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Mycotoxins as Dangerous Environmental Factors and a New Way of Their Treatment, Including the Use of Nanomaterials

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Abstract—Issues of food, feed, and renewable raw materials safety are widely covered in world literature today. Mycotoxicoses of grains, vegetables, potatoes, fruits, and other agricultural products pose a serious threat to human and animal health. Direct crop loss due to fungal contamination can reach 50% for cereals only, and the harvest may be totally unsuitable for human and animal consumption because of mycotoxin contamination. Mycotoxins are carcinogenic, mutagenic, and immunosuppressive agents which cause liver and kidney damages, nervous system effects, gastrointestinal dysfunction, blood disorders, septic sore throat, dermatitis, and also violate the reproductive function.

Keywords: mycotoxins, biosafety, destruction, nanomaterials, chelators

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Mycotoxins [derived from Greek mykes (mushroom) and toxicon (poison)] are toxic products of metabolism of microscopic fungi (molds).

More than 250 species of fungi producing several hundreds of mycotoxins are currently known of which many possess mutagenic (in particular, carcinogenic) properties. Mycotoxins differ in the chemical structure, toxicity, and mechanism of action. The common feature of mycotoxins is predominant toxicity for eukaryotic organisms [1].

The term "mycotoxins" was first used in the early 1960s, whereafter the nature and toxicity of many of the substances subsequently attributed to mycotoxins, together with the diseases resulting from mycotoxin poisoning, were collectively referred to as mycotoxicoses and described long before the term was coined. The first mentioned cases of human and animal poisoning by bread and grain contaminated with toxic fungal metabolites, specifically by ergot alkaloids (Claviceps purpurea), can be found in medieval chronicles.

Mycotoxins aroused researchers' interest in 1960, following the discovery of aflatoxins during investigation of the causes of "Turkey X Disease," in which over 100000 turkey poults died in farms of the UK. In-depth long-term studies of the peanut flour meal fed to the turkey had culminated in isolation of a colorless crystalline substance whose administration to poults allowed reproducing the symptoms of "Turkey X Disease" [2]. This substance was found to be synthesized by *Aspergillus genus* fungi (*Aspergillus flavus, Aspergillus parasiticus*), which affect peanuts, corn, soybeans, and oilseeds growing in a temperate climate. It was named aflatoxin after a producer species, *Aspergillus flavus*. Most of aflatoxins are thermally stable crystalline substances readily soluble in organic solvents.

Biosynthesis of aflatoxins proceeds via a key energy metabolite, acetyl coenzyme **A**, and typically involves a step in which one acetyl coenzyme **A** molecule is condensed with three or more malonyl coenzyme **A** molecules, i.e., has common stages with biosynthesis of lipids. For another group of mycotoxins, trichothecenes, biosynthesis proceeds via mevalonic acid lactone (structural analog of the cholesterol biosynthesis intermediate) and farnesyl diphosphate.

Some representatives of aflatoxins and their structures are shown in Table 1.

For images of some of mycotoxin producers, see Table 2.

What all the mycotoxins have in common is that they are all biocides, i.e., chemicals that kill living cells. In terms of other characteristics (tropism of action, toxicity level, and physicochemical properties) they differ very significantly, which prevents development of a uniform effective method against mycotoxins. Meanwhile, mycotoxin contamination remains a pressing biosafety concern. Annual losses of agricultural products, associated with mycotoxin contamination, exceed $15 bln worldwide. One billion tons of agricultural products are at risk of mycotoxin contamination. In a number of Asian and African countries, where cases of acute aflatoxicosis in humans are observed, a direct correlation between the liver cancer incidence in the population and the content of aflatoxins in their foodstuffs was revealed. The mycotoxin levels in food and animal feed widely vary and can reach hundreds of micrograms per kilogram. According to the generalized statistics of Europe, Canada, and the United States, grain contamination by ochratoxin is characterized by the content ranging from 5 to 360 μg kg^{-1} and the contamination frequency of ~5% [3].

The optimum toxin formation temperature ranges from 8–12°C (T-2 toxin) to 27–30°C (aflatoxins). Some countries regulate the content of the major mycotoxins in foodstuff by maximum permissible concentrations which, in particular, in Russia were set at the following levels: aflatoxin B_1 0.005, patulin 0.05, T-2 toxin 0.1, deoxynivalenol 0.5 and 1.0 (depending on the product type), and zearalenone

Table 1. Characteristics and structure of some groups of mycotoxins

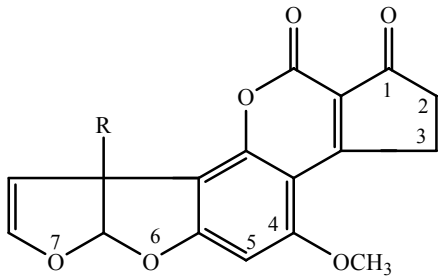
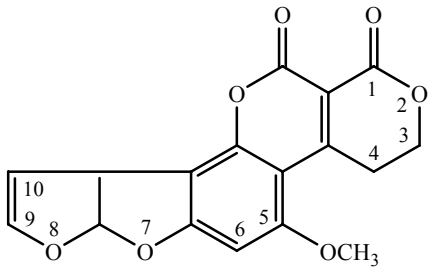
Representatives of mycotoxins and their producers	Structure and action
Aflatoxins (>15 mycotoxins); produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. Most dangerous aflatoxins: B_1, B_2, G_1, G_2, and M_1	 **I**  **II** Group **I**: aflatoxin B_1: R = H, LD_{50} = 7.8 mg kg^{-1} (macaques, orally); aflatoxin B_2: R = H, positions 8 and 9 are hydrogenated; aflatoxin M_1: R = OH. Group **II**: aflatoxin G_1; aflatoxin G_2: positions 9 and 10 are hydrogenated. Action: pronounced mutagenic, hepatocarcinogenic effects. Teratogenic and immunosuppressive effects are exerted via the reaction with the nucleophilic sites in DNA, RNA, and proteins.

Table 1. (Contd.)

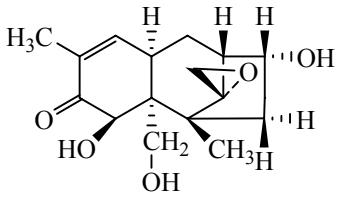
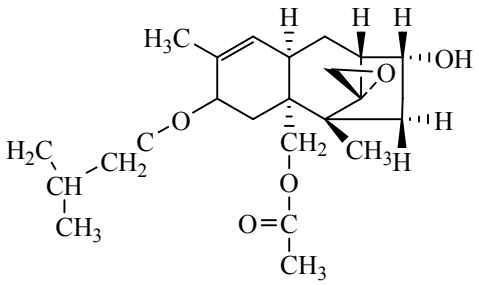
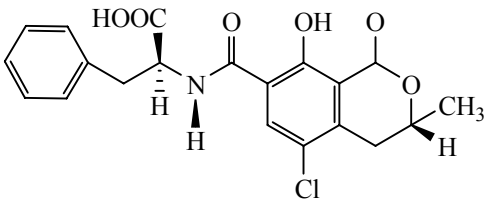
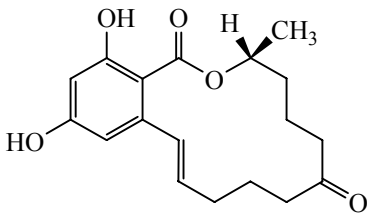
Representatives of mycotoxins and their producers	Structure and action
Trichothecenes (>60 mycotoxins); produced by *Fusarium solani* and *Fusarium graminearum* fungi; subdivided into four groups: **A, B, C**, and **D**. Group representatives: **A**: T-2 and diacetoxyskirpenol; **B**: deoxynivalenol, nivalenol; **C**: roridin **A**; and **D**: krotocin	 Deoxynivalenol  T-2 Tioxin Action: teratogenic, cytotoxic, immunosuppressive, and dermatotoxic effects; effects in blood-forming organs and central nervous system; leukopenia, hemorrhagic syndrome, and food poisoning in humans and animals. Toxicity is manifested as suppression of protein biosynthesis. LD_{50} = 6.7 mg kg^{-1} for T-2 toxin (Group **A**, mice orally). LD_{50} = 46 mg kg^{-1} mg for deoxynivalenol (Group **B**, mice, orally).
Ochratoxins; produced by *Aspergillus ochraceus, Aspergillus melleus, Aspergillus sulphureus, Aspergilluspetrakii*, and *Penicillium viridicatum* fungi (representatives of collection strains of producers: *Aspergillus ochraceus* and *Penicillium viridicatum*); subdivided into **A** (the most toxic), **B**, and **C** groups	 **A** Action: violations of basic biochemical processes, inhibition of protein and glycogen synthesis, deep pathological changes of internal organs, mainly kidneys and liver, teratogenic, carcinogenic, and immunosuppressive effects. LD_{50} =3.4 mg kg^{-1} (ochratoxin **A**, day-old chicks, orally).
Zearalenone (15 mycotoxins); produced by *Fusarium graminearum* fungus	 Exhibits interactions with estradiol-binding receptors in the target cells. Action: estrogenic and teratogenic effects, as well as antibacterial activity against gram-positive bacteria. LD_{50} = 10000 mg kg^{-1} (rats, orally).

Table 1. (Contd.)

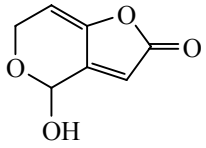
Representatives of mycotoxins and their producers	Structure and action
Patulin; produced by *Penicillium patulum* fungus	 Action: disturbances of the intestinal barrier. There exists insufficient evidence for carcinogenicity. LD_{50}: not determined; gastrointestinal inflammation and ulceration in rodents are observed at the 1 mg kg^{-1} dose.

Table 2. Images of the producers of major groups of mycotoxins

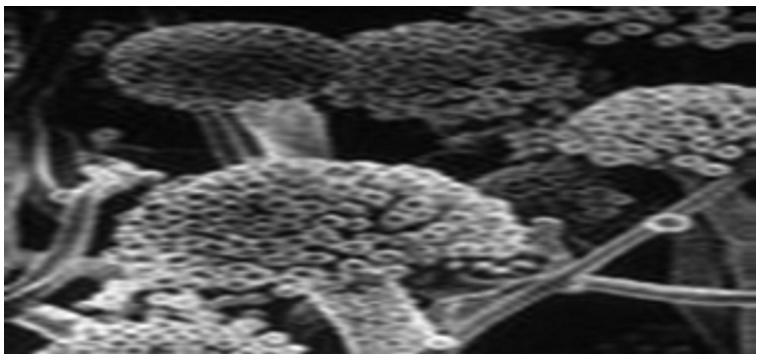
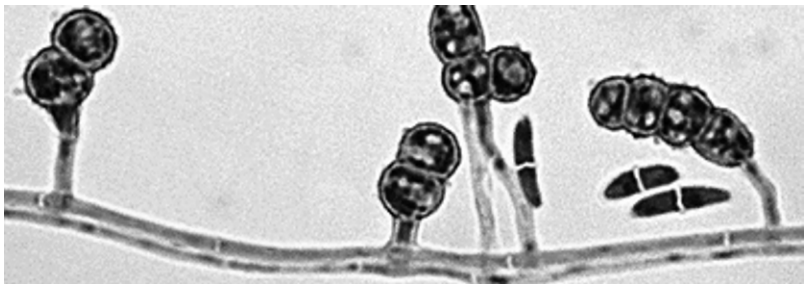
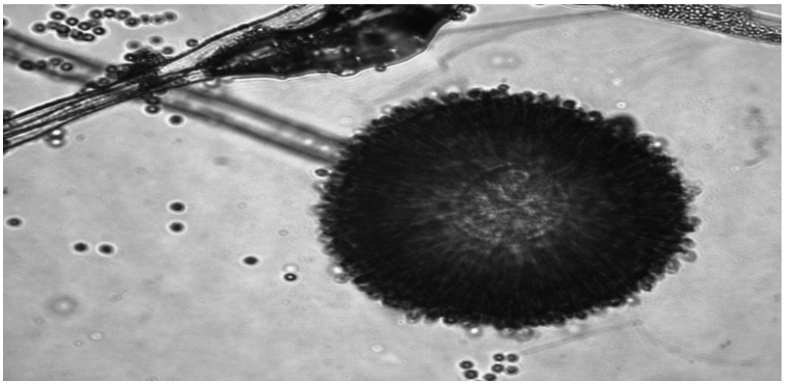
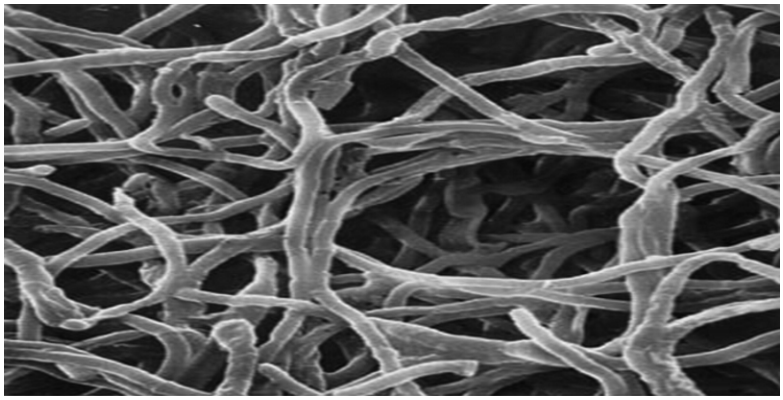
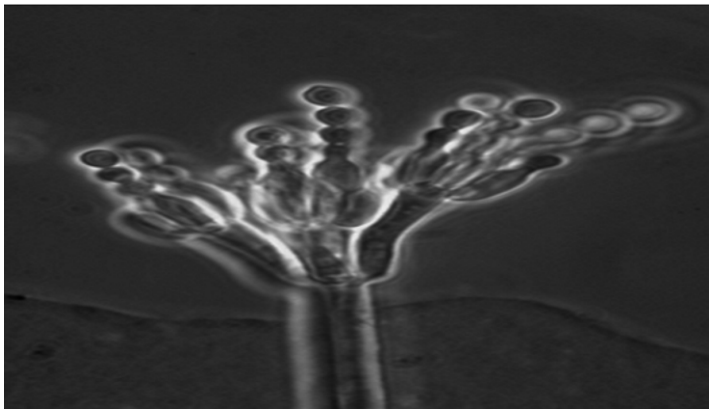
Mycotoxin type	Producer
Aflatoxins	 *Aspergillus flavus*
Trichothecenes	 *Fusarium solani*
Ochratoxins	 *Aspergillus ochraceus*

Table 2. (Contd.)

Mycotoxin type	Producer
Zearalenone	 *Fusarium graminearum*
Patulin	 *Penicillium patulum*

1.0 mg kg^{-1}. Food products mostly affected by different mycotoxin producers include: cereals, oilseeds, and legumes (aflatoxins); cereals (ochratoxins, zearalenone, and trichothecene types **A** and **B**); fibrous roughages (trichothecenes); and fruits, vegetables, and derived products (patulin).

In view of a wide occurrence of mycotoxin effects on agricultural products and big losses of agricultural production due to contamination by micromycetes, producers of dangerous toxins, detoxification of mycotoxin-contaminated agricultural products is an extremely topical issue, and for certain regions, a food safety concern. The lack of a single method for fighting mycotoxins strongly complicates the resolution of this issue and makes extremely urgent the search for new methods.

Currently, chemical and physicochemical methods are used for decontamination of agricultural products containing dangerous mycotoxins. Chemical methods are fairly effective but costly; they can lead to contamination of agricultural raw materials and animal feed by other toxic substances. In the case of food products intended for human consumption these methods are practically inacceptable for mycotoxin decontamination. Nevertheless, ammonia detoxification facilities for treating the feed at elevated temperature and pressure exist in the USA and France. In India, treatment of feed material with hydrogen peroxide was suggested. Chemical treatment methods allow reducing the dangerous aflatoxin content in animal feed below the MPC level, but the nutritional value of the feed is partially lost thereby. Some of mycotoxins are light-sensitive compounds, e.g., ochratoxin **A**, which also is efficiently oxidized by atmospheric oxygen [4].

Yet through it all, the most common method used today for mycotoxin neutralization is that based on

adsorption by organic or inorganic materials [5]. However, mycotoxin binding capacities of sorbents significantly differ because of the differences in the polarity and size of their molecules. Adsorption methods are effective in removing polar mycotoxins (mainly aflatoxins). At the same time, nonpolar toxins are virtually nonsorbable by some of the adsorbents and are sorbed inefficiently by other adsorbents. The degree of neutralization also depends on the adsorption capacity of the adsorbent and, together with the degree of contamination of the feed, determines the rate of addition of adsorbent to the feed. Essential properties of adsorbents include their operability over a broad pH range and irreversibility of mycotoxin binding. Mycotoxins are known to be sorbable by adsorbents in the stomach at acidic pH and desorbable in an alkaline medium of the intestine, which calls into question the effectiveness of this adsorbent.

Some adsorbents are able of adsorbing nutrients, vitamins, and minerals as well. There exist difficulties in assessing the efficiency of adsorbents, which greatly complicates their selection and achievement of unbiased results. The conditions of most of the classical in vitro methods do not even remotely resemble the real conditions of the gastrointestinal tract. As to in vivo experiments with sorbents, the reproducibility of their results is extremely poor. Therefore, the search for new ways of mycotoxin detoxification, as well as for models simulating the conditions most closely resembling those of real-life situations and providing the most unbiased results is underway now.

Most leading toxicologists believe that effective mycotoxin elimination from feed is only possible via the use of several complementary methods which have different mechanisms of action and are directed against different groups of mycotoxins. The maximum effect can be achieved by combining several methods and using several adsorbents, in particular, new effective adsorbents from among organic and inorganic materials to be discovered.

Now, hydrated sodium calcium aluminosilicates are recognized as the best so far inorganic adsorbents. They have adsorption capacity of 60–70 mg g^{-1} with respect to aflatoxins (cf. up to 9 mg g^{-1} for bentonites) and exhibit an optimal adsorption activity over a broad pH range of 2–10 and at temperatures of 25–42°C. Another efficient adsorbent of mycotoxins is chitosan. A new line in mycotoxin decontamination is enzymatic detoxification. This is a particularly important, and maybe the only effective approach to treating nonpolar mycotoxins, which virtually cannot be bound by adsorbents (trichothecenes, zearalenone, ochratoxins). Further development of this approach consists in selection of microorganism cultures able to actively destroy mycotoxins. A major limitation on these developments is posed by the need to preserve the quality of feed under conditions when this microflora is deliberately introduced therein and to ensure the safety of the microorganisms destroying mycotoxins to farm animals and humans.

A recent trend in mycotoxin decontamination has been the use of nanomaterials able of efficiently sorbing mycotoxins and enhancing their sensitivity to chemical agents [6].

Today, the focus of special interest is on carbon material acting as sorbents and as agents promoting degradation of mycotoxins. Among carbon nanomaterials, detonation nanodiamonds (DNDs) are the most promising for biomedical and environmental applications [7]. This is due to their hydrophilicity and low toxicity, as well as to multifunctionality predetermined by the variety of the functional groups occurring on their grain surface [8].

It was shown that DNDs are efficient adsorbents of mycotoxins, above all aflatoxins [9] which do not contain protonogenic moieties and for which reactions with the surface groups of DND grains proceed by other than ionic mechanisms. Detonation nanodiamonds are of particular interest for neutralization of aflatoxins not only because of their ability to adsorb these and other dangerous substances in the gastrointestinal tract of warm-blooded animals. Also, DNDs hold promise for more complete removal of mycotoxins from the feed during the subsequent chemical detoxification due to enhanced reactivity of the adsorbates with respect to chemical agents [10]. Thereby, DNDs are regarded as a carrier promoter at the targeted action of a chemical agent used to destroy mycotoxins. As known, oxidants, e.g., potassium permanganate, hydrogen peroxide, and ozone, cause destruction of aflatoxins. Degradation and consequently detoxification of aflatoxins most likely proceeds via destruction of the lactone ring in the aflatoxin molecule. This is the route followed by aflatoxin detoxification in vivo. As mentioned above, in vitro aflatoxin is destroyed by oxidants, but they do not provide for complete destruction of the toxic compound. With additions of both DNDs and oxidants,

e.g., potassium permanganate, it is possible to destroy over 65% aflatoxin occurring in the reaction medium. When potassium permanganate and hydrogen peroxide are cointroduced into a DND suspension containing aflatoxin, the latter is not detected in the reaction mixture after contacting the oxidants, by contrast to the blank experiment in which the toxin was treated under the same conditions but in the absence of DND additions. The catalytic activity of DNDs in aflatoxin destruction is in a good agreement with the catalytic action exerted by DNDs in electrochemical processes and reactions involving inorganic compounds, as demonstrated in a number of studies.

Thus, DNDs act not only as an enterosorbent reducing the effect concentration of xenobiotics in biological media but also as a promoter of destruction of toxins entering the body [11]. The mechanism of catalytic action of DNDs in aflatoxin degradation still remains unclear, but it may include activation of the aflatoxin molecule via the conformational change it undergoes during sorption. More efficient degradation of aflatoxin by DNDs may also be due to their ability to generate reactive oxygen species under certain conditions, as repeatedly noted by various researchers. This ability probably plays the decisive role in manifestation of diverse biological activities by this material [12].

To conclude, destruction of mycotoxins is of crucial importance for preserving the quality and ensuring the safety of agricultural products. Chemical methods used for this purpose are costly, risky, and ineffective. Biotechnological methods hold more promise for solving the mycotoxin problem, and since the appearance of biologically active nanomaterials the main focus, obviously, should be laid on nanobiotechnology methods.

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