ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

# AFLATOXIN $B_1$ REMOVAL FROM CONTAMINATED MEDIA

**Camelia Bara***

* University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea; Romania, e-mail: cameliabara@yahoo.com

***Abstract***

*The present paper has in view the method by which aflatoxin $B_1$ disappears from liquid medium by fungus mycelium. By the absorption of aflatoxin dissolved in liquid media accomplished by mycelium and clay, it is possible to detoxify contaminated solutions.*

*Many physical, chemical and microbiological methods were used in the detoxification of aflatoxin, having results ranging from less or more successful ones.*

*The present study reports the fact that absorption is the process which promotes the disappearance of the aflatoxin.*

**Key words:** aflatoxin B1, fungal mycelia, clays

## INTRODUCTION

Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds. They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. The occurence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods. Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans. As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed.

## MATERIAL AND METHODS

Mycelia used in our experiments were obtained from non-aflatoxin producing *Aspergillus flavus* strains. The mycelia were gathered from 7-days-old cultures incubated at 25°C in Czapek solution. Two grams of wet mycelium were transferred either in a coarse state or after being disintegrated into 200 ml of a saline solution containing 400 Mg aflatoxin $B_1$. After 48 h incubation at 25°C, the content of each vial was centrifuged at 27,000 g for 20 min and aflatoxin measured separately in the

supernatant and in the pellet.

One gram of clay and 400 µg of aflatoxin $B_1$ were poured into 50 ml of Sorensen buffer solution, pH 6.5. After 5 days incubation at 30ºC, each sample was centrifuged at 27,000 g for 20 min and aflatoxin measured separately in the supernatant and in the pellet.

Aflatoxin estimation was performed according to the AOAC method: each sample was extracted by chloroform and the extract evaporated to dryness in a rota-vapor, then resuspended in 2 ml chloroform. Aliquots of this volume were chromatographed on Merck silicagel GHR thin layers 0.25 cm thick, with the solvent mixture chloroform-acetone 9/1 (v/v). The silicagel of each spot was removed by scraping and washed with 4 ml chloroform. The concentration of aflatoxin $B_1$ was measured by spectrophotometry absorption at 363 nm. Values of optical density recorded from each sample were related to a standard curve previously established.

**RESULTS**

Data recorded showed that fungal mycelia put in contact with an aflatoxin solution withdrew more than 50% of the toxin from the medium.They showed also that disintegrated mycelium enhanced the removal of aflatoxin from the solution.

Extraction of the pellets by chloroform released part of aflatoxin not found in the supernatant and therefore it was clear that aflatoxin not measured in the liquid was bound onto the fungus mycelium.

Because it was assumed that the removal of aflatoxin from liquid medium was due to adsorption on the mycelial walls, we undertook a study dealing with adsorption of aflatoxin on clays. The choice of clays was justified because these materials possess well-known adsorbing power and they are widely employed in the food-processing industry.

The data also showed obviously that aflatoxin was bound onto clays; this binding was in relation to the nature of the clay. In general, bentonite, vermiculite, and hectorite bound aflatoxin so strongly that only traces were found in the supernatant. Sea sand, lacking this adsorbing property, did not bind aflatoxin.

As with the mycelium, we undertook to remove the bound aflatoxin from the pellets. The retention power of aflatoxin on the clays depended on the nature of the clays: some pellets released, by extraction with choloroform, a good deal of the bound aflatoxin, whereas others released only a very minute amount of this aflatoxin.

Hence, aflatoxin cannot diffuse again in the solution.

Treatments were applied to clays in order to check their adsorbing capacity under various conditions.

The adsorbing capacity of vermiculite and montmorillonite were tested both in their coarse state and as powders.

It was shown that fine vermiculite bound more aflatoxin than did the paillettes and that paillettes released less aflatoxin than did the fine vermiculite. It was also shown that either in its course or in its fine state, montmorillonite behaved identically towards aflatoxin $B_1$.

Three different clays.- bentonite , montmorillonite , and sepiolite were heated at 65O°C for 24 h. Their aflatoxin-binding power was then tested and compared to that of the same unheated clays.

From the data gathered, it appeared that heating at 65O°C increased the adsorbing capacity of the clays: there was much more residual aflatoxin in the supernatant of nonheated clays than in those of clays heated at 65O°C This is very obvious especially with sepiolite, where aflatoxin quantities are in the ratio of 9 to 1 for heated and nonheated clay, respectively. Released aflatoxin is higher in nonheated clays.

**DISCUSSION**

From the results of our experiments, we can state that mycelium eliminated aflatoxin from the medium by adsorption. The more the mycelium was disintegrated — i.e., the greater the contact surface — the higher was the adsorption.

From this point of view, assays were undertaken dealing with aflatoxin adsorption on clays, known for their adsorbing power. Clays can adsorb large molecules such as antibiotics, products of microbial metabolism, and pesticides. We observed that aflatoxin bound on clays according to the nature of the clays.

We have noted that the clay particle size had great influence on its adsorbing power: the finer the clay, the higher the adsorbing power.

It was established that at a temperature of about 300 -700°C, the cationic exchange capacity (CEC) of montmorillonite was on the decline and that this decline was a function of the cation (Ca, Na, Li) present in the montmorillonite. On the other hand, we have noted that clays heated at 65O°C for 24 h adsorbed more aflatoxin than did the same clays not treated at this temperature.

## CONCLUSION

Fungus mycelia and clays can remove aflatoxin from liquid media: adsorption is the phenomenon implicated in this removal. While removal of bound aflatoxin from myceiia released more than 75% of the toxin, in most clays, aflatoxin seemed to bind irreversibly.

Taking advantage of this adsorption, it would be possible to set up a convenient technique which would eliminate aflatoxin from liquid media. After binding aflatoxin, the adsorbing material would be discarded either by centrifugation or by decantation or filtration. In this respect, use of clays seems to be advantageous: clays, chiefly bentonite, are widely employed in the food-processing industry; moreover, being inert materials, clays would not interfere with the food quality.

**REFERENCES**


1. Clifford, .I., Rees, K.R., 1969, Biochem. Pharmacol. 18, 2783-2785.
2. Deribere, M., Esme, A., 1951, *La bentonite. Les argiles colloidales et leurs emplois.*
3. Dommergues, Y., Mangenot, F.,1970, *Ecologie microbienne du sol*. Paris: Masson
4. Hofmann, U., Klemen, R.,1950, Z. Anorg. Chem., 262, 95-99.
5. Kelley,W.P., Jenney,H., 1936, *Soil Science*, 41, 367-382.
6. Masimango, N., Remade, J., Ramaut, J.L.,1978, in press .
7. Moreau, C., 1974, *Moisissures toxiques dans I'alimentation*. Paris: Masson.
8. Ramaut, J.L., Vanderhoven, C, Remade, J., 1970, Rev. Perm. Ind. Alim. 25,5 :184-189.
9. Shih, C.N., Marth, E.H., 1975, Z. Lebensm. Unters. Forsch., 158**,** 361-362
10. Chen F.C., Chen C.F., Wei R.D., 1982, Acute toxicity of PR Toxin, a Mzcotoxin from Penicillium roqueforti.
11. Coman I., Constantinescu Maria, Miron L., Gogu Mihaela, 2007, Elemente de standardizare în micologie şi micotoxicologie, Editura performantica.
12. Ghergariu S., 1973, Toxicologie veterinară, Atelierele de material didactic.
13. Hura Carmen, 2006, Ghid de laborator, Metode de analiza pentru produse alimentare, Cermi, Iaşi.
14. Jon S.C., Grantt M.J., 1987, Catalogue of Fungi and Yeasts ($17^{th}$ ed.) American Type Culture Collection, Rockville, MD, 1987
15. Lee Y.H., Fang S.C., Wei R.D., 1984, The effects of Penicillium roqueforti Toxin on the activity of rat hepatic DNA polymerase. Toxicology.
16. Moreau C., 1980, Penicillium roqueforti: Morphology, Physiology, Importance in the Cheesemaking. Mycotoxins: (Bibliography Revision).
17. Moule Y., Moreaux S., Aujard, 1980, Induction of Cross Links between DNA and Protein by PRT Yoxin, a Mycotoxin from Penicillium roqueforti.
18. Ramade F., 1987, Ecotoxicologie, John Wiley.
19. Ramade F., 1987, Ecotoxicologie, John Wiley.
20. Stănescu V., Ghergaru S., Laslo C., 1977, Problema micotoxinelor şi a unor metale toxice reziduale în alimente şi furaje. Simpozionul "Ecosistemele artificiale si insemnatatea lor pentru omenire, 14 ianuarie.
21. Wei R.D., Lee Y.H.W., Wei Y.H., 1985, Some biochemical responses to PR toxin, a mycotoxin from Penicillium roqueforti. In: Trichothecenes and other Mycotoxins. J. Lacey (ed.). New York: Jonh Wiley and Sons.