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Original scientific paper

T-2 toxin adsorption by hectorite

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Abstract: The adsorption of T-2 toxin by the natural smectite mineral – hectorite at pH 3.0, 7.0 and 9.0 was investigated. The results of T-2 toxin adsorption on hectorite showed that the T-2 adsorption capacity decreased with increasing concentration of adsorbent in the suspension for all the investigated pH values. From the adsorption isotherms, an increase in T-2 toxin adsorption with increasing initial T-2 toxin concentration was observed for all the investigated pH values. The T-2 toxin adsorption by hectorite followed a non-linear (Langmuir) type of isotherm at pH 3.0, 7.0 and 9.0, with correlation coefficients (r^2) of 0.943 at pH 3.0, 0.919 at pH 7.0 and 0.939 at pH 9.0. The estimated maximum T-2 toxin adsorption by hectorite based on the Langmuir fit to the data (9.178 mg/g at pH 3.0, 9.930 mg/g at pH 7.0, and 19.341 mg/g at pH 9.0), indicated that the adsorption of T-2 toxin by hectorite is pH dependent. The obtained data suggest the existence of specific active sites in hectorite onto which the T-2 toxin is adsorbed.

Keywords: smectite; hectorite; mycotoxins; T-2 toxin; adsorption.

INTRODUCTION

The contamination of animal feed with mycotoxins represents a worldwide problem leading to economic losses in animal production. The most common naturally occurring mycotoxins are the aflatoxins, ochratoxins, fumonisins, trichothecenes, zearalenone and ergopeptine alkaloids. Trichothecenes constitute the largest group of *Fusarium* mycotoxins and are commonly found on cereals, such as wheat, barley, oats and corn. These cereals are commonly used in animal feed, thus, possibly, exposing livestock to relatively high levels of trichothecenes. Trichothecenes have a closely related sesquiterpenoid ring structure with a 12,13 epoxy ring and a number of hydroxyl, acetoxy or other substituents. T-2 toxin is one of the most acutely toxic trichothecenes and causes reduction in feed con-

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sumption and weight gain and severe oral lesions in broilers,^{1–3} The chemical structure of T-2 toxin is presented in Fig. 1.

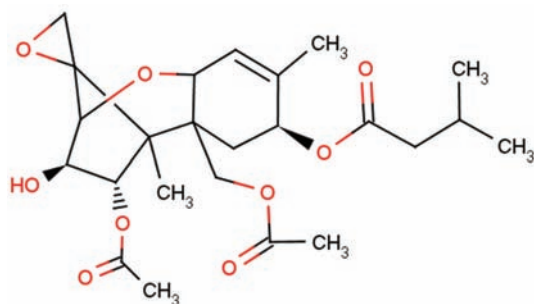


Fig. 1. Chemical structure of the T-2 toxin.

The most promising and economical approach for detoxifying mycotoxin contaminated feedstuffs is the addition of non-nutritive adsorbent materials to the diet, thus reducing gastrointestinal absorption of mycotoxins. Examples are activated carbon (AC), smectite minerals, zeolites and special polymers⁴ The efficiency of mycotoxin adsorbents differs considerably depending on the chemical structure of both the toxin and adsorbent. Thus, the binding efficacy of adsorbents is dependent on their crystal structures and physical properties (the total charge and charge distribution, the size of the pores, accessible surface area, *etc.*), as well as on the physico-chemical properties of the mycotoxins (polarity, solubility, shape, charge distribution, dissociation constants, *etc.*).⁵ It is well known that some of the extensively studied adsorbents such as smectite minerals – hydrated sodium calcium aluminosilicates (HSCAS) and zeolites – clinoptilolites are very effective in adsorbing aflatoxins, both *in vitro* and *in vivo*.^{6,7} However, their negatively-charged surfaces are not effective in preventing the toxic effects of *Fusarium* mycotoxins, such as fumonisins, trichothecenes or zearalenone. Avantiato *et al.*⁴ tested twenty-one adsorbent materials (AC, cholestyramine, Standard Q/FIS, Myco Ad A-Z, Mycofix Plus, Mycosorb, *etc.*) for the binding of fumonisin B1 (FB1), zearalenone (ZEA), and trichothecenes – deoxynivalenol (DON) and nivalenol (NIV) under *in vitro* conditions and found that AC showed the best binding activity, adsorbing 100 % of FB1 and ZEA, and a moderate amount of DON and NIV (>50 %). It is well known that chemical treatment of phyllosilicate clays and zeolites with long chain organic cations, *i.e.*, cetylpyridinium (CP), hexadecyltrimethylammonium (HDTMA) or octadecyldimethylbenzylammonium (ODMBA) ions results in an increased hydrophobicity of the mineral surface, providing a high affinity for hydrophobic organic molecules, such as the majority of the mycotoxins.^{8–10} In previous studies, it was shown that natural clinoptilolite and montmorillonite were effective in adsorbing aflatoxin B1,^{11,12} while surfactant modified zeolites had a high affinity for ZEA,¹³ ochratoxin A,¹⁴ and FB1.¹⁵

In addition to montmorillonite and zeolites, kaolinite and hectorite are commonly used industrial minerals.¹⁶ Hectorite is a 2:1 smectite clay, which is composed of units made up of two tetrahedral sheets with a central Mg octahedral sheet. It has permanent negative charges that arise due to the isomorphous substitution of Li^+ for Mg^{2+} . The negative charge is counterbalanced by the presence of inorganic cations.^{8,16,17} It is well known that hectorite has been used as an adsorbent for color removal of basic dyes and metal ions,¹⁸ while organically modified hectorites may be used as an adsorbent for the removal of anionic dyes, organic compounds and pesticides, *etc.*^{19,20} Recently, hectorite was considered as an adsorbent for ZEA detoxification, but high adsorption of this toxin was observed only when hectorite was used in combination with AC. Namely, the results of *in vitro* studies by Afriyie–Gyawu *et al.*²¹ showed that AC was the most efficient adsorbent for ZEA (adsorption index 99 %), followed by a combination of 2 parts of AC plus 3 parts of hectorite (HEC) (69 %), CP-exchanged low-pH montmorillonite (58 %), hexadecyltrimethylammonium-exchanged low-pH montmorillonite (54 %), and HEC alone (28 %). To the best of our knowledge, there are no data in the scientific literature on the efficacy of hectorite to bind trichothecenes mycotoxins.

The objective of the current study was to investigate the efficiency of the natural smectite mineral hectorite for *in vitro* adsorption of T-2 toxin at pH 3.0, 7.0 and 9.0.

EXPERIMENTAL

Hectorite (Hector, California) was obtained from the Mine-Engineering Com. (2286 E Carson St, #437 Long Beach, CA 90807, USA). The sample was dried at 60 °C and ground to yield particles smaller than 63 μm .

The cation exchange capacity (CEC) of the hectorite was measured with 1 M NH_4Cl .

The mineralogical composition of the starting sample was determined by X-ray powder diffraction analysis, XRPD, performed using a Philips PW-1710 diffractometer with monochromatic $\text{Cu-K}\alpha$ radiation, in the 2θ range 4–60°.

Investigations of the surface morphology of the hectorite were performed using a LINK AN 1000 EDS microanalyzer attached to a JEOL JSM – 6460 LV Scanning Electron Microscope (SEM). The accelerating potential was 15 kV, the beam current 3 nA, the surface electron beam measured 1 mm^2 and the counting time was 1250 s. ZAF-4/FLS software provided by LINK was used for corrections.

The hectorite was used without pretreatment for the T-2 toxin adsorption studies. A primary T-2 toxin stock solution (1000 ppm) was prepared in ethanol. The T-2 toxin test solutions for the adsorption studies were prepared by adding ethanol stock solution to 0.10 M phosphate buffer adjusted to pH 3.0, 7.0 and 9.0. Duplicate aliquots of 0.10 M phosphate buffer containing 10 ppm of T-2 toxin (10 mL) were added to 15 mL polypropylene Falcon tubes to which 100, 20, 10 or 5 mg of hectorite had been added. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.10 M phosphate buffer plus 10 mg of hectorite to Falcon tubes. For investigations of the adsorption isotherms of T-2 toxin on hectorite, a 10 mL aliquot of T-2 toxin (4.0–10 mg L^{-1}) in buffer was added to 15 mL Falcon

tubes together with 10 mg of adsorbent. The adsorption isotherms for hectorite were examined at pH, 3.0, 7.0 and 9.0 with each sample being done in duplicate. The tubes were placed on a rotatory shaker for 30 min at room temperature (based on initial studies, data not shown). Each T-2 toxin test solution and control was centrifuged at 13000 rpm for 5 min and 2 mL of the aqueous supernatant was removed for HPLC analysis. An aliquot of the original buffered T-2 toxin test solution was used as the HPLC standard. HPLC analyses were performed on Hypersil C18-BDS column ((250×4.60) mm, 5 µm particle size) using a Hitachi L-7100 pump with a Hitachi L-7200 autosampler, and UV detection with a Hitachi L-7400 UV detector ($\lambda = 212$ nm). The mobile phase was acetonitrile:water (50:50) pumped at a flow rate of 1.0 mL min⁻¹. Data were recorded and processed using a Hitachi D-7000 data acquisition package with Concert Chrom software on a microcomputer. The percent bound T-2 toxin was calculated from the difference between the initial and final T-2 toxin concentration in the aqueous supernatant after equilibrium.

RESULTS AND DISCUSSION

The cation exchange capacity (CEC) of the hectorite was 0.892 meq g⁻¹, measured with 1.0 M NH₄Cl. Sodium was the dominant ion in the exchangeable positions (0.774 meq g⁻¹), while magnesium (0.094 meq g⁻¹), lithium (0.018 meq g⁻¹), and potassium (0.006 meq g⁻¹) were present at very low levels in the exchangeable positions of the hectorite. The chemical composition of the hectorite is presented in Table I.

TABLE I. Chemical composition of the employed hectorite

Content, mass %								
SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	Na ₂ O	K ₂ O	Li ₂ O	I.L.
57.88	0.45	0.04	<0.10	23.62	2.22	0.04	1.46	15.00

The mineralogical composition of the starting sample was primarily hectorite, with smaller amounts of quartz, as measured by X-ray powder diffraction analysis. The X-ray diffraction pattern of the hectorite (Fig. 2) shows a symmetric (001) diffraction with $d_{001} = 12.49$ Å, a typical value for monovalent cation smectites with a monolayer of water molecules in the interlayer space. A similar basal spacing ($d_{001} = 12.61$ Å) for natural hectorite with a cation exchange capacity of 0.439 meq g⁻¹ was reported Baskaralingam *et al.*¹⁹

The surface morphology of the hectorite had a fluffy appearance with a fine platey structure, as can be seen from the SEM image of the hectorite presented in Fig. 3.

The T-2 toxin is a low polar organic molecule, slightly soluble in water.¹ In a preliminary study, the results obtained for T-2 toxin adsorption ($c_{0,T-2} = 10$ mg L⁻¹; $c_{susp} = 10$ g L⁻¹) by clinoptilolite (Vranje, Serbia), montmorillonite (Šipovo, Bosnia) and hectorite, at pH 3.0 showed that neither clinoptilolite nor montmorillonite were efficient in binding T-2 toxin under *in vitro* conditions (T-2 toxin adsorption indexes: clinoptilolite: 8 %; montmorillonite: 13 %). The properties of natural clinoptilolite and natural montmorillonite were given elsewhere.^{13–15}

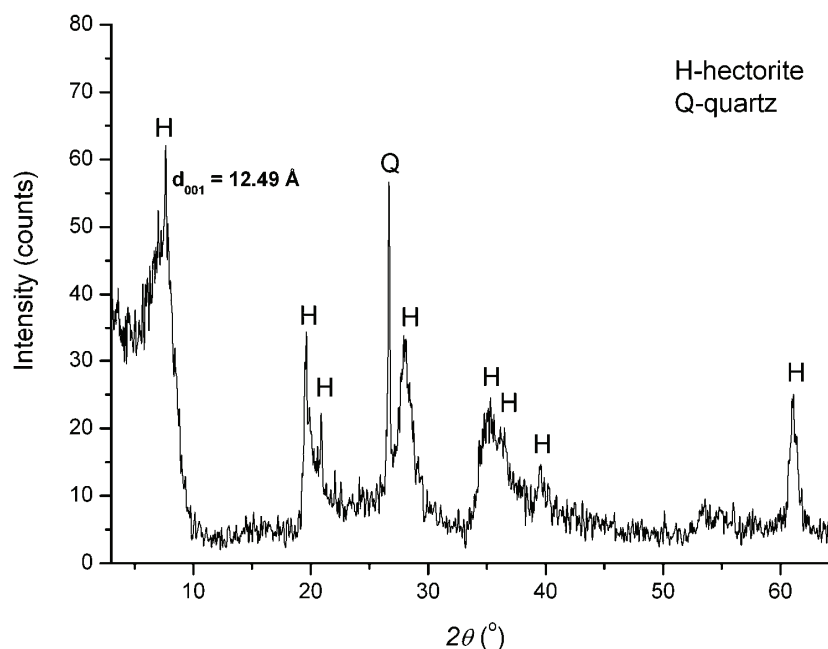


Fig. 2. XRPD Pattern of the employed hectorite.

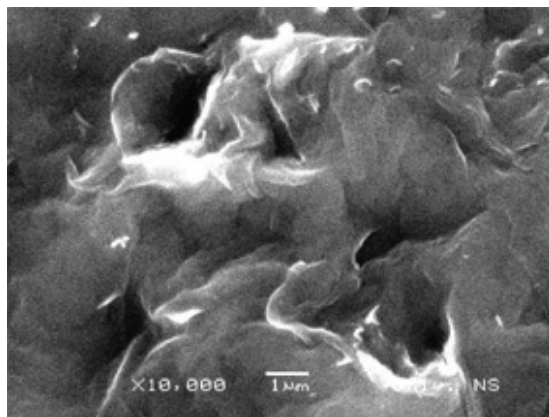


Fig. 3. SEM Image of the employed hectorite.

Compared to these minerals, the adsorption of T-2 toxin by the hectorite mineral was very high (adsorption index 95 %). The fact that montmorillonite, a di-octahedral smectite, does not adsorb T-2 toxin, while hectorite, a tri-octahedral magnesium smectite with some lithium substitution for magnesium in the octahedral layer, has a high affinity to adsorb this toxin indicates the presence of active sites in hectorite onto which this toxin may be adsorbed. Thus, to further investigate the adsorption of T-2 toxin by hectorite, experiments were performed

with different amounts of hectorite in the suspension, different initial T-2 toxin concentration and different pH values.

The effect of adsorbent mass on the amount of T-2 toxin adsorbed at pH 3.0, 7.0 and 9.0 is presented in Fig. 4.

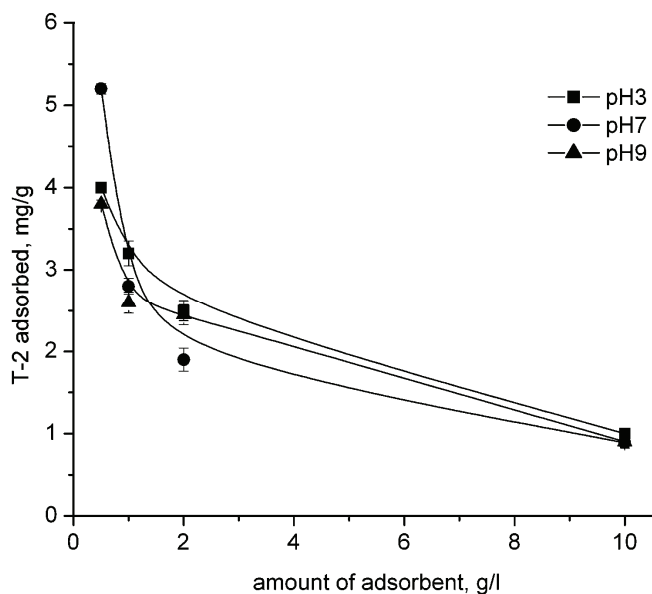


Fig. 4. T-2 toxin adsorption by hectorite vs. the amount of adsorbent in the suspension at different pH.

As can be seen from Fig. 4, a decrease in adsorption capacity with increasing adsorbent concentration was observed, at all investigated pH values. The decrease in adsorption capacity with increasing adsorbent concentration may be explained with the fact that adsorption sites remain unsaturated at higher adsorbent mass for the same initial toxin concentration solution.²⁰

The adsorption of T-2 toxin by hectorite was additionally investigated through the determination of the adsorption isotherms at pH 3.0, 7.0 and 9.0 (Figs. 5a–c). These isotherms were obtained by plotting the concentration of T-2 toxin in solution at equilibrium against the amount of T-2 toxin adsorbed per unit of weight of adsorbent.

There are only limited data in the scientific literature on the adsorption isotherms for the adsorption of mycotoxins by hectorite. Afriyie–Gyawu *et al.*²¹ reported a linear isotherm for ZEA adsorption by hectorite and suggested that hectorite appeared to sorb the low polar ZEA primarily *via* a partitioning phenomenon. Usually, it is assumed that the adsorption of low polar organic molecules to an organic-rich substrate through the partitioning mechanism obeys a linear adsorption isotherm.²² From Figs. 5a–c, T-2 toxin adsorption by hectorite followed non-linear types of isotherms at pH 3.0, 7.0 and 9.0. Additionally, an increase in

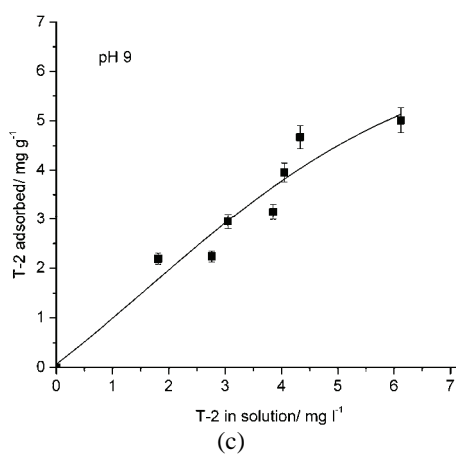
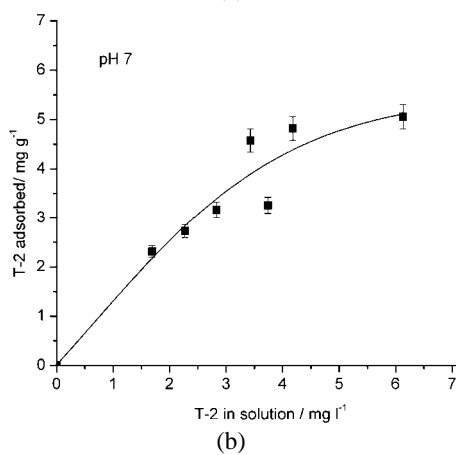
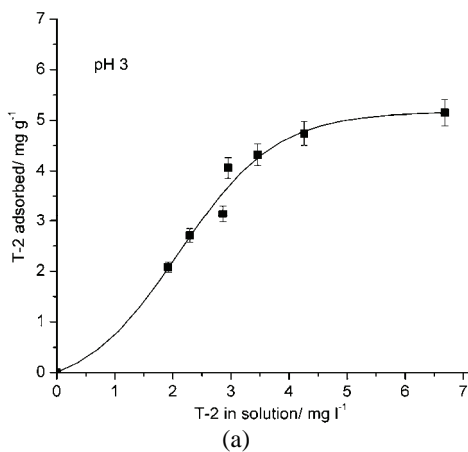


Fig. 5. T-2 toxin adsorption equilibrium data modeled by the Langmuir isotherm at pH 3.0 (a), 7.0 (b) and 9.0 (c).

T-2 toxin adsorption with increasing initial T-2 toxin concentration was observed at all investigated pH values. Non-linear (Langmuir) isotherms obtained for T-2 toxin adsorption by hectorite suggests that partition is not a relevant mechanism for its adsorption. The experimental data for T-2 toxin adsorption by hectorite at pH 3.0, 7.0 and 9.0 were accordingly fitted to the Langmuir isotherm equation:

$$c_{\text{ads}} = \frac{\alpha\beta c_{\text{eq}}}{1 + c_{\text{eq}}} \quad (1)$$

where c_{ads} and c_{eq} denote the equilibrium concentrations of T-2 toxin on the hectorite and in the aqueous phase, respectively, α is a constant related to the binding energy and β is the maximum amount of solute that can be adsorbed by the solid. It was proposed²³ that an adsorption conforms to the Langmuir model when the value of the correlation coefficient (r^2) is greater than 0.89. Thus, the calculated r^2 values of 0.943 at pH 3.0, 0.919 at pH 7.0 and 0.939 at pH 9.0 indicate that these isotherms are empirically consistent with the Langmuir model. Since a Langmuir isotherm is commonly associated with specific bonding,²⁴ the obtained results suggested the presence of specific active sites in hectorite onto which the T-2 toxin can be adsorbed. The estimated maximum T-2 toxin adsorption by hectorite, based on the Langmuir fit to the data, was 9.178, 9.930 and 19.341 mg g⁻¹ at pH 3.0, 7.0 and 9.0, respectively. This suggests that the adsorption of T-2 toxin by hectorite may depend on the solution pH. Since the T-2 toxin is non ionizable (Fig. 1), the increase in adsorption with increasing pH indicates some change at the hectorite surface that contributes to the adsorption. The fact that an increase of the solution pH increases the T-2 toxin adsorption suggests that additional interactions between the negative hectorite surface and T-2 toxin cause the greater adsorption. Based on the structure of the T-2 toxin and the obtained adsorption data, it is possible that more than one adsorption mechanism is involved in the adsorption of T-2 toxin by hectorite.

CONCLUSIONS

The effects of adsorbent mass, solution pH and the initial concentration of T-2 toxin in the solution on T-2 toxin adsorption by the natural mineral hectorite were investigated. It was found that adsorption capacity for the T-2 toxin decreased with increasing adsorbent concentration in the suspension, at all the investigated pH values. The adsorption of T-2 toxin by hectorite followed a non-linear (Langmuir) type of isotherm at pH 3.0, 7.0 and 9.0. The estimated maximum T-2 toxin adsorption by hectorite, based fitting the data to the Langmuir model, was 9.178 mg g⁻¹ at pH 3.0, 9.930 mg g⁻¹ at pH 7.0, and 19.341 mg g⁻¹ at pH 9.0, indicating that the adsorption of T-2 toxin by hectorite is pH dependent. The results presented in this paper show that hectorite possess a very high

capacity for T-2 toxin, over a wide pH range and may be suitable for potential practical applications.

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ИЗВОД

АДСОРПЦИЈА Т-2 ТОКСИНА НА ХЕКТОРИТУ

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У овом раду су приказани резултати испитивања адсорпције Т-2 токсина на минералу из групе смектита – хекториту, на различитим рН вредностима (3,0; 7,0 и 9,0). Испитивања су показала да адсорбована количина Т-2 токсина на хекториту опада са порастом садржаја чврсте фазе у суспензији на свим испитиваним рН вредностима. Са адсорпционих изотерми се примећује да са повећањем почетне концентрације Т-2 токсина расте и адсорпција Т-2 токсина, на свим испитиваним рН вредностима. Адсорпција Т-2 токсина на хекториту се може описати нелинеарним (Ленгмир, *Langmuir*) типом изотерме на рН 3,0; 7,0 и 9,0, при чему фактор корелације (r^2) износи 0,943 на рН 3,0; 0,919 на рН 7,0 и 0,939 на рН 9,0. Максимална адсорбована количина Т-2 токсина на хекториту на основу Ленгмирове изотерме (9,178 mg g⁻¹ на рН 3,0; 9,930 mg g⁻¹ на рН 7,0 и 19,341 mg g⁻¹ на рН 9,0), указује да је адсорпција Т-2 токсина на хекториту зависна од рН. Добијени резултати указују на постојање специфичних активних центара на хекториту на којима се Т-2 токсин адорбује.

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