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Kinetics of aflatoxin B_1 and G_2 adsorption on Ca-clinoptilolite

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The kinetics of aflatoxins B_1 and G_2 adsorption on Ca-clinoptilolite at pH 2 and 7, in aqueous electrolyte at 37 ºC were studied. For both aflatoxins, the adsorption process begins with a fast reaction whereby most of the toxin is adsorbed in the first few minutes. This fast process is followed by the significantly slower process of aflatoxin bonding at active centers of mineral adsorbent. The initial rate method showed that the fast adsorption process of aflatoxin B_1 and G_2, at both pH values is a first order reaction, while the slow adsorption process of these aflatoxins is a zero order reaction. The adsorption indexes and adsorption rates for both examined toxins were pH dependent. In the investigated initial toxins concentration ranges (500–3000 μg/dm^3), high adsorption indexes were achieved (> 80 %).

Keywords: clinoptilolite, adsorption, aflatoxin B_1, aflatoxin G_2.

INTRODUCTION

The aflatoxins form a group of naturally occurring fungal elaborated poisons. *Aspergillus* fungi frequently produce the aflatoxins B_1, B_2, G_1 and G_2. They commonly contaminate foodstuffs such as: peanut, peanut products, rice, wheat, corn and cereals.[1] These fungal metabolites are toxic when consumed by animals, including human beings. Aflatoxins are known as hepatotoxins and very strong mutagens, cancerogens and teratogens.[2–5]

The B series of aflatoxins contains the β-ketolactone and the G series contains the α-bislactone functionality. Also, the aflatoxins B_1 and G_1 have a 2,3 double bound in the form of a vinyl ether at the terminal furan ring. Aflatoxin B_1 is the most toxic and cancerogenic of all aflatoxins.[6] It is believed that during the complex enzymatic reactions accompanying metabolism and detoxification of aflatoxin B_1, a highly reactive intermediate 2,3-epoxide is generated. This reactive molecule might covalently binds to various nucleophilic centers in cellular macromolecules such as DNA,

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RNA and proteins, which could lead to mutagenesis or carcinogenesis.[7–9]

There are many methods for detoxification of aflatoxin contaminated crops. They include physical separation, thermal inactivation, microbial degradation and treatment with different chemicals. Many of these methods are, however, impractical, ineffective and potentially unsafe.

Mineral adsorbents based on zeolites, silicates and phylosilicates show different abilities to bind *in vitro* aflatoxins from aqueous solutions.[10] These mineral adsorbents, depending on type, posses active sites which can be located within the interlayer channels, at basal planes on the surfaces or within pores and at the edges of particles. The adsorption process is very dependent on the type of exchangeable cations. Phillips *et al.* reported that HSCAS (hydrated sodium calcium alumosilicate) might bind aflatoxins *in vitro* forming highly stable complexes.[10–12] Those authors also suggested that HSCAS has a preference for compounds containing a β-ketolactone or an α-bislactone functionality (aflatoxins B and G series). The HSCAS/aflatoxin complex was stable in water at pH 2, 7 and 10 and at temperatures of 25 ºC and 37 ºC. The proposed mechanism of aflatoxin chemisorption by the mineral adsorbent is a rapid complex formation between the ligand and mineral. These *in vitro* investigations considered aflatoxin adsorption at equilibrium (the adsorption process is usually almost complete after 30 min adsorbent/toxin contact time). Tomašević-Čanović *et al.* showed[13] that three different types of clinoptilolite (Ca^{2+}, Na^{+} and NH_4^{+}) adsorbed mycotoxins from aqueous electrolytes to different degrees. Aflatoxin B_2 was adsorbed on all types of clinoptilolite with a high efficiency (over 80 %).

In the literature, no values of aflatoxins adsorption rates at the active centers of mineral adsorbent were found. These data are very important for the practical application of mineral adsorbents.

In this paper, the kinetics of aflatoxins B_1 and G_2 adsorption on a mineral adsorbent based on natural zeolite (Ca-clinoptilolite), from aqueous electrolytes at pH 2 and pH 7 and at a temperature of 37 ºC were studied. The aim of this work was to determine the rates at which the adsorption process of aflatoxin on the active centers of the mineral adsorbent occurs . Also, the pH dependence of these adsorption processes and the influence of the medium pH on the kinetics of aflatoxin binding on the active centers of the mineral adsorbent were investigated.

Many *in vivo* experiments have shown that using mineral adsorbent in animal feed can block and immobilize aflatoxins in the gastrointestinal tracts of animals.[10–12,14–17]

EXPERIMENTAL

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations that possess infinite, three-dimensional crystal structures. They are characterized by their abilities to hydrate and dehydrate reversibly and to exchange some of their constituent cations, both without major structural changes. The zeolites consist of three-dimensional frameworks of SiO_4^{4-} tetrahedra, wherein all the O's of each tetrahedron are shared with adjacent tetrahedra. In zeolites, some of the quadrivalent Si is replaced by trivalent Al, giving rise to a deficiency of positive charge in the framework. This

charge is balanced by monovalent and divalent cations, usually Na^+, Ca^{2+}, K^+ and Mg^{2+}, elsewhere in the structure.[18,19]

The starting material used in these experiments was natural zeolite from the Zlatokop deposit near Vranjska Banja (Yugoslavia). The sample was initially ground to 100 % below 63 μm. The mineralogical determination was made by XRPD analysis. The content of clinoptilolite was over 90 % with quartz and feldspate as impurities.[20] The chemical composition of the clinoptilolite is given in Table I.

TABLE I. Chemical composition of the Ca-clinoptilolite

Component	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	CaO	MgO	Na_2O	K_2O	H_2O
Content/%	64.21	11.48	0.88	0.25	0.03	4.55	1.45	1.71	1.29	14.00
Content mol/100 g	1.07	0.12	0.006	0.003	0.0004	0.08	0.04	0.03	0.01	0.78

According to the results of the chemical analysis, the calculated Si : Al ratio is 4.81. This ratio is characteristic for clinoptilolite. The calculated unit cell composition of the clinoptilolite is:

$$Ca_{2.23}Mg_{0.98}Na_{1.52}K_{0.73}(Al_{16.09}Si_{29.28}O_{72}).23.79\ H_2O$$

The cation exchange capacity[21] of the clinoptilolite was 146 meq/100 g. The dominant ion in exchangeable positions was Ca^{2+} ion.

The kinetics of aflatoxin B_1 and G_2 adsorption were studied on Ca-clinoptilolite at pH 2 and pH 7, in time interval from 5 min to 48 h at a temperature of 37 ºC. The electrolyte used in this study had a composition similar to that of the gastric juice of animals. The electrolyte contained 0.1 mol/dm^3 HCl and 0.05 mol/dm^3 NaCl. The pH of electrolyte was adjusted with 0.1 mol/dm^3 NaOH.[22]

The aflatoxin B_1 and G_2,produced by Sigma Chemical Co, were used as received.

The content of aflatoxin B_1 of G_2 was determined in the electrolyte both without and with the mineral adsorbent. A certain amount of each toxin (500, 1000, 2000 and 3000 μg/dm^3) was added to 100 cm^3 electrolyte and an aliquot (0.4 cm^3) was taken for the determination of the total toxin concentration present in solution (c_t). Then 1 g of mineral adsorbent was added to the contaminated electrolyte solution. After a certain reaction time, the concentration of non-adsorbed toxin in the supernatant was determined (c_f). The total and non-adsorbed concentrations of aflatoxin B_1 or G_2 were determined, after chloroform extraction, by HPLC. The chromatographic analysis was performed using an LKB Broma Model 215 HPLC pump with a RHEODINE 7125 injector and a Bio-Sil C18 HL 90-5 S Column (250×4.6 mm, 5 μm particle size). Detection was with a UV detector LKB Broma 2140 Rapid Spectral Detector (λ = 365 nm). The mobile phase was ACN : MeOH : H_2O (11 : 26 : 63) and the flow rate of the mobile phase was 1 ml/min.

RESULTS AND DISCUSSION

This study was performed to determine the efficiency of a mineral adsorbent based on natural zeolite (Ca-clinoptilolite) to bind aflatoxins B_1 and G_2 *in vitro* at different pH values, as well as the aflatoxins adsorption rates.

The toxicity of aflatoxins is already well known.[23] The important characteristic for the practical application of Ca-clinoptilolite to diminish the adverse effects of aflatoxins is the adsorption index, which is defined as the ratio between the adsorbed ($c_t - c_f$) and total (c_t) toxin concentration. The determined adsorption indexes in the ivestigated aflatoxins concentrations range (500–3000 μg/dm^3), at pH 2 and pH 7, after 48 h contact time, are presented in Table II.

TABLE II. AFB_1 and AFG_2 adsorption indexes at pH 2 and at pH 7 on Ca-clinoptilolite. Contact time 48 h

Contact time h	Adsorption index/%															
	500 μg/dm³				1000 μg/dm³				2000 μg/dm³				3000 μg/dm³			
	pH 2		pH 7		pH 2		pH 7		pH 2		pH 7		pH 2		pH 7	
	B_1	G_2	B_1	G_2	B_1	G_2	B_1	G_2	B_1	G_2	B_1	G_2	B_1	G_2	B_1	G_2
48	90	90	96	86	87	91	94	84	86	91	93	85	87	93	95	89

It can be seen, from the results presented in Table II that, for both aflatoxins, high adsorption indexes (> 80 %) were achieved both at pH 2 as well as at pH 7. In the investigated concentrations range, after 48 h contact time, the aflatoxin B_1 adsorption index was highest at pH 7, while aflatoxin G_2 was adsorbed better at pH 2.

Phillips *et al.,* using HSCAS (hydrated sodium calcium alumosilicate) as adsorbent for aflatoxin B_1, performed similar *in vitro* investigations. The specific

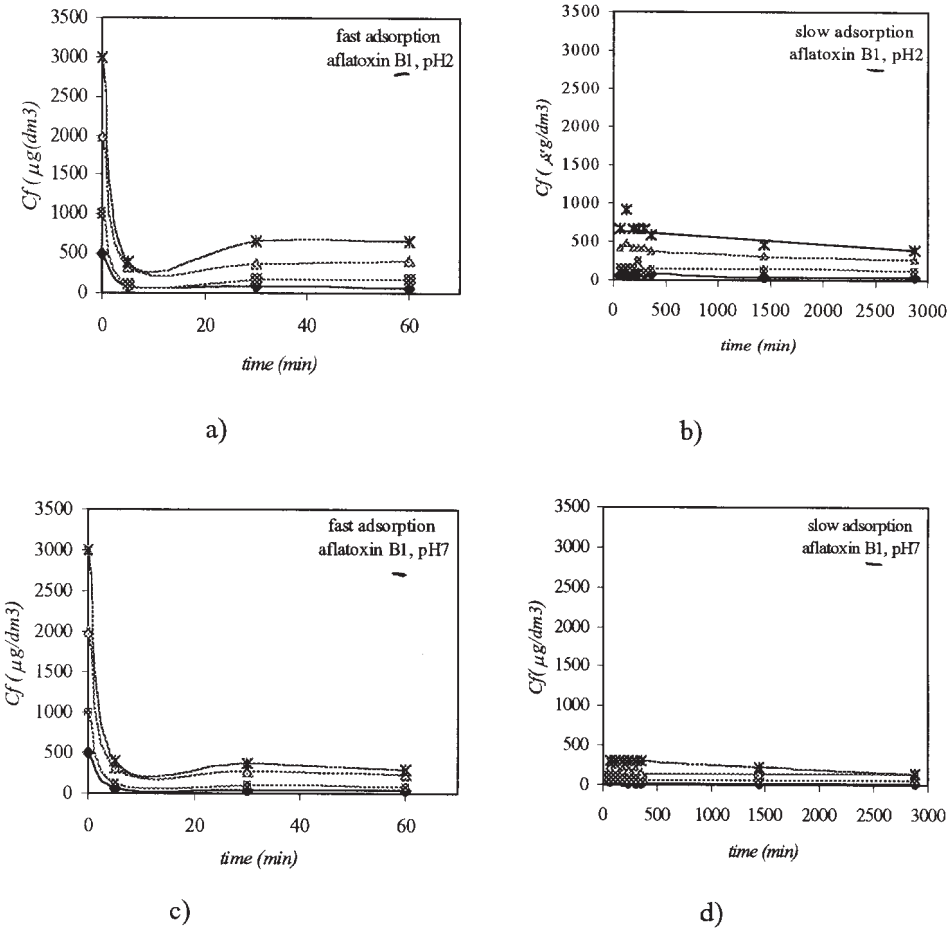

Fig. 1. The kinetic curves for aflatoxin B_1 adsorption at pH 2 (a,b) and pH 7 (c,d).

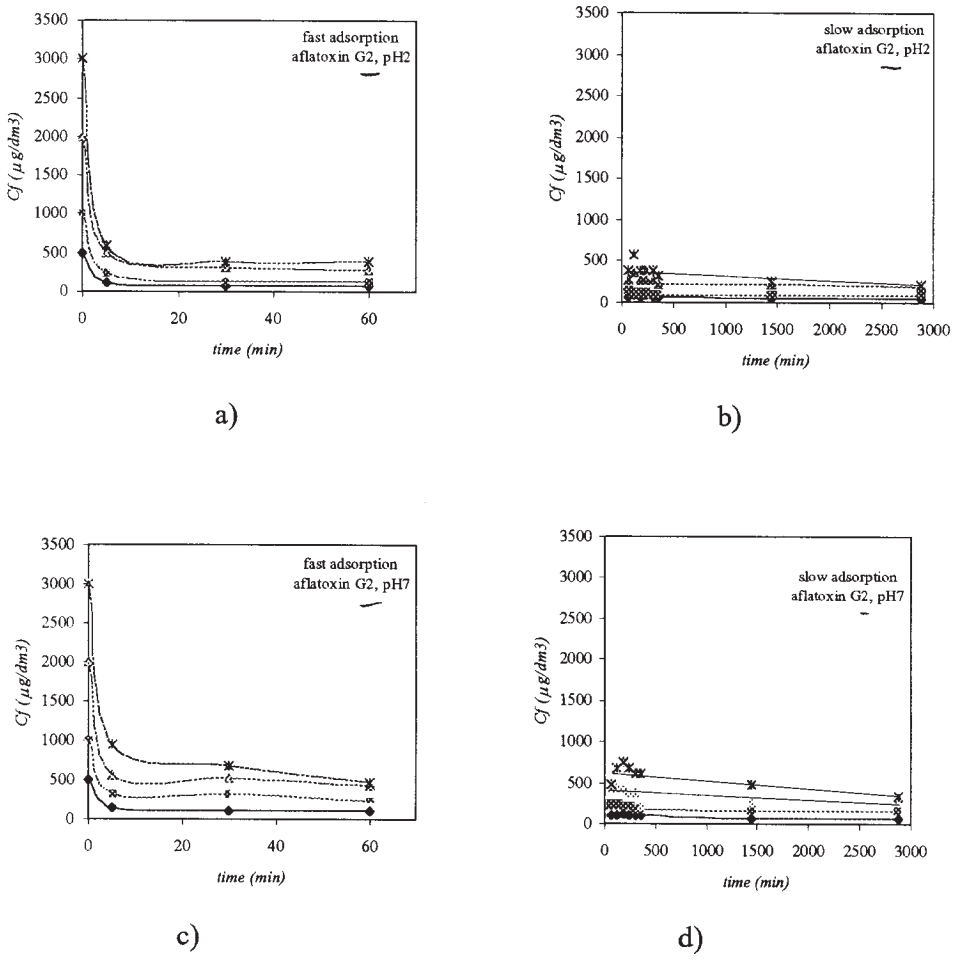

Fig. 2. The kinetic curves for aflatoxin G_2 adsorption at pH 2 (a,b) and pH 7 (c,d).

HSCAS had a high efficiency to bind aflatoxins at pH 2, 7 and 10.[10–12] These *in vitro* data are consistent with our results on Ca-clinoptilolite.

In vivo experiments have shown that the addition of HSCAS in a concentration of 0.5 % of the diet significantly diminished the toxicity of aflatoxins in chickens,[24] turkeys,[25] *etc*. When clinoptilolite was incorporated in the diets of laying hens neither the remainders of zeolite nor mycotoxins were found in samples of red and white meat, liver and eggs, although the concentration of aflatoxin G_2 and ohratoxin were fortified in the diets.[26] Also, the addition of clinoptilolite in the diet of growing broilers improved the body weight by 1.5 and 1.7 %.[27]

The rates and the order of the aflatoxins adsorption reaction were calculated from the kinetic curves. The kinetic curves for aflatoxin B_1 and G_2 adsorption on Ca-clinoptilolite at pH 2 and 7 are presented in Figs. 1 and 2. These results show

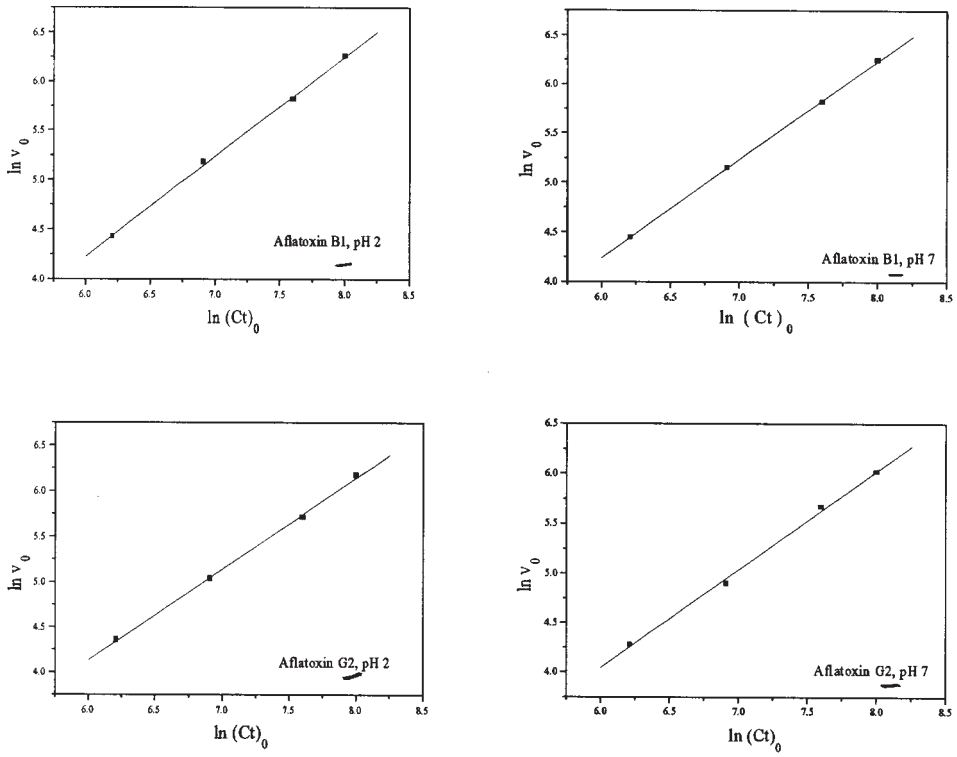

Fig. 3. The initial rate method for the fast adsorption of aflatoxin B_1 and G_2 at pH 2 and pH 7.

that, for both toxins, the adsorption process begins with a fast reaction and that most of the toxin is adsorbed in first few minutes. The fast adsorption process for both the examined toxins is followed by a significantly slower process. The slow adsorption was studied in the time interval from 1 h to 48 h.

The rate constants (k) and the order (n) of the fast adsorption process were determined by the initial rate method.[28] The initial rates of adsorption for different total toxin concentrations were calculated from:

$$(\upsilon_0)_i = -\mathrm{d}(c_{t,0})_i / \mathrm{d}t = k(c_{t,0})_i^n$$

Putting this equation in the logarithmic form yields:

$$\ln (\upsilon_0)_i = \ln k + n \ln (c_{t,0})_i$$

When the $\ln \upsilon_0$ values are plotted against the $\ln (c_{t,0})$ values, a straight line is obtained with a slope equal to n and intercept equal to $\ln k$ (Fig. 3). This method

showed that the fast adsorption of aflatoxin B_1 and G_2 is a first order reaction at pH 2 and 7.

The calculated adsorption rate constants for the fast aflatoxin B_1 and G_2 adsorption reaction are presented in Table III.

TABLE III. Rate constants of the fast aflatoxins adsorption processes

	k /min^{-1}	
	pH 2	pH 7
Aflatoxin B_1	0.16	0.17
Aflatoxin G_2	0.15	0.15

As can be seen from the results presented in Table III, the fast adsorption process of aflatoxin G_2 is independent on pH and is slightly slower than the fast adsorption of aflatoxin B_1. The adsorption rate constants for aflatoxin B_1 are insignificantly different at the two different pH values. The rate constant value for aflatoxin B_1 is slightly greater at pH 7. Bearing in mind the complexity of the possible reaction mechanism of aflatoxin B_1, it is possible that changes of medium pH leads to the formation of different toxin intermediates that could be more efficiently adsorbed at pH 7.

During the slow adsorption process a continuous decrease in the aflatoxin B_1 or G_2 concentration in the supernatant can be observed at pH 2 and 7. This part of the kinetic curve for the time interval 60 min to 48 h can be represented as a straight line implying that the slow adsorption process is a zero order reaction. The slope of the curve $c_f = f(t)$ equals the rate constant of this slow process. The rate constants for the slow aflatoxin B_1 and aflatoxin G_2 adsorption at different pH were calculated for an initial toxin concentration of 3000 μg/dm^3. The results are presented in Table IV.

TABLE IV. Rate constants of the slow aflatoxins adsorption processes: c_t = 3000 μg/dm^3

	k/μg dm^{-3} min^{-1}	
	pH 2	pH 7
Aflatoxin B_1	0.12704	0.05685
Aflatoxin G_2	0.08138	0.10931

Concerning the slow adsorption process, the influence of pH is obvious for both examined toxins. At sufficiently long contact times, differences in the rate constants are visible for aflatoxin B_1, as well as for aflatoxin G_2. Comparing the rate constant values for both toxins at different pH values, it can be seen that aflatoxin B_1 is adsorbed faster at pH 2, while aflatoxin G_2 is bonded faster at pH 7. It is possible, that the slow adsorption process occurs by the formation of various toxin intermediate species, which are then bound to the active centers of the clinoptilolite. Also, a slight dealumination of the zeolite at pH 2 might give rise to the faster aflatoxin B_1 adsorption. On the contrary, the dealumination slowed down the adsorption of aflatoxin G_2. It is possible, that the dealumination leads to the formation of new centers in the clinoptilolite onto which aflatoxin B_1 adsorption is much faster than the adsorption of aflatoxin G_2.

The continual increase of aflatoxin B_1 and G_2 adsorption on the Ca-clinoptilolite, at different pH values, over the entire reaction time, indicates that at these initial toxin concentratios (500, 1000, 2000 and 3000 μg/dm^3) saturation of all the active centers of the mineral adsorbent is not achieved.

CONCLUSION

The obtained results are very important for the practical application of Ca-clinoptilolite. This mineral adsorbent, based on natural zeolite, may be used for detoxification of fodder contaminated with aflatoxins. The kinetics of aflatoxin B_1 and G_2 adsorption showed that the aflatoxin adsorption is very fast and that most of the toxin is bonded in first few minutes of adsorbent/toxin contact. This means that poisoning will probably not occur because of its prevention by the fast adsorption rate. Also, the adsorption process of these toxins occur without desorption.

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ИЗВОД

КИНЕТИКА АДСОРПЦИЈЕ АФЛАТОКСИНА B_1 И G_2 НА Ca-КЛИНОПТИЛОЛИТУ

АЛЕКСАНДРА ДАКОВИЋ*, МАГДАЛЕНА ТОМАШЕВИЋ-ЧАНОВИЋ, ВЕРА ДОНДУР[1], АЛЕКСАНДРА ВУЈАКОВИЋ и ПРЕДРАГ РАДОШЕВИЋ

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Праћена је кинетика адсорпције афлатоксина B_1 и G_2 на Ca-клиноптилолиту, у воденом раствору електролита, на pH 2 и pH 7 и на температури 37 °C. Добијени резултати показују да процес адсорпције оба токсина започиње брзом реакцијом и да се највећи део токсина адсорбује у првих неколико минута. После овог брзог процеса следи знатно спорији процес у коме се наставља везивање токсина за активне центре минералног адсорбента. Показано је методом почетних брзина да је брзи процес адсорпције оба токсина реакција првог реда, док је спори процес реакција нултог реда. Индекси адсорпције и брзине адсорпције зависе од pH средине. У испитиваном опсегу концентрација (500–3000 μg/dm^3) постигнути су високи индекси адсорпције како афлатоксина B_1 тако и афлатоксина G_2 (> 80 %).

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