IN VITRO REMOVING OF MYCOTOXINS BY USING DIFFERENT INORGANIC ADSORBENTS AND ORGANIC WASTE MATERIALS FROM SERBIA

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ABSTRACT: Aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZON), deoxynivalenol (DON) and T-2 toxin are the most extensively studied toxic fungal metabolites. Once mycotoxins enter the food/feed production chain keeping their toxic characteristics, it is very difficult to remove or eliminate them. One of promising methods to reduce mycotoxins in contaminated food/feedstuffs is the use of mycotoxin binders. This paper presents the results of *in vitro* investigations of mineral mycotoxin binders (bentonite - BEN, diatomite – DIA and zeolite - ZEO), and organic mycotoxin binders - agricultural waste materials (*Myriophillium spicatum*, peach and sour cherry pits). Chemical compositions of the adsorbents have showed that they do not consist of elements toxic to the animals. Inorganic adsorbents (BEN, DIA and ZEO) tested *in vitro* were better binders of AFB1 (94.97% - 96.90%), while the biosorbents were more efficient in adsorption of OTA (19.98% - 66.66%), ZON (33.33% - 75.00%) and T-2 toxin (16.67% - 50.00%). Inorganic adsorbents and organic waste materials expressed similar binding capacity for DON *in vitro*, with the exception of *M. spicatum* that did not at all adsorb this type B trichothecene. Our results indicate that feed contamination with different types of mycotoxins might be diminished by a product that combines different inorganic and organic adsorbents with diverse mycotoxin binding properties.

Key words: *mineral adsorbents, biosorbents, mycotoxins, in vitro binding*

INTRODUCTION

Mycotoxins, secondary metabolites of filamentous fungi, are considered to be a major risk factor affecting human and animal health. They are small and stable molecules with different chemical structures that have diverse biological effects, such as mutagenicity, cancerogenicity, teratogenicity, oestrogenicity, neurotoxicity, immuno-modulation, etc. Scientists have already identified about 400 toxic metabolites biosynthesized by about 100 fungal species. Among them, most extensively investigated are mycotoxins, such as aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZON), deoxynivalenol (DON), T-2 toxin (T-2) and fumonisins (FB_1 and FB_2). It is extremely difficult to remove or eliminate these substances when they enter the feed production chain while keeping their toxic pro-

perties (Kolosova and Stroka, 2011).

Although there is a lot of innovative strategies for the reduction of mycotoxins in food/feed (Shanakhat et al., 2018) one of the most common approaches to their detoxification includes the use of diverse, non-nutritive mycotoxin binders, i.e. sorbent materials.

These agents are supposed to detoxify feedstuffs as they pass through the digestive system of animals by adsorbing and/or degrading mycotoxins selectively under pH, temperature and moist conditions in the digestive system (Döll and Dänicke, 2004). This mycotoxin-binder complex is eliminated by means of feces (Devreese et al., 2013). These additives reduce mycotoxin uptake and subsequent distribution to blood and target organs.

Adsorbent efficacy depends on the properties of both the binder and the mycotoxin - physical structure of the bin-der (total charge and charge distribution, pore size, surface accessibility, etc.) and physical and chemical characteristics of the mycotoxin (polarity, solubility, molecular size, shape charge distribution, etc.) (Jard et al., 2011; Di Gregorio et al., 2014). In the Commission Regulation No 386/2009/EC, a new functional group of feed additives was established, including products for suppression and reduction of mycotoxins adsorption, promotion of their excretion or modification of their mode of action.

SUBSTANCES FOR REDUCTION OF MYCOTOXIN CONTAMINATION

Mineral adsorbents

Some of the most common feed additives are aluminosilicate minerals, such as bentonites, diatomaceous earth and zeolite materials.

Bentonites (BENs) are hydrated aluminasilicates of volcanic origin. The impure clay consists of minerals from the smectite group, mostly of montmorillonite (50-90%). The crystal structure of these mineral adsorbents is composed of SiO_2 tetrahedrons and Al_2O_3 octahedrons, linked to build three-layer plates with a negative charge, while the edges of the lamellae have positive charges. In the presence of water, lamellae are separated and their volume is increased. In the feed industry, BENs are used in the pelleting process since they increase the hardness and toughness of pellets. It is well known that they can adsorb some mycotoxins (Huwig et al., 2001), as well as radionuclides, toxic metals and ammonia (Adamović et al., 2009).

Diatomite (DIA) is sediment formed in lacustrine and marine environments. It is composed of very small (from 0.01 to 0.4 mm) shells of silicon, unicellular algae (*Diatomeae*), whose number amounts to 10-30 millions in cm^3. DIA has small mass (0.5-0.8 g/cm^3) and high porosity.

Due to a high content of silicon dioxide, this adsorbent has large porosity and, consequently, a high adsorption capacity. DIA is used as a component for the production of certain mycotoxin adsorbents (Whitlow, 2006), for remediation of diarrhoea in animals and uranyl ion adsorption (Adamović et al., 2011).

Zeolites (ZEOs) are hydrated aluminasilicates of alkaline and alkaline earth metal ions, which possess an infinite three-dimensional crystal structure. Natural ZEOs are characterized by their ability to lose or receive water and change cations without major changes of the structure.

A primary building unit in the structure of zeolites is a tetrahedron, in the centre of which is the silicon or aluminium atom, while at the top there are oxygen atoms shared by two tetrahedra.

These minerals are rich in channels and cavities in one, two or three directions. The cations placed in the channels can be replaced with other metal ions.

The ability of natural ZEO with a high proportion of clinoptilolite (over 80%) to adsorb (more or less successfully) specific mycotoxins on its negatively charged surface is based on this fact (Tomašević-Čanović et al., 2003). This mineral adsorbent can also bind radionuclides, toxic metals and ammonia (Adamović et al., 2003 and 2011).

Biosorbents

Biosorption has proved to be an efficient, low cost and sustainable treatment with cheap and abundant biomaterials, usually declared as waste. These organic materials are used for removing heavy metals l., 2012a and 2012b; Lopičić et al., 2013a and 2013b).

Biosorbents can usually be: primary agricultural waste (straw, chaff, husk, corn cobs, pea pods, etc.), by-products of the food industry (sugar beet pulp, brewery spent grain, seeds and pulp of fruits, shell walnuts, hazelnuts, almonds, coconuts, etc.) or wood industry (bark, sawdust, wood chips, leaves, pine needles, moss, etc.).

Agricultural waste materials mostly consist of cellulose, lignin, hemicellulose, pectin, lipids and other organic compounds that are rich in differrent functional groups, responsible for binding of pollutants. Biosorbents also have a multilayer, porous structure, filled with ca-vities and channels that provide a huge volume per unit of sorbent surface area, which is favourable in the process of bio-sorption. The average pore diameter of less than 1 µm might be beneficial for diffusion and adsorption of mycotoxins (Hubbe et al., 2011). The mechanisms underlying the biosorption process are single or multiple ion exchange, building complexes, adsorption, electrostatic interaction, deposition and building chelates.

There are many different types of modifications that can be used for improving adsorption capacities of biomaterials, such as physical, chemical, thermal or combined treatments. In most cases, chemical modification of cellulosic materials improves the absorbing capacity of the materials (Sun, 2010). Acid pre-treatment that removes some soluble organic impurities also changes the structure of functional cell compounds and results in much more binding sites.

Table 1.
Chemical composition (% w/w) of the investigated mineral adsorbents (Bočarov Stančić et al., 2011)

Ingredient	Adsorbent		
	Bentonite	**Diatomite**	**Zeolite**
SiO_2	48.48	79.79	65.69
Al_2O	22.39	9.41	14.03
CaO	5.86	0.63	3.57
Fe_2O_3	4.73	1.11	2.34
MgO	1.71	0.14	1.09
K_2O	0.40	0.79	1.39
Na_2O	0.07	0.08	1.41
TiO_2	0.34	0.21	0.17
Loss by ignition	16.02	7.84	10.29
CEC mEq/100 g	141.23	42.75	142.24

CEC – cation enhance capacity

Table 2.
Chemical composition (% w/w) of tested unmodified biosorbents (Milojković et al., 2012; Lopičić et al., 2013b)

Parameter	Biosorbent		
	M. spicatum	**Peach pits**	**Sour cherry pits**
Crude protein	17.95	1.26	1.48
Crude fat	1.28	0.05	0.04
Crude cellulose	23.33	58.05	54.74
Ash	17.64	0.42	0.33
NFE	30.91	32.45	37.50
NDF	33.38	71.12	65.18
ADF	30.96	66.12	62.47
Dry matter	91.11	92.23	94.09
Moisture	8.98	7.77	5.91
Lignin	6.33	16.54	17.47

NFE-nitrogen free extracts, NDF-neutral detergent fiber, ADF-acid detergent fiber

PHYSICAL AND CHEMICAL CHARACTERISTICS OF INVESTIGATED ADSORBENTS

Mineral sorbents

Calcium bentonite was obtained from Šipovo, Bosnia and Herzegovina; diatomite was originated from the diatomite mine Kolubara - Lazarevac, in Baroševac, field "B", while zeolite (with over 80% of clinoptilolite) was obtained from Igroš, Kopaonik, Serbia.

The preparations of the samples were done at the Institute for Technology of Nuclear and Other Mineral Raw Materials in Belgrade, following the previously described procedures (Bočarov Stančić et al., 2011).

All three inorganic adsorbents had a high content of SiO_2 (48.48 to 79.79%) (Table 1). The highest content of SiO_2 was found in DIA. On the other hand, DIA had a significantly lower content of other cations, mainly Al_2O_3, CaO and MgO, which had a great impact on its cation exchange capacity (CEC) (Table 1). Despite differences in the chemical composition, the common trait of all the investigated mineral adsorbents was their porosity. Their cavities and channels spread in different directions, allowing them to exchange cations and thus to adsorb certain mycotoxins.

The particle size of all mineral adsorbents was below 63 μm. The smallest particles were found in DIA (95.45% of mass was below 12 μm). BEN had 75.00% of particle mass below 15 μm, while ZEO had 49.04 % of particle mass smaller than 13 μm. These data indicate that, to some extent, there was a difference in distribution of particle sizes.

Biosorbents

The biomass of aquatic weed *Myriophyllum spicatum* L. (MS) was obtained from Sava Lake (Belgrade), while agricultural waste materials - peach pits (PP) and sour cherry pits (CP), were obtained from "Vino Župa" company from Aleksandrovac, Serbia, where they had been disposed as a by-product waste from the juice factory. All biosorbents (unmodified and modified with hydrochloric acid) were prepared at

the Institute for Technology of Nuclear and Other Mineral Raw Materials in Belgrade, following the procedures described in detail in some previous papers (Milojković et al., 2012; Lopičić et al., 2013b). Only the hard part of the fruit pits was used in the experiments. The diameter of the particles was less than 100 μm.

The data presented in Table 2 indicate that the content of crude protein, fat and cellulose in *M. spicatum* was very similar to the content of the same substances in the leguminous plants from *Fabaceae* family (alfalfa - *Medicago sativa* L. and clover - *Trifolium repens* L.), grown in Serbian agricultural areas. These plants are often used as animal feed – fresh, dried (hay) or preserved (silage or haylage).

Peach and sour cherry pits (Table 2) had a very similar or approximate chemical composition to the composition of peanuts or sunflower husks. Both materials had an extremely small content of crude proteins (1.26 and 1.48%, respectively) as well as fat (0.04 and 0.05%, respectively). On the other hand, the content of crude cellulose, NDF (cellulose+hemicellulose+lignin) and ADF (cellulose+lignin) was the dominant one (amounted to > 54%). Lignin content was also rather high (amounted to > 16%). The presence of these three biological polymers (cellulose, lignin, and hemicellulose) makes peach pits rich in hydroxyl and phenol groups, which can be further chemically modified in order to produce adsorbent materials with improved adsorbing properties (Lopičić et al., 2013a and 2013b).

Peach and sour cherry pit particles contained several important micro- and macro elements, in the first place calcium (0.14% and 0.52%, respectively) and potassium (0.089% and 0.073%, respectively) (Lopičić et al., 2013a and 2013b). They did not consist of elements that could be toxic to living organisms. Thus they can be used in animal feedstuffs as energetic material. They can even be used as carriers of certain active substances or ingredients of complex mycotoxin-binding additives for food and animal feed, which at the same time can have double effect (fungistatic and bacteriostatic), as des-

cribed in the Patent Application Number US20120070516 (2012).

IN VITRO ADSORPTION OF MYCOTOXINS

In the *in vitro* adsorption experiments, crude extracts of following six mycotoxins were used : aflatoxin B_1 (AFB$_1$), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZON), diacetoxyscirpenol (DAS) and T-2 toxin (T-2) (Bočarov Stančić et al., 2007, 2009a, 2009b and 2010).

The efficacy of the investigated natural mineral adsorbents and biosorbents (unmodified and modified) for binding mycotoxins was evaluated in the electrolyte 0.1M K_2HPO_4, pH 3.0 and 6.9 or 7.0, respectively. These pH values were chosen having in mind the fact that pH in the major part of the digestive system of animals (stomach and intestines) is in the range of tested values, because mycotoxin-adsorbent complex should be stable to prevent desorption of the toxin during digestion. The mass ratio of individual mycotoxin and particular adsorbent was 1:5000. The experimental mixture was incubated for 1 hour on a rotary shaker (185 rpm) at room temperature (22-25 $^{\circ}$C). After the incubation, the extraction of non-adsorbed mycotoxin from the filtrate was performed with an organic solvent, and its quantification was done by thin-layer chromatography (TLC), as described in detail in the previous paper (Bočarov-Stančić, 2011).

The adsorption efficiency of the natural adsorbents was expressed as the adsorption index, where C_i is the initial and C_{eq} is the equilibrium concentration of a particular mycotoxin:

Adsorption index = $[(C_i - C_{eq})/C_i] * 100$.

Table 3.
Adsorption indices of investigated natural mineral adsorbents at different pH values (Bočarov Stančić et al., 2011 and 2012b)

Adsorbent	pH	Adsorption index of individual mycotoxins (%)				
		AFL	OTA	DON	ZON	T-2
Bentonite	3.0	96.90 ± 0.20	0	50.03 ± 0.86	37.03 ± 0.76	25.00 ± 0,42
	6.9	96.90 ± 1.44	0	0	24.97 ± 1.16	33.33 ± 0.73
Diatomite	3.0	95.00 ± 1,14	66.67± 1.27	24.97 ± 1.45	24.93 ± 1.43	33.33 ± 1.12
	6.9	94.97 ± 1.50	0	0	25.00 ±.60	33.33 ± 1.06
Zeolite	3.0	95.50 ± 0.65	0	49.98 ± 1.23	12.20 ± 2.05	0
	6.9	95.50 ± 0.92	0	0	12.30 ± 0.92	16.67 ± 0.92

Presented data are the average values of three independent determinations rounded to two decimal points ± standard deviation

Table 4.
Adsorption indices of investigated biological adsorbents at different pH values (Bočarov Stančić et al., 2012a; Lopičić et al., 2013a and 2013b; Milojković et al., 2012)

Biosorbent	pH	Adsorption index of individual mycotoxins (%)				
		AFL	OTA	DON	ZON	T-2
Myriophillum spicatum	3.0	95.97 ±0.57	49.97 ±1.67	0	70.00 ±1.43	16.67 ±0.75
	6.9	94.68 ± 1.18	30.00 ± 1.57	0	75.00 ± 0.99	33.33 ± 0.90
Peach pits (PP)	3.0	58.82 ± 0.88	42.85 ± 0.48	23.08 ± 1.56	50.00 ± 1.32	25.00 ± 0.88
	7.0	58.82 ± 0.85	33.32 ± 0.87	39.97 ± 1.05	33.33 ± 1.03	39.98 ± 1.00
Modified peach pits (MPP)	3.0	41.18 ± 0.92	42.85 ± 0.60	40.03 ± 1.17	33.33 ± 1.04	50.00 ± 0.98
	7.0	41.18 ± 1.12	33.32 ± 1.08	49.98 ± 1.24	58.34 ± 0.65	39.97 ± 1.22
Cherry pits (CP)	3.0	58.82 ± 1.30	66.66 ± 0.73	21.97 ± 1.52	33.33 ± 1.06	50.00 ± 1.16
	7.0	41.18 ± 1.18	19.98 ± 1.14	30.00 ± 1.27	33.33 ± 1.70	40.00 ± 1.40
Modified cherry pits (MCP)	3.0	58.82 ± 1.05	76.20 ± 0.49	22.03 ± 0.30	50.00 ± 1.08	49.97± 0.73
	7.0	58.82 ± 0.72	20.00 ± 0.20	21.88 ± 0.95	50.00 ± 0.86	40.00 ± 0.84

Presented data are the average values of three independent determinations rounded to two decimal points ± standard deviation

Aflatoxin B$_1$

The adsorption of this mycotoxin by all natural (inorganic and organic) adsorbents has been detected when tested *in vitro* (Tables 3 and 4). The highest adsorption indices of applied AFB$_1$ were recorded when it came to mineral binders - BEN, DIA and ZEO, and biosorbent - *M. spicatum* (> 94.5%). Unlike the results of Thimm et al. (2001), during these experiments, the pH value of the electrolyte had no significant influence on the binding of this mycotoxin. Results similar to ours were reported by following authors. At all 3 investigated pH values (pH 3, 7, and 10) different BEN agents bound more than 95% of AFB$_1$ (Diaz et al., 2002). The investigations of Spotti et al. (2005) also showed that ZEO was very efficient in binding this mycotoxin. Last authors found that 100% of AFB$_1$ was adsorbed in rumen juice. Modirsanei et al. (2008) demonstrated that tectosilicate - DIA reduced some toxic effects of AFB$_1$ in broilers (decrease in body weight gain and feed intake). The obtained results are not surprising, since it is known that the surface of alumosilicate adsorbents when saturated with water attracts polar functional groups of AFB$_1$ and other polar mycotoxins (Tomašević-Čanović et al., 2003; Kollosova et al., 2009). Biagi (2009) cited that different clays (zeolite, bentonite etc.) improved animal performances in an experiment with piglets diets contaminated with aflatoxins.

With the exception of *M. spicatum* (Table 4), other tested biosorbents (modified and unmodified fruit pits) bound less AFB$_1$ (from 41.18% to 58.82%) than tested mineral binders. Hydrochloric acid modification of peach pits was unfavourable for the process of adsorption.

Ochratoxin A

When it comes to OTA, the highest adsorption indices of binders were recorded in case of modified and unmodified sour cherry pits (76.20% and 66.66%, respectively), at pH 3.0 (Table 4).

Among alumosilicate binders, only DIA adsorbed this toxin – the adsorption index was 66.67% at pH 3.0 (Table 3). The fact that most mineral binders showed higher adsorption indices of OTA at pH 3.0 than at pH 6.5 was noted by other authors as well (Thimm et al., 2001). According to the literature available (Whitlow, 2006; Manafi et al., 2009), besides binding AFL and OTA, diatomaceous earth has a potential to adsorb *in vitro* other mycotoxins as well (ZON, T-2 toxin and sterigmatosystin). Contrary to the results of Kurtbay et al. (2008), in which bentonite and montmorillonite could bind from 40 to 100% of OTA from wine, BEN used in our investigation did not express that ability.

Opposite to sour cherry pits, the modification of peach pits did not improve the biosorption capacity of that material. Adsorption indices were the same in both cases - at acidic pH (42.85%) and at neutral pH (33.32%), respectively. The effect of the pH value on the binding capacity of organic adsorbents was very much expressed (Table 4). As in the case of DIA, higher adsorption indices *in vitro* were detected at pH 3.0. The presented data on OTA biosorption capacity (Table 4) match the data on the possibility of mycotoxin biosorption of indigestible dietary fibres. Aoudia et al. (2008 and 2009) demonstrated significant protective effects of micronized wheat fibres against toxicity of the same mycotoxin *in vivo* for rats and piglets, by reducing OTA intestinal absorption and increasing its faecal excretion.

Deoxynivalenol

Adsorption indices of DON varied from 21.88% to 50.03%, depending on the type of natural adsorbents (Tables 3 and 4). The best binding capacity of this type B trichothecene was expressed by BEN, ZEO and modified peach pits. The effect of electrolyte pH value on the adsorption of DON was quite different. In the case of mineral adsorbents, *in vitro* binding of this fusariotoxin was detected only at pH 3.0 (Table 3), while biosorbents - modified cherry pits, bound larger amounts of DON at a neutral pH (Table 4). The modification of peach pits resulted in an increased adsorption capacity of DON (from 23.08% to 40.03% at pH 3.0, and from 39.97% to 49.98% at pH 7.0, respectively) while in

the case of sour cherry pits, the adsorption capacity remained almost unchanged. *M. spicatum* did not show any capacity for binding this mycotoxin.

Contrary to our results, other authors (Döll et al., 2004; Sabater-Vilard et al., 2004) found that most of the commercially available mineral adsorbents were not able to bind DON in appreciable percentage. According to them, activated carbon was the best adsorbent of this mycotoxin. Although we used not activated, only raw or acid-treated biosorbents with a high content of cellulose (PP - 58.05% and CP - 54.74%), they showed a similar adsorption capacity as activated carbon as stated by Döll et al. (2004).

Zearalenone

In most cases, agricultural waste materials showed a higher binding capacity of this oestrogenic mycotoxin (from 33.33% to 75.00%) than inorganic adsorbents (from 12.20% to 37.03%) (Tables 3 and 4). The effect of the electrolyte pH value on the adsorption of ZON differed, although in most cases it was not expressed at all. In the case of mineral adsorbent, the best one was BEN (37.03% at pH 3.0) (Table 3). Other investigators (Bueno et al., 2005) also observed that bentonite can bind zearalenone to some extent, although ZON adsorption rates were higher. Adsorption indices that were up to 100% have been detected only in the case of organophylic bentonites (Dakovic et al., 2001; Sabater-Vilard et al., 2004)**.**

The best binder of ZON *in vitro* was aquatic weed *M. spicatum* – 70.00% at pH 3.0 and 75.00% at pH 6.9, respectively (Table 4). The presented data on ZON biosorption capacity match the data on the possibility of the same mycotoxin biosorption by similar plant materials, such as alfalfa (Smith, 1980). PP and CP modified by hydrochloric acid demonstrated a higher binding capacity of ZON (58.34% and 50.00%, respectively) than untreated material (33.33%) at pH 7.0 (Table 4).

Type A trichothecenes

In the investigated *in vitro* conditions, mineral adsorbents (BEN, DIA, and ZEO) did not bind diacetoxyscirpenol (Table 3). Ra-

ther small quantities of this mycotoxin were adsorbed only by some of organic materials: peach pits (16.67% at pH 7.0), modified peach pits (16.67% at pH 3.0, and 33.33% at pH 7.0, respectively) and modified cherry pits (16.67% at pH 7.0). Little or no beneficial effect on DAS binding by different mineral adsorbents was also shown in the results of other authors (Devegovda and Aravind, 2002).

In most cases, agricultural waste materials demonstrated a higher adsorption capacity of T-2 toxin (from 16.67% to 50.00%) than inorganic adsorbents (from 16.67% to 33.33%) (Tables 3 and 4). With the exception of DIA, higher indices of mycotoxin adsorption by mineral materials were recorded at pH 6.9 (Table 3). On the other hand, most of the investigated plant materials bound more T-2 toxin at the acidic pH (Table 4). Our finding is not surprising because T-2 toxin is a non-polar compound. Bočarov Stančić et al. (2000a and 2000b) and Nešić et al. (2007) observed similar *in vitro* binding capacities of commercial mineral adsorbents. A much higher adsorption index (95%) of T-2 toxin *in vitro* conditions was obtained by hectorite, another natural mineral adsorbent (Stojanović et al., 2008). *In vivo* experiments of Carson and Smith (1983a) demonstrated a slightly positive effect of the addition of BEN in T-2 toxin contaminated diet for rats.

The best *in vitro* T-2 toxin binders were unmodified sour cherry pits and modified fruit pits of both kinds (Table 4). In the available literature there are reports that some similar plant materials can reduce, to a greater or lesser extent, toxic effects of this type A trichothecene. *In vivo* experiments with rats demonstrated that alfalfa and indigestible dietary fibres can decrease the effects of T-2 toxin (Carson and Smith, 1983b).

CONCLUSIONS

Although many siliceous materials are efficient binders of aflatoxin, in the case of other mycotoxins, such as OTA, ZEA or trichothecenes of types A and B, their options are very often limited. The chemical composition of mineral adsorbents varies greatly, resulting in differences in their

affinities and capacities for mycotoxins. An alternative to inorganic adsorbents can be organic binders, which, among others, include waste materials from agriculture (aquatic plant *M. spicatum* and various fruit pits). Some studies in the available literature, and our own results presented in this paper, indicate that these natural plant adsorbents are effective against a large range of mycotoxins. Mineral adsorbents (BEN, DIA and ZEO) tested *in vitro* were better binders of AFB1 (94.97% - 96.90%), while the biosorbents were more efficient in adsorption of OTA (up to 66.66%), ZON (up to 75.00%) and T - 2 toxin (up to 50.00%).

Following *in vitro* studies, it is also necessary to perform *in vivo* studies of efficiency, safety, and potential interactions of the adsorbent with nutrients, as well as purity of adsorbent, i.e. the absence of hazardous contaminants.

Although many siliceous materials are efficient binders of aflatoxin, in the case of other mycotoxins such as OTA, ZEA or trichothecenes of types A and B, their options are limited. So, in the case of contamination of feed with a number of mycotoxins the solution could be the product which is a combination of different inorganic and organic adsorbents with diverse mycotoxin binding properties.

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IN VITRO УКЛАЊАЊЕ МИКОТОКСИНА КОРИШЋЕЊЕМ РАЗЛИЧИТИХ НЕОРГАНСКИХ АДСОРБЕНАТА И ОРГАНСКИХ ОТПАДНИХ МАТЕРИЈАЛА ИЗ СРБИЈЕ

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Сажетак: Афлатоксин Б1 (АФБ1), охратоксин А (ОТА), зеараленон (ЗОН), дезоксиниваленол (ДОН) и Т-2 токсин су највише изучавани токсични метаболити гљива. Када микотоксини уђу у производни ланац за храну/храну за животиње, задржавајући своје токсичне карактеристике, тешко их је уклонити или елиминисати. Једна од обећавајућих метода за смањење нивоа микотоксина у контаминираној храни/храни за животиње је коришћење микотоксинских везива. Овај рад представља резултате *in vitro* истраживања минералних микотоксинских везива (бентонит - БЕН, диатомит - ДИА и зеолит - ЗЕО) и органских везива микотоксина – пољопривредног отпадног материјала (*Myriophillium spicatum*, коштице брескве и вишње). Хемијски састави адсорбената показали су да не садрже елементе токсичне за животиње. Неоргански адсорбенти (БЕН, ДИА и ЗЕО) тестирани *in vitro* боље су везивали АФБ1 (94,97% - 96,90%), док су биосорбенти били ефикаснији у адсорпцији ОТА (19,98% - 66,66%), ЗОН-а (33,33% - 75,00%) и Т-2 токсина (16,67% - 50,00%). Неоргански адсорбенти и органски отпадни материјали су показали сличан капацитет *in vitro* везивања ДОН-а, са изузетком *M. spicatum* који уопште није адсорбовао овај трихотецен типа Б. Наши резултати који су приказани овде показују да загађивање хране и хране за животиње различитим врстама микотоксина може бити смањено додавањем препарата добијеног комбинацијом различитих неорганских и органских адсорбената који поседују различите карактеристике везивања микотоксина.

Кључне речи: *минерални адсорбенти, биосорбенти, микотоксини, in vitro везивање*