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JOURNAL	World Science
p-ISSN	2413-1032
e-ISSN	2414-6404
PUBLISHER	RS Global Sp. z O.O., Poland

ARTICLE TITLE	RESEARCH OF ADSORPTION AFLATOXINS BY TECHNICAL LIGNIN
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ARTICLE INFO	R. Z. Uridia, N. G. Karkashadze, L. T. Tatiashvili, N. P. Tserodze, I. I. Mikadze, R. P. Tsiskarishvili. (2021) Research of Adsorption Aflatoxins by Technical Lignin. World Science. 10(71). doi: 10.31435/rsglobal_ws/30112021/7703
DOI	https://doi.org/10.31435/rsglobal_ws/30112021/7703
RECEIVED	24 September 2021
ACCEPTED	22 November 2021
PUBLISHED	26 November 2021
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RESEARCH OF ADSORPTION AFLATOXINS BY TECHNICAL LIGNIN

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DOI: https://doi.org/10.31435/rsglobal_ws/30112021/7703

ARTICLE INFO

Received: 24 September 2021
Accepted: 22 November 2021
Published: 26 November 2021

KEYWORDS

Aflatoxin, mycotoxin, technical lignin, adsorption.

ABSTRACT

Aflatoxins represent aggressive group of mycotoxins. They are really toxic, carcinogenic and dangerous for human health. There are mechanical, physical and chemical methods for their detoxification. Aflatoxins could be also neutralized by means of various adsorbents as well. We do some research work in the direction of aflatoxin adsorption by presence of lignin, which is quite affordable and gives an effective result.

Citation: R. Z. Uridia, N. G. Karkashadze, L. T. Tatiashvili, N. P. Tserodze, I. I. Mikadze, R. P. Tsiskarishvili. (2021) Research of Adsorption Aflatoxins by Technical Lignin. *World Science.* 10(71). doi: 10.31435/rsglobal_ws/30112021/7703

Introduction. Alkaline lignins and lignosulfonated find a wide variety of applications. They are used as: dispersants (for carbon black, insecticides, herbicides, pesticides, clays, dyes, pigments, ceramic materials); emulsifiers, stabilizers and fillers (for soils, road surfaces, asphalt, waxes, rubbers, soaps, latexes, fire extinguishing foams); compounds that bind metals (in process water, agricultural micronutrient fertilizers). Lignin contains highly reactive hydroxyl groups.

Mycotoxins belong to one of the dominant group of biogenic poisons, which contaminates cattle food and worsens their nutritional value and quality. Humans and animals are poisoned when they consume these products. Mycotoxicosis have negative influence on endocrinal gland secretion, it weakens immune system and causes internal organ damages [1].

Increased import-export scale of wheat, the world climate changes, use of fungicide and pesticide, leads to 100 times increased formation of mycotoxins and to the cattle food saturation with various mycotoxins. At the end, all of these leads to human and animal health disorders and the general economic situation. Mostly Fusarium and Aspergillus are the types of fungus, which pollute the cattle food. They correspondingly produce quite aggressive mycotoxins T-2 and aflatoxin B-1 [2,3].

Recently, all the effort is directed to the developing of fast methods of analysis of mycotoxins, which allows us to diagnose the samples as we collect them. It is obvious, that the immuno- and immuno-ferment analysis are the best for these purposes, because they give opportunity to visualize the results.

Aflatoxin B-1 and other toxins of this group bond irreversibly with protein molecules in the liver and produce DNA adducts (excepting Aflatoxin B-1-lizine in albumin molecule).

Objects, that have herbal, animal, microbiological and mineral origins and are used to produce food products, are considered as consumer raw materials.

Main ways of pollution are the following:

- Agricultural crops and livestock products pollution with pesticides, which are used to protect the plants and animals from pests.
- Violation of hygienic norms while using plant fertilizer.
- Frequent use of unauthorized supplements in plant and animal feeding process, such as: various preservatives, paints, growth stimulating and therapeutic-preventive supplements.
- Migration of toxic substances in food products: raw, food equipment, tableware, inventory and unauthorized polymers, usage of rubber and metal wrapping.
- Violation of food producing and preserving sanitary rules.
- Food product pollution from the environment with toxic substances and radionuclides: atmosphere, soil and water reservoir.

Mold fungus produce mycotoxins, causing decrease the sanitary quality of consumer raw materials. These mycotoxins are especially dangerous substances. When raw and food products are polluted, they get in the human and animal body and lead to different kinds of poisoning, which cause specific disease – mycotoxicosis. Besides, some mycotoxins are carcinogenic [4,5].

More than 250 various species of microscopic fungi are known, which produce more than 100 various toxic metabolites.

From the above-mentioned spectrum, aflatoxins represent one of the most aggressive and dangerous group of mycotoxins, which have strong carcinogenic characteristics. Aflatoxins are derived from two stains of species: Aspergillus Flavus (Link) and Aspergillus Parasiticus (spare). Above mentioned funguses belong to mesophilic microscopic order. They have the capability to develop at 6-8°C.

Amoung the genius of Aspergillus fungi, there are separate group of aflatoxins, which has similar chemical structure. It is established, that they are sensitive to the action of oxidation reagent. Aflatoxins are soluble in moderate polar solvent, such as: acetone, chloroform, dichloromethane, dimethyl sulfoxide, ethanol, isopropanol, and etc. and insoluble in diethyl ether. In pure form, they are sustainable during the warm up in the air (Melting Point: 268-269°C). They degrade easily in polar solvents under light. They are preserved in benzene and chloroform in dark environment for several years [6, 7, 8].

Four groups of aflatoxins are identified (B-1, B-2, G-1, G-2), which differ from each other with some characteristics and the level of toxicity. B-1 and B-2 aflatoxins are produced in the cell of A. Flavus, while G-1 and G-2 – in the cell of A. Parasiticus [9].

A significant amount of high toxicity and carcinogenic aflatoxins are discovered in main food product all over the world. This made necessary to develop the methods of detoxification for raw, food products and corm.

The methods of aflatoxin detoxification are: mechanical, physical and chemical. The method of mechanic detoxification lies in removing of polluted raw material with manual and electro-colorimetric distinguishing methods. The physical method lies in material thermal processing with autoclave, ultraviolent radiation and ozonation. The chemical method lies in material treatment with strong oxidants [10].

Every single one of the above mentioned methods have its flaws. Mechanical and physical processing are not highly effective, while chemical processing causes degradation of not justaflatoxins, but beneficial nutrients as well.

The usage of various adsorbents is very effective for protecting vegetables and wheat raw materials, consumer and raw foods, various dry fruits, breads and confectionary from aflatoxins. The special advantage of adsorption method is its availability and effectiveness [11,12]. Aflatoxin adsorbents are divided in several groups: mineral, carbon containing, polysaccharide, and mixed.

Research. The work was conducted in the following sequence: purifying the filtrate, preparing the standard solutions, identifying aflatoxin and quantitative analyze. The objective of our work was to determine the quantitative and qualitative analyse of aflatoxins derived from genus Aspergillus. We were observing adsorption of aflatoxins by the sulfate lignin parallel to microorganisms' growth phase. The following method have been used: Potato was artificially infected by the microorganisms from the genus Aspergillus. Accordingly, once per week during 9 weeks, sulfate lignin was analyzed and humidity was determined. Aflatoxins were determined with thin layered chromatography and quantitative analyze was determined [13]. The dehydrated Na_2SO_4 was placed on a glass filter on chromatographic column and silicagel-chorophorm suspension was added on the formed layer. After the sedimentation were carefully transfared the dehydrated Na_2SO_4 in the column. Exceed amounts of chlorophorm was expelled from faucet and we were putting the sample momentarily and slowly. The vial walls were washed with chloroform, which was transferred to the column. Once the liquid level was equal to the upper layer of Na_2SO_4, we carefully added a chloroform and methanol solution (97:3) in portions. The waste was washed 3 times. These extracts were combined and evaporated to dry weight. The remaining waste was dissolved in chloroform and thin layer chromatography was performed. The aflatoxin was identified by the method of fluorescence. Its quantitative determination was evaluated due to fluorescence intensity of aflatoxin spots in extracts. It was calculated in the certain amount of time. Peak height (h) was determined with the following formula:

$$C = \frac{V1 \cdot V3 \cdot V5 \cdot m(st)}{V2 \cdot V4 \cdot V6 \cdot M \cdot h(\mathrm{st})} \cdot K \ (0.01)$$

Research results. Fungus from the genus Aspergillus, which was artificially entered to the potato, created toxin, for which the peak during chromatography was 22.8 ± 4.7 mm. The results for calculation aflatoxin concentration are 0.0021 ± 0.0007 mcg/l. Simultaneously, sulfate lignin was processed with the solvent system, where the concentrated toxin was elevating weekly and humidity was determined in parallel. The results are displayed in the table 1 figures (1 and 2).

Table 1. Results of representatives of Aspergillus genus

Weeks	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Humidity on sulfate lignin (g, %)	2.12 ≈4	1.77 ≈3	1.14 ≈2	2.85 ≈4	3.44 ≈5	4.65 ≈7	6.72 ≈9	7.42 ≈10	8.52 ≈11
Toxin quantity (mcg/l)	0.0021	0.0025	0.0032	0.0046	0.0054	0.0063	0.0068	0	0

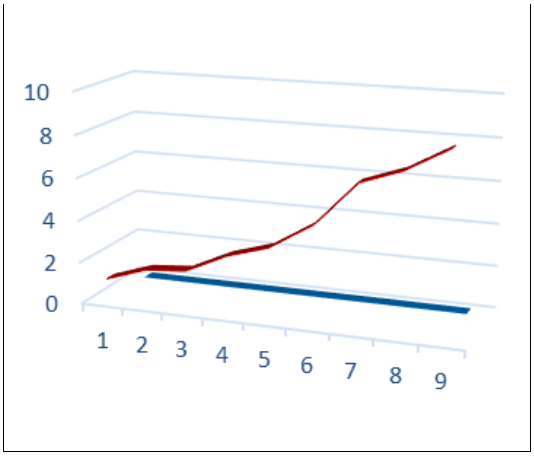

Fig. 1 Quantitative analysis of aflatoxins and moisture according to the growth phase of the microorganism

Results have shown that humidity was elevating during the 8 week period and simultaneously the quantitative indicator of the toxin was elevating. The humidity elevation was continued in the following weeks, while the quantitative indicator of the toxin was decreased. Above-mentioned issue

requires further research in this direction. Research results showed, that sulfate lignin is distinguished with high quality of toxin adsorption. Especially high ability of assimilation was revealed in microorganism exponential growth phase.

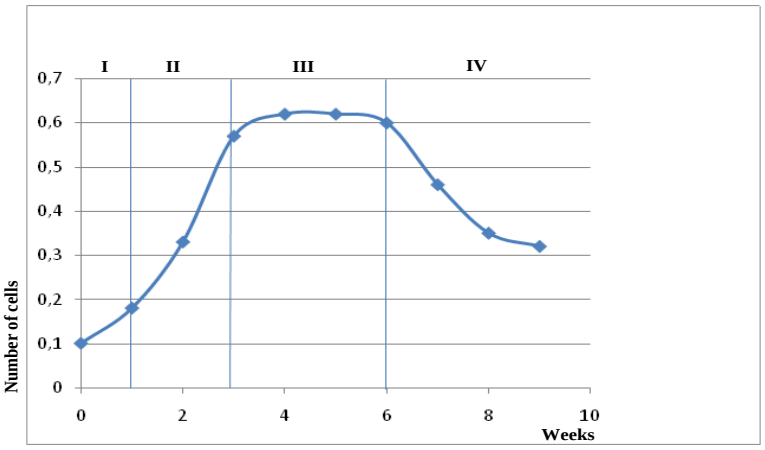

Fig. 2. Aspergillus growth phase (I-Lag-phase; II-Exponential phase; III-Stationary phase; IV-decline phase)

Conclusions. Mineral adsorbents consist minerals mined in different places such as: bentonite, montmorillonite, zeolite and etc. Mixed groups are those, that could be consisted as source of minerals, organic components and mold inhibitors. Considering the above mentioned factors and its topicality, our work is conducted to develop technical, sulfate free lignin from the sawdust, to use it for aflatoxin adsorption in the future. Among the solutions used for aflatoxin detoxification, we choose adsorbent produced on sawdust base – technical lignin, which stands out with its low cost and high effectivity. Sulfate lignin is brown powder in the dry condition. The particle size of such lignin varies between 10 mcm – 5 mm. Sulfate lignin is considered as nontoxic compound, which is used as wet paste. It's not flammable. Examples of sawdust are coniferous, deciduous and their mixture. Considering the urgency of the matter and the factors mentioned above, a number of works are underway, to obtain technical sulfate lignin for subsequent use in the adsorption of aflatoxins.

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