Report C00-3563-70

CONF-7805193--1

Bacteria and Plutonium in Marine Environments

Anne E. Carey

and

Vaughan T. Bowen

Woods Hole Oceanographic Institution, MA

MASTER

DISCLAIMER

This book was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Presented at American Society for Microbiology Annual Meeting, Las Vegas, Nevada, May 1978

(Only Abstract to be published)

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT

Microbes are important in geochemical cycling of many elements. Recent reports emphasize biogenous particulates and bacterial exometabolites as controlling oceanic distribution of plutonium. Bacteria perform oxidation/reduction reactions on metals such as mercury, nickel, lead, copper and cadmium. Redox transformations or uptake of Pu by marine bacteria may well proceed by similar mechanisms. On R/V KNORR cruise 69 in Sept-Oct 1977, along the continental shelf off Nova Scotia and in the Gulf of St. Lawrence, we obtained profiles of water samples, and sediment cores. Epifluorescent microscopy was used to view bacteria (from water or sediment) after concentration on membrane filters and staining with acridine organge. Radiochemical analyses measured Pu in sediments and water samples. Studies of ^{237}Pu uptake used a strain of Leucothrix mucor isolated from a macroalga. Enumeration shows bacteria to range 10^4-10^5 cells/ml in seawater or 10^7-10^8 cells/gram of sediment. These numbers are related to the levels and distribution of Pu in the samples. In cultures of L. mucor amended with Pu atom concentrations approximating those present in open ocean environments, bacterial cells concentrated ^{237}Pu slower and to lower levels than did clay minerals, glass beads or phytoplankton. These data further clarify the role of marine bacteria in Pu biogeochemistry.

INTRODUCTION

Microbes are important in the geochemical cycling of many elements. Recent reports emphasize biogenous particulates and bacterial exometabolites as controlling oceanic and sediment distributions of plutonium. From field studies that had been conducted in our laboratory at Woods Hole Oceanographic Institution, we believed that sedimentation of plutonium was mediated by biogenous particulates. Higher inventories of fallout plutonium are found in areas of active bioturbation where the particle-associated plutonium is quickly removed from the possibility of horizontal transport. Sediments in areas of elevated bioturbational activity have higher organic content than sediments in areas of lower bioturbational activity. In these areas of organic-rich sediments, one would expect greater numbers of bacteria.

This field study was conducted to determine whether bacteria which may have been associated with detrital particles affected the distribution of plutonium in sea water or could act as indicators of the processes affecting plutonium retention in marine sediments. Laboratory experiments were undertaken to assess the effectiveness of bacteria, whether alive or killed, in plutonium uptake from seawater media, and to compare the bacterial performance with that of algae or of various inorganic detritus-simulants.

ACKNOWLEDGEMENTS

This work would not have been possible without the kind cooperation of many of our colleagues, in particular Lyn Brady, Barbara Brockhurst, John Andrews and William Clarke, who performed the radiochemical analyses of sediment and water samples, and Brenda Olson, who shared her data from the uptake experiments with phytoplankton and glass beads. The program has been supported by the Department of Energy under contracts EY-76-S-02-2379 and EY-76-S-02-3563 with the Woods Hole Oceanographic Institution.

Field Studies

All sampling was done on R/V KNORR cruise 69, leg 2, from Halifax, Nova Scotia, to Woods Hole, Massachusetts, in September and October 1977. Water samples were obtained with a 140-liter Bodman sampler (Bodman et al., 1961). Sediment cores were obtained using a 21-cm diameter gravity coring device in a tripod frame (Burke and Hamblin, in prep.). Immediately after collection, water samples for radiochemistry were pumped into 60-liter linear polyethylene Deldrums and 10-ml aliquots were removed, fixed with 0.1 ml 8% glutaraldehyde and refrigerated. Cores were extruded immediately after collection. Sediment aliquots of 1-cc were obtained using a 3-cc plastic syringe whose end had been cut off with a clean razor blade. These samples were diluted with 9 ml of sterile 3% NaCl, fixed with 0.1 ml 8% glutaraldehyde and refrigerated. Sediment samples for radiochemical analysis were stored in 16 ounce polystyrene jars whose screw caps were sealed with plastic tape.

Radiochemical analyses of water and sediment were performed according to the methods described by Livingston et al. (1975). Plutonium was electrodeposited on polished stainless steel discs and determined by alpha spectrometry.

Epifluorescent microscopy was used to make direct counts of acridine orange-stained bacteria, according to the method of Hobbie et al. (1978).

Sampling locations are shown in Figure 1. Water samples were taken at the stations indicated by the crosses, and sediment cores were taken at the stations indicated by the dots. Station 8 is located in a small, shallow basin.

Figure 2 shows the results of the bacterial counts and ^{239}Pu concentrations in the water profiles. The plutonium concentrations and shapes of the vertical profiles are typical of the nearshore North Atlantic at these latitudes. The error bars represent 1-sigma error from the counting statistics. The profiles of ^{239}Pu concentrations show subsurface maxima, while the bac-

terial numbers are maximal at the surface, decline to subsurface minima, and increase toward the bottom. If the bacteria were directly determining plutonium concentrations and distributions in the water column, then we would expect the profiles to appear different than they do.

Figure 3 shows sediment-Pu profiles and bacterial profiles. The vertical error bars represent the sampling interval, and the horizontal error bars represent 1-sigma errors, as in the previous figure. Depth of the overlying water is given for each core, and the plutonium inventories are given for cores T-18 and T-13. The higher inventory of plutonium for core T-18 we attribute to its location in the small shallow basin at station 8 that is probably trapping sediment representing a larger area. The decline of Pu concentration at depth most likely reflects the variety of biological mechanisms (bioturbation) which move the plutonium through the sediment. Bacterial numbers show a nearly constant level at all depths sampled.

In T-22, T-13 and T-18, the surface plutonium values range from 50 to 150 dpm/kg, yet the bacterial numbers are essentially the same at all three stations. The plutonium inventory at T-13 is 1.1 mCi/km^2 and at T-18 is 2.5 mCi/km^2, a difference of a factor of 2, yet the bacterial numbers are the same at both stations. The highest bacterial counts were found at T-29, which was a coarse, sandy sediment exhibiting the lowest plutonium levels.

Laboratory Experiments

Experimental studies of ^{237}Pu uptake were designed to determine the role of various kinds of particles in the uptake and transport of plutonium. In these experiments, we used plutonium atom concentrations comparable to those our laboratory has measured in the open ocean environment. In these studies, we used bacterial and phytoplankton cultures, glass beads, and clay minerals.

The strain of Leucothrix mucor used in uptake experiments was isolated by streaking decaying macroalgae, found on Nobska Beach, Woods Hole, Mass., onto agar plates of medium composed of 0.04% yeast extract, 0.02% beef extract, and 0.02% Na acetate in 75% seawater. An estuarine clone of Thallassiosira pseudonana (3H) used in these studies was obtained from Robert Guillard of Woods Hole Oceanographic Institution.

Clay mineral reference standards were obtained from John Hathaway of the U. S. Geological Survey, Woods Hole. Standard density glass beads (density 2.06) which ranged in diameter from 9-44 μ, were obtained from Fisher Scientific Company.

Standardized tracer solutions of ^{237}Pu (obtained from Oak Ridge National Laboratories) were prepared in filtered Sargasso Sea water adjusted to pH 1.5 with HCl, and sterilized by overnight UV-irradiation in quartz tubes. The volume of the Pu solution was adjusted as the plutonium decayed to produce solutions with 5-10 counts/second/gram in our gamma counting geometry. Disposable, sterile, 3 ml syringes were employed to weigh plutonium aliquots of approximately 1 gram each to be added to experimental flasks. Syringes were tared after the solution was dispensed.

In order to prevent plutonium from polymerizing or associating with particles, media preparation was done in this manner. Prepared media were adjusted to pH 8.5, filtered through 0.22 μ sterile Millipore filters, and then dispensed into sterile flasks. Upon addition of the acidic plutonium solution, the pH of the medium returned to 7.6. Addition of base after plutonium was in the flask was avoided to prevent development locally of very basic areas in which the plutonium might irreversibly change its form to a polymer or an insoluble salt. These polymers and salts are formed

in unpredictable amounts in the presence of strong base, and their formation would result in change in plutonium-uptake kinetics and difficulty in reproducing experimental conditions.

^{237}Pu decays by electron capture to ^{237}Np, emitting X-rays of 101 KeV. These were measured by a Harshaw 3-inch thallium-doped sodium iodide well crystal and data were stored in a Nuclear Data 130 512-channel pulse height analyzer which measured 10-820 KeV over the first 128 channels.

Sampling the flasks was achieved by periodically pipetting a homogeneous aliquot from each flask and filtering it by vacuum through a 0.2 μ Nuclepore filter for bacteria and clays and a 1.0 μ Nuclepore filter for algae and glass beads. Each filter was placed in a separate soda lime glass gamma-counting tube and covered with 5 ml 8N HNO_3.

Cells of Leucothrix mucor used for these experiments were grown in 75% Sargasso seawater supplemented with 0.1% casamino acids and a vitamin mixture. Cells of Thallassiosira pseudonana were grown in medium f/2 of Guillard and Ryther (1962). Mid-log phase cells were harvested by centrifuging in sterile centrifuge bottles of linear polyethylene. Dead cells were prepared by heating the cell suspension in a water bath. Bacterial cells were heated at 47°C for 30 minutes, and algal cells were heated at 45°C for 15 minutes. After heating, bacterial cells were no longer viable, as determined by plating on agar of the same medium used for growing cells. After heat treatment, phytoplankton cells no longer took up oxygen, determinded manometrically. Both bacterial and phytoplankton cells were intact after heat treatment, determined microscopically.

Dry weights of Leucothrix mucor cultures averaged 15 mg in each experimental flask, and cells continued to divide throughout the course of the experiments. 20 mg of clay mineral was used per flask. Phytoplankton weights averaged 3.2 mg per flask, and did not divide. Glass bead weights

averaged 2.6 grams per flask. Direct counts of phytoplankton cells and glass beads were made using a Speirs-Levy Eosinophile counting chamber.

Figure 4 shows the results of an uptake of ^{237}Pu experiment by dead and by growing cells of Leucothrix mucor. The dead cells took up the plutonium to a greater extent and at a faster rate than did the live cells, which showed Pu levels nearly at background for most of the course of the experiment.

Figure 5 shows the results of a ^{237}Pu-uptake experiment comparing live bacterial cells and clay minerals. For the first 6 hours of the experiment, the cells took up little, if any, of the plutonium which is consistent with the results shown in Figure 4. Yet by 6 hours, the montmorillonite had taken up 37% of the added plutonium, and the kaolinite had taken up 60% of the plutonium. At the termination of the experiment, the cells had taken up 45% of the plutonium, the montmorillonite had taken up 57%, and the kaolinite had taken up 76%.

Figure 6 shows the results of ^{237}Pu uptake by live and by dead cells of Thallassiosira pseudonana. The results of uptake by 10^5 cells/ml, both live and dead, are similar, but the 10^4 cells/ml took up much less of the plutonium.

Figure 7 shows the result of uptake by ^{237}Pu by phytoplankton cells and glass beads. Rate of uptake by 10^5 cells/ml is comparable to that by 10^5 glass beads/ml, and the rate of uptake by 10^4 cells/ml is comparable to the rate of uptake by 10^4 glass beads/ml.

CONCLUSIONS

The results of these experiments seem to indicate that among the types of particles examined, bacterial detritus would be least effective in plutonium uptake in the marine environment. Attachment of bacterial cells

to other sorts of detrital particles, both biogenous and inorganic, might even decrease the efficiency of plutonium uptake by these particles.

These results were corroborated by the field study described at the beginning of this report. These results are contrary to opinions expressed by several investigators, including one of us, who felt that bacterial detritus should be important in the vertical transport of plutonium in the water column and that bacterial numbers should coincide with the distribution and concentration of plutonium in the water column.

Higher inventories of plutonium are found in areas of active bioturbation. These biologically reworked sediments are generally high in organic matter and because of the high organic content, one would expect to find large numbers of bacteria. The metabolic products of these bacteria, especially organic acids, should serve as important factors in the solubilization and remobilization of the particle-associated plutonium. The zone of production of these organic acids is in areas of low oxygen tension. Diffusion of these bacterially-produced organic acids would result in plutonium transport through the sediment column. Thus the metabolic activity of the bacteria, rather than their total numbers, should reflect the plutonium inventories.

REFERENCES

Bodman, R. H., L. V. Slabaugh, and V. T. Bowen, 1961, A multipurpose large volume sea-water sampler. J. Mar. Res. 19: 141-148.

Burke, J. C. and R. E. Hamblin, A 21-cm diameter sphincter tripod corer. In preparation.

Guillard, R. R. L. and J. H. Ryther, 1962, Studies on marine planktonic diatoms I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Gran.Can.. J. Microbiol. 8: 229-239.

Hobbie, J. E., R. J. Daley, and S. Jasper, 1977, Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Env. Microbiol. 33: 1225-1228.

Livingston, H. D., D. R. Mann, and V. T. Bowen, 1975, Analytical procedures for transuranic elements in seawater and marine sediments. In: Advances in Chemistry Series, No. 147, Analytical Methods in Oceanography, pp. 124-138.

FIGURE LEGENDS

Fig. 1 Station locations.

Fig. 2 ^{239}Pu data and bacterial counts from water samples at Station 8 (same location as core T18) and Station 14 (same location as core T22). The error bars represent 1-sigma errors from the counting statistics.

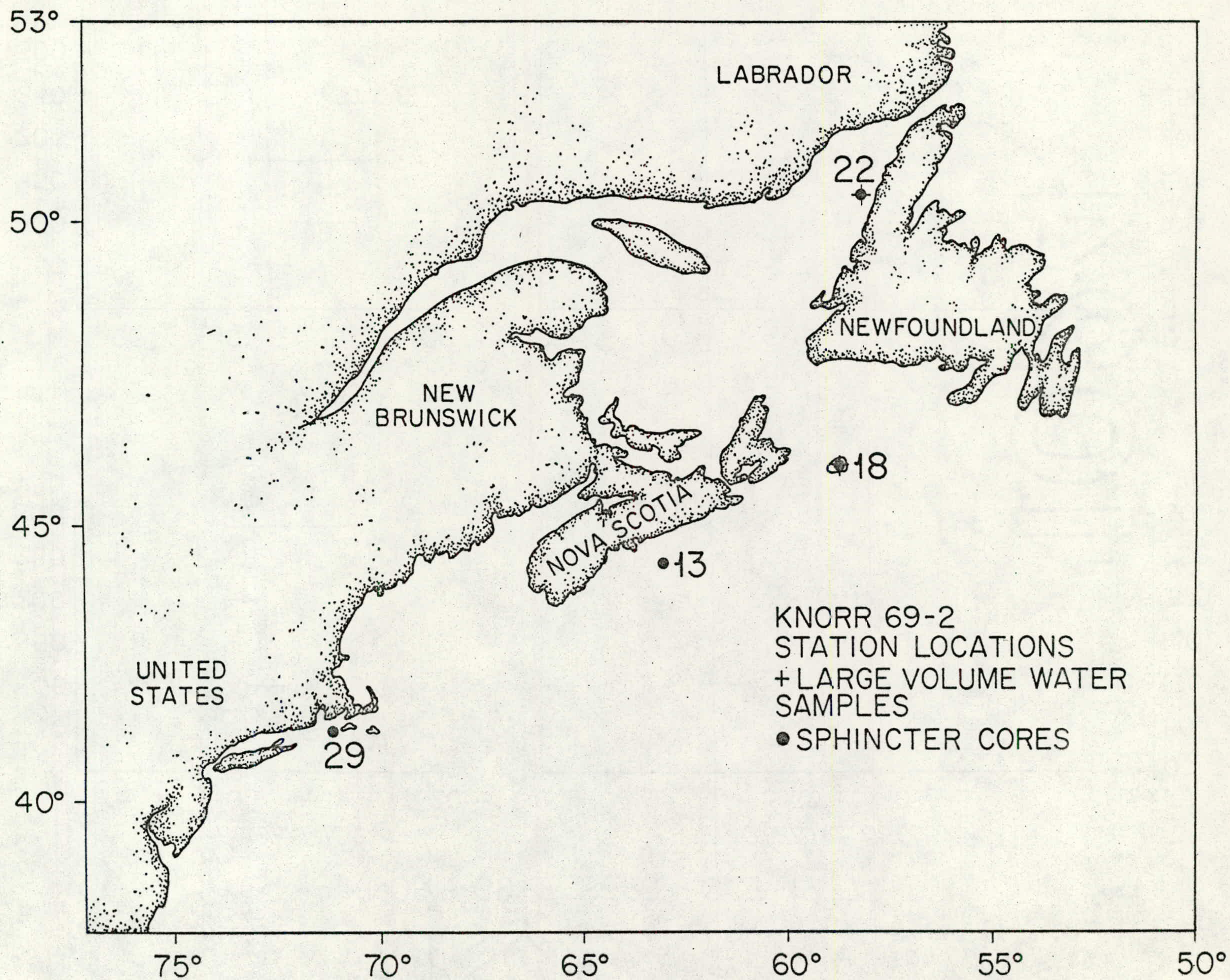
Fig. 3 ^{239}Pu data and bacterial counts from sediment cores. Depth of the overlying water is given for each core, and the plutonium inventories are given for cores T13 and T18. The vertical error bars represent the sampling interval and the horizontal error bars represent 1-sigma errors from the counting statistics.

Fig. 4 Uptake of ^{237}Pu by Leucothrix mucor. Error bars represent 1-sigma errors from the counting statistics.

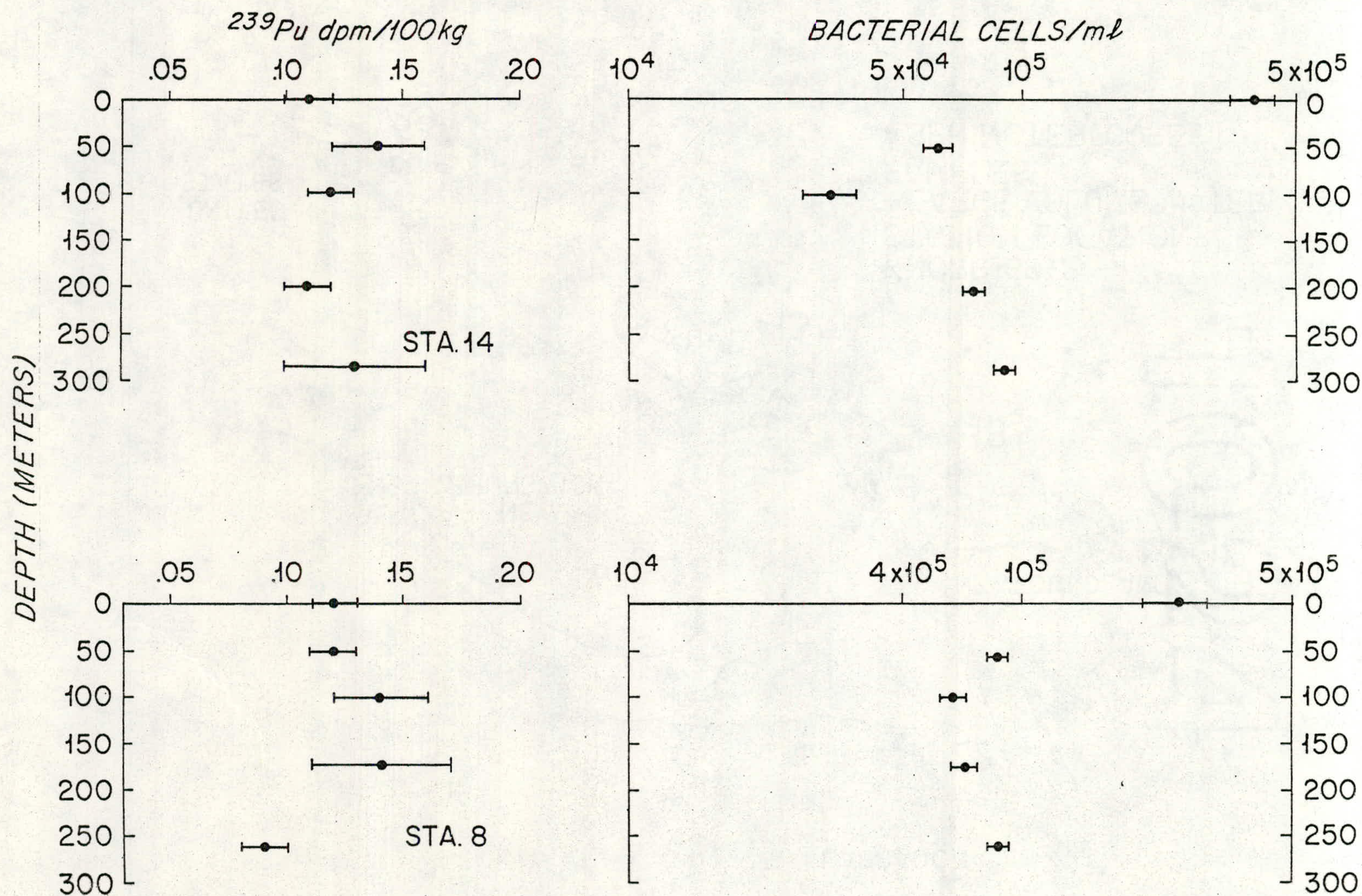
Fig. 5 Uptake of ^{237}Pu by Leucothrix mucor, kaolinite, and Ca-montmorillonite Error bars represent 1-sigma errors from the counting statistics.

Fig. 6 Uptake of ^{237}Pu by live and dead cells of Thalassiosira pseudonana. X-axis is incubation time in hours. Error bars represent 1-sigma errors from the counting statistics.

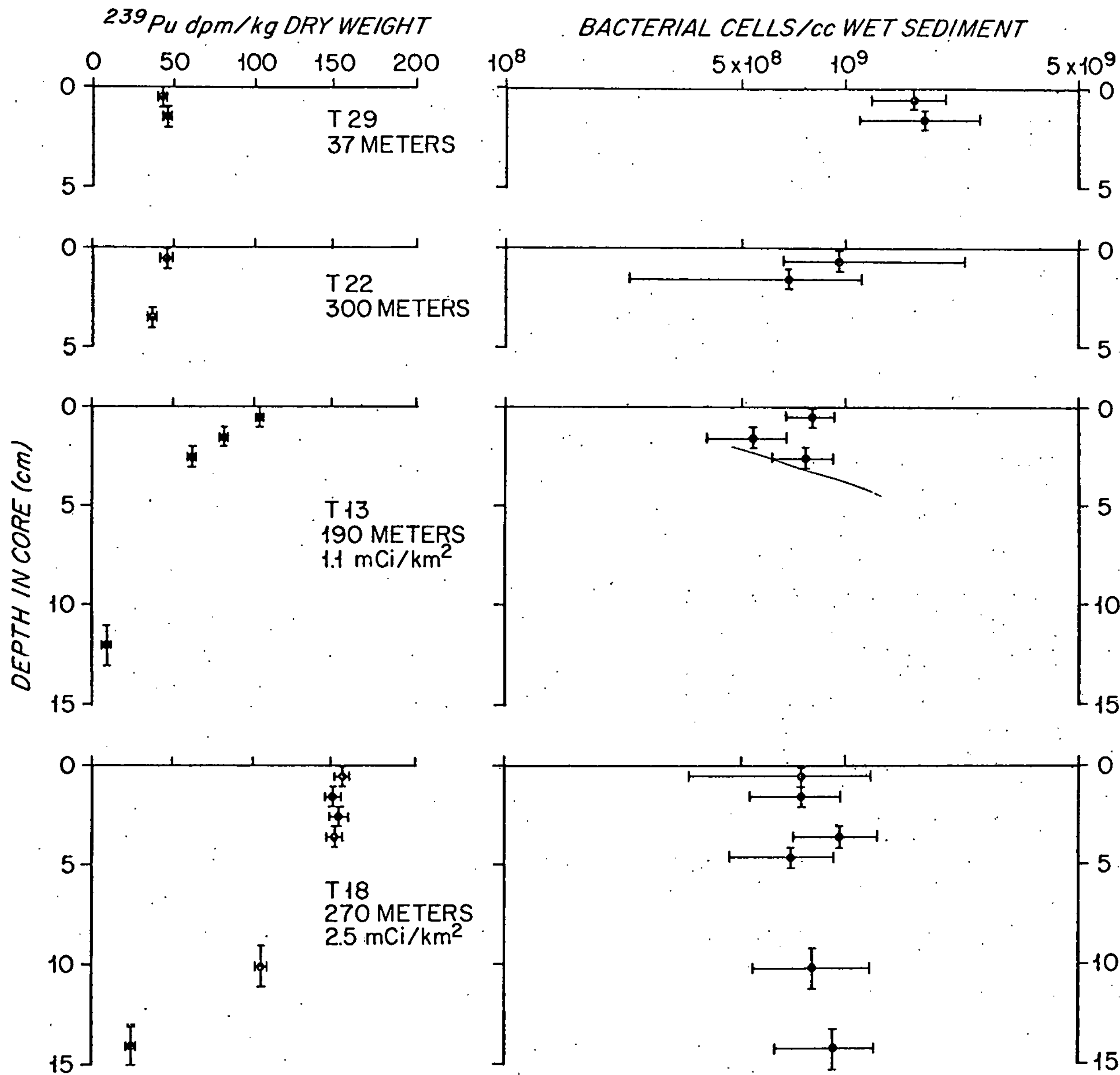
Fig. 7 Uptake of ^{237}Pu by Thalassiosira pseudonana cells and glass beads. Error bars represent 1-sigma errors from the counting statistics.

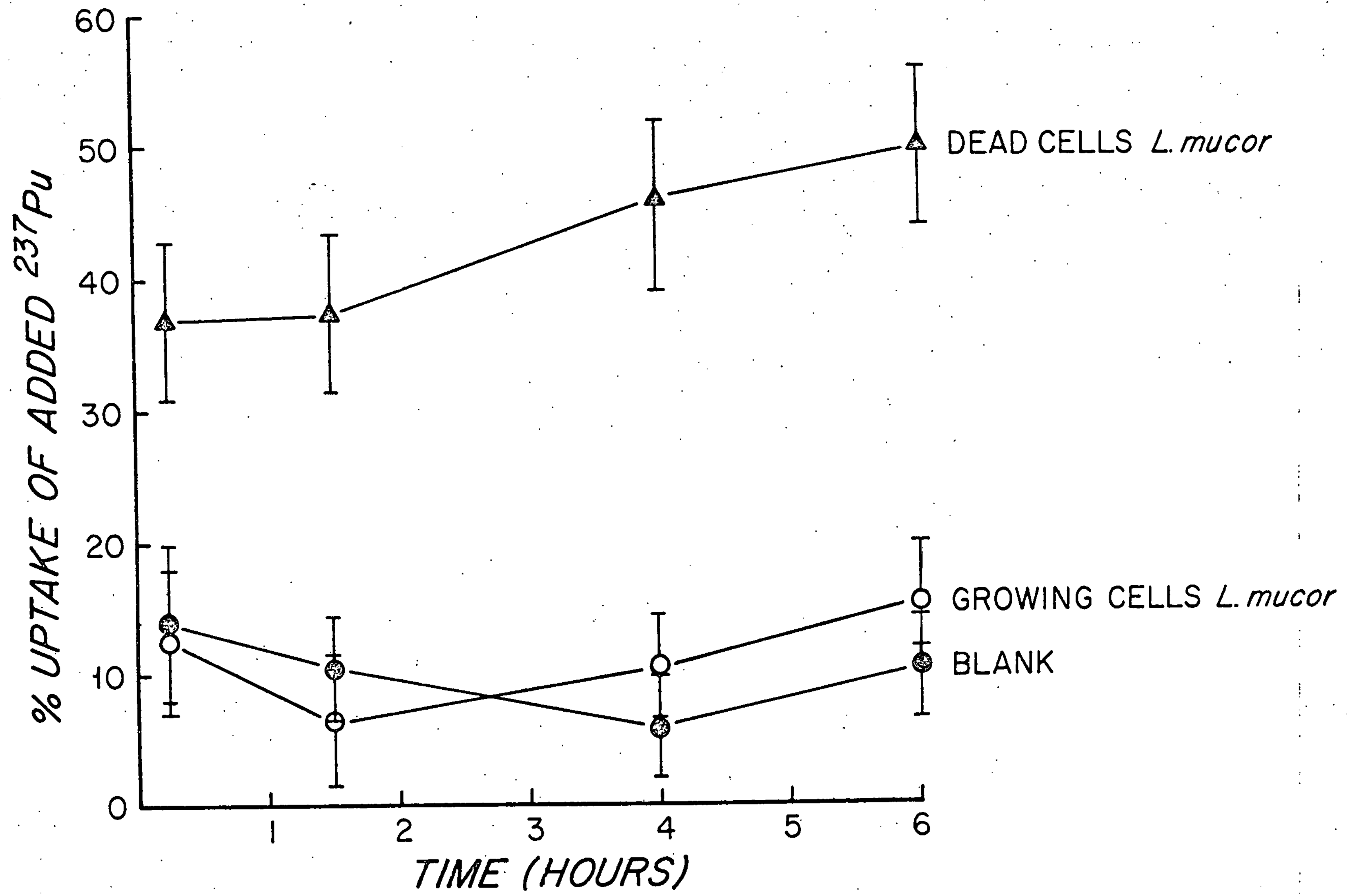

53°
50°
45°
40°
75°
70°
65°
60°
55°
50°
LABRADOR
NEWFOUNDLAND
NEW BRUNSWICK
NOVA SCOTIA
UNITED STATES
22
18
13
29
KNORR 69-2
STATION LOCATIONS
+ LARGE VOLUME WATER SAMPLES
● SPHINCTER CORES

KNORR 69-2 SEPT. - OCT. 1977

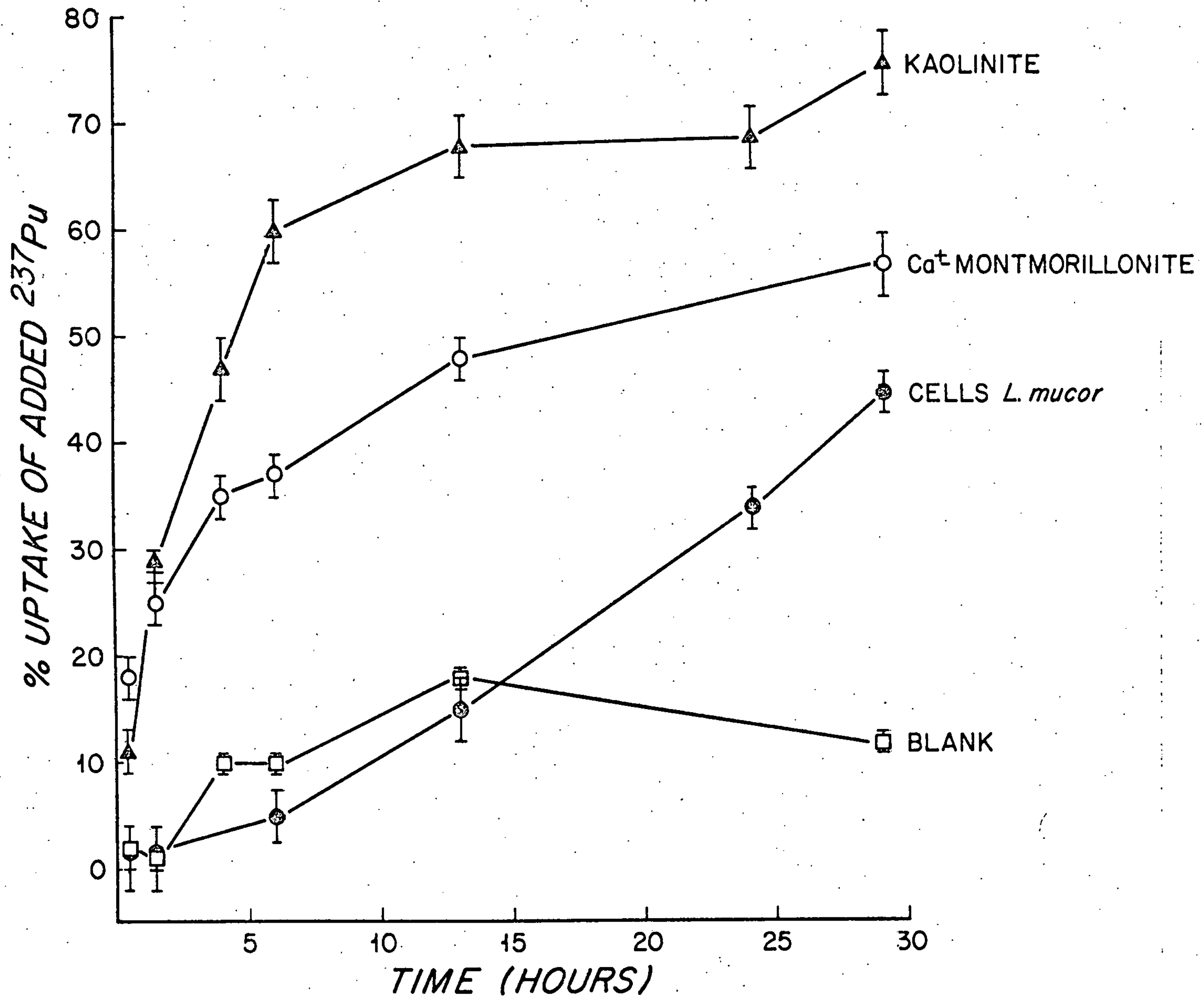


KNORR 69-2 SEPT. - OCT. 1977

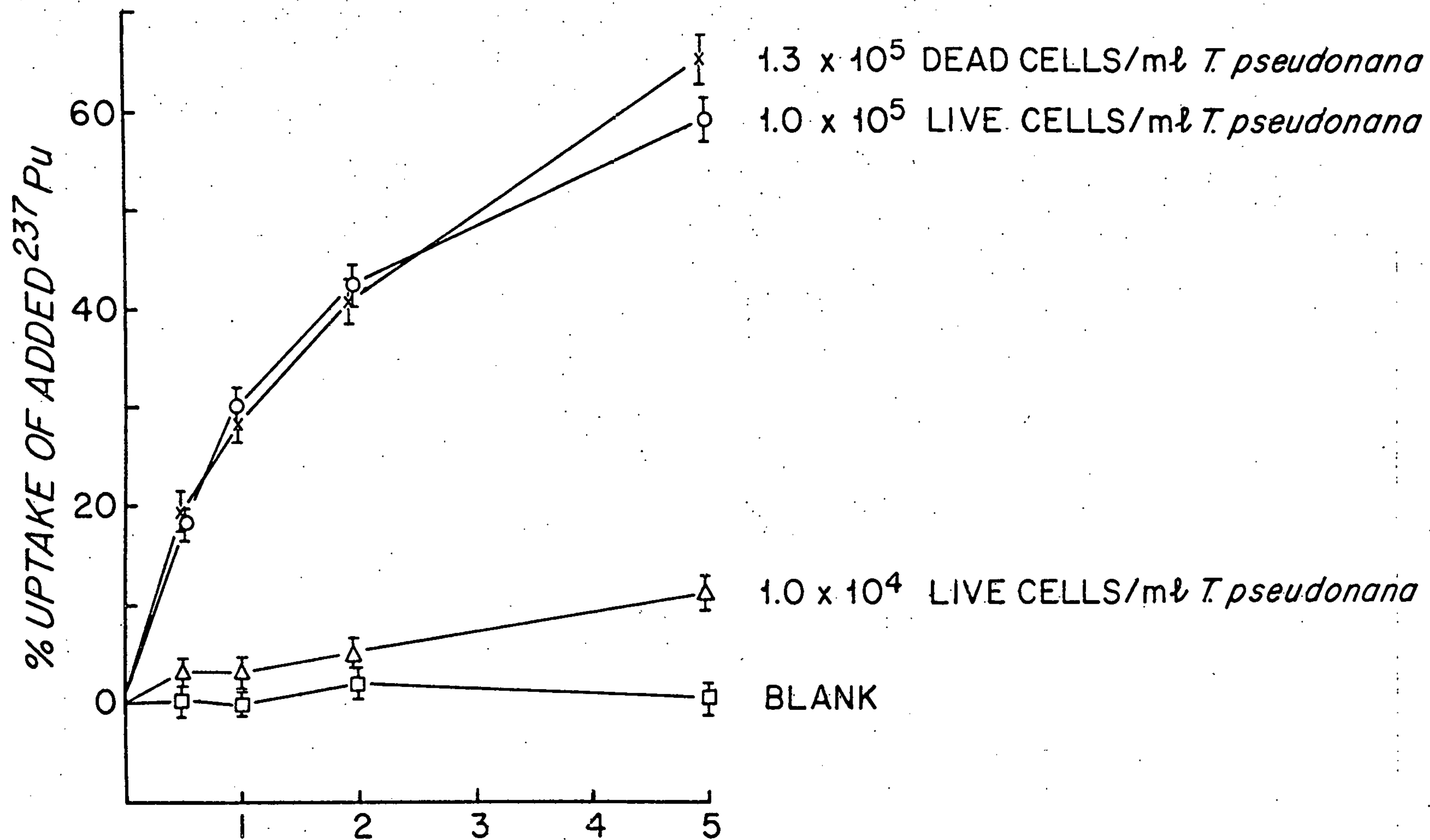


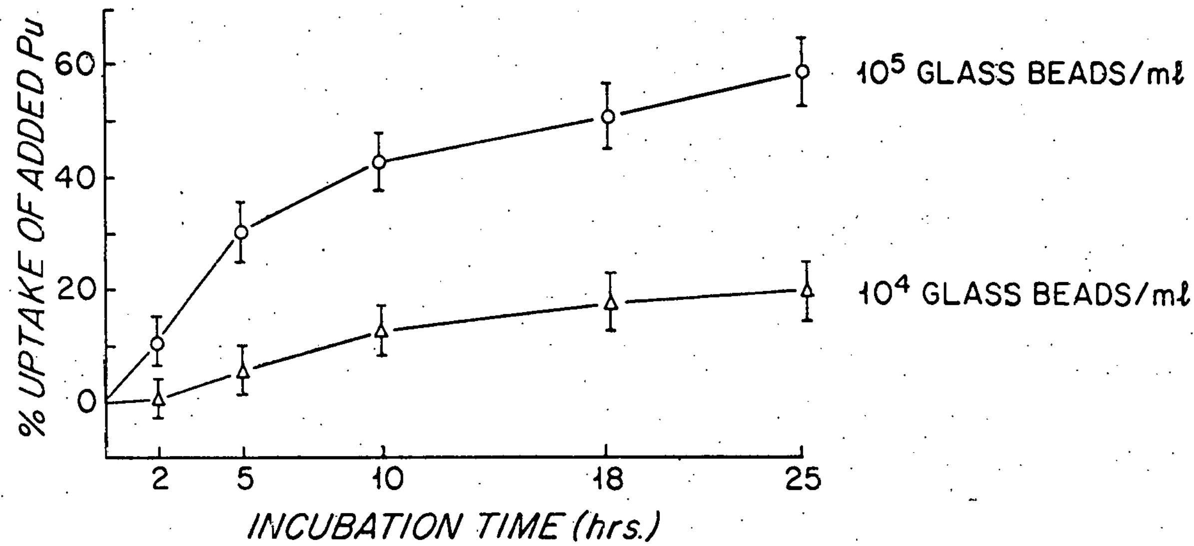


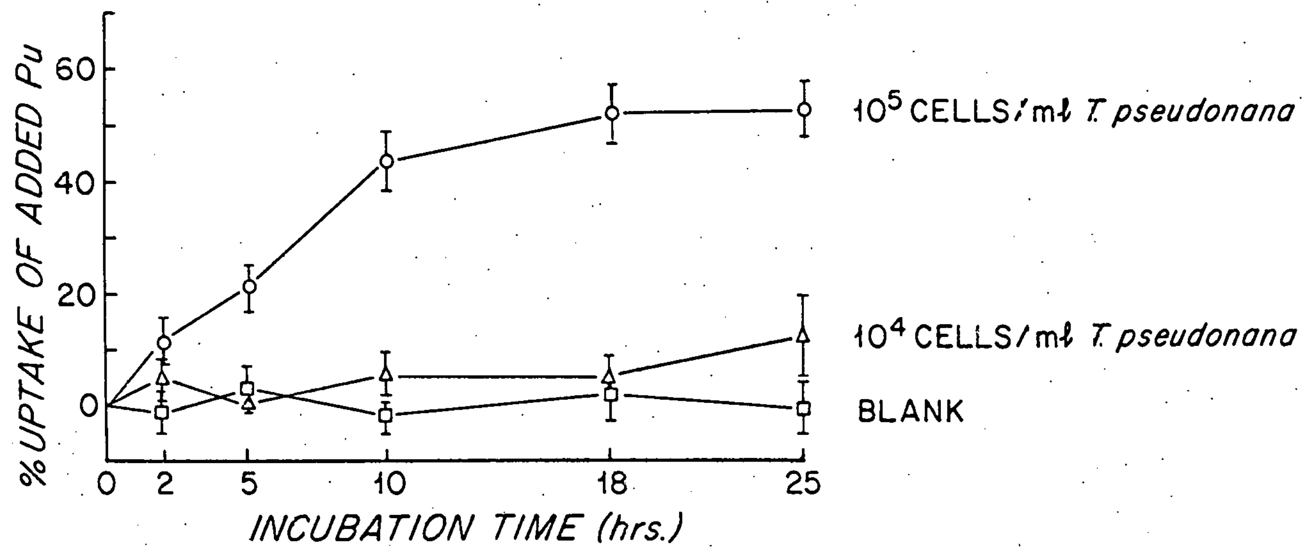
% UPTAKE OF ADDED ^{237}Pu
60
50
40
30
20
10
0
1
2
3
4
5
6
TIME (HOURS)
DEAD CELLS *L. mucor*
GROWING CELLS *L. mucor*
BLANK



% UPTAKE OF ADDED 237Pu
TIME (HOURS)
KAOLINITE
Ca+ MONTMORILLONITE
CELLS L. mucor
BLANK
0
10
20
30
40
50
60
70
80
5
10
15
20
25
30


1.3 x 10^5 DEAD CELLS/mℓ T. pseudonana
1.0 x 10^5 LIVE CELLS/mℓ T. pseudonana
1.0 x 10^4 LIVE CELLS/mℓ T. pseudonana
BLANK
% UPTAKE OF ADDED 237Pu
60
40
20
0
1
2
3
4
5


% UPTAKE OF ADDED Pu
60
40
20
0
2
5
10
18
25
INCUBATION TIME (hrs.)
10^5 GLASS BEADS/ml
10^4 GLASS BEADS/ml


% UPTAKE OF ADDED Pu
60
40
20
0
0
2
5
10
18
25
INCUBATION TIME (hrs.)
10^5 CELLS/ml T. pseudonana
10^4 CELLS/ml T. pseudonana
BLANK