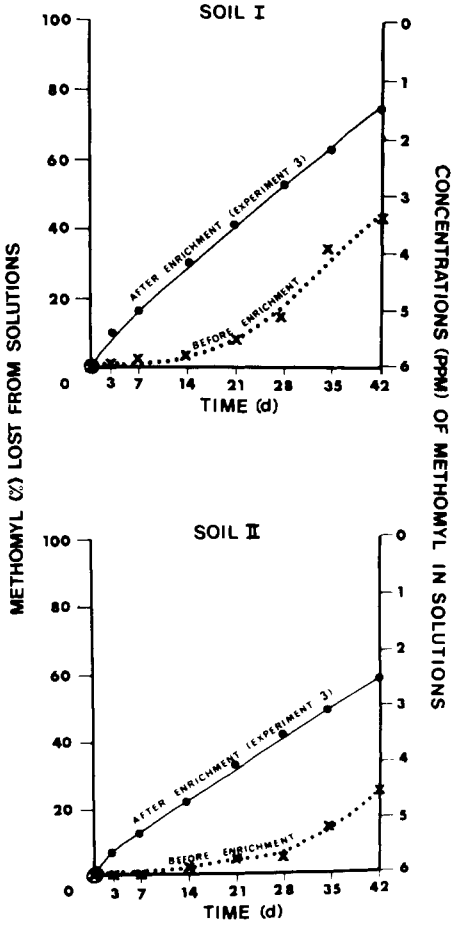

Figure 3. Effects of microbial transformation on rate of loss of methomyl and on changes in concentration of methomyl in solutions before and after enrichment of soils I and II.

(e.g., value of n). Cognizance should also be taken of the fact that the polyvinyl alcohol in the soils could affect the degree of adsorption of methomyl.

(c) Experiment 3. Since this experiment was run after experiment 1, most sites of adsorption for methomyl in the soils were probably occupied and the contribution of adsorption to the loss of methomyl from solution was expected to be small (certainly less than 5%). Therefore, the rapid loss of methomyl in experiment 3 (Figure 3) can be attributed largely to microbial transformation. Since virtually no lag phase was detected it would appear that the treatment of the soils with methomyl in experiment 1 resulted in enrichment. However, after about 3 days the rate of loss of methomyl was constant, and after about 21 days the rate of loss was similar to that before enrichment (Figure 3). Whereas in another situation (Kearney et al., 1965) where enrichment has been described, not only has the duration of the lag phase decreased but also the rate of transformation has increased. Therefore, in the absence of control soils (i.e., soils untreated with methomyl) in experiment 3, it is not absolutely certain if the soils used in the experiment were enriched. The important issue here, with reference to the field situation, is that the time required for the concentration of methomyl to be reduced to half of the initial concentration will probably be less in soils recently treated with methomyl (Figure 3).

CONCLUSION

The contribution of adsorption to the dissipation of methomyl from solutions was small when compared with that of microbial transformation. Therefore the results of the perfusion studies, although of limited applicability to the field situation, show that microbial transformation is likely to be of major importance in determining the behavior of methomyl in soils.

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A Clean-Up Procedure for HPLC Analysis of Aflatoxins in Agricultural Commodities

A minicolumn method has been modified as a simple, efficient clean-up procedure for use of high-pressure liquid chromatography for determination of aflatoxins in peanuts, rice, and corn. The procedure, which utilizes a heavy metal salt precipitation combined with a short alumina filtration under vacuum, can be easily adapted to various sample sizes and provides adequate clean-up for use with both microparticulate and pellicular solid support columns.

The analysis of aflatoxins by high-pressure liquid chromatography has been reported by Rao and Anders (1973), Seiber and Hsieh (1973), Seitz (1975), and Pons (1976). None of these authors reported a clean-up procedure to remove interferences. A procedure, which Holaday and Lansden (1975) used to qualitatively de-

termine total aflatoxin in peanuts and rice, has been modified to provide clean-up of aflatoxin extracts from these commodities for HPLC analysis.

EXPERIMENTAL SECTION

Equipment. The liquid chromatographic system is a Waters Associates Model ALC 202 fitted with a 6000-psi pump and a Waters 350-nm ultraviolet absorption detector. Chromatograms were recorded on a 10-mV Texas Instrument Servo Riter II Recorder. Samples were injected with a Valco CV-6UHPA-C-20 (7000 psi) injection valve fitted with a 100-μL sample loop (Valco Instruments, Houston, Tex.).

Precision bore 2 ft × 0.125 in. i.d. stainless steel columns were dry-packed with Corasil II, 37–50 μm (Waters Associates, Milford, Mass.). The analytical columns were fashioned into 4-ft sections by a 2 × 0.020 in. i.d. connecting tube and conditioned with the eluting solvent of $CHCl_3$:heptane:MeOH (54.5:45:0.5 v/v). Analyses were made at a flow rate of 1.0 mL/min.

Chemicals and Supplies. Aflatoxins B_1, B_2, G_1, and G_2, as a standard mixture for TLC quantitation, were obtained from Southern Regional Research Center, New Orleans, La. Aflatoxins were also obtained from solid state fermentation of rice by *Aspergillus parasiticus*, NRRL no. 2999, according to the procedures outlined by Hesseltine (1973) and Shotwell (1966). Chloroform and methanol were Mallinckrodt spectro grade and heptane was Mallinckrodt AR grade (Mallinckrodt Chemical Works, St. Louis, Mo.).

The following standard laboratory items are used for the clean-up procedure of the aflatoxin extracts: 1-qt. Waring blender, graduated cylinders, Buchner funnels, glass fiber filter paper, 500-mL filter flasks, 250-mL separatory funnels, and a rotary-evaporator system. The alumina column is a 60-mL sintered glass funnel layered with 30 g of basic alumina, grade IV (aluminum oxide W 200 basic, ICN Pharmaceuticals, Cleveland, Ohio), and then with 30 g of anhydrous sodium sulfate powder. The extraction solvent is methanol:water (80:20). The salt solution is prepared by dissolving 150 g of zinc sulfate, 150 g of sodium chloride, and 3.75 mL of glacial acetic acid in distilled water in a 1-L volumetric flask. All chemicals and solvents are ACS grade unless otherwise specified.

Procedure. A sample of 100 g is blended with 200 mL of the extraction solvent for 2 min at high speed. The entire mass is vacuum filtered and 50 mL of the salt solution is added to 100 mL of the filtrate. The resulting precipitate is filtered out and 100 mL of filtrate is extracted with three 25-mL portions of chloroform. The chloroform extract is pulled through the alumina column under vacuum and the column is washed with two 10-mL portions of chloroform. The chloroform eluent and washes are collected and evaporated to dryness on a rotary evaporator (40–45 °C). The residue is redissolved in 1 mL of spectral grade chloroform from which a 50-μL sample is injected into the chromatograph.

DISCUSSION

The HPLC pellicular silica gel column system used was developed as an alternate to the microparticulate silica gel systems. The microparticulate columns, as described by Seitz (1975) and Pons (1976), have a more efficient and sensitive response than pellicular columns. However, the microparticulate columns are more expensive (pellicular columns are about a sixth the cost of microparticulate columns) and require partial deactivation by equilibration with water-wet solvents. Water saturated chloroform, Seitz (1975) and Pons (1976), requires daily preparation since

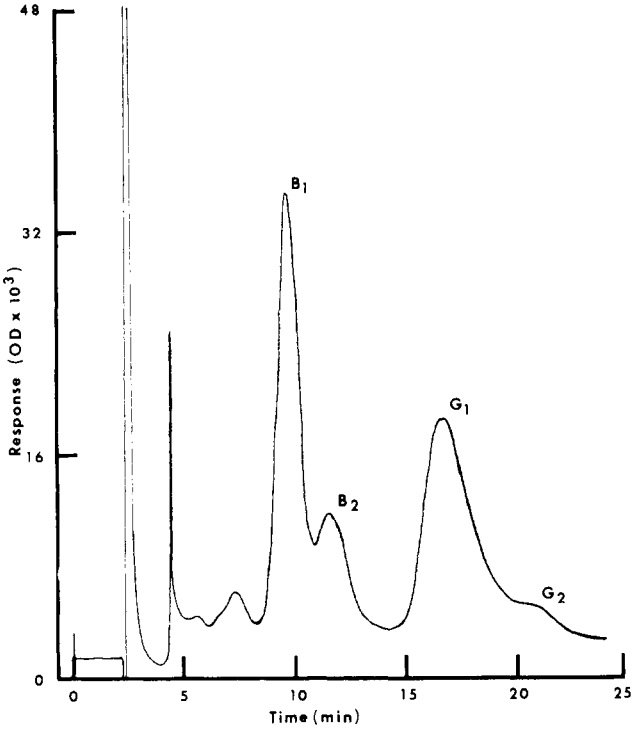

Figure 1. HPLC analysis of aflatoxins in naturally contaminated peanuts (0.45 μg of B_1; 0.23 μg of B_2; 0.76 μg of G_1; 0.34 μg of G_2).

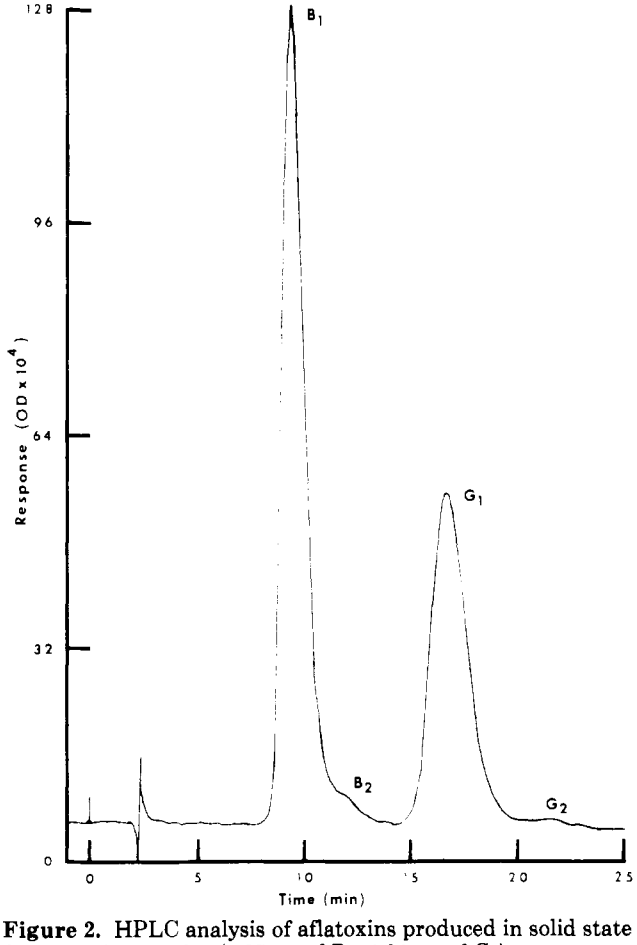

Figure 2. HPLC analysis of aflatoxins produced in solid state fermentation on rice (1.35 μg of B_1; 1.91 μg of G_1).

chloroform degradation occurs even under excellent storage conditions. This laboratory uses both the microparticulate systems of Seitz (1975) and the pellicular system presented here; however, the pellicular system is used for most

routine application because of the cost and storage factors. The clean-up procedure works well with both columns. The chromatograms presented are from typical analyses utilizing the pellicular columns.

Overall recovery of aflatoxins is 90% as determined from peanut samples spiked with known amounts of aflatoxin B_1. The minimum linear limits of the pellicular HPLC system are 20 ng of aflatoxin B_1, 20 ng of B_2, 30 ng of G_1, and 50 ng of G_2. For the 100-g sample, these limits, which convert to 12 ppb for aflatoxin B_1 and B_2, 18 ppb for aflatoxin G_1, and 30 ppb for aflatoxin G_2, may be further lowered by increasing the sample size or injection volume, provided there are no major interferences. The sample mass/extraction volume ratio must be retained for larger samples. A typical chromatogram obtained from a 100-g sample of naturally contaminated peanuts is presented in Figure 1.

If samples are known to have a high aflatoxin content/interference ratio, the alumina column may be omitted thereby reducing the sample preparation time from approximately 30 to 20 min. The chromatogram shown in Figure 2 was taken from a trace mineral study on aflatoxin production by NRRL no. 2999 in solid state fermentation. The 50-μL injection sample was taken directly from the 75-mL chloroform extract.

Presently the clean-up system is being enlarged to include multimycotoxin analysis which will undoubtedly require microparticulate columns and gradient elution.

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Amino Acid Composition of Dried Citrus Sludge and Its Potential as a Poultry Feedstuff

Three sludges, aerobic, anaerobic, and sun-dried, formed as citrus processing waste water by-products were analyzed for their protein and amino acid contents. Comparisons were made between amino acids present in these citrus sludges and those listed as nutrient requirements of broilers. The limiting amino acids were taken into account in considering the sludges as poultry feed supplements.

Citrus sludge is a decomposition product from plant materials discarded during the processing of citrus. An estimated 83 million lb/year of citrus sludge is produced in the United States (Ratcliff, 1976; U.S. Department of Agriculture, 1975). Managing large quantities of sludge generated by individual waste treatment systems is both expensive and technically difficult. Since excess sludge can adversely affect the efficiency of waste digesters it must be removed and discarded periodically. This sludge contains proteinaceous material formed during the digestion process involving microbial decomposition in both aerobic and anaerobic digesters. The use of citrus sludge as an animal feed supplement would eliminate a waste management problem as well as create a new product from the citrus industry. As part of investigations into the use of this by-product as a source of nutrition for animal feeds, the protein and amino acid contents need to be determined and compared with known nutrient requirements.

Some studies on the composition and use of citrus sludge as an animal feed have been reported. Dougherty and McNary (1958) analyzed a laboratory-created activated citrus sludge and speculated about its use as a poultry feed supplement. Jones et al. (1975) reported on the costs of drying a citrus sludge and suggested that dried sludge as an ingredient in a properly formulated poultry diet may be a viable means of reducing the cost of sludge handling. Damron et al. (1974, 1977) used a dried citrus sludge as a feed supplement in broiler feeding trials. None of these studies reported a detailed amino acid profile on the sludge used.

The current study reports the protein contents and amino acid profiles for three types of citrus sludges (by-products of commercial citrus processors) and compares the amino acid profiles with the nutritional requirements for poultry reported by the National Academy of Sciences (1971).

MATERIALS AND METHODS

Sludge samples were obtained from waste treatment facilities of commercial citrus processors. The following sludge samples were dried in a vacuum oven at 105 °C under 31 in. Hg for 2 days, 4 days, and 1 day, respectively.

Aerobic sludge (ca. 1% moisture) was prepared from the gelatinous material (ca. 90% moisture) separated with a "skimmer/clarifier" from the surface of an aerobic digester.

Anaerobic sludge was prepared from wet sludge (ca. 93% moisture) which had accumulated in the bottom of an anaerobic digester.

Sun-dried sludge was obtained from the waste spray field of a processor whose waste treatment system con-