plateau; perennial ice has appeared on the continent, extending well down into middle latitudes.

If the latitudinal heat diffusivity is reduced, with a lowering of S, there is no sharp drop in temperature when the winter snow line approaches the north side of the middle-latitude plateau (curve A in Fig. 1). There is, instead, a sharp increase in the rate of cooling with lowering S; the appearance of perennial ice on the continent is roughly coincident, but the explosive southward advance is replaced by a more retarded expansion. The rapid expansion of continental ice with large diffusivity occurs as a result of the reduced latitudinal temperature gradients; for a given reduction of mean temperature, a much greater surface area can be affected than when a larger temperature gradient is present.

The surface albedo values for snow-ice used in these experiments are very high and are more appropriate for polar latitudes (*2, 6*). Additional experiments were carried out with the use of a fresh snow value of 0.75 and an old snow value of 0.55; the response was similar to curve A in Fig. 1, displaced toward somewhat lower values of S. A more comprehensive test of the topographic enhancement shoud allow for albedo dependence on the terrain type, which is not easily incorporated into the model used here.

The transition to increased sensitivity with the lowering of S, at 286 K, is about 2 K below the present temperature of the Northern Hemisphere (*3*). This model estimate may be low because of an underprediction of summer temperatures, as seen in Fig. 2.

The model's zonal symmetry may result in excessive sensitivity; different distributions of topography with longitude, each with the same zonal average, can produce different rates of increased snow cover with a displacement of the snow line. The absence of the Asian monsoon in the model rules out consideration of the large seasonal fluctuation of the snow line over Asia, and in winter the large deviation from the zonal mean (*7*). Another missed feature is the large area of permanent snow over the Himalayas. Topographic enhancement of feedback may also be exaggerated in the model, because of the use of snowfall rate independent of longitude, the snowfall and hence the mean albedos over the Asian landmass being overestimated.

Although these numerical experiments are only suggestive of topographic enhancement of albedo feedback, the establishment of such a link between climate and topography would provide a potential mechanism for climate change on the geologic time scale. It is likely, for example, that most of the features making up the middle-latitude feature achieved roughly their present elevations in the early Pleistocene, about 2 million years ago (*8*), that is, at the beginning of Northern Hemisphere glaciations.

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Removal of Uranium(VI) from Solution by Fungal Biomass and Fungal Wall–Related Biopolymers

Abstract. Penicillium digitatum *mycelium can accumulate uranium from aqueous solutions of uranyl chloride. Azide present during the uptake tests does not inhibit the process. Killing the fungal biomass in boiling water or by treatment with alcohols, dimethyl sulfoxide, or potassium hydroxide increases the uptake capability to about 10,000 parts per million (dry weight). Formaldehyde killing does not enhance the uranium uptake. The inference that wall-binding sites were involved led to the testing of uranium uptake by chitin, cellulose, and cellulose derivatives in microcolumns. All were active, especially chitin.*

Although bioaccumulation of the heavy elements has been well known and well documented for more that 30 years (*1*), our recent discovery of the capability of *Penicillium digitatum* isolates to absorb uranyl chloride (UO_2Cl_2) (*2*) is one of the few examples in which fungi exhibit the behavior (*3, 4*).

This capability is all the more significant in light of the additional demonstrations that (i) the absorbed uranium can be quantitatively recovered if one strips the metal-loaded mycelium with aqueous solutions of alkali carbonates and (ii) the biosorbent property does not require living hyphae (*5*). Our study of the fungal absorption system indicates that uranium uptake by *P. digitatum* preparations can be greatly enhanced by boiling and other treatments and that the basis for uranium binding resides mainly in wall macromolecules.

General methods for the preculture and handling of fungal biomass have been detailed in (*2, 5*), as has the procedure for uranium analysis, "delayed neutron release" (*6*). In principle, neutrons released by fission products formed after sample irradiation with thermal neutrons are counted. The sensitivity is ~ 0.1 μg at natural isotopic composition with an accuracy of ± 5 percent. For the delayed neutron procedure, 100 parts per million (ppm) of $UO_2Cl_2 \cdot 2H_2O$ standards correspond to 61.7 ppm of uranium; hence the correction factor 0.617 has been applied to all data presented here. The results are based on at least duplicate determinations.

Two specific test procedures were used in the experiments reported here. For the study of uptake enhancement, control mycelial charges of 3 g fresh weight (0.125 g dry weight) were incubated with 20 ml of 100 ppm UO_2Cl_2 solution (61.7 ppm uranium) for 4 hours at 23° to 24°C. Additional charges of mycelial biomass comparable in size were

pretreated by boiling or with the chemical agents listed in Table 1. They were then washed and incubated in UO_2Cl_2 solution as set forth above. After the 4-hour incubation period, mycelial masses were harvested, washed, dried at 90°C for 24 hours, and analyzed for uranium. Other sets of mycelia were exposed to 0.012 percent sodium azide during incubation as a "metabolic control."

In the second procedure we essentially tested the performance of mycelium and wall-related biopolymers packed in microcolumns. For that purpose, 5-ml plastic syringes were filled with the material to be tested (see Table 2 for charge weight), washed several times with distilled water, and filled dropwise with 5 ml of 100 ppm UO_2Cl_2 solution. Each column was then eluted with 50 ml of water; its adsorbents were removed, dried, and analyzed for uranium. The wall-related biopolymers tested included purified chitin (Mann Research Laboratories); cellulose phosphate (Sigma Chemical); carboxymethyl cellulose (Schuchardt, Munich); and cellulose powder (Sigma Chemical).

Although hyphae can grow while accumulating various metals, working conditions are limited by the sensitivities of the living protoplasts (*4*). The testing of killed mycelium was done as a matter of course, but the observation of an elevation of uranium uptake after boiling or after selected chemical pretreatment was completely unexpected (Table 1). Those treatments leading to elevated uptake are associated with solvent or denaturant properties. These agents may expose uranium binding sites either by configurational change or by removal, in solution, of masking groups. Formaldehyde denatures but also cross-links and masks binding sites. Sodium azide, an inhibitor of electron transport, does not affect the uptake process significantly. The absence of metabolic involvement in the process and the general response of the mycelium to treatments tending to expose active groups suggest that those groups might be located in the fungal wall (*7*).

When wall-related biopolymers were tested (Table 2), they appeared to be active in uranium binding (retention). Chitin, cellulose, and modified celluloses were active.

The functional groups capable of serving as binding sites for UO_2^{2+} may include virtually any anionic centers plus a variety of ligands capable of coordinating with the uranium(VI) ion. Further experimentation will be required for the precise characterization and optimization of the uptake process.

Irrespective of mechanistic details, the phenomenon itself has both biogeochemical significance for the localized accumulation of uranium and other heavy elements and biotechnological significance for wastewater decontamination and metals recovery.

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Table 1. Enhancement by pretreatment of subsequent mycelial uptake of uranium. Mycelial charges of 3 g fresh weight (0.125 g dry weight) were pretreated in one of the ways listed below. [All the pretreatments were applied prior to incubation in UO_2Cl_2 solution except for sodium azide, which was present at 0.012 percent (1.75 m*M*) during the 4-hour incubation with UO_2Cl_2.] Then the treatment chemicals were removed by washing, and the fungal mass was incubated for 4 hours in 20 ml of 100 ppm UO_2Cl_2 solution (61.7 ppm uranium) at 23° to 24°C, then washed, dried, and analyzed by the delayed neutron method. Uptake data are given as means ± standard deviations or as means of duplicate determinations.

Pretreatment	Number of trials	Mycelial uptake of uranium (ppm dry weight)
None (control)	10	3890 ± 220
Boiled for 15 minutes	3	6590 ± 260
Ethanol (80 percent) for 1 hour	5	7960 ± 140
Methanol (80 percent) for 1 hour	2	7890
Formaldehyde (10 percent) for 10 minutes	2	4610
Dimethyl sulfoxide (100 percent) for 90 minutes	2	9240
Potassium hydroxide (5 percent) for 10 minutes	2	9860
Sodium azide (0.012 percent)	3	4270 ± 110

Table 2. Uptake of uranium from aqueous UO_2Cl_2 solutions by cell wall–related biopolymers. Commercial biopolymers were packed in 5-ml syringe barrel microcolumns, filled with 5 ml of 100 ppm UO_2Cl_2 solution (61.7 ppm uranium), and flushed with 50 ml of water. Column contents were then removed and analyzed for uranium. Values are based on the averages of duplicate determinations.

Biopolymer	Microcolumn charge (g dry weight)	Amount of uranium retained	
		Micrograms	Percent*
Chitin	0.98	305	98.7
Cellulose phosphate	0.76	230	74.4
Carboxymethyl cellulose	0.93	254	82.2
Cellulose	1.86	214	69.2

*Based on 308.65 μg of uranium provided to each column.

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