

The effect of exchangeable cations in clinoptilolite and montmorillonite on the adsorption of aflatoxin B₁

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The adsorption of aflatoxin B₁ (AFB₁) by cation-exchanged clinoptilolite zeolitic tuff and montmorillonite was investigated at 37 °C and pH 3.8 from an aqueous electrolyte having a composition similar to that of gastric juices of animals. Both minerals were exchanged from the natural form to the sodium form and then to the Cu²⁺, Zn²⁺ and Co²⁺-rich forms. The cation exchange was different for the different cations, but in all cases the exchanges were larger on montmorillonite than on clinoptilolite. The degree of exchange on montmorillonite was 76 % for copper (from a total of CEC 0.95 meq/g, Cu²⁺ –0.73 meq/g) and 85 % for zinc and cobalt. Under the same conditions (concentration, temperature, pH, contact time), the degree of exchange on zeolitic tuff was 12 % for Cu²⁺ (from a total CEC of 1.46 meq/g, Cu²⁺ –0.17 meq/g), 8 % for Zn²⁺ and 10 % for Co²⁺. Both groups of mineral adsorbents showed high AFB₁ chemisorption indexes (c_{α}). For the montmorillonite forms, c_{α} ranged from 0.75 for the Cu-exchanged montmorillonite to 0.89 for the natural Ca-form, 0.90 for the Zn-exchanged form and 0.93 for the Co-exchanged montmorillonite. The adsorption of AFB₁ on the different exchanged forms of clinoptilolite gave similar values of c_{α} for the Cu and Ca forms (0.90) and values of 0.94 and 0.95 for the Zn- and Co-exchanged form. The impact of the mineral adsorbents on the reduction of essential nutrients present in animal feed (Cu, Zn, Mn and Co) showed that the Ca-rich montmorillonite had a higher capability for the reduction of the microelements than the Ca-rich clinoptilolite.

Keywords: clinoptilolite, montmorillonite, cation exchange, adsorption, aflatoxins.

INTRODUCTION

Aflatoxins comprise a diverse group of pervasive, naturally occurring, fungal elaborated poisons that have been strongly implicated in animal diseases. Aflatoxin B₁ (AFB₁) is the most toxic and cancerogenic of the aflatoxins.¹

Phillips *et al.*^{2–4} and Tomašević-Čanović *et al.*⁵ have shown that certain natural aluminosilicates (bentonite and zeolite) may be used, in animal diets, to prevent signs of

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certain mycotoxigenes. These mineral materials bind aflatoxins, forming highly stable complexes. Hydration of the exchangeable cations creates a hydrophilic environment on the surface of zeolites and on the surface and in the interlayer region of montmorillonite. This parameter has an influence on the adsorption of different organic molecules, including mycotoxins, on zeolite and montmorillonite particles and on the stability of the adsorbed complexes.^{4,6,7} A proposed mechanism of aflatoxin chemisorption by mineral adsorbents involves the rapid formation of a complex between a ligand and the mineral.⁸

Although these mineral adsorbents are added to animal rations to prevent the negative effects of aflatoxins, their impact on other components of the feed (vitamins, aminoacids, and microelements) is not well known. Chung and Baker⁹ reported effects of montmorillonite on phosphorous and Chung *et al.*¹⁰ on Zn, Mn, vitamin A and riboflavin. They noted no reductive effects on phosphorous, Mn, vitamin A, and only a slight reduction in Zn utilization. The addition of 0.2–0.5 % clinoptilolite to basal diets did not impair the utilization of tryptophane, phenylalanine, vitamin A, D and E.¹¹ The *in vitro* adsorption of vitamin B₆ on different mineral adsorbents showed that the Ca and H form of clinoptilolite adsorbed this vitamin to about 20 %. On the contrary, vitamin B₆ was tightly bound to hydrate sodium calcium aluminosilicate (HSCAS)-mineral adsorbent based on montmorillonite (98 %).¹²

The objective of the present study was to evaluate the affinity of different cation-exchanged forms of clinoptilolite and montmorillonite for aflatoxin B₁ *in vitro*. In addition, the impact of natural Ca-rich clinoptilolite and Ca-rich montmorillonite on essential microelements (Cu, Zn, Co and Mn) present in animal feed were examined.

EXPERIMENTAL

Preparation of the different forms of the adsorbents

The natural zeolite sample used in this study was a clinoptilolite-rich tuff from the Zlatokop deposit (Vranjska Banja, Yugoslavia). From X-ray diffraction analysis, the content of clinoptilolite was ≥ 90 %. The chemical composition of this sample was as follows (wt. %): SiO₂ – 64.21, Al₂O₃ – 11.48, Fe₂O₃ – 0.88, CaO – 4.55, MgO – 1.45, Na₂O – 1.71, K₂O – 1.29, L.I. – 14.0. The cation exchange capacity (CEC) was 1.46 meq/g. The sample was crushed and sieved, and the fraction $<63 \mu\text{m}$ was selected for study.

The Ca-rich clinoptilolite tuff (10 % suspension) was reacted with 2 M NaCl solution to obtain a Na-rich clinoptilolite tuff. The suspension was continuously stirred for 24 h at room temperature, then the supernatant was decanted off and the residue washed with deionized water until Cl⁻ ions were no longer detectable. The obtained sample was air dried at 105 °C.

A natural Ca-rich bentonite from the Šipovo deposit in Bosnia with average particle size $< 5 \mu\text{m}$ was used. It contained about 90 % montmorillonite and a small amount of quartz and calcite (X-ray diffraction analysis). The chemical composition was as follows (wt. %): SiO₂ – 55.36, Al₂O₃ – 22.94, Fe₂O₃ – 3.65, CaO – 3.58, MgO – 3.27, Na₂O – 0.11, K₂O – 0.32, L.I. – 12.31. The total CEC was 0.95 meq/g.

The Na-exchanged form of montmorillonite was obtained by passing a 5 % suspension of Ca-rich bentonite through a glass column filled with Woffatit KPS resin.

Cation exchange of both clinoptilolite and montmorillonite was carried out in an aqueous suspension after the addition of inorganic salts: CuSO₄ · 5H₂O, ZnSO₄ · 7H₂O and CoCl₂ · 6H₂O (sup-

plied by Merck). The initial concentration of the metal ions before ion exchange was 1.50 meq/g for clinoptilolite and 1.00 meq/g for montmorillonite. Those concentrations satisfied the CEC of the starting zeolitic tuff and of Ca-rich montmorillonite. The contents of exchangeable cations in all the mineral adsorbents used for AFB₁ adsorption are listed in Table I.

TABLE I. The contents of exchangeable cations in different forms of clinoptilolites and montmorillonites

Mineral adsorbent	Exchangeable cations/(meq/g)						
	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cu ²⁺	Zn ²⁺	Co ²⁺
Ca-rich (natural) clinoptilolite	0.95	0.13	0.22	0.16	–	–	–
Na-exchanged clinoptilolite	0.38	0.14	0.81	0.16	–	–	–
Cu-exchanged clinoptilolite	0.39	0.13	0.59	0.16	0.17	–	–
Zn-exchanged clinoptilolite	0.39	0.14	0.65	0.16	–	0.12	–
Co-exchanged clinoptilolite	0.37	0.14	0.65	0.16	–	–	0.15
Ca-rich montmorillonite	0.89	0.04	0.006	0.01	–	–	–
Cu-exchanged montmorillonite	0.08	0.04	0.046	0.01	0.73	–	–
Zn-exchanged montmorillonite	0.08	0.04	0.016	0.01	–	0.81	–
Co-exchanged montmorillonite	0.08	0.04	0.006	0.01	–	–	0.81

From the results presented in Table I, it can be seen that the amounts of Cu, Zn and Co exchanged by montmorillonite were much higher than by clinoptilolite. The degree of ion exchange for each cation was calculated as $(x/\Sigma) \cdot 100$ where x represents the amount of cation (Cu, Zn or Co) in an exchangeable position and Σ is the sum of the exchangeable cations in the starting sample (clinoptilolite tuff or montmorillonite). For the different cationic forms of montmorillonite, the degree of ion exchange was 76 % for Cu and 85 % for Zn and Co. For the different forms of clinoptilolite, the degree of ion exchange was 12 % for Cu, 10 % for Co and 8 % for Zn. The obtained cation-exchanged clinoptilolites and montmorillonites were used for AFB₁ adsorption.

Adsorption of aflatoxin B₁

Aflatoxin B₁ (AFB₁) was obtained from Sigma Co. The solution from which the adsorption was to be examined was chosen to simulate the gastric juice of animals (electrolyte). It contained: 0.1 mol/dm³ HCl and 0.05 mol/dm³ NaCl. The content of AFB₁ was determined in the AFB₁ containing electrolyte both without and with a mineral adsorbent. The experiment was carried out at pH 3.8 and at a temperature of 37 °C. A certain amount of AFB₁ (200 µg) was added to 100 cm³ electrolyte and an aliquot (0.4 cm³) was taken for the determination of the total toxin concentration present in the solution (c_t). Then, 1 g of mineral adsorbent was added to the contaminated electrolyte solution. At the end of the reaction time (2 h), the concentration of non-adsorbed AFB₁ was determined in the supernatant (c_f). The total and non-adsorbed concentrations of AFB₁ were determined, after chloroform extraction, by the HPLC method. The chromatographic analysis was performed on a Bio-Sil C18 HL Column (250 × 4.6 mm; 5 µm particle size) using a LKB Broma Chromatograph, Model 215 HPLC Pump with a RHEODINE 7125 injector.

Reduction of the microelements

To satisfy the physiological needs of the animals, cattle fodder must contain 10 ppm Cu, 100 ppm Zn, 80 ppm Mn and 0.8 ppm Co. At the same time, for the prevention of mycotoxicosis, about 0.5 % of a mineral adsorbent should be added. In this way, a certain ratio between mineral adsorbent–microelement is achieved requiring the determination of any possible reduction in the amounts of the available microelements present.

The following procedure was used: A certain amount of each microelement: Cu–2 mg, Zn–20 mg, Mn–16 mg and Co–0.16 mg was added to 100 cm³ of the electrolyte and an aliquot was taken for the determination of the starting concentrations of the cations present in the solution (c_g). Then, 1 g of mineral adsorbent was added to the electrolyte. After gentle shaking for 2 h, the concentrations of the non-exchanged microelements were determined in the supernatant (c_f). The total and non-exchanged concentrations of the microelements were determined by atomic absorption spectrophotometry using a Perkin Elmer, Model 703 instrument.

RESULTS AND DISCUSSION

The chromatograms of AFB1 (200 µg) determined in the electrolyte without (1) and with different ion exchanged clinoptilolites (2–6) are presented in Figure 1, while

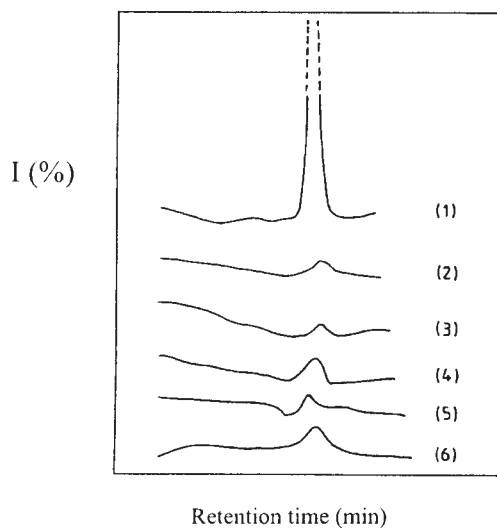


Fig. 1. Adsorption of AFB1 by different forms of clinoptilolite (cli): (1) without adsorbent; (2) with Ca-rich cli; (3) with Na-exchanged cli; (4) with Cu-exchanged cli; (5) with Zn-exchanged cli; (6) with Co-exchanged cli.

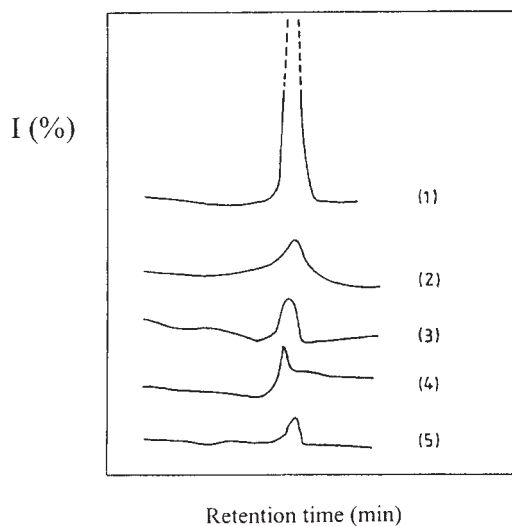


Fig. 2. Adsorption of AFB1 by different cation exchanged montmorillonites (mont): (1) without adsorbent; (2) with Ca-rich mont; (3) with Cu-exchanged mont; (4) with Zn-exchanged mont; (5) Co-exchanged mont.

similar chromatograms without (1) and with different ion exchanged forms of montmorillonites (2–5) are presented in Fig. 2.

The chemisorption index⁴ for the different mineral adsorbents was calculated as:

$$c_{\alpha} = \frac{c_t - c_f}{c_t}$$

where c_t = total concentration of AFB1 and c_f = the concentration of non-adsorbed AFB1; $c_{\alpha} = 1$ represents total binding of AFB1.

The determined AFB1 chemisorption indexes (c_{α}) for the different ion exchanged clinoptilolites and montmorillonites are presented in Table II.

TABLE II. Aflatoxin B₁ chemisorption indexes (c_{α}) for different cation exchanged forms of clinoptilolite and montmorillonite

	c_{α}	
	Clinoptilolite	Montmorillonite
Ca-rich (natural)	0.90	0.89
Na-exchanged	0.93	–
Cu-exchanged	0.90	0.75
Zn-exchanged	0.94	0.90
Co-exchanged	0.95	0.93

For all the used ion exchanged mineral adsorbents, high adsorption indexes were found. These results show that both groups of minerals have a high efficiency for *in vitro* binding of AFB1. The chemisorption indexes for different cation exchanged montmorillonites were between 0.75 for the Cu-exchanged form and as high as 0.93 for the Co-exchanged montmorillonite. All the clinoptilolite materials had greater adsorption values ($c_{\alpha} \geq 0.90$) than the different forms of montmorillonite. The Ca-rich and Cu-exchanged clinoptilolite showed chemisorption indexes of 0.90, the Na-exchanged clinoptilolite 0.93 and Co-exchanged clinoptilolite 0.95.

The Cu-exchanged form of montmorillonite had the lowest binding affinity for AFB1 ($c_{\alpha} = 0.75$), but the Co-forms of both minerals were the best (c_{α} from 0.93 to 0.95).

The presented *in vitro* data clearly demonstrate that all of the used mineral adsorbents greatly diminished the toxicity of AFB1 at a concentration of 200 µg per g of adsorbent. This value corresponds to 1 mg/kg of AFB1 in the diet, if the adsorbent is incorporated into the diet at a level of 0.5 %. Considering the toxicity of AFB1, most countries allow a concentration in animal feed of no more than 20 ppb (0.020 mg/kg).

The results of the reduction of the concentrations of the microelements (Cu, Zn, Co and Mn) by cation-exchange on Ca-rich clinoptilolite and Ca-rich montmorillonite, at pH 3.8 (the same electrolyte as for the AFB1 adsorption) are presented in Table III. The initial microelement concentration (milligram per gram of adsorbent) (c_i) was obtained based on the addition of an adsorbent to the diet at the 5 g/kg level.

TABLE III. The chemisorption indexes (c_{α}) of the microelements (Cu, Zn, Mn, Co) on Ca-rich clinoptilolite and Ca-rich montmorillonite

Micro element	c_1 /(mg/g)	c_{α}	
		Ca-rich clinoptilolite	Ca-rich montmorillonite
Cu	2	0.23	0.80
Zn	20	0	0.18
Co	0.16	0.10	0.87
Mn	16	0.03	0.15

The results presented in Table III suggest that Cu and Co showed high chemisorption indexes, $c_{\alpha} \geq 0.80$, for Ca-rich montmorillonite, but for Ca-rich clinoptilolite tuff $c_{\alpha} \leq 0.20$. The chemisorption indexes for Zn and Mn on both minerals were ≤ 0.20 .

Examination of the impact of the mineral materials on the microelements clearly show a greater reduction of the concentrations of the microelements by montmorillonite materials than by clinoptilolite materials. The results indicate that Cu and Co are highly adsorbed by Ca-rich montmorillonite ($c_{\alpha} \geq 0.80$), whereas on Ca-rich clinoptilolite, copper has a chemisorption index of 0.23 and cobalt a value of 0.10. Zinc and manganese were not bound as strongly on montmorillonite as Cu and Co ($c_{\alpha} \leq 0.20$). Also, Zn and Mn showed negligible adsorption by clinoptilolite materials; for Mn $c_{\alpha} = 0.03$. Clearly Ca-rich montmorillonite has a greater capability of reducing the amounts of microelements in animal feed than Ca-rich clinoptilolite.

CONCLUSION

The obtained results show that different exchanged forms of clinoptilolites and montmorillonites adsorbed substantial amount of aflatoxin B₁. Generally, the different exchanged forms of clinoptilolite have higher aflatoxin B₁ chemisorption indexes than the same forms of montmorillonite. For both minerals, the Co-exchanged forms showed the greatest adsorption and the Cu-exchanged form of montmorillonite the lowest. The results of microelement reduction suggest that the Ca-rich montmorillonite material would greatly reduce the amount of micronutrient present in the animal feed, compared with Ca-rich clinoptilolite.

ИЗВОД

УТИЦАЈ ВРСТЕ ИЗМЕНЉИВОГ КАТЈОНА КОД КЛИНОПТИЛОЛИТА И МОНТМОРИЛЛОНИТА НА АДОРПЦИЈУ АФЛАТОКСИНА В₁

МАГДАЛЕНА ТОМАШЕВИЋ-ЧАНОВИЋ, АЛЕКСАНДРА ДАКОВИЋ, ВЕСНА МАРКОВИЋ И ДРАГАН СТОЛШИЋ¹

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У овом раду је испитивана адсорпција афлатоксина В₁ на различитим облицима клиноптилолита и монтморилонита на 37 °С и рН 3–8 у воденом раствору електролита.

Полазни (Са) облици клиноптилолита и монтморилонита су прво преведени у Na-облик а затим модификовани јонима бабра, цинка и кобалта. Степен јонске измене код монтморилонита износи 76 % за Cu и 85 % за Zn и Co. Под истим условима, код клиноптилолита је постигнут нижи степен измене и то: 12 % за Cu, 8 % за Zn и 10 % за Co. Испитивања адсорпције афлатоксина B₁ су показала да обе групе минерала ефикасно адсорбују AFB₁. Код монтморилонита постигнут индекс адсорпције овог токсина износи 0,75 за Cu-монтморилонит, 0,89 за Ca-монтморилонит, 0,90 за Zn-монтморилонит и 0,93 за Co-монтморилонит. На различитим облицима клиноптилолита су постигнуте нешто виши индекси адсорпције: 0,90 за Ca-и Cu-клиноптилолит, 0,94 за Zn- и 0,95 за Co-клиноптилолит. Испитивања утицаја минералних адсорбената (Ca-клиноптилолит и Ca-монтморилонит) на редукцију микроелемената (Cu, Zn, Co и Mn) неопходних за животињску исхрану су показала да монтморилонит ефикасније адсорбује микроелементе него клиноптилолит.

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