Soil as Natural Reservoir for Human Pathogenic Fungi

Libero Ajello

Medical mycologists have long been interested in questions regarding the natural habitats of human pathogenic fungi. They have been uncertain whether these organisms are obligate parasites of man and lower animals, or whether they are saprophytes that possess the specialized ability to infect susceptible persons under certain conditions.

As early as 1893, Sabouraud (*1*) postulated that the dermatophytes are primarily soil saprophytes on the basis of their ease of cultivation on synthetic media, vegetable matter, and especially soil. A few years later, Sabouraud (*2*) went on to predict that the systemic fungi would also be found to exist as saprophytes in nature. Although several decades were to elapse before saprophytism was demonstrated among the dermatophytes, the second prophesy already had come to pass, for in 1894, Sanfelice (*3*) had recovered *Cryptococcus neoformans* from peach juice. Previously *Aspergillus fumigatus* (*4*) as well as several species of Mucorales, (*5–8*) which were not yet recognized as agents of systemic mycoses, had been isolated from nonliving substrata. Since that time, an impressively large group of pathogenic fungi of medical importance has been isolated from the environment (Table 1). With knowledge that 21 pathogenic, or potentially pathogenic, fungi have been recovered from nonliving substrata, the concept that soil is the ultimate source of many of the organisms that cause mycoses commands serious attention.

Such occasional pathogens as *Aspergillus fumigatus, Absidia corymbifera, Absidia ramosa,* and *Rhizopus oryzae* are commonly recovered from soil and air and even used, in one instance, in the manufacture of food (*9*). The saprophytic existence of *Sporotrichum schenckii* has been established by the detection of macroscopic colonies of that fungus on mine timbers (*10*). *Phialophora verrucosa* has been isolated from old boards (*11*), and the vegetative spores of *Histoplasma capsulatum* (*12–14*) and *Microsporum gypseum* have been found in soil (*13*).

Thanks to the pioneering work of Emmons (*15*), it has been demonstrated that soil surveying is one of the most fruitful means for discovering the natural habitats of the fungi that are capable of causing human disease. During the past 5 years, groups of soils collected in widely scattered geographic areas have been examined in our laboratory for pathogenic fungi. The purpose of this report (*16*) is to present the results obtained from that study.

Materials, Methods, and Results

Since 1950, through the cooperation of several individuals (*17*) and by personal collection, 1215 soil specimens have been gathered in the following areas. In the United States, 710 samples were collected in Tennessee, 79 in Georgia, 48 in Arizona, 44 in Michigan, 16 in West Virginia, 11 in Maryland, and 10 in Alabama. One hundred specimens each were obtained from the Territory of Hawaii and the Republic of Panama, including the Canal Zone; 76 samples were obtained from Nigeria, 12 from Canada, 5 from Venezuela, and 4 from Peru.

Most of these soils were examined for the presence of *Histoplasma capsulatum* and other systemic fungi by injecting the supernatant from soil suspensions intraperitoneally into mice with subsequent culturing of their livers and spleens. Dermatophytes were sought according to a procedure developed by Vanbreuseghem (*18*) by baiting plates of moistened soil with pieces of sterilized human hair. Details of these procedures have been described previously (*19, 20*).

From 1141 of the soils, through use of the mouse procedure, 73 isolates of *Histoplasma capsulatum* were obtained: 62 from Williamson County and 5 from Shelby County in Tennessee, 1 from soil gathered in the Republic of Panama, 4 from soil specimens collected in a Venezuelan cave, and 1 from a Peruvian cave. *Allescheria boydii* was recovered from 19 samples, 15 from Williamson County, Tennessee, 2 from Georgia soil, and one each from material collected in the Republic of Panama and the Territory of Hawaii. *Cryptococcus neoformans* was isolated in 12 instances: 5 from Williamson County, Tennessee, 2 from Georgia as well as Alabama, and one each from Maryland, the Territory of Hawaii, and Nigeria. One isolate of *Candida albicans* was recovered from a soil sample gathered in Shelby County, Tennessee, while *Coccidioides immitis* was found in a soil specimen that was collected at the mouth of a rodent burrow in Tucson, Arizona.

By baiting 439 of the soil samples with filaments of hair, 126 isolates of the dermatophyte *Microsporum gypseum* were recovered. These positive soils had the following geographic distribution: Panama, 36; Tennessee, 30; Hawaii, 23; Georgia, 13; Nigeria, 6; Michigan, 5; Alabama, 3; West Virginia, 2; and Canada, 2. These data are summarized in Table 2.

Discussion

Surveys similar to the ones just described serve a useful function. They lead to the discovery of the natural habitats of human pathogenic fungi and to the factors that influence their growth in a given environment. By correlating such observations with the mode and extent of infection in man and other animals, effective measures for control of the mycoses may be developed.

Our surveys, although they are limited in geographic coverage and numerical scope, have served to emphasize that soil in many parts of the world harbors a wide variety of medically important fungi. However, the same array of species will not be encountered in all areas of the world, for some of these fungi have such critical ecological requirements that their geographic distribution is necessarily limited. This fact is well illustrated by *Coccidioides immitis,* whose physical and physiological requirements are such that it is known to occur solely in certain of the semiarid regions of North, Central, and South America. These factors are so restrictive that despite the widespread distribution of contaminated plant materials and products from its endemic areas and the broad dispersal of spores of this fungus by dust storms, no evidence exists to indicate that *C. immitis* has become established beyond its classic domain.

In contrast, such species as *Allescheria boydii, Cryptococcus neoformans, Histoplasma capsulatum, Microsporum gypseum,* and others would be expected to occur throughout the world in appropri-

The author is senior scientist in charge of the Mycology Unit, U.S. Public Health Service, Communicable Disease Center, Chamblee, Ga.

ate habitats. Thus, through use of Vanbreuseghem's keratin-baiting technique, *M. gypseum* has been recovered by Fuentes (*21*) from Cuban soils and by Durie (*22*) from Australian soils; and *H. capsulatum* has been recovered by mouse passage from Mexican soils by Gonzalez-Ochoa (*23*).

Both negative and positive correlations exist between the prevalence of a given fungus in soil and the number of human infections caused by that organism. The basic saprophytism of *Allescheria boydii* and *Microsporum gypseum* is reflected by the contrast between their abundance in soil and the rarity of human infections caused by these two ubiquitous molds.

On the other hand, paralleling the 72-percent level of reactivity to histoplasmin among the inhabitants of Williamson County in Tennessee (*24*) is the frequent recovery, in soils frequented by chickens, of *Histoplasma capsulatum*, which reached 38.9 percent (Table 3). This positive correlation undoubtedly stems both from the high infectivity of this parasite, which in mice has been shown to initiate infections through a single spore (*19*), and from the inherent susceptibility of human beings to *H. capsulatum*.

Table 1. Pathogenic or potentially pathogenic fungi isolated from nonliving sources.

Fungus	Reference
Absidia corymbifera	Lichtheim, 1884 (*5*)
A. ramosa	Lindt, 1886 (*6*)
Allescheria boydii	Emmons, 1950 (*33*); Ajello, 1952 (*39*)
Aspergillus fumigatus	Renon, 1897 (*4*)
Candida albicans	Negroni *et al.*, 1941 (*30*); di Menna, 1955 (*29*)
C. guilliermondii	Lodder *et al.*, 1952 (*40*)
C. krusei	Lodder *et al.*, 1952 (*40*)
Coccidioides immitis	Stewart *et al.*, 1932 (*41*)
Cryptococcus neoformans	Sanfelice, 1894 (*3*)
Epidermophyton floccosum	Ajello *et al.*, 1954 (*42*)
Histoplasma capsulatum	Emmons, 1949 (*12*)
Phialophora pedrosoi	Trejos, 1954 (*43*)
Microsporum gypseum	Mandels *et al.*, 1948 (*44*)
Nocarida asteroides	Gordon *et al.*, 1946 (*45*)
Phialophora jeanselmei	Trejos *et al.*, 1954 (*43*)
P. verrucosa	Melin *et al.*, 1934 (*46*); Conant, 1937 (*47*)
Rhizopus arrhizus	Rabenhorst, 1892 (*7*)
R. oryzae	Went *et al.*, 1895 (*8*)
Sporotrichum schenckii	DeBeurmann *et al.*, 1908 (*48*)
Trichophyton mentagrophytes	Muende *et al.*, 1937 (*49*); Lurie *et al.*, 1955 (*50*)
T. rubrum	Ajello *et al.*, 1954 (*42*)

Table 2. Soil study data.

Locality	Sample (No.)	Pathogenic species isolated					
		Allescheria boydii	*Candida albicans*	*Coccidioides immitis*	*Cryptococcus neoformans*	*Histoplasma capsulatum*	*Microsporum gypseum*
Tennessee*	710	15	1		5	67	30
Hawaii	100	1			1		23
Panama	100	1				1	36
Georgia†	79	2			2		13
Arizona‡	48			1			
Michigan§	44						5
Nigeria	76				1		6
W. Virginia§	16						2
Canada§	12						2
Maryland‡	11				1		
Alabama	10				2		3
Venezuela‡	5					4	
Peru‡	4					1	
Totals	1215	19	1	1	12	73	120
No. of soils tested for each fungus		1,141	1,141	48	1,141	1,141	439
Percentage positive		1.7	0.08	2.0	1.1	6.4	27.3

* Only 73 tested for *M. gypseum*. † Only 44 tested for *M. gypseum*. ‡ Not tested for *M. gypseum*. § Not tested for *H. capsulatum*.

Discovery and characterization of the ecological requirements of human pathogenic fungi can result from large-scale soil-screening programs. Studies carried out in Williamson County, Tennessee, have revealed that areas frequented by chickens are especially favorable for the growth and proliferation of *Histoplasma capsulatum* (*25*). It was found that approximately 39 percent of soils gathered in chicken houses and chicken yards yielded *H. capsulatum*; this is in contrast to a 13.0-percent yield from soils gathered in other habitats (Table 3). In addition, it was learned that protection of the soil from the elements favorably influenced the occurrence of this fungus. Chicken-house soils were 46.2-percent positive for *H. capsulatum*, while chicken-yard soils only gave a 20.0-percent recovery (Table 4).

The predilection of *H. capsulatum* for chicken and other avian habitats has been strikingly borne out by the epidemiological studies of Emmons (*11*) and those of Grayston *et al.* (*26*).

It would seem, therefore, that chickens and shelter, in a manner yet undetermined, favorably influence the development of *H. capsulatum* in soil. This influence must be an indirect one, for laboratory tests and field observations indicate that chickens are not carriers of *H. capsulatum*.

This conclusion is based on failure to discover cases of histoplasmosis among chickens in Williamson County and the inability to infect chickens following injection of heavy suspensions of the tuberculate spores of *H. capsulatum* (*25*).

The desire to determine what indirect influence, if any, that chickens exert on the growth of this fungus in soil prompted two types of investigations: (i) chemical and physical analysis of positive and negative soils (*27*) and (ii) a comparative study of the mycoflora of soils that yield *H. capsulatum* and of soils that are negative for this mold.

Physical and chemical analysis of a group of 100 soils revealed that chicken area soils were more acid than negative soils and, as expected, had a significantly higher organic carbon content and moisture-holding capacity than soils from other habitats. It may be postulated that these and other properties of chicken-contaminated soils may create an environment more favorable for the growth of *H. capsulatum* than occurs in other types of soils. It is well known that the number and kinds of microorganisms occurring in a soil are determined, to a large extent, by the nature of the soil itself.

Table 3. Influence of habitat on occurrence of *Histoplasma capsulatum* in Williamson County, Tennessee soil. Data are derived from a group of soil specimens collected for chemical analysis.

Habitat	Samples (No.)	Recovery of *H. capsulatum*	
		Positive samples (No.)	Positive (%)
Chicken areas	54	21	38.9
Other	46	6	13.0
Total	100	27	27.0

An analysis of the mycoflora of chicken-area soils and that from non-chicken habitats has been initiated in an effort to determine what role competition among soil fungi may play in influencing the occurrence and development of *H. capsulatum* in soil. It remains to be seen what this type of study will reveal.

Agents other than chickens, however, must play a role, for the geographic distribution of *H. capsulatum* does not parallel that of poultry. Basic soil types as postulated by Zeidberg (*28*) may have some bearing on this matter, but the problem is very complex and its solution does not seem to be imminent.

The recovery of *Candida albicans* from the grounds of an abandoned house, di Menna's isolations from several New Zealand soils (*29*) and Negroni's original isolation from decaying vegetation (*30*) indicate that this yeastlike organism may exist as a saprophyte in nature. Since *C. albicans* is thought to be a part of the normal flora of the body of human beings and other animals, whether it exists passively in soil following introduction by animal carriers, as di Menna (*29*) has speculated, or whether it actually grows in soil, is a question still to be determined.

Isolation of *Cryptococcus neoformans* from soils collected in four states of the United States, Nigeria, and Hawaii indicates that this unicellular fungus has a wide distribution in nature and probably is a normal component of the soil's mycoflora. The basis for such a belief was provided by Sanfelice in 1894 (*3*) and was confirmed by Emmons in 1951 (*31*).

Analysis of the nature of the sites from which *Cryptococcus neoformans* was isolated reveals that eight came from chicken areas, one from a pigeon nest (*32*) and one each from the edge of a pond, a barn, and a tractor shed. Our isolations, however, are too few in number to enable us to determine what significance to give, if any, to the preponderant recovery of *C. neoformans* from bird sites. It should be borne in mind, however, that Emmons (*11*) has found *C. neoformans* to be unusually prevalent in pigeon droppings.

Table 4. Influence of shelter upon the occurrence of *Histoplasma capsulatum* in soil.

Source of sample	No. of samples	Isolations of *H. capsulatum*	
		No.	(%)
Chicken house	39	18	46.2
Open chicken yards	15	3	20.0
Under house	26	5	19.2
In open	15	1	6.7
Under barn	3	0	
Other	2	0	
Total	100	27	27.0

Convincing proof is on hand that *Allescheria boydii,* one of the common causes of mycetomas and rare cases of systemic disease, is widespread in nature. It has not only been recovered during the course of our studies from the localities previously mentioned—Tennessee, Georgia, Hawaii, and Panama—but Emmons (*33*) discovered it in Maryland and Virginia soils, and Cain (cited by Emmons, *11*) considers it to be of common occurrence in Canadian soils. Cooke (*34*) has frequently isolated *A. boydii* from a polluted stream in Ohio as well as from several sewage treatment plants. It is not surprising, therefore, that spores of this organism have been recovered from the air by Morrow (cited by Emmons, *11*) in Texas and that it appeared as a contaminant in a culture tube observed by Adams (*35*) in South Carolina. The wide distribution of airborne spores of the organism undoubtedly accounts for Blank's (*36*) isolation of this organism from ear scrapings.

It is truly remarkable that only one dermatophyte, *Microsporum gypseum,* is regularly isolated from soil. The ease with which dermatophytes grow in soil under laboratory conditions, as well as the recovery of several species of *Trichophyton* and *Epidermophyton floccosum* from inanimate substrata, encourages one to believe that these too may exist as saprophytes in nature. Yet these species, as well as others of the genera *Trichophyton* and *Microsporum,* remain elusive. It may be that these keratinophilic fungi have become so specialized in their growth requirements that they can survive and maintain themselves only on living animal hosts; or are suitable techniques lacking with which to detect and isolate them from the environment? In time, most, if not all, of the fungi that cause human disease will probably be found to be soil saprophytes.

Knowledge about where these fungi exist in nature and how they interact with the chemical, physical, and microbiological elements of their environment should provide information applicable to the control of the mycoses. Already during World War II, the armed forces were able to reduce the incidence of coccidioidomycosis among trainees in the endemic areas by instituting dust-control measures (*37*). It should be possible to prevent acute epidemics of histoplasmosis by disseminating information concerning the infectivity of soil and debris from chicken and pigeon habitats (*38*).

It is obvious that an ecological approach to the science of medical mycology promises to provide not only knowledge regarding the habitats of human pathogenic fungi, but also the basis for the development of rational control measures.

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Roy W. Miner, Naturalist and Marine Biologist

The thin and thinning ranks of invertebrate zoologists were further depleted by the death on 13 December 1955, of Roy Waldo Miner, curator emeritus of marine biology at the American Museum of Natural History and editor of the publications of the New York Academy of Sciences.

Dr. Miner's background and training provided the basis for the accomplishments of his mature years. Endowed with stamina derived from pioneer New England ancestors, he was born at North Adams, Massachusetts, 24 February 1875. A studious and diligent boy, he was graduated from the Drury Academy in 1893 and from Williams College in 1897. As an undergraduate he was elected to Phi Beta Kappa, and 30 years later his alma mater awarded him the degree of doctor of science *honoris causa*. Meanwhile, he had completed the requirements for the degree of doctor of philosophy, which he received from Columbia University in 1923.

On graduation from college, he entered the General Theological Seminary in New York, from which he was graduated in 1900. At that time he chose to be a teacher rather than a clergyman, but his classical education and training in homiletics were to prove of inestimable value throughout his life. He taught Latin and biology at the Berkeley School in New York from 1900 to 1904, when he became instructor and associate headmaster at the Kelvin School in New York.

An ardent student of nature, his interest in biology led to association with members of the American Museum of Natural History, and, in 1905, at the invitation of the director, the late Herman C. Bumpus, he joined the staff of the museum as assistant curator of invertebrate zoology. For the next 38 years, his talents were devoted to research and to the preparation of educational exhibits, which both instructed and delighted the multitudes that thronged the halls of the museum. He was associate curator, 1917–21; curator of living invertebrates, 1922–43; and curator emeritus since 1943.

To obtain ideas and material for the realistic, accurate, and artistic reconstructions that he designed for the museum, Dr. Miner made repeated trips to the Bay of Fundy, the wharves and tidepools of New England, and the coral reefs of the Caribbean and the South Pacific. His careful and detailed notations, both written and photographic, provided the information for precise and colorful reproductions of marine life as viewed through the eyes of a trained observer. Forty tons of coral from the West Indies was built into the framework of the Hall of Ocean Life, and smaller but equally charming and accurate exhibits portrayed the plants and animals in the tidepools and on the wharf piles of New England. He designed and supervised the construction of many invertebrate groups, aided by the loyal and enthusiastic cooperation of talented workmen who made models of many kinds, from individual protozoans to garguantuan reproductions of the life of a coral reef.

On his retirement from the staff of the American Museum of Natural History, Dr. Miner began to edit the publications of the New York Academy of Sciences. For 12 years his careful supervision and critical judgment have stamped the issues of the *Annals* and *Transactions* of the academy and the parts of the *Scientific Survey of Puerto Rico and the Virgin Islands,* published since 1943. He did this work as an unpaid officer of the academy, the welfare of which was one of his dearest concerns.

In addition to his contributions as curator of marine life at the American Museum of Natural History and editor of the publications of the New York Academy of Sciences, Dr. Miner published many magazine and journal articles, reports of expeditions, and books on natural history. Among the more important may be listed: *Animals of the Wharf Piles* (1912), his dissertation on *The Pectoral Limbs of Eryops and Other Primitive Tetrapods* (1925), *Diving in Coral Gardens* (1933), *The Kingdom of the Tides* (1934), *Exhibition Halls of the American Museum of Natural History* (1939), and his beautifully illustrated *Field Book of Seashore Life* (1950), which depicts the invertebrates of the Atlantic Coast of North America.

The central theme of his writing and of his designs for groups in the American Museum was the idea of evolution in nature; this was expressed particularly in the arrangements for Darwin Hall. For him, the interrelations of plants and animals were not haphazard associations but the meaningful result of the ecological factors whose operation had determined the development and character of each biotic area. His concept of unity in nature, his belief in the virtue of goodness and the triumph of right, his simple and straightforward honesty, integrity, kindliness, generosity and fine sense of humor were significant manifestations of his character and personality.

Dr. Miner was a member of Sigma Xi, and fellow of the New York Academy of Sciences, the New York Zoological Society, and the Consular Law Society. He was a friendly man, who enjoyed the company of his associates and the conviviality of social affairs. His passing has removed a meticulous scholar, a fine gentleman, and a loyal friend. The world is richer because of his contributions.

HORACE W. STUNKARD
Department of Biology,
New York University,
University Heights, New York