A DIRECT OBSERVATION TECHNIQUE FOR THE STUDY OF MICROORGANISMS IN SEDIMENT SAMPLES

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Abstract. A technique is described for the incubation of sediment samples under controlled conditions and the examination of the sediment by direct microscopy after percolation with a fluorescent stain—the ammonium salt of 8-anilino-1-naphthalenesulfonic acid. The incubation chamber employed enables the sediment sample to be treated by percolation with nutrients or with toxic chemicals. The response of the sediment microorganisms to these amendments can be analyzed using pattern determination statistics or direct counts at intervals during incubation.

1. Introduction

The types and distribution of microorganisms in sediments have been studied using a number of different techniques. In most cases, the microorganisms have been isolated with plating techniques (Bell and Dutka, 1972; Wetzel, 1975) or have been examined microscopically with a variety of staining agents (Sorokin and Kadota, 1972; Rodina, 1972; Francisco et al., 1973). In all of these techniques, disturbance of the structural integrity of sediment samples has meant that the distribution patterns on a small, micro-site scale have been difficult or impossible to determine. In addition, the response of such sediment microorganisms to potentially toxic materials present in sediment has been examined in pure cultures, or by examining various sediment activities such as respiration, radioactive glucose uptake rates or release of CO2 from such substrates. No direct microscopic method has been available which allows continued examination of sediment samples in the laboratory under conditions which can be varied so as to approximate those occurring in situ in the sediment. Such a technique has been proposed for use in soil samples (Polonenko et al., 1978) and, coupled with statistical analysis of the data using a two-within-four randomization test originally suggested by Mead (1974), it has been possible to determine changes in bacterial distribution patterns in soils incubated for relatively long periods in the laboratory. In the present study, the soil incubation technique used in the soil studies was modified for use with sediment samples. By staining with the ammonium salt of 8-anilino-1-naphthalenesulfonic acid (Mayfield and Polonenko, 1979) the effects of various carbon and nitrogen sources on the growth and distribution patterns of bacteria in the sediments was examined. In this manner, colony development was followed and the effect of the various treatments upon the bacteria was assessed. The potential application of this technique to toxicological studies in sediments was also examined.

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2. Materials and Methods

The sediment samples were collected in late summer from the Bay of Quinte of Lake Ontario (44° 2' 48" N, 77° 7' 10" W) from a water depth of 9 m by a scuba diver using plexiglass corers. The cores were transported to the laboratory immersed in ice. The sediment is organic in nature, containing 5.5% organic carbon (in the surface layers) and 0.75% total nitrogen (Kemp et al., 1972). The area is productive, with algal blooms in late summer and fall.

The slide perfusion chambers used in these studies were modified from those developed for soil studies (Polonenko et al., 1978). Each unit contained three separate compartments with three chambers in each compartment (Figure 1). Each chamber had the internal dimensions of 24 mm × 24 mm × 4 mm deep. The external dimensions of each unit were 88 mm × 88 mm.

![Fig. 1. Sediment incubation chamber.](image)

The sediment samples were placed in the three inner compartments so that the solution in the outer compartments could be percolated through the sediment samples. In the original method detailed by Polonenko et al. (1978), the slide incubation chamber was smaller and held only one sample. The present system is equivalent to three of those chambers, so that three replicate samples could be examined without removing the incubation chamber from the microscope during observation. The sediment in the compartments was maintained in a saturated condition during all experiments. Solutions were transferred from the outer chambers via glass fibre filter material joining the outer and inner chambers. This process was controlled by the amount of solution in the outer chambers. A cover slip over the compartment containing the sediment allowed microscopic examination of the microorganisms in the