Ecological Frontier

Subsurface soil and water are home to microbes living in tightly about the organisms or how they survive in their nutrient-poor,

By CHERYL SIMON

In 1916, Selman A. Waksman published a paper asserting that below the root zone, soil is nearly devoid of life. As the years passed, that conclusion grew in importance, following the reputation of Waksman who went on to discover streptomycin antibiotics. For generations the finding went unchallenged; virtually no one studied life below the surface or in underground water.

All that changed five years ago when the Environmental Protection Agency (EPA) asked microbiologist John Wilson to take another look. Using every available tool of microbial ecology, he established several lines of evidence showing that water-saturated soils and rocks — the aquifers that supply the vast majority of fresh water — support rich communities of microbes. Further, he found, densities of the populations are comparable to or greater than those in rivers and lakes.

As Wilson, now with EPA's Environmental Research Laboratory in Ada, Okla., published his results, other researchers began to speak up. "I've had several people come to me and say, 'I saw the same thing but I was afraid to publish it,'" Wilson says. "A lot of people have seen it. It's sort of the emperor's new clothes in reverse."

The fledgling study of groundwater microbiology is of basic scientific interest, but it is the changing view of groundwater that spurs the investigation. Only a decade ago, groundwater was consumed with utmost confidence in its purity. People were secure in the assumption that undesirable bacteria and chemicals would be filtered out as they passed through the soil en route to the water table, and that the abundant microbial life in the surface layer would degrade what remained into harmless compounds. For the most part, this mechanism works. But while most of the groundwater still is pristine, tongues of highly toxic contaminants have penetrated into some aquifers, making them unfit for use.

"Unfortunately, most of the water that's polluted is water that we're interested in using," Wilson says. "Some of the water probably never will be contaminated. Some probably is ruined for the next millenium. The more honest work we do here, the more water...will be saved for potable consumption."

The fact that microbes live in aquifer soil in concentrations from one thousand to one million bacteria per gram of soil provides a basis for studies of how microbes may affect, or be affected by, contaminants. Microbial density is a critical condition because the potential for biological degradation of any substance is "the time of exposure times the density of organisms," Wilson says. More microbes over more time would have more opportunity to consume a pollutant. Groundwater flowing at a rate of five feet per day is moving at breakneck speed, and in some places groundwater may move only five feet per year. (This also explains why it has taken decades for some pollutants to appear in aquifers.) Thus a plume of pollution moving slowly 10 to 100 feet through an aquifer stands more chance of being biologically degraded than the same pollutant travelling the entire length of the Mississippi. The density of microbes in the river is not much greater than in groundwater, but movement occurs far more quickly.

Other than their presence, precious little is known about groundwater organisms. "We are looking at groundwater as an environment that hasn't been looked at before," says William Ghiorse of Cornell University in Ithaca, N.Y. "What do the organisms do? How do they interact and grow? How do they move away?"

With David Balkwill of Florida State University in Tallahassee, Ghiorse has been working to characterize the types and structures of microbes in saturated groundwater soils, as well as in laboratory cultures. The effort has been enhanced by new techniques that allow samples to be extracted from aquifers without their encountering surface soils with enormously different biology. The researchers also have developed special staining procedures that allow them to distinguish cellular material from noncellular particles of the same shape and size.

In Santa Fe, N.M., at the recent Sixth International Symposium on Environmental Biogeochemistry, Ghiorse and Balkwill reported that the total number of microorganisms does not decrease with greater depths — further refuting Waksman's conclusion. And while the number of microbes is consistent in all of the aquifers they've checked, they said, the kinds of organisms may vary considerably. Since different types of microbes eat different food, the fates of specific pollutants well may depend on an aquifer's residents and their respective diets.

The range of microbial types also might prove significant, they said. "If you stress a groundwater environment with pollutants, if it kills the microorganisms, you cannot expect the pollutant to degrade," Ghiorse says. A pollutant might kill all microbes in an aquifer inhabited by only a few types of bacteria, whereas a more diverse microbial community would be more likely to include a species that could survive or even break down a pollutant compound.

As studies of groundwater microbiology advance, it is clear that in some ways groundwater is unlike any other microbial ecosystem. Wells, for instance, while linked to aquifers, rapidly may form an ecosystem separate and different from the parent because the water has contact with surface soils and the atmosphere. Microbes in aquifers have other living standards. For example, samples taken from groundwater systems reveal few, if any, predators — yeasts or other fungi, protozoa, or higher animals that usually feed on bacteria. This leaves the groundwater microbes free to focus on using their food — mostly organic carbon — in the most effi-

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Prospect for Biodegradation of Selected

Class of Compounds
Halogenated Aliphatic Hydrocarbons
Trichloroethylene
Tetrachloroethylene
1,1,1-Trichloroethane
Carbon Tetrachloride
Chloroform
Methylene Chloride
1,2-Dichlorethane
Brominated methanes
Chlorobenzenes
Chlorobenzene
1,2-Dichlorobenzene
1,4-Dichlorobenzene
1,3-Dichlorobenzene
Alkylbenzenes
Benzene
Toluene
Dimethylbenzenes
Styrene
Phenol and Alkyl Phenols
Chlorophenols
Aliphatic Hydrocarbons
Polynuclear Aromatic Hydrocarbons
Two and three rings
Four or more rings

*Possible, probably incomplete.
†Probable, at high concentration.

If an organic pollutant is present in concentrations greater than 100 micrograms/liter, microbes may increase their numbers to exploit the nutrient source. At concentrations lower than 10 μg/l, the microbes may not find the compound valuable, even if they are

packed communities. But little is known predator-free world.

cient manner.

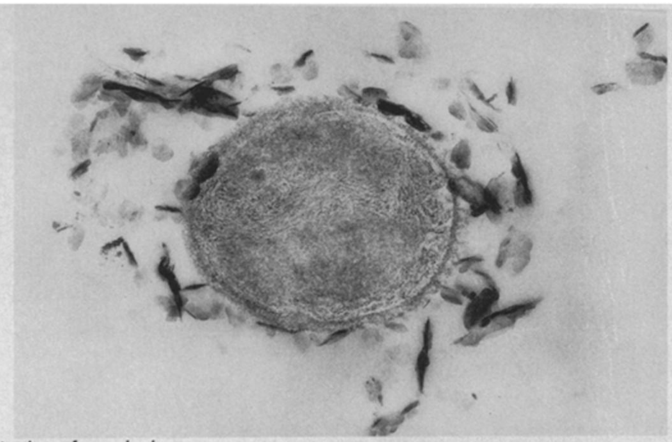
"If the predators are missing from an ecosystem, then that allows the bacteria to compete with each other, and to specialize on metabolizing and living on very low concentrations of compounds, or on compounds that are very difficult to degrade," Wilson says.

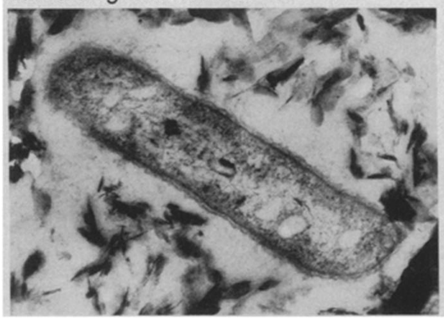
The nature of the subsurface food supply also separates the groundwater microbes from those on the surface. Most bacteria break down and degrade biopolymers, like cellulose or lignin, that biochemically are similar to their own protoplasm. In groundwater though, the dominant forms of organic carbon are bound up in the soil in a complex organic material that is much more difficult to degrade. "Its biochemistry is much different from the central biochemistry of the cell," Wilson says. By nature, he says, the subsurface microbes are "forced to exist on the stuff that no one else bothered to eat. That's why it got down there in the first place."

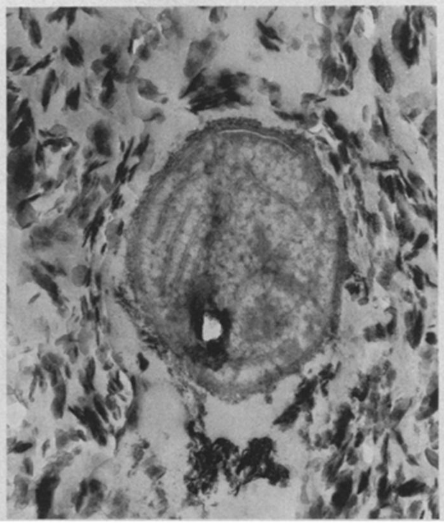
Organic Pollutants in Water-Table Aquifers

Aerobic Water, Concentration of Pollutant micrograms/liter		
>100	<10	Anaerobic Water
none	none	possible*
none	none	possible*
none	none	possible*
none	none	possible*
none	none	possible*
possible	improbable	possible
possible	improbable	possible
improbable	improbable	probable
probable	possible	none
probable	possible	none
probable	possible	none
improbable	improbable	none
probable	possible	none
probable	possible	none
probable	possible	none
probable	possible	none
probable	probable	probable†
probable	possible	possible
probable	possible	none
possible	possible	none
improbable	improbable	none

capable of degrading it. Compounds in high concentrations may be only partly degraded if oxygen becomes entirely depleted (anaerobic). Further degradation will await mixing of contaminated water with oxygenated (aerobic) water.







Right: Nearly round cell found at Lula, Okla. site dominates the groundwater ecosystem. Strands in the middle of the cell are the organism's DNA. Below right: Wavy internal membranes in this organism found in a Ft. Polk, La., aquifer may indicate the cell's metabolic capabilities. Below left: Rod-shaped bacterium from Lula reveals granules within the cell. The granules are produced by bacteria as food becomes scarce, and are used in the way that humans use fat. Oddly shaped particles outside the cell in each photo are clay minerals and other non-biological material.

Groundwater microbes probably can eat a number of foods, a skill that may explain their ability to attack and degrade many of the chemical compounds that infiltrate their homes. But the persistence of some pollutants, such as trichloroethylene, shows that some compounds are impervious even to the most voracious bugs. Any attempt to predict how adept groundwater microbes will be at breaking down a given substance requires an understanding of which microbes live where, and of their nutritional requirements.

The exploration of groundwater ecology has led researchers to develop a system that allows them to describe quantitatively the total microbial community. "Many kinds of biodegradation require a community, or a consortium of organisms," says David C. White of Florida State. "Usually one can't do it. You have to have a whole group."

Instead of trying to isolate particular microbial species, White measures the whole community by looking for the presence and ratios of cellular constituents, such as lipids and amino sugars [sugars in which one or more hydroxyl group is replaced by an amino group], that are restricted to a particular group of organisms. Over the years, as a catalog of the unique biochemical signatures of organisms builds up, the technique may make it possible, for instance, to tell a pristine aquifer from one whose biology has already responded to the introduction of a foreign compound. The ratios of these constituents can reveal which groups of organisms dominate an ecosystem.

Groundwater hydrologists and microbiologists have fond hopes that someday, genetically engineered bacteria may be developed to give nature a hand in aquifers where the microbes lack interest in a particular contaminant or the ability to degrade it. It may be relatively easy to create bugs that eat noxious materials in a laboratory; to make the same compounds look appetizing in a natural system where other food is available may prove a more difficult problem.

A microbe introduced into an aquifer also will face the traumas of competing and surviving in an alien environment already populated by organisms that have been living there for years, giving them a selective advantage over any newcomer.

White is skeptical of the potential for success of a genetically engineered bug in an aquifer and likens it to putting a partridge in a forest. "The one you put out is the first one the fox will eat," he says. "It doesn't know where to hide. It's not the right color." An alternative to genetic engineering, he suggests, is to make up a mixture of organisms that are very closely related to the ones in the aquifer, but can degrade the targeted chemicals. "Put them in your aquifer along with some nutrients that they require, and get them to clean it up for you," he says. "That's our dream." □