SUBSURFACE HYDROBIOLOGY

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Summary

The terrestrial subsurface is an extremely important region for the sustainability of this planet. Given the burgeoning human population, it is no wonder that many subsurface environments across the world are now contaminated by a variety of pollutants, ranging from radioactive materials to human and animal wastes. In both the developed and the developing countries, groundwater contamination is a reality. It has been estimated that nearly 5 billion waterborne infections occur annually in Africa, Asia, and Latin America alone. Our curiosity about the microbiology of subsurface environments has come about partly because of our interest in the remediation of contaminated sediments and groundwater, and the need to protect our drinking water resources from human and animal pathogens. Studies over the last 50 to 60 years have provided much information about the occurrence of the different microbial groups in the subsurface, the factors controlling their survival, and their means of transport within the subsurface, as well as their role in the biogeochemical transformations taking place there. Our need to monitor *in situ* bioremediation has spawned the development of improved drilling and extraction tools, as well as of molecular microbial detection tools. Many of these methods are currently being used around the world to detect and characterize microbial pathogens

that may be present in groundwater. These tools have provided the ability not only to dissect the genetic composition of microbial communities but also to examine their spatial orientation with respect to one another. Our basic concepts of microbial diversity, the origins and survival of microbial populations within deep geological formations, and the possibility of chemosynthetic primary production operating within subsurface ecosystems have already undergone radical change. These findings are already paying dividends in terms of public health, as well in commercial applications.

1. Introduction

Basic understanding of the microbiology of subsurface environments has improved significantly since about 1990. The advances that we have witnessed have been spectacular considering that before this period there was still skepticism about the terrestrial subsurface's ability to harbor microbial populations. The realization that many microorganisms are naturally indigenous to the subsurface—including the deep subsurface—and the investigations that have followed have led to a better understanding of the roles of microbial populations in subsurface chemical transformations, as well as the potential fate of introduced microbial populations. Considering the importance of public health in the overall realm of global sustainability, this contribution will discuss aspects related to introduced and indigenous microbial populations, and issues concerning microbial pathogens in the subsurface.

2. Subsurface Environment

Groundwater makes up 4% of the global water volume. Most of the groundwater in the subsurface is interstitial: in other words, it is present within a matrix of minerals with varying physical and chemical characteristics. Thus, the geological medium in the subsurface has a major influence on the microbial flora. The large surface area provided by the sediment material strongly influences the surrounding physical and chemical conditions of the groundwater by adsorbing the microbial cells, and by altering concentrations of dissolved aqueous constituents at the surfaces. The thickness of the unsaturated zone can also have a significant effect on the microbiology of the saturated zones. In arid regions, the vadose zone may be tens of meters thick and rainfall may be insufficient to percolate through to the saturated zone. Even though there have been a number of studies pertaining to the surficial soil layers, not much work has investigated the deeper tracts of the vadose zone. Understanding the microbiology of this zone can have a significant impact on understanding the fate of pollutants in soil.

2.1. Microbiology

Groundwater in general is characterized by wide variations in nutrient levels. There are certain situations where increased nutrient levels may be found: for example, beneath landfills and in some tropical areas with shallow water tables. In a number of instances, the groundwater is considered oligotrophic, and the microbial populations that are indigenous to it have adapted themselves to survive in these conditions. A heterotrophic mode of nutrition predominates, involving reliance either on nutrients that percolate downward or on organic material present on sedimentary material. Chemoautotrophy has been documented in some subsurface environments. Photosynthesis does not occur.

There have been instances where algal cells have been detected in groundwaters, especially in regions with elevated water tables. In such situations the groundwater is regarded as being “under the direct influence of surface water,” and technically is not considered true groundwater. The relative abundance of each of these different groups does, however, depend on the thickness of the vadose zone and the possibility of nutrients percolating from the surface to the populated strata.

The physical and chemical stratification of the subsurface has played a major role in the microbiology of the subsurface. The indigenous microorganisms that have been identified in the subsurface (i.e. saturated zones) have mostly been unicellular bacteria and protozoa. Actinomycetes and fungi are found in the vadose zone at concentrations ranging between 10^0–10^1 CFU g^{-1}. It is interesting that endospore-forming bacteria have not been observed in large numbers. This is surprising, considering that endospore formation endows cells with long-term survival capabilities. The bacterial numbers range from approximately 10^7 CFU g^{-1} at the surficial layers to approximately 10^2 CFU/g in the deeper regions. It must be mentioned, however, that many of these counts are based on culturable, numbers and not necessarily on actual viable numbers. A number of studies have reported on the occurrence of “viable but unculturable” microbial populations in oligotrophic conditions. Protozoa tend to be present in low numbers in the vadose zone, as compared to the capillary fringe. Approximately 50–100 protozoan cysts per gram of sediment have been isolated at a depth of 8 m below the surface. Studies suggest that these free-living protozoa tend to graze on bacterial cells, and that their occurrence is closely linked to the abundance of bacterial cells in the various lithologies. Cyst-forming flagellates and amoebae appear to be the principal types of subsurface protozoa.

There have been isolated reports of the occurrence of bacteriophages in the subsurface. Most of the recent information concerns the presence/absence of coliphages, since their occurrence in groundwater would suggest fecal contamination. Even though the occurrence of bacteriophages in open aquatic systems such as oceans is widely appreciated, the abundance and role of phages in groundwater is still poorly understood. Larger organisms occur only rarely, in subterranean caves or cavernous aquifers that are connected to the surface. The origins of subsurface-associated microbial populations are still controversial, however. The organisms could be descendants of those that were present during the initial surface deposition of geological material, or they may have originated from bodies that percolated through the soil profile. It is, however, almost impossible to determine the “age” of these isolated organisms. The lack of appropriate sampling and incubation methodologies to study *in situ* microbial activity has also hampered detailed investigations. Experimental evidence suggests that a majority of subsurface environments possess climax ecological communities. These communities are genetically diverse, possess trophic structure, and exhibit material cycling and energy transfer. These adaptations enable them to disperse and survive within such ecosystems. Even within a single area, there is significant spatial variability in microbial composition. A current limitation on analysis is that the precise flow paths of groundwater are difficult to define. The inability to define exact flow paths makes it hard to predict the movement of nutrients and microbial cells.

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Biographical Sketches

Dr. Suresh D. Pillai is currently Associate Professor of Food Safety and Environmental Microbiology in the Poultry Science Department of Texas A&M University. He also serves as the Associate Director of the Texas A&M University's multidisciplinary Institute of Food Science and Engineering. Dr. Pillai's research interests include the occurrence, fate, transport, and activity of microbial pathogens both in human-created ecosystems and environments, such as food production and processing systems, and in natural settings, including groundwater, surface water, and bioaerosols. As part of his research program he is also involved in the development and testing of rapid diagnostic molecular assays for microbial pathogens, and in the evaluation of public health risks from microbial pathogens. He received his M.Sc. in industrial microbiology from the University of Madras, India, and his Ph.D. in microbiology and immunology from the University of Arizona.

Dr. Dirk Schulze-Makuch is Assistant Professor and chief scientist of the GeoBiological Groundwater Research Group at the Department of Geological Sciences of the University of Texas at El Paso (UTEP). His interests range from hydrogeology to contaminant transport, and from geobiology to astrobiology. Prior to his employment at UTEP he worked at the Wisconsin Branch Office of Envirogen, Inc. as Senior Project Hydrogeologist, where his major tasks were investigating and coordinating the remediation of hydrocarbon spills in the subsurface. During that time he also taught as Adjunct Professor at the University of Wisconsin-La Crosse. Dr. Schulze-Makuch received his doctoral degree in geosciences from the University of Wisconsin-Milwaukee, and a Diploma and Vordiploma degree (M.Sc. and B.Sc. equivalent, respectively) from the Justus-Liebig-University in Giessen, Germany and USDA-CSREES grant 2001-34461-10403.