Aflatoxins in Municipal Solid Wastes Compost? A First Answer

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The occurrence of potential mycotoxins producing *Aspergillus* molds in municipal solid waste (MSW) and MSW compost was investigated. Among the strains isolated, five were potential mycotoxin producers and three of them actually produced aflatoxins or sterigmatocystin *in vitro* when cultured in liquid complex medium. However, no mycotoxin was recovered from MSW composts. A possible interaction between aflatoxin B_1 and compost components was suggested. A single chloroform extraction allowed 32% of added aflatoxin B_1 to be recovered while 73% was recovered after eight extractions. Because of the possible interaction between compost and aflatoxin B_1 pointed out by this experiment, it was impossible to conclude that aflatoxin B_1 was actually absent in MSW compost. This should be investigated further in order to assess the risks associated with MSW composting and mycotoxins exposure.

Keywords: *Municipal solid waste; compost; mycotoxins; humic acids; adsorption*

INTRODUCTION

Composting transforms organic matter into a stable product containing humus-like substances. This end product is available for agricultural use. Raw matter for composting is various, from yard waste and manure to sewage sludge (SS). Because of the high load of organic matter in municipal solid wastes (MSW), composting can be used to treat and valorize MSW (Stratton et al., 1995). Because composting is a biological process, there is a risk for the end product to be contaminated by microorganisms metabolites such as mycotoxins, a group of fungal metabolites found in a wide variety of foods and feeds. Mycotoxins as a whole have been recognized within the last two decades as a potential threat for human and animal health (OMS, 1980). Among them, aflatoxins produced by several *Aspergillus flavus* and *Aspergillus parasiticus* strains and structural analogs (such as sterigmatocystin) have been established to be hepatocarcinogens and mutagens (Stark, 1980; IARC, 1993). Aflatoxins have been also associated with lung tumors (Burg and Shotwell, 1984) and therefore have been looked for and found associated with feed dust (Lafontaine et al., 1994). If aflatoxins occur in MSW compost, different populations might be exposed to the mycotoxins through several pathways (Figure 1). When compost is used, humans are exposed to aflatoxins through inhalation of dust aerosolized in the atmosphere by compost handling (Millner et al., 1994). Compost can be also ingested by hand−mouth contact. This ingestion has been evaluated to 60−100 mg/day (Calabrese et al., 1989; Sheppard et al., 1992). If compost is used on pastures, animals, which ingest 6% of soil when grazing, might be contaminated (Fries, 1995).

Risk assessment from compost contaminents must take into account compost dilution when it is spread. Dilution in soil is evaluated to 1/10 when compost is used for gardens, park, or sport fields and 1/100 for agricultural use (Déportes et al., 1995).

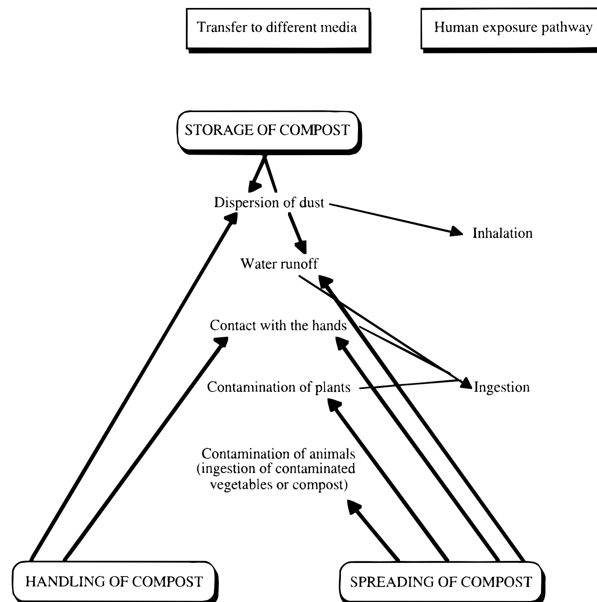

Figure 1. Theoretical transfer media and human exposure pathways for a possible compost contaminant (adapted from Déportes et al., 1995).

The possible occurrence of aflatoxins in SS compost has been discussed by two authors (Clark et al., 1984; Epstein and Epstein, 1985). In a speculative discussion, they disagree on the possible occurrence of aflatoxins in compost. However, it is important to look for these mycotoxins in MSW compost, because of the health threat they present and as mycotoxins can theoretically occur in MSW composts. To our knowledge, the occurrence of aflatoxin in compost has never been checked in a laboratory.

In this paper, we have first examined the possibility of aflatoxins occurring in MSW by looking for the presence of mycotoxinogenic strains at the different steps of the composting process and their aflatoxin production ability in liquid medium. Then, we have

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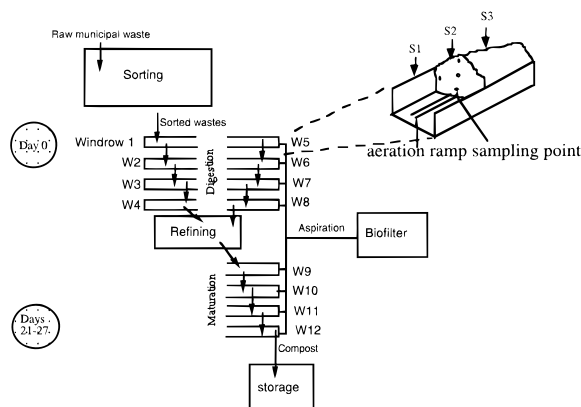

Figure 2. Composting plant and sampling strategy.

investigated the actual presence of aflatoxins and sterigmatocystin in compost and their possible interactions with compost.

MATERIALS AND METHODS

Sampling. Samples were collected at a new composting plant (Siloda, OTVD, France) treating since February 1994 sorted MSW of a 400 000 inhabitant metropolitan area (Grenoble, France). Incoming wastes are sorted before they are delivered to the composting plant. This facility receives the fermentable part of MSW, which is sorted a second time to remove most of the inert particles and only then sent on to the composting process. Degradation occurs in longitudinal silos placed side by side. Ambiant air is sucked through the piles.

MSW is forwarded through two series of windrows (Figure 2). At first, the digestion phase takes place in four windrows. After this digestion phase, waste is refined by mechanical sorting of the inerts (mostly plastics and glasses). Then, the product passes through a second series of four windrows for the maturation phase. Due to the seasonal pattern of the compost market, most of the end product is stored several months in a roofed hall. There are two parallel rows of degradation windrows (W_1–W_4, W_5–W_8). W_1 and W_5 are filled alternately. Only one series of windrows was studied, because both series are similar in terms of MSW origin and composting process. There is only one series of maturation windrows W9–W12 which lumps together W_4 and W_8 material.

Windrows are turned by the Siloda wheel, and compost is forwarded from one windrow to the next every 2–5 days. Finally, the end product is sent to the storage area after 3–4 weeks of processing.

Samples were collected when the windrows were turned. The Siloda wheel was stopped a few minutes and driven backward. In the cross section, samples were collected at four locations in order to account for heterogenicity of the material (Figure 2). The temperature was recorded with a thermic probe plunged 50 cm deep into the heap. This operation was repeated thrice in the windrow, every 8–9 m. Samples were collected in freezer bags, designed for food use and thus presumed to be sterile. At each collection point, 500 g of material was collected and manually blended at the laboratory to obtain a mean sample (about 5 kg) representative of the windrow. Samples for analysis were taken from the mean sample.

A waste cohort was followed through the composting chain, and sample characteristics are summarized in Table 1.

Isolation of Fungi from MSW and Compost Samples. Isolation of fungi from compost samples collected at different steps of composting was accomplished by using the soil plate method of Warcup (Parkinson and Waid, 1960): compost samples were placed into Petri dishes (90 mm diameter), and sterile malt extract (Difco, France), 1.5%; agar (Difco, France), 1.5%; chloramphenicol (Cooper, France), 0.05%) medium was poured over it. After solidification, dishes were incubated at 22 and 30 °C, and fungi were isolated as soon as they appeared. Each sample was analyzed in triplicate.

Table 1. Time of Sampling along the Composting Chain (Days) and Sample Characteristics

age (days)	composting step when collected	mean temp (°C)	H_2O (%)
	During the Active Composting Process		
d 0	W_1 was filled	16.7	46
d 5	W_1 was turned	54.0	47
d 21	W_4 was turned	66.0	36
d 27	W_{11} was turned	56.0	31
	During Storage		
d 174	stored heap is returned	22.3	16
d 242	stored heap is returned	13.2	18

Mycotoxin Production by Isolated Fungi. Sucrose yeast extract (SY) medium was used for fungal growth and toxin production (Stewart et al., 1977). The medium contained (g/L) sucrose (Prolabo, France), 40.0; yeast extract (Difco, France), 20.0. Sterilization was carried out by autoclaving for 15 min at 121 °C. The pH after sterilization was 6.5. Before cultivation in liquid medium, fungi were grown on solid malt extract medium for 1 week to obtain sufficient inoculum, inoculated in 50 mL of SY medium in 250 mL flasks, and grown for 7 days at 30 °C with 180 rpm agitation (diameter of shaking 25 mm). Each strain was analyzed in triplicate.

Mycotoxins Extraction from Fungal Cultures. The fungal mycelia were filtered off, and the culture media were analyzed directly or after extraction (Krivobok et al., 1987). Culture media were extracted with three 20 mL aliquots of chloroform. Mycelia were extracted with hot chloroform for the characterization of sterigmatocystin (Scott et al., 1972). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness at 30 °C under vacuum. The residues were dissolved in 1 mL of chloroform.

Mycotoxin Extraction from MSW and Compost Samples. Fifty grams of the mean sample was mixed and extracted during 10 min with 1 L methanol:water (70:30, v/v). The results given by the analysis of interactions between compost and aflatoxins have driven us to use five serial extractions. After filtration, 40 mL of the extract was passed through immunoaffinity column for aflatoxins B_1, B_2, G_1, and G_2 analysis (RIDA, Transia-Diffchamb SA, France). The column was washed with 15 mL of water and then eluted with 0.5 mL of methanol. The eluate was evaporated to near dryness and the volume adjusted to 0.5 mL of methanol.

TLC Analysis. Thin layer chromatography (TLC) was carried out on a silica gel 60 plate (Merck, Darmstadt, Germany) using a mixture of chloroform:ethyl acetate:90% formic acid (6:3:1, v/v/v) as development solvent (Olivigni and Bullerman, 1977). The aflatoxins were visualized in UV light at 366 and 254 nm (UV_{366} and UV_{254}) before treatment and after $AlCl_3$ treatment for sterigmatocystin (Stoloff et al., 1971).

HPLC Analysis. The presence of aflatoxins was confirmed by high-performance liquid chromatography (HPLC). HPLC analyses were performed with a Shimadzu LC-6A instrument and a Shimadzu C-R6A Chromatopac data processor equipped with a 10 μm C_{18} ODS-Hypersil column (250 × 4.6 mm i.d.) (Shandon HPLC, France). The mobile phase was water: methanol:acetonitrile (4:3:3, v/v/v) at a flow rate of 1 mL/min. Eluted compounds were detected with a Shimadzu RF-530 fluorescence detector (excitation 365 nm, emission 440 nm). They were identified by comparison with pure commercial samples used as external and internal standards (t_R aflatoxins: AFB_1 = 11.1 min; AFB_2 = 9.4 min; AFG_1 = 6.5 min; AFG_2 = 5.6 min).

Interaction between Compost and Aflatoxins. One hundred milligrams of compost was contaminated and mixed with 50 μg of aflatoxin B_1 (50 μL of standard solution, 1 μg/μL). The mixture was put in an Eppendorff vial, filled with 1 mL of $MeOH:H_2O$ (7:2, v/v) solution and blended for 10 min. After centrifugation at 2000 rpm for 2 min, the supernatant was collected for HPLC analysis. The solid fraction was dissolved again in 1 mL of $MeOH:H_2O$ (7:2, v/v) and treated eight times as described above. The same experiment was

Table 2. Likelihood of Aflatoxin Production in MSW[a]

factors associated with aflatoxins production	likelihood in MSW
optimum temperature: 20−35 °C	possible before heat increase of the composting process
humidity: 80-85%	punctually possible
cereals (oats, wheat, corn, rice)	possible
oil-producing seeds (peanut, cotton, walnut, pistachio)	possible
dried fruits	possible

[a] Adapted from Betina (1989), Scudamore (1993), and Stoloff (1976).

performed on 50 μL of aflatoxin B_1 standard solution (1 μg/μL) in an Ependorff vial without compost to evaluate the recovery rate.

Mycotoxins Standards. Aflatoxins mixture (B_1 25 μg, B_2 7.5 μg, G_1 25 μg, G_2 7.5 μg) and aflatoxin B_1 (1 mg) (Sigma Chemicals Co., St. Louis, MO) were solubilized in benzene: acetonitrile (98:2, v/v) to prepare separate stock solutions containing 1 and 2.5 μg of B_1/mL. Stock solution are used to prepare when necessary aflatoxin standard in benzene:acetonitrile (98:2, v/v) at suitable concentration. Sterigmatocystin (1 mg) (Acros Organics, Pittsburgh, PA) was solubilized in 1 mL of benzene:acetonitrile (98:2, v/v). Solutions were kept frozen.

RESULTS AND DISCUSSION

Fungal Strains Isolation and Mycotoxin Detection from MSW and Compost. Among the strains isolated from MSW and compost samples, we found five potentially mycotoxigenic strains: *A. flavus* (2 strains), *A. parasiticus* (2 strains), and *A. sydowii* (1 strain). *A. flavus* and *A. parasiticus* were found in the first step of the composting process (MSW, d 0) and during compost storage (d 174 and 242). *A. sydowii* was found only in stored compost. In spite of the presence of these strains, no mycotoxin (aflatoxins or sterigmatocystin) was found in MSW and compost samples even after five extractions. MSW is an unusual medium for aflatoxin-producing molds which have mainly been described in harvested or stored cereals (Betina, 1989). It is heterogeneous and therefore can punctually supply the alimentary needs of a wide panel of microorganisms. During composting, high temperatures decrease dramatically *A. flavus* and *A. parasiticus* populations and aflatoxin production is very unlikely to occur. But, MSW are collected, stored in bins, and wait for sorting before getting composted.

During this period, as MSW is highly heterogeneous, environmental conditions favorable for mycotoxins production are likely to occur, at least in some places (Table 2). Mycotoxins are heat resistant and thus will remain until the end product is obtained (Jones, 1977).

Production of Mycotoxins by the Fungal Strains Isolated from MSW and Composts. A potential aflatoxigenic strain does not always produce aflatoxin. Among the five potential mycotoxin producers, only three actually produced mycotoxins in liquid complex SY medium after 5−7 d growth: *A. flavus* (aflatoxin B_2), *A. parasiticus* (aflatoxins B_1, B_2, G_1, and G_2), and *A. sydowii* (sterigmatocystin) (Table 3 and Figure 3). Others had noticed that various aflatoxins were produced: aflatoxin B by *A. flavus* and aflatoxins B and G by *A. parasiticus* (Eppley, 1966; Jacquet and Tantaoui-Elaraki, 1977). Sterigmatocystin, mainly intracellular mycotoxin, is only found in the mycelium as described

Table 3. Mycotoxins Production in Liquid Culture Medium from *Aspergillus* Strains Isolated from MSW Compost[a]

strains	sample	mycotoxins	mycotoxins quantification (μg/L SY media)
A. parasiticus Speare	d 0		
A. parasiticus Speare	d 242	aflatoxins B_1, B_2, G_1, G_2	40
A. flavus Link:Fr.	d 0	aflatoxin B_2	2
A. flavus Link:Fr.	d 242		
A. sydowii (Bain. & Sartory) Thom & Church	d 242	sterigmatocystin	NT

[a] Mycotoxins detection was by TLC analysis. Aflatoxins were confirmed by HPLC analysis with internal standards. NT: not tested.

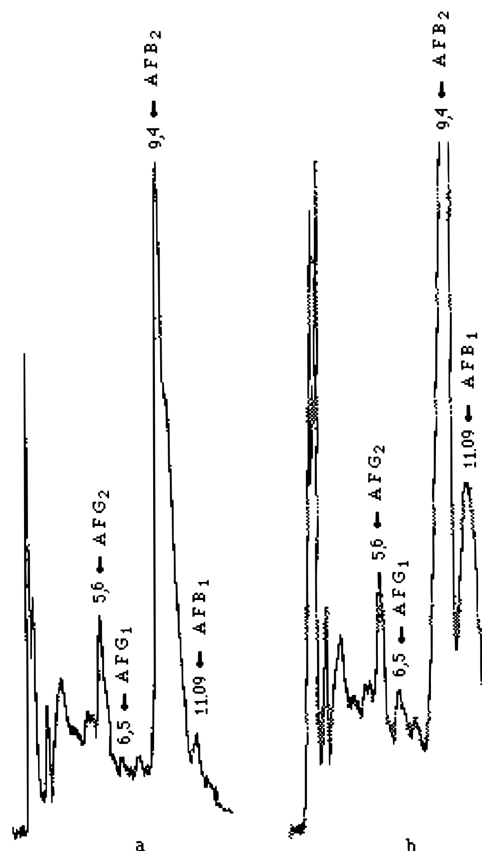

Figure 3. Aflatoxins B_1, B_2, G_1, and G_2 produced by *A. parasiticus* recovered from the first stage of composting. HPLC parameters: flow rate, 1.25 mL/min, water:methanol:acetonitrile (4:3:3, v/v/v); fluorometric detection (excitation 365 nm, emission 440 nm). (a) 10 mL of medium extract injected. (b) 10 μL of medium extract and 15 μL of aflatoxin B_1 (AFB_1) standard (10 ng/10 μL) injected.

by Filtenborg et al. (1983). It was never detected at any step of composting.

Interaction between Compost and Aflatoxins. However, in our study, aflatoxins were not found in MSW or in compost samples. Interaction between compost and polyaromatic molecules (McCarthy and Jimenez, 1985; Cozzi et al., 1993) or benzo[*a*]pyrene (Sato, 1987) was previously observed. In order to look for a possible interaction between aflatoxins and humic acids, the main components of compost, a compost sample was contaminated with aflatoxin B_1 and treated by serial extractions. Only 32% of aflatoxin B_1 was recovered after the first extraction. After five serial extractions, 70% of aflatoxin B_1 was recovered and 73%

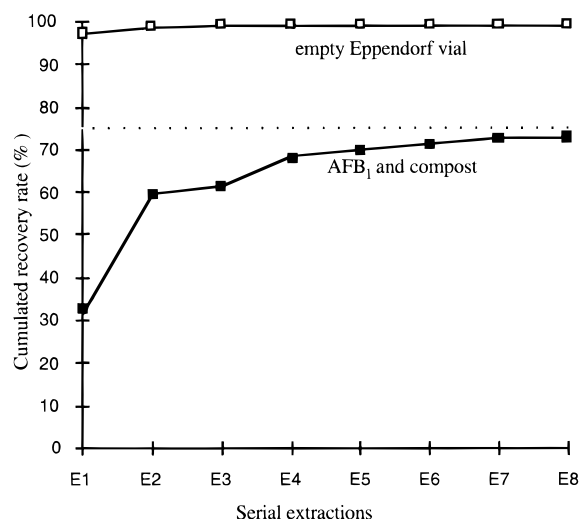

Figure 4. Relation between the recovery of aflatoxin B_1 (AFB_1) and the number of extractions.

after eight extractions (Figure 4). This result suggests that there is actually an interaction between aflatoxins and compost components. This interaction is partially reversible, as serial extractions allowed 73% of aflatoxin to be recovered from contaminated compost, but the real nature of this interaction is still unknown. After serial extractions of benzo[*a*]pyrene from humic acids, Sato et al. (1987) supposed that the interaction was weak, reversible, and could be an adsorption. This interaction could explain the very low recovery rate of a single extraction. It also could account for the lack of aflatoxins in extraction of compost samples in spite of the occurrence of aflatoxigenic strains. Recently, Madden and Stahr (1995) have shown that the addition of soil to a highly aflatoxin B_1-contaminated chicken diet significantly reduced aflatoxin B_1 levels in liver. As mycotoxins can occur in MSW compost, interaction should be more investigated in order to establish the bioavailability of the mycotoxins for humans and animals when compost is ingested or compost dust inhaled.

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LITERATURE CITED

Betina, V. *Bioactive molecules. Vol 9: Mycotoxins: chemical, biological and environmental aspects*; Elsevier: Amsterdam, 1989.

Burg, W. R.; Shotwell, O. L. Aflatoxin levels in airborne dust generated from contaminated corn during harvest and at an elevator in 1980. *J. Assoc. Off. Anal. Chem.* **1984**, *26*, 309−312.

Calabrese, E. J.; Barnes, R.; Stanek, E. J.; Pastides, H.; Gilbert, C. E.; Veneman, P.; Wang, X.; Lasztity, A.; Kostecki, P. T. How much soil do young children ingest: an epidemiologic study. *Regul. Toxicol. Pharmacol.* **1989**, *10*, 123−137.

Clark, C. S.; Bjornson, H. S.; Schwartz-Fulton, J.; Holland, J. W.; Gartside, P. S. Biological health risks associated with the composting of wastewater treatment plant sludge. *J. Water Pollut. Control Fed.* **1984**, *52*, 1269−1276.

Cozzi, R.; Nicolai, M.; Perticone, P.; De Salvia, R.; Spuntarelli, F. Desmutagenic activity of natural humic acids: inhibition of mitomycin C and maleic hydrazide mutagenicity. *Mutat. Res.* **1993**, *299*, 37−44.

Déportes, I.; Benoit-Guyod, J.-L.; Zmirou, D. Hazard to man and the environment posed by the use of urban waste compost: a review. *Sci. Total Environ.* **1995**, *172*, 197−222.

Eppley, R. M. Aflatoxins: a versatile procedure for assay and prepatory separation of aflatoxins from peanut products. *J. Assoc. Off. Anal. Chem.* **1966**, *XLIX*, 1218−1223.

Epstein, E.; Epstein, J. I. Health risk of composting: a critique of the article “Biological health risks associated with the composting of wastewater plant sludge”. *BioCycle* **1985**, May/June, 38−40.

Filtenborg, O.; Frisvad, J. C.; Svendsen, J. A. Simple screening method for molds producing intracellular mycotoxins in pure cultures. *Appl. Environ. Microbiol.* **1983**, *45*, 581−585.

Fries, G. F. Transport of organic environmental contaminants to animal products. *Rev. Environ. Contam. Toxicol.* **1995**, *141*, 71−109.

IARC. *IARC monograph on the evaluation of the carcinogenic risks to human: some naturally occuring substances: some food, tons and constituents, heterocyclic aromatic amines and mycotoxins*; IARC: Lyon, 1993; No. 56.

Jacquet, J.; Tantaoui-Elaraki, A. Sur la notion du pouvoir toxinogène des souches d'*Aspergillus* du groupe *flavus*. (The toxicogenic power of *Aspergillus flavus* group strains.) *Ann. Nutr. Aliment* **1977**, *31*, 563−574.

Jones, B. D. Aflatoxins and related coumponds: chemistry. In *Mycotoxic fungy, mycotoxins, mycotoxincoses, vol. 1*; Willye, Th. D., Morehouse, L. G., Eds.; Dekker: New York, 1977; pp 136−143.

Krivobok, S.; Seigle-Murandi, F.; Steiman, R.; Marzin, D. J. Screening methods to detect toxigenic fungi in liquid medium. *Microbiol. Methods* **1987**, *7*, 29−36.

Lafontaine, M.; Delsaut, P.; Morele, Y.; Taiclet, A. Aflatoxines: prélèvements et analysis dans une filière de fabrication d'aliments pour animaux. (Exposure to aflatoxins in a branch of the animal feed manufacturing industry. Sampling and analysis.) *Cah. Notes Doc.* **1994**, *156*, 297−305.

Madden, U. A.; Stahr, H. M. Retention and distribution of aflatoxin in tissues of chicks fed aflatoxin-contaminated poultry ration amended with soil. *Vet. Hum. Toxicol.* **1995**, *37*, 24−29.

Mc Carthy, J. F.; Jimenez, B. D. Interactions between polycyclic aromatic hydrocarbons and disolved humic acid material: binding and dissociation. *Environ. Sci. Technol.* **1985**, *19*, 1072−1076.

Millner, P. D.; Olenchock, S. A.; Epstein, E.; Rylander, R.; Haines, J.; Walker, J.; Ooi, B. L.; Horne, E.; Maritato, M. Bioaerosols associated with composting. *Compost Sci. Util.* **1994**, *2*, 4−57.

Olivigni F. J.; Bullerman L. B. Simultaneous production of penicillic acid and patulin by a *Penicillium* species isolated from cheddar cheese. *J. Food Sci.* **1977**, *42*, 1654−1657.

OMS. *Mycotoxines. Critères d'hygiène de l'environnement. II*; Organisation Mondiale de la Santé: Genève, 1980.

Parkinson, D.; Waid, J. S. *The ecology of soil fungi*; Liverpool University Press: Liverpool, England, 1960.

Sato, T.; Ose, Y.; Nagase, H.; Hayase, K. Mechanism of the desmutagenic effect of humic acid. *Mutat. Res.* **1987**, *176*, 199−204.

Scott, P. M.; van Walbeek, W.; Kennedy, B.; Anyeti, D. Mycotoxins (ochratoxin A, citrinin and sterigmatocystin) and toxigenic fungi in grains and others agricultural products. *J. Agric. Food Chem.* **1972**, *20*, 1103−1109.

Scudamore K. A. Mycotoxins in stored products: myth or menace. *Int. Biodeterior. Biodegrad.* **1993**, *32*, 191−203.

Sheppard, S. C.; Gaudet, C.; Sheppard, M. I.; Cureton, P. M.; Wrong, M. P. The development of assessment and remediation guidelines for contaminated soils, a review of the science. *Can. J. Soil Sci.* **1992**, *72*, 359−394.

Stark, A. A. Mutagenicity and carcinogenicity of mycotoxins: DNA binding as a possible mode of action. *Annu. Rev. Microbiol.* **1980**, *34*, 235−263.

Stewart, R. G.; Wyatt, R. D.; Ashmore, M. D. The effect of various antifungal agents on aflatoxin production and growth characteristics of *Aspergillus parasiticus* and *Aspergillus flavus* in liquid medium. *Poult. Sci.* **1977**, *56*, 1630−1635.

Stoloff, L.; Neisheim, S.; Yin, L.; Rodricks, J. V.; Stack, M.; Campbell, A. D. A multimycotoxin detection method for aflatoxins, ochratoxins, zearalenone, sterigmatocystin and patulin. *J. Assoc. Off. Anal. Chem.* **1971**, *54*, 91−97.

Stoloff, L. Occurence of mycotoxins in foods and feed. In *Mytcotoxins and other fungal related food problems*; Rodricks, J. V., Ed.; Advances in Chemistry Series 149; American Chemical Society: Washington, DC, 1976.

Stratton, M. L.; Braker A. V.; Rechcigl, J. E. Compost. In *Soil amendments and environmental quality*; Rechcligl, J. E., Ed.; CRC: Boca Raton, FL, 1995.

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