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ULTRA-CLEAN TECHNIQUES IN ASSESSING THE EFFECTS OF METALS ON PHYTOPLANKTON

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EXECUTIVE SUMMARY

Ultra-clean Techniques in Assessing Metal Effect on Phytoplankton

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The necessity of using ultra-clean techniques in measuring primary productivity in water from Batchewana Bay, Lake Superior and Turkey Lake, Ontario, were evaluated. There was no significant difference in productivity in the waters collected with Van Dorn bottle (with metal components) and with the rubber-and-plastic hand pump (metal free). Incubation of waters in polycarbonate flasks gave much higher productivity values than in the glass flasks. The decrease in values in glass flasks was not due to the leaching of metals from the glass flasks. The increase in productivity in polycarbonate flasks could be due to the increase of light penetration through the polycarbonate bottles.

The enhanced metal toxicity observed in polycarbonate flasks could be explained by less metal adsorption onto the walls of the flasks and thus more available to the phytoplankton for causing toxic effects.

Additions of metals (Cu,Zn,Ni,Pb) at the Great Lakes Water Quality Objective levels greatly suppressed the primary productivity of waters of Lakes Superior and Turket Lake. The use of Ultra-clean techniques in measuring productivity of oligotrophic waters is still recommended.

SOMMAIRE PRINCIPAL

Utilisation de méthodes de propreté extrême pour évaluer les effets des métaux sur le phytoplancton

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On a évalué s'il était nécessaire d'avoir recours à des méthodes de propreté extrême (sans métal) pour mesurer la productivité primaire dans les eaux de la baie Batchaouana, du lac Supérieur et du lac Turkey, en Ontario. On n'a pas constaté de grande différence de productivité entre les eaux prélevées grâce à la bouteille de Van Dorn (avec métal) et celles prélevées grâce à une pompe à main en caoutchouc et plastique (sans métal). L'incubation de l'eau dans les bouteilles de polycarbonate a entraîné une productivité plus grande que dans les bouteilles en verre. L'augmentation de la producvité dans les bouteilles de polycarbonate pourrait être due à l'augmentation de la pénétration de la lumière dans ces bouteilles.

L'augmentation de la toxicité des métaux observée dans les bouteilles de polycarbonate pourrait être expliquée par le fait que l'adsorption des métaux sur les parois des bouteilles a été moins importante et, par conséquent, qu'il y a eu présence plus grande de métaux pouvant être adsorbés par le phytoplancton, entraînant des effets toxiques.

L'ajout de métaux (Cu, Zn, Ni, Pb) à raison de doses conformes à l'objectif de qualité de l'eau des Grands Lacs a diminué de beaucoup la productivité primaire des eaux du lac Supérieur et du lac Turkey. On recommande quand même le recours à des méthodes de propreté extrême pour mesurer la productivité des eaux oligotrophes.

Utilisation de méthodes de propreté extrême pour évaluer les effets des métaux sur le phytoplancton

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On a évalué s'il était nécessaire d'avoir recours à des méthodes de propreté extrême (sans métal) pour mesurer la productivité primaire dans les eaux de la baie Batchaouana, du lac Supérieur et du lac Turkey, en Ontario. On n'a pas constaté de grande différence de productivité entre les eaux prélevées grâce à la bouteille de Van Dorn (avec métal) et celles prélevées grâce à une pompe à main en caoutchouc et plastique (sans métal). L'incubation de l'eau dans les bouteilles de polycarbonate a entraîné une productivité plus grande que dans les bouteilles en verre. La diminution des valeurs dans les bouteilles de verre n'était pas due à la lixiviation des métaux dans les bouteilles en verre, car la quantité de Cu, Pb, Ni et Zn se séparant par dissolution dans ces deux types de bouteille ne variait pas beaucoup. La contamination des eaux par les métaux pourrait entraîner une réduction considérable de la productivité, comme l'ajout d'un mélange de métaux (Cu, Zn, Ni et Pb) dans l'eau le prouve.

Ultra-clean techniques in assessing the effects of metals on phytoplankton

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The necessity of using ultra-clean techniques (metal-free) in measuring primary productivity in waters from Batchewana Bay, Lake Superior and Turkey Lake, Ontario was evaluated. There was no significant difference in productivity in waters collected with a Van Dorn bottle (which has metal components) and with a metal free rubber-and-plastic hand pump. Incubation of waters in polycarbonate flasks gave much higher productivity than in glass flasks, but the decrease in values in glass flasks was not due to the leaching of metals from the glass flasks since there was no significant difference in the quantity of Cu, Pb, Ni and Zn leached from the two types of flasks. However, contamination of waters by the addition of ~~a~~ mixture of metals (Cu (5 μg/L), Zn (30 μg/L), Ni (25 μg/L), and Pb (25 μg/L)) resulted in a drastic decrease in productivity.

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Introduction

Phytoplankton are known to be sensitive to low concentrations of metal contaminants in the waters (Wong et al., 1982; Rai et al., 1981). For example, Fitzwater et al., (1982) have shown that small amounts of metal contaminants introduced during sampling and handling procedures could adversely affect ^{14}C-primary productivity measurements in the open-ocean waters. Since sampling and incubation techniques in the Great Lakes rely on standard methods similar to those in oceans, the possibility of metal contamination is equally great, particularly in the oligotrophic Upper Great Lakes, where the trace metal and nutrient concentrations are extremely low. Our objective was to determine if ultra-clean sampling and experimental procedures are necessary in Lake Superior and in Turkey Lake.

Materials and Methods

Sampling sites -

We chose Batchewana Bay (latitude 46° 49'N; longitude 84° 39'W) in Lake Superior and Little Turkey Lake (latitude 46° 45'N; longitude 84° 30'W) in Ontario for our July-August, 1983 studies because both are oligotrophic and accessible (Vollenweider et al., 1974).

Sampling water -

We examined several types of water samples for possible contamination from their metal components. The most commonly used Van Dorn bottle (General Oceanics) has a number of metal parts and fittings which would be difficult to remove or replace with non-metal parts. The Niskin bottle (GM Manufacturing) has fewer metal parts (2 pins, 1 spring and 1 clamp), but is available only in a 2.5 L size. The Go-Flo ball-valve bottle (General Oceanics) was difficult to operate manually especially in a small boat. The "March" pump (March Manufacturing Inc.) could be used to pump a large volume of water at a specific depth. The pump has no metal component. Unfortunately a generator is required for pumping. The rubber-and-plastic hand-operated pump (ITT Industries) has no metal part, is light, and can be operated manually. After experimenting with various bottles in the field, we decided to use the Van Dorn bottle as the conventional method of taking water samples, and the rubber-and-plastic hand pump as the metal-free method of taking water samples.

Water was collected at a depth of 4.9 m, mixed in a plastic carboy and dispensed into 500 mL erlenmeyer glass or polycarbonate flasks. Four flasks were housed in one flask holder and lowered into 4.9 m depth for in-situ incubation (Figure 1) with $^{14}CO_3$.

Mooring -

Three types of mooring were compared for ease in handling and retrieval of the samplers (Figure 1). The spar buoy had a radar reflector attached to a plastic buoy on a 6 foot spar. The sampler was stabilized with a half concrete block. We found that it was difficult to retrieve the sampler because of the weight of the spar buoy itself, and the necessity to detach and reattach the spar buoy to the anchor line in order to retrieve the sampler. This was found to be very awkward for two people in a small boat in rough weather. In addition, the radar reflector was not essential to our work. The tether buoy had a similar problem in that retrieval of the samples required detaching and reattaching the anchor rope each time. The dumbell buoy was convenient for retrieving the sampler simply by undoing the knot from one end of the pipe. The concrete block was subsequently replaced with a smaller, plastic-coated lead weight. The dumbell buoy was used in all our experiments.

Primary productivity -

To reduce metal contamination from ^{14}C-bicarbonate, we used high activity stock (56 mC/m mole or 10 mC/5mL) from Amersham Co. This was diluted to a working solution of 50 μC/mL with double distilled water and stored in acid-cleaned Teflon bottles. For experiments, we added 0.2 mL of 50 μC/mL to 500 mL of sample i.e., 10 μC/sample. The flasks were tightly capped with rubber stoppers which were wrapped with teflon tape. After 4-hour incubation, 500 mL sample was filtered on a 0.45 μ cellulose acetate filter, and rinsed rapidly with 100 mL of double distilled water. Filters were dissolved in 15 mL PCS scintillation counting fluor (Amersham/Searle) and counted in a liquid scintillation counter (Beckman model LS8100).

Cleaning -

All bottles, flasks, graduated cylinders, pipettes and tubings were sequentially soaked overnight in Decon (BDH Ltd.), dilute nitric acid (4-10%), 0.02 M EDTA, and rinsed several times with double distilled water.

Metal analyses -

Cu, Pb, Ni and Zn were solvent-extracted and analyzed in a graphite furnace, atomic absorption spectrophotometer with a detection limit of 1 µg/L (Environment Canada, 1979).

Results and Discussion

Fitzwater et al., (1982) identified several potential sources for metal contamination that could adversely affect the productivity measurement in the waters. These included water samplers, incubation flasks, and radioactive isotopes. To test the effects of possible metal contamination from the sampling bottles, we compared the primary productivity in water from Batchewana Bay taken with Van Dorn bottle or rubber-and-plastic hand pump and incubated with ^{14}C in polycarbonate flasks. The results in Table 1 indicate that the productivity in water taken with the "metal-free" technique was slightly higher than in water taken with the conventional Van Dorn bottle. The results are not statistically different as analyzed by Student-Newman-Keuls test at 95% confidence limits. Similarly, there was no significant difference in productivity in water from Turkey Lake using two water sampling techniques (Table 2). Hence, the leaching of metals, if any, from the Van Dorn bottle did not inhibit the phytoplankton significantly.

In the course of the experiments, we realized that the dispensing of water into the incubation flasks on board a diesel fuel boat could lead to the contamination of water by metals and organic compounds from the vapor of the diesel fuel. To avoid this atmospheric contamination, we devised an in-situ incubation technique. We connected the 4 incubation flasks with 2 glass U-tubes (Figure 2), stoppered tightly and lowered the samplers to

4.9 m depth. A porcelain messenger was sent down to break the U-tubes and allowed the water to enter the flasks by hydrostatic pressure. This technique avoided metal contamination from the water-sampling bottles and from the atmosphere.

Metal contamination could also come from the incubation flasks. Fitzwater et al., (1982) reported that even after vigorous cleaning, significant amounts of Cu leached from the glass flasks and significantly depressed primary productivity. To verify this observation, we compared the productivity in water incubated in glass or polycarbonate flasks using the above in-situ technique. The productivity in waters from Lake Superior and Turkey Lake incubated in polycarbonate flasks was indeed much higher than in glass flasks (Table 3). A similar observation was reported by Carpenter and Lively (1980) and was attributed to the relatively low metal leaching from the polycarbonate flasks.

Experiments were carried out to determine whether the low productivity in glass flasks was due to the leaching of toxic metals from the walls of glass flasks. Water samples from Batchewana Bay were collected either with Van Dorn bottle or rubber-and-plastic hand pump and incubated in polycarbonate and glass flasks for 4 hours. The concentrations of Cu, Pb, Ni and Zn in the waters were determined. Results (Table 4) indicate that Cu, Pb and Ni levels were below detection limits of 1 μg/L in all the samples. There was no significant difference in the Zn concentrations of 6.0 - 7.5 μg/L. Similarly, water samples from Turkey Lake contained <1 μg/L of Cu, Pb and Ni and 16.0-17.5 μg/L of Zn (Table 5). Extending the incubation time from 4 hours to 28 days did not increase the concentration of the metals.

Since there was no significant difference in productivity in water collected by conventional and relatively "metal-free" sampling techniques and in the concentrations of metals leaching from the flasks, the observed increase in the productivity in polycarbonate flasks could be attributed to the more effective light penetration into the polycarbonate bottles (Ilmavirta and Hakata, 1972).

The emphasis by Fitzwater et al (1982) on using the ultra-clean technique in measuring productivity in oligotrophic waters is still valid. As shown in Table 6, addition of a metal mixture of Cu, Zn, Ni and Pb at the Great Lakes Water Quality Objective levels (IJC, 1976) reduced the productivity by 39 to 74%. The enhanced metal toxicity in polycarbonate flasks could be explained by lower adsorption of the metals onto the inner walls of the flasks (Robertson, 1968).

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Table 1. Effect of water sampling techniques on primary productivity in Lake Superior water

Water sampling techniques	No. of samples	Primary productivity dpm/500 mL (mean ± S.E.)
Conventional**	9	6,834* ± 302
Metal-free***	5	7,301* ± 614

* No significant difference in Student-Newman-Keuls test at 95% confidence limits

** Van Dorn bottle

*** Rubber-and-plastic hand-operated pump.

Table 2. Effect of water sampling techniques on primary productivity in Turkey Lake water

Water sampling techniques	No. of samples	Primary productivity dpm/500 mL (mean ± S.E.)
Conventional**	8	18,100* ± 3,526
Metal-free***	8	21,872 ± 828

* No significant difference in Student-Newman-Keuls test at 95% confidence limits

** Van Dorn bottle

*** Rubber-and-plastic hand-operated pump.

Table 3. Comparison of primary productivity in waters from Lake Superior and Turkey Lake using in-situ technique[1] with glass and polycarbonate flasks

Location	No. of Samples	Primary productivity (dpm/500 mL; mean ± S.E.) glass flask	Primary productivity (dpm/500 mL; mean ± S.E.) polycarbonate flask	P.C./glass
Lake Superior	10	4,125 ± 205	13,715 ± 1,076	3.3
Turkey Lake	13	42,403 ± 5,271	110,902 ± 20,839	2.6

Table 4. Effects of water collection methods and incubation flasks on Cu, Pb, Ni and Zn concentrations (μg/L) in water from Batchawana Bay

Water Collection Method	Type of flask	Sample Number	Cu	Pb	Ni	Zn ($\bar{x}$ ± S.D.)
Metal-free	polycarbonate	2	<1	<1	<1	6.0 ± 1.4*
Conventional	polycarbonate	2	<1	<1	<1	7.5 ± 0.7*
Metal-free	glass	2	<1	<1	<1	7.5 ± 2.1*
Conventional	glass	2	<1	<1	<1	6.5 ± 0.7*

*No significant differences in Student-Newman-Keuls test at 90% confidence limits

Table 5. Effects of water collection methods and incubation flasks on Cu, Pb, Ni and Zn concentrations (μg/L) in water from Turkey Lake

Water Collection Method	Type of flask	Sample Number	Cu	Pb	Ni	Zn ($\bar{x}$ ± S.D.)
Metal-free	polycarbonate	4	<1	<1	<1	16.5 ± 2.1*
Conventional	polycarbonate	4	<1	<1	<1	16.0 ± 0 *
Metal-free	glass	4	<1	<1	<1	17.5 ± 0.6*
Conventional	glass	4	<1	<1	<1	16.3 ± 1.0*

*No significant differences in Student-Newman-Keuls test at 90% confidence limits

Table 6. Effects of a metal mixture on primary productivity in waters from Lake Superior and Turkey Lake

Water	Addition	Flask	Primary productivity (dpm/500 mL)	% of control
Lake Superior	None	Glass	4,125	100
"	metals*	"	2,425	59
"	none	Polycarbonate	13,715	100
"	metals	"	3,704	27
Turkey Lake	None	Glass	42,403	100
"	metals	"	25,935	61
"	none	Polycarbonate	110,902	100
"	metals	"	29,058	26

*Metal mixture contained Cu (5), Zn (30), Ni (25) and Pb (25) µg/L.

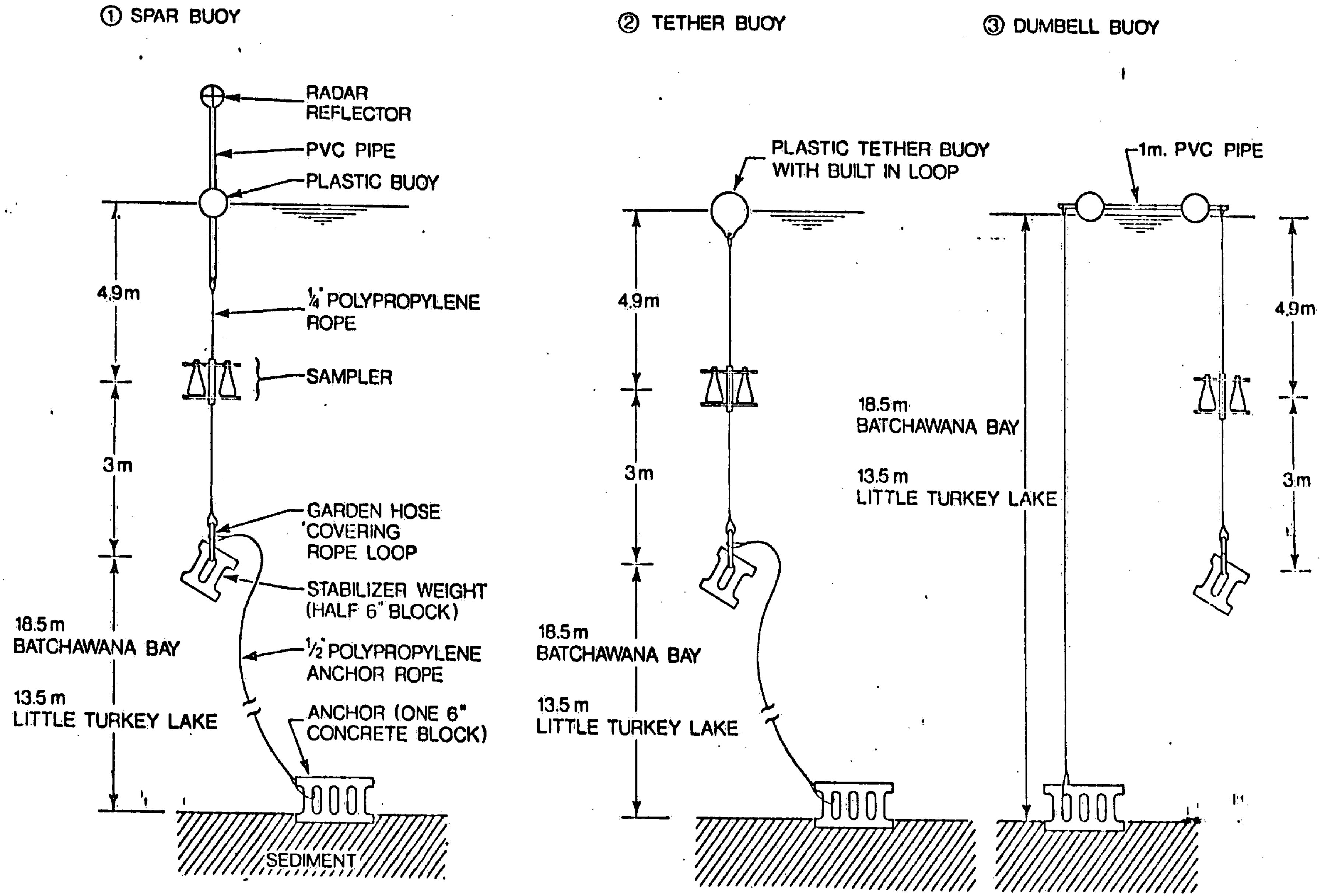

FIG 1 THREE TYPES OF MOORING

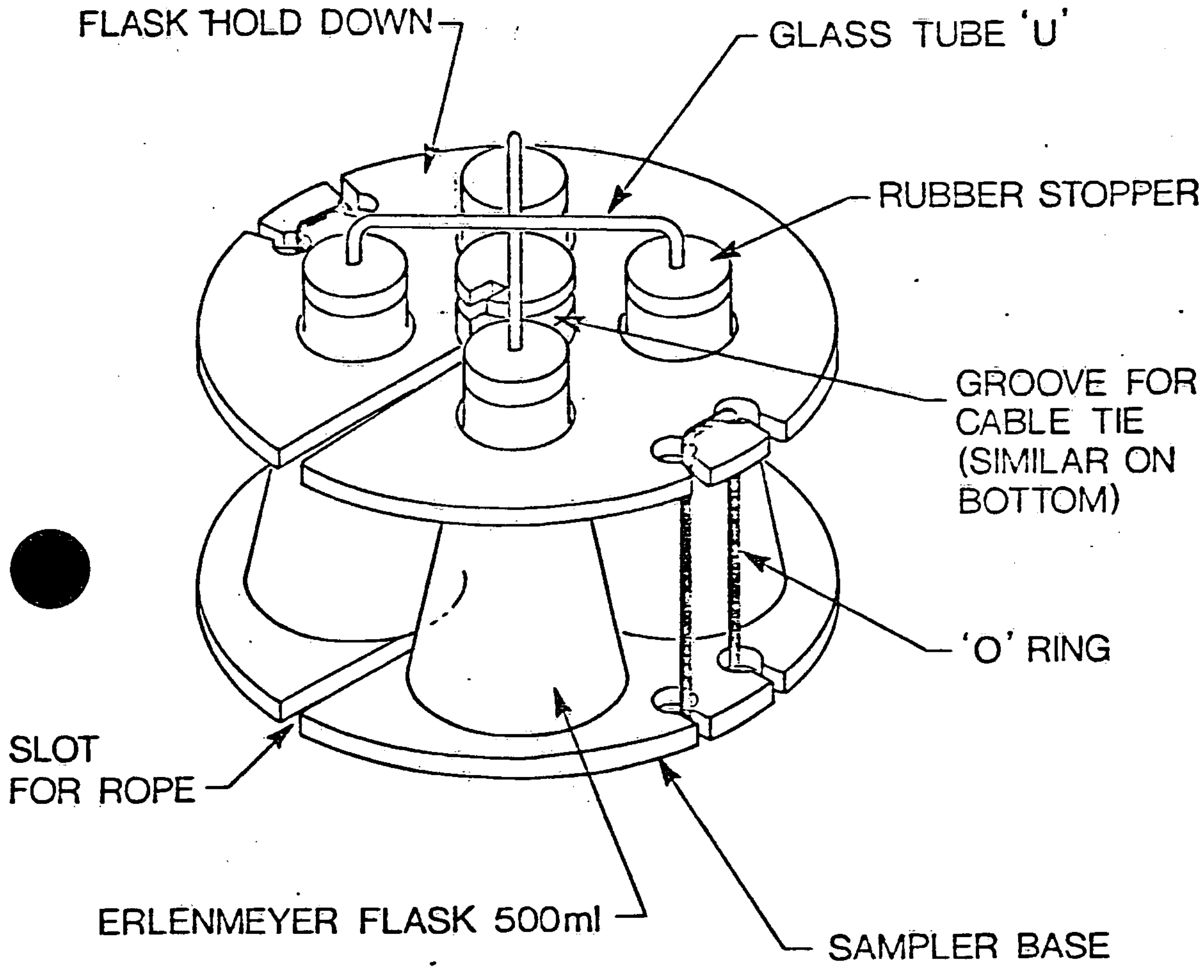

FIG 2 IN-SITU SAMPLER