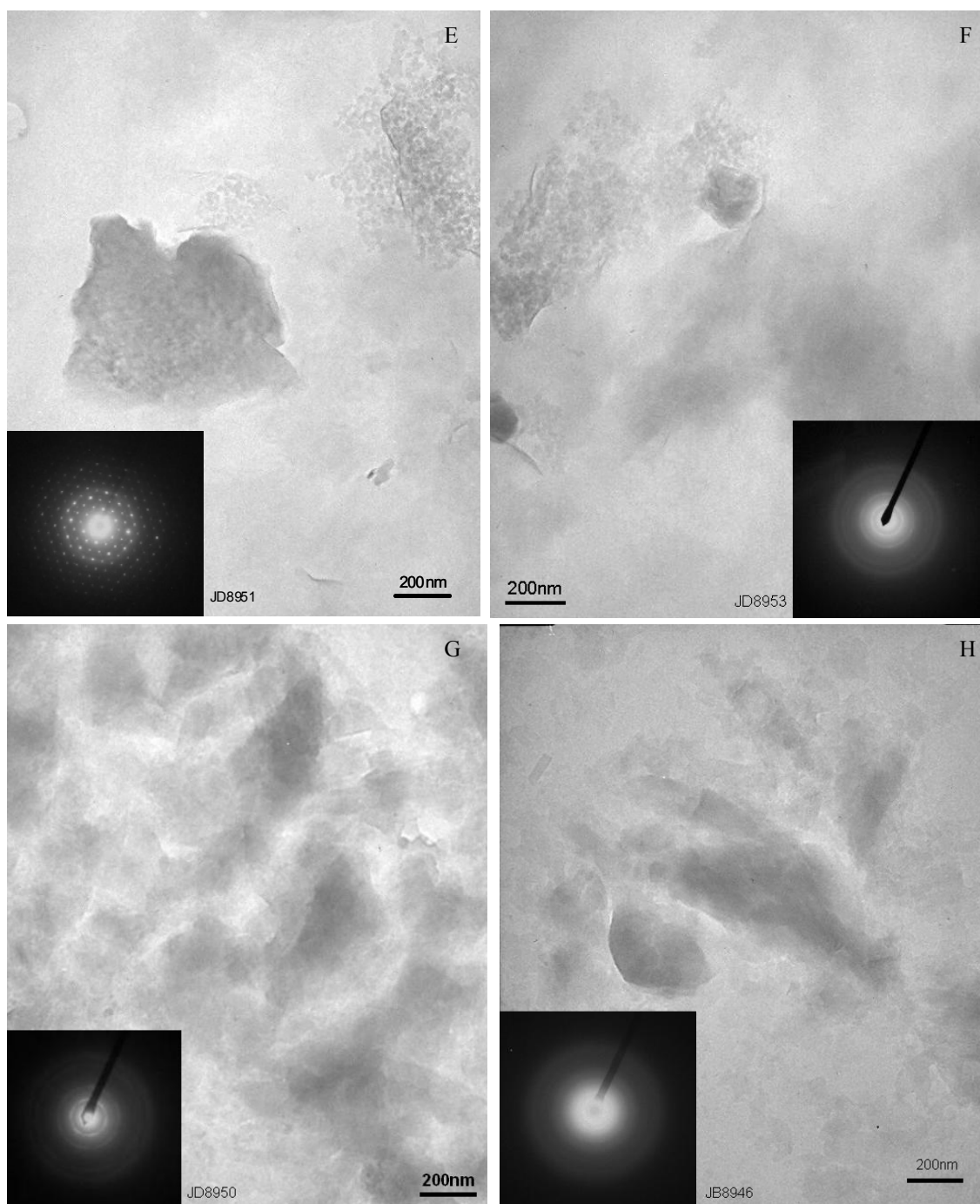
Fig. 15b: TEM micrographs and SAED pattern of samples 11ID (E, F) and 5OK (G, H).

alkaline earth cations furnished the remaining cations within the structures. All samples contained structural iron, on average 4.1%, with sample 5OK having the lowest amount of structural iron (1.1 atomic %). Sample 11ID contained large amounts of iron (8.1 atomic%) and potassium, aluminium and silicon as the only other elements, suggesting that the 3.4% K^+ are the major interlayer cation and maybe include illitic material.

4.4.5. FTIR

The FTIR patterns obtained for the four untreated samples show the same absorption bands around wavenumbers 913, 840 and $795cm^{-1}$, which represent OH-bending bands for octahedral aluminium, octahedral magnesium and aluminium, and the SiO_2-streching band of amorphous silica (Farmer, 1974; Gates, 2004; Madejová and Komadel, 2004). The amorphous silica band is less distinct for samples 1MS and 5OK. At $624cm^{-1}$ all samples display a shoulder or a slight band indicating the presence of tetrahedral aluminium. Additional to these bands, FTIR pattern of samples 8TX, 1MS and 11ID show a OH-bending absorption at $884cm^{-1}$, indicating the presence of octahedral iron, which is missing completely in sample 5OK (Figure 16). This finding correlates well with the results obtained from the EDS obtained during TEM analysis, in which sample 5OK contained the lowest amount of iron. The three good AfB_1-adsorbents contained octahedral iron. This observation was supported by a survey of the 16 other smectite samples in which samples 16MX and 7AZ, which had low AfB_1-sorption capacity, lacked the expression of AlFeOH-bending bands. Additionally, the three low AfB_1-sorbing samples had only a weak to no expression of amorphous SiO_2-stretching bands.
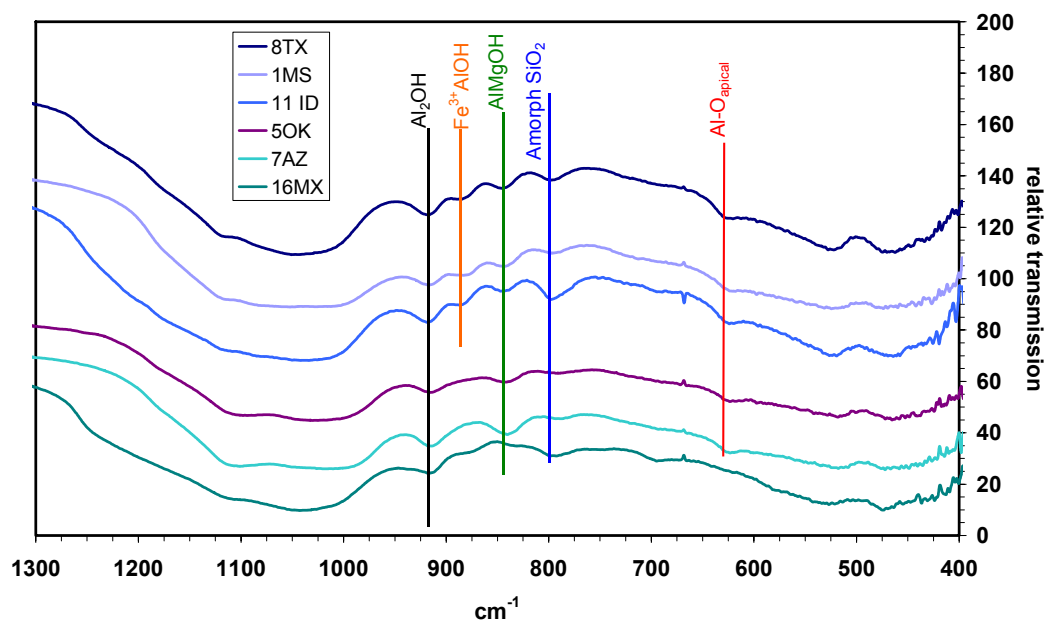
Fig. 16: FTIR pattern of bulk samples with similar octahedral cations. Sample 5OK, 7AZ and 16MX (additional data from Tenorio Arvide et al., 2006) are not good AfB$_1$ adsorbents and show no or only little octahedral iron.

4.5 Discussion

The loss of sample material during the conventional fractionation procedure can partly be assumed to result from the carbonate removal. This explanation is supported by XRD data of whole materials in which all samples exhibited calcite peaks. Sample 8TX exhibited dolomite peaks in XRD of the sand fraction even after the pre-treatment, which gave an indication of the presence of carbonates for that sample.

The fractionation procedure included steps to remove binding agents from the samples so the clay fractions could be dispersed more easily. Na_2CO_3 was employed as a dispersion agent in order to use the creation of a high degree of hydratation by the hydrated Na ions and the development of negative charges on the particle edges for repellent action (Jackson, 1985). The sodium saturation and drying of the smectites during the fractionation treatments seemed to reduce the sorption capacities of fractions since their adsorption capacities for AfB_1 were consistently lower in the fractions than in the bulk untreated samples (Table 9). Probable causes are the dispersion of the smectite particles to thin crystallites, and decrease in stacking order and thickness, leading to the reduction in sorption of AfB_1. Differences in interlayer cation might also have contributed to differences in adsorption behaviour.

The relatively large portion of the sand (Table 8) can mostly be explained by the presence of coarse well-crystallized minerals indicated by the narrow XRD peaks and by a small amount of undispersed sand size smectite aggregates in sample 8TX. The silt fractions contain prismatic minerals and undispersed smectite; the silt-size smectite is most abundant in the 11ID sample. Thus, the dispersion procedure was not successful to completely remove smectite from the silt and sand fractions. It also reduced the adsorption capacity of the smectites. The drying from aqueous suspension during the fractionation procedure may have contributed to aggregation and loss of colloidal properties. The concentration of smectites in the CC and FC fractions by the conventional fractionation did not enhance their sorption capacity but the treatment artificially reduced AfB_1 sorption capacities of the natural smectites.

Particle-size distribution using LDPSA might help identifying potentially good AfB_1 adsorbents when comparing their amounts of particles <10μm (Figure 10 and 11). As indicated by results summarized in Table 8b, more testing and calibrations might help make this an effective tool for sorbent sample analysis in the future, but an appropriate methodology was not yet developed at this stage.

Since 5OK has different properties (higher CEC, low amount of structural Fe) compared to the more efficient adsorbents it might be considered an outlier in Figure 12. A low amount of small particles in sample 5OK suggests greater depletion by weathering of the finer particles of smectite (Jackson, 1948). Its exclusion would enhance the linear correlation.

The difference in data obtained from fractionation and LDPSA is not yet understood and probably due to problems with incomplete or differing degrees of dispersion. The more invasive sample pretreatment used in the fractionation procedure may have lead to an overall more complete dispersion of the samples. Carbonates, that may function as flocculating agents (Kunze and Dixon, 1986) that were apparently completely removed in the fractionation prcedure in contrast to the pretreatment for the LDPSA in which samples were only Na-saturated, shaken and treated in an ultrasonic bath for dispersion. During the sample preparations it was extremely difficult to disperse 5OK and that it settled out easily.

Adsorption capacities, Q_{max}, for the effective sorbent samples 8TX, 1MS and 11ID were slightly (11ID) to clearly (8TX) higher in the CC than in FC fractions (Table 9). This observation seems to indicate that the order and extent of stacking of the smectites is of importance to the adsorption process and also emphasizes that the main adsorption depends upon the availability of intact interlayers. According to these implications, edge sites and outer surface sites, which are more abundant in the fine clay fraction, might play a less important role in AfB_1 adsorption. On the other hand the C-type isotherms of the clay fractions have a linear shape and have a better fit to a straight line than to the Langmuir equation (Fig. 13). Essington (2004) described this adsorption behaviour of linear partitioning as common for nonpolar or hydrophobic organic compounds,

indicating that the Freundlich model may be a more appropriate model. Nonetheless, the Langmuir function is useful to obtain comparative values for maximum adsorption.

Palygorskite is a Si- and Mg-rich fibrous clay mineral with low CEC and large specific surface area, which has been reported to adsorb nonpolar organic compounds (Singer, 2002). They are in general known as useful adsorbents for different purposes (e.g. decolorizing agent, animal bedding; Grim, 1962), yet were not effective for AfB_1 adsorption. Assuming that for AfB_1 sorption the interlayer region is of pivotal importance, the low sorption by the palygorskite structure might be expected, since they do not possess continuous interlayers but instead rectangular channels that lie between the backs of opposing 2:1 ribbons.

The structures of vermiculite and smectites resemble each other closely, and they have the same type of edge and outer-surface sites. The main difference lies in the higher layer charge of vermiculites of 0.6 to 0.9 per formula unit, which is balanced by hydrated exchangeable cations (Malla, 2002). Adsorption of AfB_1 is assumed to be inhibited by the higher layer charge of vermiculite. This gives another hint that the interlayer region is of pivotal importance to the adsorption of AfB_1. The illitic characteristics of components of the CC and FC fraction of sample 11ID (figure 15E and F) indicate that the higher layer charge associated with illite compared to smectite might have reduced the sorption ability of that sample by steric hindrance due to less opening of interlayer spaces compared to the overall more highly smectitic samples 8TX and 1MS. Layer charge seems to be one of the mineralogical factors influencing adsorption.

Contrary to these findings, suggesting predominance of interlayer adsorption over edge site and outer surface adsorption, Phillips (1999) suggested multiple sites for reaction based on studies using Langmuir, Freundlich, Toth and modified equations.

Differing mineralogy of the associated minerals in the samples as indicated by XRD patterns (Figure 12) of their sand and silt fractions indicate, that these smectites were formed under different environmental conditions (Galán, 2006). They can form in soils, as weathering crusts, in oceans and through hydrothermal activity. Most commercial

bentonites form mainly by alteration of volcanic ash deposits (Senkayi et al., 1984) or authigenically in alkaline continental basins (Grim and Güven, 1978; Borchart, 1989; Galán, 2006). The presence of dolomite in sample 8TX as well as calcite suggests formation from volcanic ash in shallow marine environments, which is common for bentonites. The presence of dolomite in sample 8TX only may indicate environmental conditions of formation leading to mineralogical properties that are responsible for its outstanding AfB_1 adsorption capacity.

One hypothesis was that with the pure smectite phase the sorption potentials for AfB_1 between samples should be similar since no other phases dilute the sorbent material. Adsorption isotherms for each fraction showed that this assumption was not justified and suggest that differences in smectite properties, not that of other mineral phases that dilute the smectite, can influence their relative AfB_1 adsorption capacities.

Electron-optical examination of the samples gave information on sample structural properties and an indication of sample evolution. Sample 8TX and 1MS both showed frequently folded particles (Figure 15 A-D), as well as rather diffuse particles which were common with all four sample. These amorphous features indicate evolution from volcanic ash deposits, as from particles resembling broken glass in sample 11ID (Figure 15E and F). Sample 11ID contained well crysatllized illitic material as indicated by the spot patterns and the presence of potassium according to EDS data. The use of EDS facilitated important observations of the chemical composition regarding the samples, and showed clear differences between materials (Table 10). Samples 8TX and 1MS both displayed a variety in alkali and alkaline earth metals, besides silicon and aluminium. Except for sample 5OK, which was a poor AfB_1 adsorbent, all samples contained iron in their layer silicate structures. Observations with FTIR on an extended population of poor AfB_1 -sorbing smectites (Tenorio Arvide et al., 2006) showed a lack of the AlFeOH-bending bands and only a weak to negligible expression of amorphous SiO_2-stretching bands. This might indicate that AfB_1 participates in a specific bonding mechanism with the smectite, possibly forming complexes with iron or other metals. The depletion in iron and silica are characteristic of advanced weathering of those minerals (Kittrick, 1986;

McBride, 1995). For smectites containing no iron, deep-sea hydrothermal alteration is another possible path of formation but they must be Mg-free as well (Galán, 2006). Grim and Güven (1978) describe that loss of alkali metals and downward migration of silica can occur during alteration of parent material (volcanic ash), which can be accompanied by leaching. Accordingly, the low AfB_1-sorbing samples were highly weathered bentonites from which silica and iron had been leached. Although aluminous smectites with low Fe content are less frequent than the Fe and Mg containing smectites with favourable sorbent properties, they occur worldwide, e.g. in many states of the USA, Mexico and France (Ross and Hendricks, 1945; Grim and Güven, 1978). Although they are uncommon they are widely distributed and deserve recognition in looking at potential sorbent smectites on a world scale. More AfB_1 sorption investigations are needed on a larger set of samples that include aluminous smectites.

4.6. Conclusions

Concentration of smectite by separation of particle-size fractions was ineffective in improving the AfB_1-adsorption capacity. Incomplete dispersion occurred. It is an ill advised procedure for sorption investigations because it disperses the thin crystallites, increases stacking disorder, and reduces sorption of AfB_1.

Smectites that did not contain Fe in the structure tended to be poorer sorbents of AfB_1. Such smectites are widely distributed throughout the world, yet they are less common than the Fe and Mg containing smectites with more favorable sorbent properties.

Coarse clay smectite sorbed more AfB_1 than fine clay smectite, yet other factors such as stacking order also influence, which complicates the influence of particle size.

Vermiculite and palygorskite were not effective as sorbents of AfB_1.

5. SUMMARY AND CONCLUSIONS

Several chemical and mineralogical properties of twenty smectite clays have been found to influence their sequestration of aflatoxin molecules. AfB_1 was demonstrated to be adsorbed to the interlayer region of smectites. Orderly stacking increased the amount of AfB_1 desorption. Although questions remain regarding the smectite-AfB_1 interaction, the extensive characterization of three of the best smectite sorbent samples (1MS, 8TX, 11ID) by mean of standard chemical methods, x-ray diffraction, infrared analysis, transmission electron microscopy and AfB_1-adsorption isotherms can be used as a preliminary model to establish quality criteria. One of these three samples was previously shown to be an effective aflatoxicosis suppressant in many animal feeding studies (e.g. Phillips, 1995).

To initiate public use of smectite clays as aflatoxin B_1 detoxifiers amended to animal feeds, each industrially available batch of clay should be carefully examined to guarantee consistency of smectite properties that can vary within their large natural deposits (Dixon et al., 2006) and aflatoxin B_1 adsorption capacity. Twenty years of animal feeding studies support the assumption that smectites will not negatively affect animal health. Only the consumer's evaluation of animal health and performance will determine eventual success of smectite amended animal feed. Quality labels by state authorities should be confined to samples that fulfill the following selection criteria, which have been found pertinent based on results with the twenty smectites utilized in the current study:

1. pH near neutral in water, between 6.5 and 8.5.
2. Cation exchange capacity > 75$cmol_c$/kg.
3. Organic carbon content below 2.5 g/kg to avoid misleading CEC interpretation.
4. First order XRD peak near 1.3nm and expansible to ~1.7nm and preferentially a visible third order peak.

5. FTIR analysis is recommended and should show expression of the AlFeOH-bending band in the 868-889 cm^{-1} region (Tenorio, et al., 2006, manuscript in review).

6. Langmuir adsorption capacity for AfB$_1$ sorption should be 0.40 mol/kg or higher.

Continued research is needed to minimize potential risks by low AfB$_1$-adsorbing smectites and learn more about the factors controlling for AfB$_1$ sorption. Promising results from FTIR analysis should be pursued further.

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APPENDIX

Sample	Sample specification	Munsell Color			
		Dry	Name	Wet	Name
16 MX	Bentonite, Michocan, MX	10YR 7/1	light gray	2.5Y 5/2	grayish brown
5 OK	Camargo 1, Oklahoma	5YR 8/2	white	10YR8/2	white
7 AZ	Ca-Montmorillonit, AZ, Mt18	5YR 8/1	white	10YR8/2	white
9 ID	Muldoon 27, OTSC	10YR 8/1	white	5Y 5/2	olive gray
12 ID	Muldoon 30, OTSC	10YR 8/1	white	5Y 5/2	olive gray
2 MS	Novasil 13, OTSC	5Y 7/2	light gray	5Y 4/2	olive gray
10 ID	Muldoon 26, OTSC	10YR 8/1	white	5Y 5/2	olive gray
4 MS	Novasil 14, OTSC	5Y 7/2	light gray	5Y 4/2	olive gray
15 MX	Bentonite, Durango, MX	5YR 8/1	white	5YR 7/2	pink grayish
T8	Milwhite	10YR 8/2	white	10YR 6/3	pale brown
6 WY	Montmorillonit, WY #25, Mt03	5Y 8/2	white	5Y 7/2	light gray
3 MS	Novasil 16, OTSC	5Y 7/2	light gray	5Y 4/2	olive gray
13 ID	Muldoon 29, OTSC	10YR 8/1	white	5Y 5/2	olive gray
17 TX	Ca-Bentonite, Southern Clay Products. Acid bentonite.	10YR 8/1	white	10YR 8/3	very pale brown
14 MS	Ca-Bentonite, Aberdeen, MS	5Y 7/2	light gray	5Y 5/2	olive gray
T4	Novasil Plus	5Y 6/2	light olive gray	5Y 4/2	olive gray
11 ID	Muldoon 28, OTSC	10YR 8/1	white	5Y 5/2	olive gray
T7	Astra Ben, Tx	10YR 8/2	white	10YR 6/2	light brownish gray
1 MS	Novasil 15, OTSC	5Y 7/2	light gray	5Y 4/2	olive gray
8 TX	Ca-Bentonite, TX, Mt22	5Y 8/1	white	5Y 8/3	pale yellow

| Sample | pH | Cole Value | NH₄OAc extr bases | | | | Total | CEC in NaOAC | Base Sat | Calcite | Dolomite | CaCO3 EQ | ORGN C |
| | | | Ca | Mg | Na | K | | | | | | | |
			cmol$_c$/Kg								%		
16 MX	7.6	1.1	20.0	22.6	12.1	2.0	56.7	68.3	83	_1	_	_	0.21
5 OK	7.3	0.3	78.5	23.4	1.1	0.4	103.4	101.3	100	_	_	_	0.16
7 AZ	7.6	0.7	96.9	19.2	1.0	1.2	118.3	90.1	100	_	_	_	0.15
9 ID	7.5	1.3	50.0	18.3	9.4	0.7	78.4	77.8	100	_	_	_	0.17
12 ID	7.4	1.1	58.3	15.3	8.9	0.7	83.2	75.9	100	_	_	_	0.17
2 MS	8.7	1.0	93.3	9.2	0.4	1.7	104.6	85.4	100	2.3	0.3	2.6	0.17
10 ID	7.8	1.8	44.7	18.5	11.9	0.8	75.9	75.1	100	_	_	_	0.13
4 MS	8.5	0.9	115.0	14.7	0.4	1.6	131.7	93.3	100	2.5	1.1	3.7	0.04
15 MX	8.7	~3	39.0	8.9	59.7	1.3	108.9	93.7	100	_	_	_	0.23
T8	7.8	1.0	43.1	14.5	12.1	0.9	70.6	74.2	95	_	_	_	0.16
6 WY	9.6	1.8	15.5	4.5	83.6	1.1	104.7	83.3	100	_	_	_	0.24
3 MS	8.6	0.8	90.6	9.8	0.4	1.5	102.3	94.3	100	2.2	1.0	3.3	0.04
13 ID	7.5	0.7	59.5	16.5	9.2	0.7	85.9	79.7	100	_	_	_	0.25
17 TX	4.7	2.2	36.8	17.4	10.7	1.0	65.9	78.4	84	_	_	_	0.07
14 MS	7.7	0.6	97.9	13.8	0.5	1.9	114.1	84.3	100	3.3	0.4	3.7	0.11
T4	8.5	0.9	92.1	12.9	0.6	1.5	107.1	99.9	100	_	_	_	0.50
11 ID	7.4	1.3	56.6	15.6	9.2	0.7	82.1	76.4	100	_	_	_	0.18
T7	8.8	1.2	51.7	15.3	43.5	0.5	111.0	87.6	100	_	_	_	0.27
1 MS	8.3	0.6	94.1	9.4	0.4	1.5	105.4	85.8	100	2.1	0.6	2.6	0.18
8 TX	7.1	1.3	54.6	21.8	8.3	1.0	85.7	84.1	100	_	_	_	0.12

[1] no analysis available

Sample	Langmuir Isotherm				Sorption in each point of the isotherm					
	Equation	Maximum sorption capacity Qmax (mol/kg)	K_d	R^2	0.4	1.6	3.2	4.8	6.4	8
							mol/kg			
16 MX	y = 16.635x + 6E-05	0.0601	277315	0.96	0.0087	0.0292	0.0425	0.0523	0.0442	0.0565
5 OK	y = 7.6826x + 0.0002	0.1301	38432	0.89	0.0047	0.0138	0.0375	0.0328	0.0747	0.0745
7 AZ	y = 6.6429x + 0.0004	0.1506	16600	0.69	0.0023	0.0068	0.0119	0.0196	0.0280	0.0362
9 ID	y = 4.8006x + 6E-06	0.2083	800128	0.91	0.0314	0.1556	0.2079	0.1663	0.2150	0.1914
12 ID	y = 4.16x + 1E-05	0.2403	416146	0.93	0.0328	0.1366	0.1541	0.2171	0.1779	0.2475
2 MS	y = 3.546x + 7E-06	0.2822	506226	0.94	0.0443	0.1397	0.2228	0.1842	0.2682	0.2780
10 ID	y = 3.5323x + 4E-06	0.2831	883080	0.97	0.0393	0.1676	0.2549	0.2553	0.2735	0.2600
4 MS	y = 3.5152x + 4E-06	0.2844	876043	0.86	0.0456	0.1240	0.2741	0.2358	0.3313	0.2503
15 MX	y = 3.4709x + 2E-06	0.2881	1735580	0.89	0.0639	0.1492	0.2802	0.2712	0.2875	0.2783
T8	y = 3.4905x + 9E-06	0.2897	383538	0.94	0.0282	0.1545	0.2287	0.2422	0.2154	0.2740
6 WY	y = 3.4041x + 1E-05	0.2937	340483	0.95	0.0327	0.1209	0.1913	0.1971	0.2846	0.2526
3 MS	y = 3.1428x + 8E-06	0.3181	392958	0.97	0.0319	0.1583	0.2218	0.2631	0.2508	0.2946
13 ID	y = 2.819x + 9E-06	0.3547	313253	0.97	0.0295	0.1585	0.2549	0.2585	0.2864	0.3103
17 TX	y = 2.6647x + 9E-06	0.3752	296138	0.98	0.0299	0.1488	0.2472	0.2626	0.3093	0.3159
14 MS	y = 2.4799x + 7E-06	0.4032	354308	0.93	0.0450	0.1522	0.2528	0.2400	0.3539	0.3911
T4	y = 2.4754x + 1E-05	0.4040	247524	0.92	0.0359	0.1172	0.1654	0.2397	0.3032	0.3533
11 ID	y = 2.0773x + 5E-06	0.4814	415454	0.97	0.0373	0.1832	0.3208	0.3492	0.4365	0.4035
T7	y = 1.9414x + 5E-06	0.5151	388274	0.99	0.0411	0.1815	0.3254	0.4000	0.4276	0.4443
1 MS	y = 1.8999x + 3E-06	0.5263	633352	0.95	0.0520	0.1994	0.3329	0.3590	0.4232	0.5376
8 TX	y = 1.4776x + 5E-06	0.6767	295551	0.98	0.0443	0.1941	0.3498	0.4275	0.4878	0.5995

Sample	Water	K saturated	K saturated 550° C	Blank (clay + benzene:acetonitrile 98:2) Temperature (°C)					Saturated (100ppm AfB$_1$ in benzene:acetonitrile 98:2) Temperature (°C)				
				25	100	150	200	245	25	100	150	200	245
				d-spacing (Å)									
16 MX	15.78	12.28	10.40	14.61	13.1	≈12.03	10.92≈	10.53	15.64	14.85	14.85	13.7	14.03
5 OK	15.24	11.87	9.99	15.64	15.24	13.81	14.49	≈10.85	15.64	15.78	14.98	14.26	14.03
7 AZ	14.49	12.28	10.16	15.37	14.14	12.19	12.28	12.9	15.78	14.85	15.24	14.49	14.03
9 ID	14.26	11.41	9.88	15.11	13.19	12.03	11.33	9.99	15.92	14.49	14.14	13.39	13.19
12 ID	14.85	11.48	9.56	15.37	13.19	12.45	13.19	≈11.63	15.92	15.64	14.49	13.92	13.6
2 MS	14.85	11.63	10.16	15.51	12.45	10.53	10.72	10.28	15.24	14.14	13.6	13.81	13.7
10 ID	14.14	11.12	10.05	15.37	13.5	12.19	13.19	≈11.12	15.92	14.49	14.14	NA	13.19
4 MS	14.30	11.19	12.36	15.37	12.54	11.63	10.65	10.22	15.51	14.37	13.6	13.7	12.9
15 MX	15.24	12.11	9.77	13.81	12.19	11.48	10.72	≈9.77	14.98	14.61	14.14	13.6	13.39
T8	17*	12.36	10.04	14.73	13.1	11.56	10.78	9.94	16.07	15.64	14.73	14.14	13.92
6 WY	12.63	11.19	9.67	12.9	10.98	9.94	9.67	9.94	13	13.19	12.81	12.72	12.9
3 MS	14.85	11.41	10.59	15.24	12.11	11.19	10.98	10.53	15.37	14.14	13.5	13.81	13.19
13 ID	14.98	11.33	9.88	15.51	14.14	12.19	12.63	10.98	16.22	14.85	14.49	13.92	13.7
17 TX	14.73	12.90	10.16	15.51	14.26	12.28	9.88	9.99	16.37	15.78	15.11	14.14	13.81
14 MS	14.73	11.41	10.11	15.51	13.81	12.28	10.98	10.11	15.37	13.92	14.03	13.39	13.39
T4	13.60	12.45	9.77	15.11	14.61	12.81	12.45	11.33	15.92	14.26	14.03	13.6	13.92
11 ID	15.11	11.12	9.94	15.37	14.14	12.19	≈11.26	12.11	15.11	14.14	13.7	13.6	13.39
T7	14.61	11.71	9.83	14.98	12.28	11.26	12.9	10.28	14.61	13.7	13.5	13.13	13.1
1 MS	13.81	11.50	10.22	15.51	13.7	13.39	≈12.72	13.6	14.73	13.92	13.6	13.6	13.39
8 TX	13.92	11.26	9.88	15.64	14.03	13.6	≈10.28	≈11.26	15.11	14.49	14.03	13.6	13.5

Sample	Water	K saturated	K saturated to 550° C	Blank (clay in benzene:acetonitrilo 98:2)					AfB$_1$ Saturated				
				Temperature (ºC)									
				RT	100	150	200	250	RT	100	150	200	250
				Particle thickness from XRD smectite peak breadth (Å)									
16 MX	103.3	69.81	228.43	186.34	75.66	NSP[1]	NSP	88.83	186.32	75.62	97.31	81.70	NSP
5 OK	233.95	134.93	228.48	228.06	293.92	120.32	413.24	NSP	293.89	228.06	157.50	186.37	157.54
7 AZ	220.99	134.91	294.45	228.06	157.54	120.36	81.74	88.85	228.06	186.35	186.34	186.35	186.39
9 ID	227.31	156.11	344.14	413.18	120.35	75.68	65.91	88.91	186.31	120.30	120.32	40.00	157.56
12 ID	180.79	126.37	294.41	293.89	120.33	120.38	107.61	NSP	228.01	120.29	107.59	107.60	107.61
2 MS	172.93	106.15	294.38	228.06	136.49	65.93	55.21	65.92	228.08	120.32	107.59	120.32	120.32
10 ID	137.12	118.86	228.40	293.90	97.34	107.65	120.34	NSP	157.48	120.31	120.32	NA	157.56
4 MS	159.11	97.1	146.43	228.06	136.49	88.89	75.72	75.73	186.32	120.30	120.32	136.46	413.46
15 MX	233.95	185.12	294.38	120.31	97.37	58.36	75.72	NSP	157.51	107.61	107.59	120.33	47.45
T8	128.26	136.5	157.74	186.35	97.35	65.91	70.48	97.46	157.48	107.58	97.31	88.82	107.60
6 WY	227.4	144.76	344.05	413.41	75.71	157.79	294.47	294.41	294.05	228.16	413.43	294.08	413.39
3 MS	220.97	97.09	186.34	228.08	107.65	61.91	70.47	97.43	228.41	120.32	136.45	157.54	136.45
13 ID	227.28	134.95	294.41	293.89	120.32	120.38	120.36	75.72	157.49	120.29	120.30	120.33	157.56
17 TX	220.97	185.11	228.50	293.90	186.31	120.29	136.43	157.56	256.78	136.45	97.38	120.52	228.46
14 MS	176.79	134.95	294.42	186.32	107.61	81.74	88.91	58.39	186.33	157.54	186.41	136.46	228.16
T4	118.68	126.34	228.49	293.92	120.30	75.66	70.43	49.81	228.05	120.31	120.32	186.41	136.46
11 ID	256.59	107.63	228.49	293.92	107.60	75.67	47.49	45.37	157.52	136.43	157.56	157.56	157.57
T7	220.99	134.94	294.45	157.51	136.49	65.92	107.76	88.96	136.42	157.57	186.39	228.16	186.41
1 MS	172.93	97.12	294.39	228.06	107.61	97.33	61.88	58.32	186.34	186.39	186.38	186.39	186.41
8 TX	120.55	120.64	294.41	228.06	88.83	61.86	107.76	NSP	157.51	136.43	157.56	186.39	157.56

[1] NSP = no smectite peak

Isotherm sorption points: Percentages of AfB$_1$ in solution and adsorbed to 100µg clay

Sample	0.40	1.60	3.20	4.80	6.40	8.00	% left in solution of x ppm aflatoxin B$_1$						% adsorbed to clay of x ppm aflatoxin B$_1$					
	conc.(µg aflatoxin/mL) left in solution of original conc. (µg aflatoxin/mL)																	
	0.38	1.63	3.08	4.84	6.50	8.13	0.38	1.63	3.08	4.84	6.50	8.13	0.38	1.63	3.08	4.84	6.50	8.13
16 MX	0.35	1.42	2.94	4.48	6.13	7.65	91.3	87.0	95.4	92.5	94.2	94.0	8.7	13.0	4.6	7.5	5.8	6.0
5 OK	0.39	1.54	2.99	4.62	5.96	7.56	103.0	94.1	97.1	95.4	91.6	92.9	-3.0	5.9	2.9	4.6	8.4	7.1
7 AZ	0.36	1.56	3.05	4.60	6.07	7.70	95.4	95.5	99.2	95.0	93.4	94.7	4.6	4.5	0.8	5.0	6.6	5.3
9 ID	0.24	0.66	1.94	3.80	5.09	6.84	62.8	40.6	62.9	78.5	78.3	84.1	37.2	59.4	37.1	21.5	21.7	15.9
12 ID	0.23	0.78	2.27	3.48	5.32	6.49	60.0	47.8	73.8	71.9	81.8	79.8	40.0	52.2	26.2	28.1	18.2	20.2
2 MS	0.12	0.72	1.80	3.64	4.72	6.26	30.9	44.2	58.6	75.3	72.6	76.9	69.1	55.8	41.4	24.7	27.4	23.1
10 ID	0.15	0.55	1.60	3.20	4.68	6.37	38.5	33.4	52.0	66.1	72.0	78.3	61.5	66.6	48.0	33.9	28.0	21.7
4 MS	0.10	0.81	1.47	3.31	4.32	6.42	26.3	49.6	47.9	68.4	66.4	78.9	73.7	50.4	52.1	31.6	33.6	21.1
15 MX	0.00	0.67	1.45	3.11	4.60	6.26	0.2	40.9	47.1	64.2	70.8	77.0	99.8	59.1	52.9	35.8	29.2	23.0
T8	0.21	0.62	1.75	3.27	5.04	6.27	53.9	37.8	56.9	67.5	77.4	77.1	46.1	62.2	43.1	32.5	22.6	22.9
6 WY	0.22	0.87	2.03	3.59	4.65	6.45	58.0	53.3	66.0	74.3	71.5	79.3	42.0	46.7	34.0	25.7	28.5	20.7
3 MS	0.21	0.62	1.83	3.17	4.84	6.17	55.7	38.2	59.3	65.5	74.5	75.9	44.3	61.8	40.7	34.5	25.5	24.1
13 ID	0.17	0.56	1.56	3.14	4.56	6.02	44.3	34.5	50.7	64.9	70.2	74.0	55.7	65.5	49.3	35.1	29.8	26.0
17 TX	0.21	0.66	1.65	3.15	4.46	6.02	54.3	40.7	53.6	65.2	68.6	74.0	45.7	59.3	46.4	34.8	31.4	26.0
14 MS	0.28	0.83	1.78	2.87	3.98	6.04	72.8	50.7	57.7	59.2	61.3	74.2	27.2	49.3	42.3	40.8	38.7	25.8
T4	0.17	0.86	2.16	3.29	4.50	5.78	43.5	52.5	70.1	68.0	69.1	71.1	56.5	47.5	29.9	32.0	30.9	28.9
11 ID	0.18	0.47	1.21	2.63	3.68	5.49	46.5	28.6	39.2	54.3	56.6	67.5	53.5	71.4	60.8	45.7	43.4	32.5
T7	0.31	0.91	1.63	2.82	4.31	5.96	80.4	55.5	52.8	58.3	66.2	73.3	19.6	44.5	47.2	41.7	33.8	26.7
1 MS	0.05	0.33	1.10	2.53	3.73	4.62	13.3	20.2	35.6	52.3	57.4	56.8	86.7	79.8	64.4	47.7	42.6	43.2
8 TX	0.13	0.39	1.02	2.13	3.35	4.26	32.8	23.8	33.0	44.0	51.6	52.3	67.2	76.2	67.0	56.0	48.4	47.7

VITA

Ines Kannewischer was born to Wolfgang and Sabine Kannewischer on February 4th, 1982 in Datteln, North Rhine-Westphalia, Germany.

She attended grade schools in Waltrop, North Rhine-Westphalia and Bredenbeck, Lower Saxony, before attending high school in Gehrden, Lower Saxony. She attended Leibnitz University of Hannover in Hannover, Germany from 2001 to 2004, completing all undergraduate and parts of her graduate studies with success, including several field trips, amongst others to Italy. She received her Vordiplom in geosciences in 2004. An internship of three months duration in the Volcanology Laboratories of the CNRS (Centre National de la Recherche Scienitfique) at Clermont-Ferrand, France and ten month of participation in a research project in the Soil Mineralogy Laboratories at A&M University, College Station Texas, followed in the years from 2004 to 2005. She then continued her graduate studies at Texas A&M University of College Station starting in fall 2005 to pursue a M.S. degree in the Soil and Crop Science Department. She received her degree in December 2006.

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