Uptake of Mercuric Chloride and Methylmercury Chloride from Liquid Media by *Aspergillus niger* and *Penicillium notatum.*

by

J. E. HARDCASTLE and NARA MAVICHAKANA

Chemistry and Biology Departments
Texas Woman's University
Denton, Tex. 76204

Excess mercury in the environment represents a hazard to many forms of life. Fungi may absorb mercury and other environmental contaminents from their soil and water environments. Since fungi can be in the food chain of man, their absorption of various environmental contaminents (such as mercury, cadmium and radionuclides) should be studied. The objective of the research reported in this paper was to measure the uptake of an inorganic and an organic form of mercury by two common soil fungi, Aspergillus niger and Penicillium notatum.

EXPERIMENTAL

Preparation of cultures

Spore suspensions of Aspergillus niger and Penicillium notatum (WARDS NATURAL SCIENCE ESTABLISHMENT, INC. ROCHESTER, NEW YORK) were prepared from two week old cultures on agar slants. Slants were washed with 10 ml portions sterile Czapek broth. The washings were pooled in a 125 ml sterile flask. Counts of spore suspensions were made by means of a Neubauer Counting Chamber. The final concentration was adjusted to 2×10^7 spores per ml. Flasks containing 150 ml of sterile Czapek media were inoculated aseptically with one ml of standard spore suspension. The flasks were then incubated at 25C in the dark for four days.

The inorganic mercury stock solution was prepared from $HgCl_2$ (crystal, reagent ACS), and was tagged with Hg-203. The specific activity of the mercury in this solution was 50 μ Ci/mg. Dilutions were made from this stock solution and 50 ml aliquots from these dilutions were added to the culture flasks after the 4 day incubation period. The final concentrations of mercury in the culture flasks were 0.1, 1.0, 2.0, 5.0, 10.0, and 20.0 ppm. A control was made by adding 50 ml distilled water to the culture flasks. Each treatment was replicated 3 times, and all the cultures were incubated for 10 more days.

The organic mercury stock solution was prepared from methylmercury chloride (CH_3HgCl), and was tagged with Hg-203 (as methylmercury chloride). The specific activity of this stock solution was 250 μ Ci/mg. Dilutions were made from this stock solution and 50 ml aliquots from these solutions were added to the culture

Bulletin of Environmental Contamination & Toxicology,
Vol. 11, No. 5 © 1974 by Springer-Verlag New York Inc.

flasks after the 4 day incubation. The final amounts of mercury in the culture flasks were 0.025, 0.050, 0.075, 0.100, 0.200, 0.500 ppm, and 1.00 ppm. A control was made by adding 50 ml distilled water to the culture flasks. Each treatment was replicated 3 times, and all the cultures were incubated for 10 more days.

Radioassay of the fungal tissues

The fungal tissues were harvested and washed 3 times with distilled water to remove excess nutrient solution. After washing the fungal tissues were placed on tared aluminum pans, and dried in an air current oven at 70° C for 4 hours. The dried tissues were cut in small pieces with scissors, and 0.50 gm samples of the tissues were made into uniform wafers 1-1/4" in diameter using a hydraulic press (FRED S. CARVER, INC., MENOMONEE FALLS, WISCONSIN). The wafers were then assayed for radioactivity with the BECKMAN WIDE BETA II.

The counting efficiency of the instrument was determined by adding different amounts of Hg-203 to 0.50 gm samples of the dried, macerated non-radioactive fungal tissues. The tissues were then allowed to dry at room temperature for 24 hours. Uniform wafers 1-1/4" in diameter were made from the dried tissues and were assayed for radioactivity using the BECKMAN WIDE BETA II. The counting efficiencies for each level of mercury were calculated by dividing the observed cpms by dpms. The counting efficiencies were linear in the range of activities used.

From the cpm data of the fungal tissues grown in Hg-203 media, the counting efficiencies, and the specific activities, the amounts of Hg in the fungal tissues were calculated. Graphs were made of μ gm Hg per gram of fungal tissues versus ppm Hg in the nutrient medium (Graph 1 & 2).

RESULTS

The two test fungi appeared to grow normally in the nutrient media containing up to 10 ppm Hg as $HgCl_2$, and up to 0.50 ppm Hg as CH_3HgCl. The mass of tissue produced did not appear to be related to the amount, and chemical form of mercury in the nutrient solution. No abnormalities in fungal pigmentation and morphology were observed in these experiments. However, the fungi did not grow in nutrient media containing 20 ppm Hg as $HgCl_2$ and 1.0 ppm Hg as CH_3HgCl.

The quantitative results of these uptake studies are presented in Tables 1 and 2. At the 10 ppm level of Hg as $HgCl_2$ in the nutrient media *Aspergillus niger* absorbed more than 700 μ gm Hg, and *Penicillium notatum* absorbed more than 500 μ gm Hg per gram of fungal tissue. However, at the 0.50 ppm level of Hg as CH_3HgCl *A. niger* absorbed 25 μ gm Hg, and *P. notatum* absorbed about 20 μ gm Hg per gm of fungal tissue. Thus, the maximum amount of mercury absorbed by each organism as $HgCl_2$ was about 25 times that

absorbed as CH_3HgCl. Thus, the fungal tissue could tolerate much more of the inorganic mercury than the organic mercury, and methyl mercury must be about 25 times as toxic to the mycelia of these two fungi as is mercuric chloride.

TABLE I

Absorption of Mercuric Chloride by
Aspergillus niger and *Penicillium notatum*

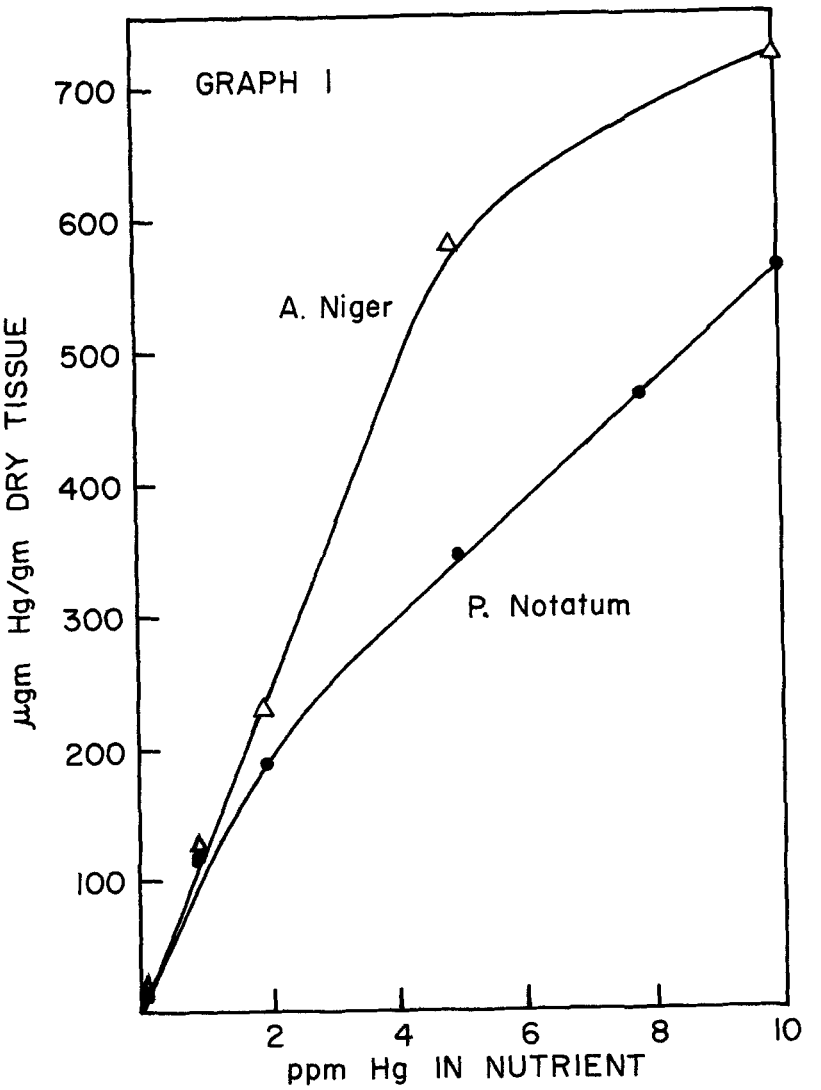
ppm Hg in nutrient	μ gm of Hg absorbed per gram of dry tissue	
	A. niger	*P. notatum*
0.1	14	18
1.0	128	126
2.0	230	193
5.0	580	341
10.0	720	553

TABLE II

Absorption of Methylmercury Chloride by
Aspergillus niger and *Penicillium notatum*

ppm Hg in nutrient	μ gm of Hg absorbed per gram of dry tissue	
	A. niger	*P. notatum*
0.025	6.8	0.7
0.050	11.1	2.5
0.075	14.0	4.5
0.100	18.2	6.5
0.200	19.0	11.2
0.500	25.2	19.1

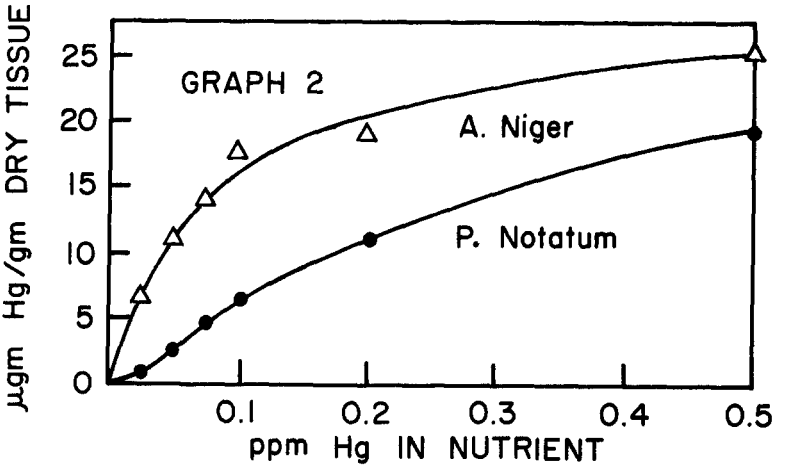
The values listed in Tables 1 and 2 are the averages of 3 replicates. The differences between organisms at each treatment level, and between treatments for each organism were highly significant ($P<0.01$). The amounts of mercury (both organic and inorganic) absorbed by *A. niger* were significantly greater than those absorbed by *P. notatum*, and, thus, the mycelia of *A. niger* were more tolerant to the mercury than those of *P. notatum*. Tolerance to mercury by the fungi used in this study is in agreement with results reported by several researchers (ASHWORTH AND AMIN, 1964: JACKSON, 1963; MACFARLANE AND NADEEN, 1948; BONALY et al., 1961; GERARDIN AND KAYSER, 1958).



GRAPH I Uptake of Hg as mercuric chloride

DISCUSSION

The results of this research indicate that Aspergillus niger and Penicillium notatum do have a certain tolerance for mercury, both inorganic and organic, and are able to grow and reproduce with certain levels of this element in their tissues. Because of their abundance, and ability to concentrate mercury, the fungi's role in the metabolism of mercury in the environment is important, and should receive further study. Although the levels of mercury in the environment are usually less than 0.5 ppm (BOEGEN et al., 1971; WILLISTON, 1968; CRANSTON AND BUCKLEY, 1972) fungi could pass considerable quantities of this element on to man when



GRAPH 2 Uptake of Hg as methylmercury chloride

they are in the food chain of man.

ROGERS, et al. (1972) report the use of certain fungi (among them are species of Aspergillus and Penicillium) to produce edible protein from waste cellulosics and wood pulp. The protein would be extracted from the fungal cells, and fed to animals or even to human beings. These waste cellulosics and wood pulp may contain mercury, or other heavy metals that could be absorbed and concentrated by the fungal cells. Some of the mercury would be bound to the proteins in the fungal cells. Thus, if any proposal such as this is to be seriously considered for the production of human food then the metabolism of mercury in fungi must receive more study.

REFERENCES

ASHWORTH, L. J., and J. V. AMIN: Phytopathology 54, 1459 (1964).
BOEGEN, J. G., H. T. SHACKLETTE, R. L. TURNER: U. S. Geological Survey, circular 644 (1971).
BONALY, R., F. KAYSER, and A. VARNEY: Bull. Soc. Pharm. Nancy 51, 11 (1961).
CRANSTON, R. E., and D. E. BUCKLEY: Envir. Sci. Tech. 6, 274 (1972).
GERARDIN, C., and F. KAYSER: Soc. Biol. Paris, Compt. Rend. 152, 1780 (1958).
JACKSON, C. R.: Plant Dis. Rept. 47, (1963).
MACFARLANE, E. W., and L. V. NADEEN: Soc. Exptl. Biol. Med. Proc. 67, 118 (1948).
ROGERS, C. J., E. COLEMAN, D. F. SPINO, T. C. PURCELL, P. V. SARPINO: Envir. Sci. Tech. 6, 715 (1972).
WILLISTON, S. H.: J. Geophys. Res. 73, 7051 (1968).