oculation replaced up to 39 kg of fertilizer N per hectare, comparable to the 42 kg/ha replaced in the millet.

These results indicate that some fertilizer N was required to induce a response to inoculation. This might not be necessary where residual soil nitrogen is not extremely low, as it is in the sandy soil in which our experiments were conducted. We consider that this nitrogen may be required to stimulate plant growth and photosynthesis: the plant supplies energy to the bacteria for nitrogen fixation, and this requires a reasonable rate of plant metabolism at the outset.

In Fig. 1, B and C, the plots of dry matter yield against fertilizer N concentration are linear for uninoculated plots and curvilinear for inoculated plots. This may be because low concentrations of fertilizer N promote the establishment of nitrogen-fixing associations, giving a greater than normal growth response, while higher concentrations reduce the response by repressing nitrogenase (*8*).

The data reported here show that increased dry matter yields or reduced fertilizer N requirements can be obtained with grass-*Spirillum* systems. Nitrogen fixation by these systems is efficient and is achieved at a reasonable energy cost to the plant.

REX L. SMITH, J. H. BOUTON
S. C. SCHANK, K. H. QUESENBERRY
Department of Agronomy, Institute of Food and Agricultural Sciences, University of Florida, Gainesville 32611
M. E. TYLER, J. R. MILAM
Department of Microbiology, University of Florida
M. H. GASKINS
Agricultural Research Service, U.S. Department of Agriculture, University of Florida
R. C. LITTELL
Department of Statistics, Institute of Food and Agricultural Sciences, University of Florida

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7. *Spirillum lipoferum* strains used in these studies were obtained from J. Dobereiner, Empresa Brazileira de Pesquisa Agropecuaria, Rio de Janeiro, Brazil.
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10. We thank S. H. West and D. Hubbell for their assistance. This work was jointly supported by the Institute of Food and Agricultural Sciences, the Agricultural Research Service, and the Agency for International Development. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product, and does not imply its approval to the exclusion of other products that may also be suitable. This publication is Florida Agricultural Experiment Station Journal Series No. 9013.

8 March 1976; revised 3 May 1976

Acoustic Tracking of Ocean-Dumped Sewage Sludge

Abstract. *With a modified 200-kilohertz acoustic echo sounder, it has been possible to detect and map sewage dumped into the ocean over several hours. The three-dimensional distribution of suspended material and its rate of diffusion are indicated after digital processing of the data.*

Until now, the real-time tracking of sewage sludge dumped into the ocean has been difficult because in situ identification techniques were lacking. Chemical sampling is complicated by the inherent delay between the time the sample is taken and the time the analysis is complete, and also by the lack of reliable "tag" substances with which to distinguish samples inside the sludge "cloud" from those outside it. Partial success in the chemical sampling of ocean-dumped sewage sludge has been reported by Duedall *et al.* (*1*) and Callaway *et al.* (*2*). Shipboard measurements with a light beam transmissometer, whether towed or lowered, are made along a horizontal or vertical line and require extensive sampling of the sludge cloud to provide a reasonably complete three-dimensional

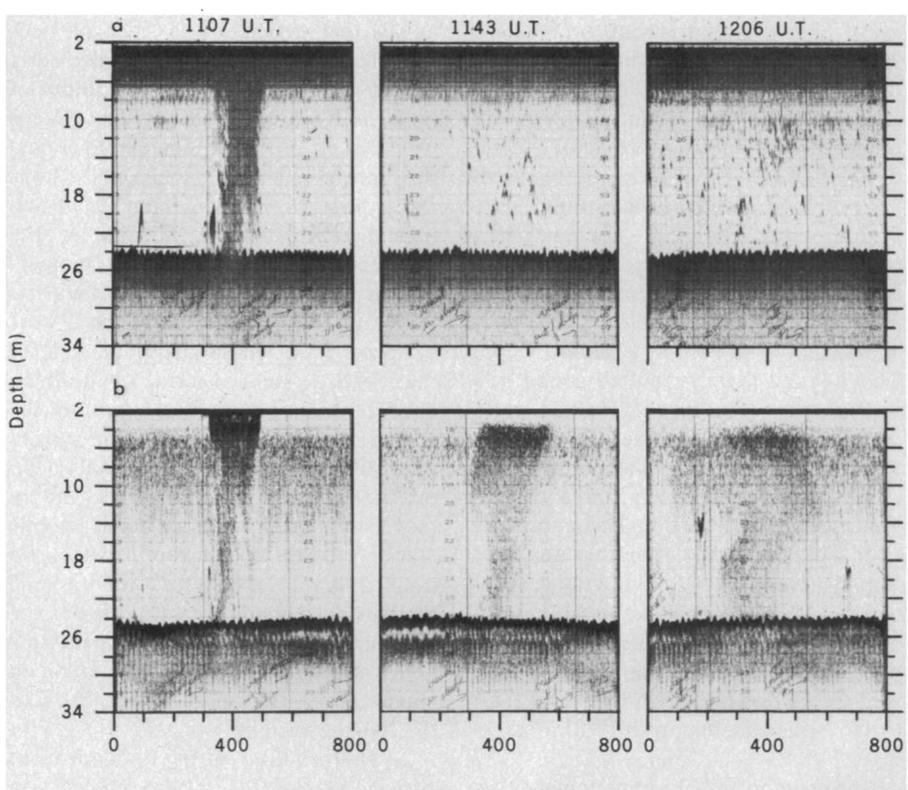
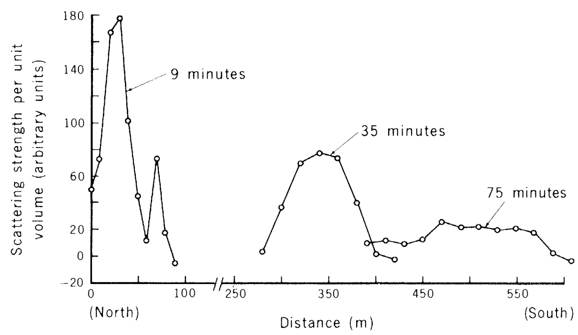


Fig. 1. Three segments of the (a) 20-khz and (b) 200-khz acoustic records for traverses of the 1045 E.D.T. line sewage sludge dump, 22 September 1975, during the sludge-tracking acoustical experiment. The distance indicated is along the ship track. The universal time (U.T.) of each traverse is given above the respective record. The first traverse shown (1107 U.T.) occurred approximately 9 minutes after the sewage sludge was dumped (that is, 9 minutes after that portion of the sewage sludge through which the traverse occurred was dumped).

Fig. 2. Net scattering strength per unit volume due to sludge for three 45° traverses of the east-west line sludge dump. The distance scale represents a projection of the distance along the ship track against a north-south line to show the actual width of the sludge cloud and its southerly drift. A negative value of the ordinate means that the scattering strength deviated below the average background level. Ordinate units are linear.



map of concentrations. The time required for such extensive sampling is probably long compared to the time interval in which significant changes take place. We report here the use of a modified 200-khz echo sounder, on board ship, to detect sewage sludge dumped into the ocean and to monitor in real time the evolution of a sewage sludge dump over a period of several hours.

In September 1975, as part of the Marine Ecosystems Analysis Project of the National Oceanic and Atmospheric Administration (NOAA), we conducted a 3-day pilot sludge-tracking experiment aboard the NOAA ship *George B. Kelez* in the New York Bight sewage sludge dump area. This dump area is a 4.5-km square centered 24 km from the Rockaway–Sandy Hook transect. An 80-watt, 200-khz echo sounder with the receiver modified to give 100 db of maximum gain and a 1-kw, 20-khz echo sounder were operated continuously throughout the 3-day period (*3*). Paper strip chart recorders provided real-time data output from both systems. Each echo sounder had a transducer which was towed within a streamlined hydrodynamic towbody. The two towbodies were towed on opposite sides of the ship at a depth of 2 m. The 200-khz system emitted sound in a nearly conical beam; the angular width of the cone varied between 12° and 18°, that is, the sound cone was not symmetrical. The detected signal from each system was tape-recorded in analog form for later digitization and computer analysis. Finally, we obtained temperature profiles with expendable bathythermographs and a conductivity-temperature-depth sensor. The water column was well mixed down to 18 m with a 4°C (19° to 15°C) thermocline to the bottom at 25 m.

A well-controlled experiment was made possible because the Environmental Protection Agency (EPA), Region II, prohibited all sludge dumping for the first 48 hours of the experimental period (1000 E.D.T., 20 September, to 1000 E.D.T., 22 September) so that an acoustic background survey could be made in the sludge dump area at the same time that water samples were being taken. During the next 24 hours (1000 E.D.T., 22 September, to 1000 E.D.T., 23 September), specific vessels dumped sewage at designated times according to instructions that we gave through the EPA. After 1000 E.D.T., 23 September, conventional dumping procedures were resumed.

The first dump during the controlled period was made (1812 m^3 from the Owl's Head sewage treatment plant) from 1045 to 1111 E.D.T., 22 September, along a 3-km line from west to east in the sludge dump area. We traversed the length of the line dump several times by making a zigzag track at alternating 45° angles to the tanker's straight-line motion. The fresh sludge dump was clearly visible on the paper recorder output of both the 200-khz and the 20-khz systems (Fig. 1) and produced detectable acoustic return above background level throughout the water column (23 m), an indication that some portion of the sludge sank to the bottom in 20 minutes or less. After 1 hour the sludge was no longer directly detectable in the 20-khz system. It was indirectly detectable in the 20-khz system in virtue of the presence of many point scatterers in the same regions where the 200-khz system was still detecting sludge directly. We presume that the point scatterers are either fish or other types of highly mobile marine biota or floccules of sewage. We tracked this line dump for 1 hour and 40 minutes with good signal on the 200-khz system. We passed over the line dump 4 hours after the sludge had been dumped and found the sludge still detectable with the 200-khz system.

At another time during the controlled period (1450 E.D.T., 22 September) a tanker dumped its entire contents (1456 m^3 from the Nassau County Bay Park sewage treatment plant) at one location. We made many transects of this dump site over a period of more than 2½ hours. During this time we observed that the dump mass moved slightly northward with the upper portion becoming displaced farther to the north than the lower portion, an indication that a current shear was present. As with the line dump, the 20-khz system no longer gave detectable acoustic return from the sludge after approximately 1 hour. Once again, a higher concentration of point scatterers was observed in the water column occupied by the sewage sludge than in surrounding water.

Additional sludge dumps were tracked after dark on 22 September, but the background acoustic scattering throughout the water column increased significantly during the measuring period, and, as a result, detection and tracking for periods greater than 2 hours became possible. This increased background scattering level was evident on the 200-khz system in the form of increased continuous background signal amplitude (when compared to the background level measured during morning or early afternoon hours). Acoustic return from the 20-khz system indicated that the water column had become densely populated with point scatterers.

In all of our measurements point scatterers appeared rapidly (within 2 to 3 minutes) in the water column in and near the sewage sludge. They could be floccules of sewage of sufficient size to be detectable acoustic targets. Flocculation of ocean-dumped sewage sludge has been reported by Jenkinson (*4*). The objects could also be biota. Very small fish with swim bladders 0.5 mm in diameter or larger would be detectable scatterers at 20-khz but would be below noise level at 200 khz (Fig. 1). Such fish (for example, anchovies) are known to populate New York Bight waters (*5*). Whether the scatterers originate within the sludge or in the marine environment, their appearance apparently is an effect of the dumping of the sludge and as such should be investigated further.

From the analog tape, we have digitized portions of the 200-khz data from the sludge dump events. Averages of successive 10-second time intervals yield profiles of acoustic return which are reasonably noise-free and represent a sampling interval of approximately 20 m along the ship's track. Since the acoustic beam is essentially conical, the intensity of the transmitted pulse and subsequent echo decreases as the square of the depth of the scatterer. We therefore multiplied the averaged profiles by the square of the depth to give profiles of actual scattering strength per unit volume as a function of depth. The scattering strength per unit volume is a linear measure of the number of suspended particles per unit volume for a given particle size distribution. To discover how much scattering was due to the sludge alone, we subtracted the averaged profile representing scattering strength per unit volume just outside the sludge dump from each averaged profile obtained along the ship's track within the dump region. For a given transect of a dump region, we noted the value of each resultant profile (of net scattering strength per unit volume due to sludge alone) at a prescribed depth and plotted these values (Fig. 2) as a function of distance through the dump region. Figure 2 shows the scattering strength at a depth of 14 m for three crossings of the line dump.

The progressive displacement of the curves in Fig. 2 along the axis represents the southerly drift of the sludge cloud during the tracking period. The decreasing amplitude and increasing width of the curves can be used to estimate the rate of diffusion of the sludge. We compared these data with those presented by Bowden *et al.* (*6*) in a discussion of diffusion from a continuous source at sea. Bowden *et al.* found that the lateral variance of a dye plume increases according to t^m, where t is time and m varied from 1.2 to 2.7. We found that the lateral variance of the line dump increased according to $t^{1.5}$. It therefore seems reasonable to attempt to calculate the local horizontal diffusivity from this kind of data.

Our results indicate that sewage sludge dumped in the ocean is detectable for several hours with commercially available acoustic equipment that has been modified somewhat. The signal-to-noise ratio is higher during daylight than at night because of the lower biological activity in the water column. The space-time evolution of a sludge dump can be followed if regular traverses of the dump area are made, and the three-dimensional distribution of suspended material can be determined with digital processing. Chemical sampling for research or monitoring purposes can be accurately guided in real time by acoustic tracking because the sludge cloud boundaries are easily discernible from the paper strip chart alone.

JOHN R. PRONI, FRED C. NEWMAN
RONALD L. SELLERS
Atlantic Oceanographic and Meteorological Laboratories, National Oceanic and Atmospheric Administration, Miami, Florida 33149
CHARLES PARKER
Marine Ecosystems Analysis, New York Bight Project, Marine Science Center, State University of New York, Stony Brook 11790

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Selective in vitro Growth of T Lymphocytes from Normal Human Bone Marrows

Abstract. *Selective growth of T lymphocytes occurred when unfractionated normal human bone marrow cells were cultured with conditioned medium obtained from phytohemagglutinin-stimulated normal human lymphocytes (Ly-CM). Cultures of up to 90 percent T cells have been maintained for more than 9 months. The T cells exhibited a strict growth dependence upon Ly-CM and were consistently negative for Epstein-Barr viral information.*

Human lymphoblastoid cell lines have been derived from normal bone marrows (*1*); all of these lines express characteristics of bone marrow–derived (B) lymphocytes (*2*) and invariably contain Epstein-Barr virus (EBV) information (*3*). The relatively few cell lines with characteristics of thymus-derived (T) lymphocytes are of neoplastic origin (*4*). The long-term maintenance of murine (*5*) and normal human (*6*) T cells has been reported. Mixed leukocyte cultures consisting of cells from two normal donors have resulted in the growth stimulation of responder T cells up to 9 months upon repeated exposure to allogeneic lymphocytes. We describe here our development of a system in which rapidly proliferating, EBV-negative cultures consisting of more than 90 percent T lymphocytes have been selectively grown from unfractionated normal human bone marrow cell suspensions from several donors. Initiation and long-term maintenance of the lymphocytes is strictly dependent on the continuous presence of conditioned medium from phytohemagglutinin (PHA)-stimulated human blood lymphocytes (Ly-CM).

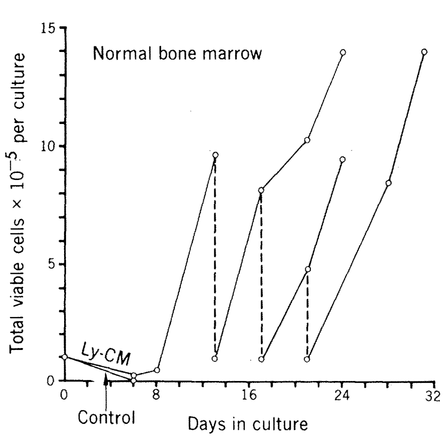

Fig. 1. Growth profile of cells derived from human bone marrow. Growth was induced by PHA-stimulated lymphocyte conditioned medium (Ly-CM). Control cultures are without Ly-CM. The vertical broken lines indicate a culture split.

The Ly-CM was prepared (Associated Biomedic Systems) by culturing pooled normal human lymphocytes. The final concentration of cells was 1×10^6 per milliliter of RMPI (Roswell Park Memorial Institute) medium containing 1 percent autologous serum and 1 percent PHA-M (Difco). After 3 days the medium was collected, concentrated fivefold, dialyzed against ten volumes of 0.15*M* NaCl by ultrafiltration (Amicon), and stored at 4°C until use as the source of Ly-CM. Bone marrow aspirates from normal donors were subjected to hypotonic shock to hemolyze the erythrocytes. The remaining cell pellets were resuspended in 0.15*M* NaCl and distributed into culture tubes (16 by 125 mm; Falcon) at a final concentration of 1×10^5 per milliliter of RPMI 1640 medium containing 20 percent fetal calf serum and 20 percent Ly-CM. The cultures were then incubated at 37°C in 5 percent CO_2 and a humidified atmosphere. Cell growth was monitored at selected intervals by counts of viable cells, at which time morphological staining of cytocentrifuge preparations was routinely done.

The growth curve (Fig. 1) is representative of nine out of nine samples. In both the control cultures, which had received only medium, and the supplemented cultures (Ly-CM) a decline in the viable population to less than 10 percent