

Measurements of Mercury Sorption by Algae

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13. ABSTRACT

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Mercury sorption to Millipore filters was greater in the presence of dilute culture medium than in a 3.5% NaCl solution. To circumvent the problems associated with this phenomenon, a procedure was devised whereby the activity of isotopically tagged cells could be determined without collecting them on a filter.

Less than 2% of the mercury sorbed to *Phaeodactylum* cells was in the pigments.

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ABSTRACT

This report describes the results of laboratory experiments concerning mercury sorption by two marine algae, *Phaeodactylum tri-cornutum* and *Chaetoceros galvestonensis*. Measurements of mercury uptake have been made with the use of isotopes (carrier-free ^{197}Hg , and ^{203}Hg incorporated in mg mercury/liter concentrations). Significant mercury concentrations were found in cells grown in media prepared from chemically pure reagents; concentrations of 14 to 116 mg/kg were present in cells harvested from 10% and 100% concentrations of culture medium. *Phaeodactylum* cells grown in a 10% culture medium in the presence of 0.05 mg mercury/liter contained 2400 mg mercury/kg but their growth was inhibited only 55% in a 4-day period. *Chaetoceros* cells had a greater affinity for mercury and contained over 7400 mg mercury/kg when cultured for the same time period in a 25% culture medium containing 0.10 mg mercury/liter. They had not grown, as determined by fluorescence analysis.

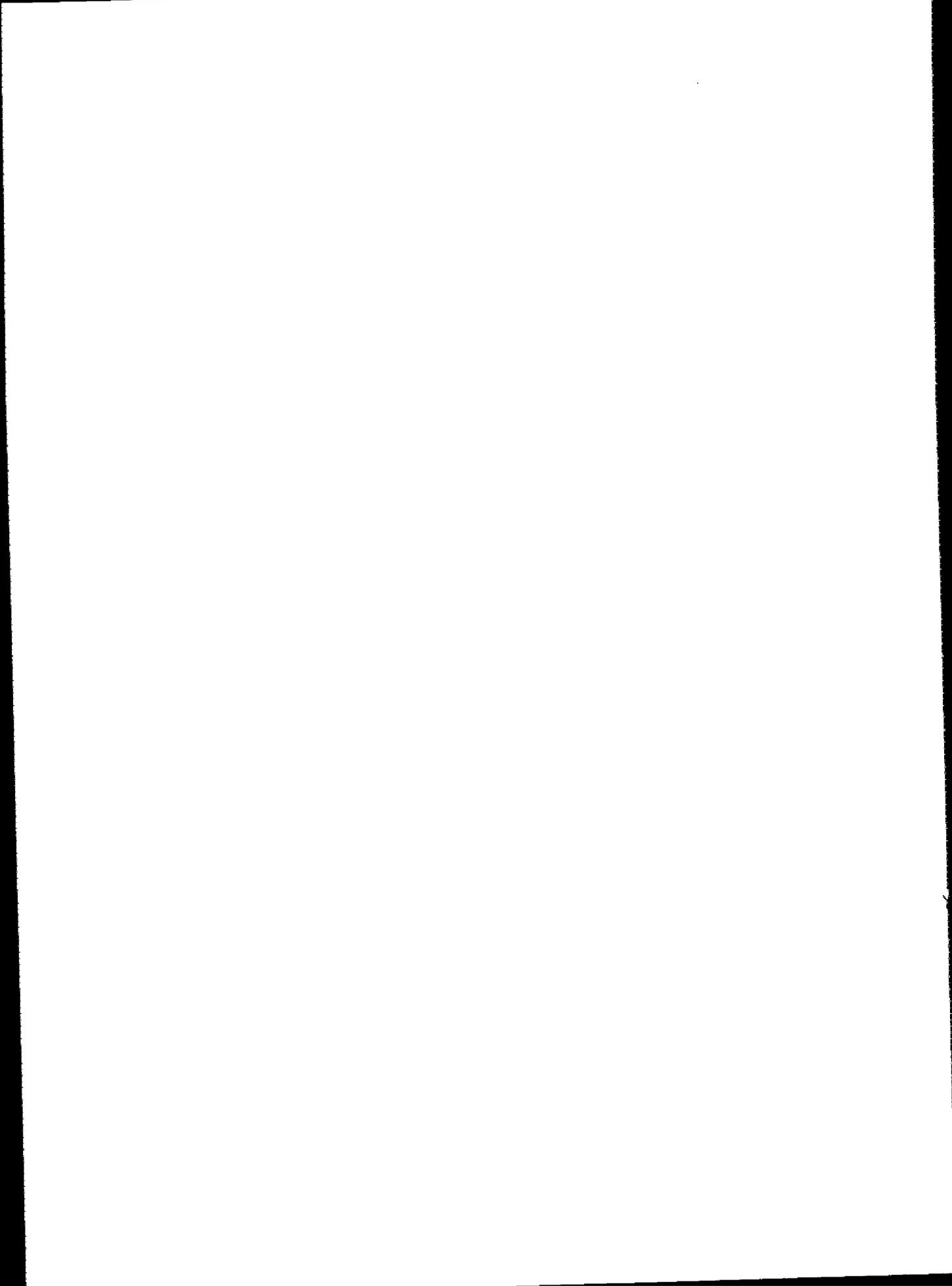
Mercury sorption to Millipore filters was greater in the presence of dilute culture medium than in 3.5% NaCl solution. To circumvent the problems associated with this phenomenon, a procedure was devised whereby the activity of isotopically tagged cells could be determined without collecting them on a filter.

Less than 2% of the mercury sorbed to *Phaeodactylum* cells was in the pigments.

PREFACE

This report concerns research spanning a period of several years. It includes some studies which are relatively complete and others which are fragmentary but which were sufficient for the purpose at the time. All were undertaken as part of a study of the effects of mercury on algal growth rates and the magnitude of mercury sorption by algal cells.

Future studies will be based on the techniques used here and will stress the kinetics of mercury uptake in conditions more closely approximating those found in nature.



MEASUREMENTS OF MERCURY SORPTION BY ALGAE

INTRODUCTION

There has been relatively little research on the mercury content of algae despite their importance as the initial scavengers of heavy metals in the sea. Some reasons for this lack are (a) the difficulty in collecting sufficiently large samples for analysis, (b) the few years for which sophisticated trace metal analysis techniques have been available, and perhaps (c) the limited number of institutions equipped with both the biological and chemical expertise required for such research. Because of the publicity generated by the deaths sustained in the Minimata Bay incident (1) and the finding that many tuna and swordfish contained mercury in parts-per-million (ppm) concentrations, more research is being done.

Keckes and Miettinen (2), in their review on mercury as a marine pollutant, cited numerous references pertinent to the "concentration effect" in which successive stages in the food chain contain increasing quantities of pollutants. Wojtalik (3) and Hannerz (4) discussed the accumulation of mercury by various organisms. Knauer and Martin (5) reported that the average mercury concentration of the phytoplankton 10 mi offshore in Monterey Bay was 207 ng/g dry weight. Nielsen (6) and Glooschenko (7) attributed most mercury uptake by phytoplankton to surface adsorption but did not present data on the actual concentration in terms of dry-weight analyses.

Most investigations of the mercury content of microorganisms concern analyses made on samples collected at one point in time and shed little light on the kinetics of the growth processes. The need for such data is demonstrated by the results obtained by Ben-Bassat et al. (8), who showed a decreasing mercury content in the freshwater alga *Chlamydomonas reinhardtii* during an 8-day growth period. Because constant aeration with CO₂ was required for its growth, there was a greater possibility for the loss of mercury from this system than from a culture medium of the pH associated with marine organisms, where sufficient CO₂ is dissolved initially to provide growth for several days. Regardless of the mechanism by which the mercury was lost, the data show the importance of continuous measurements of cell biomass and the mercury associated with it. No mention was made of the method by which the cells were collected.

The purpose of this report is to describe the effects of various procedures on analyses of algal cells for mercury. Some of the factors studied were the adsorption of mercury onto Millipore filters as a function of the composition of the solution being filtered, the adsorption of mercury onto particulates in distilled water, and the rate of adsorption onto glass (by difference). Included are studies with carrier-free ¹⁹⁷Hg and higher concentrations of mercury in which either ¹⁹⁷Hg or ²⁰³Hg were used as tracers. A significant result of this investigation has been the development of a sensitive and rapid method for the measurement of mercury bound to algal cells based on the centrifugation of suspensions containing tracers. There is still a need for methods to estimate, with precision, small masses of algal cells and work is under way in this Laboratory toward that end.

MATERIALS AND METHODS

Organism and Growth Conditions:

Phaeodactylum tricoratum and *Chaetoceros galvestonensis* were grown in stock cultures in the medium described by Guillard and Ryther (9); because of the unavailability of natural seawater at NRL, the synthetic salt mixture known as Instant Ocean was substituted for it in the preparation of the medium. No additional aeration was provided beyond that received through the cotton plugs covering the Erlenmeyer flasks. The temperature was 15°C and the light intensity was continuous at approximately 500 ft-c. Before being used in studies of growth and mercury sorption, the cells were harvested from the stock culture by centrifugation and then washed three times with 3.5% NaCl before being resuspended in freshly prepared dilute culture medium. The dilutions used are described for each experiment. Growth was estimated by changes in fluorescence of the cells (Fluoro-Microphotometer, product of American Instrument Co., Silver Spring, Md.); the instrument was calibrated with suspensions which had been prepared from serial dilutions of known suspension density.

Mercury Addition and Radioisotope Preparation

A stock solution of 0.338 g HgCl₂/ℓ was the source generally used for the introduction of mercury into the test suspensions. The concentrations in the assay tests were calculated from the amounts added, and analyses of the solutions were made only for those instances noted.

Details of the preparation of ¹⁹⁷Hg are described by Wilkniss, Beach, and Marlow (10). Essentially it consists in irradiating gold foil to produce mercury by the ¹⁹⁷Au (p,n) ¹⁹⁷Hg reaction; upon heating, the mercury is released from the foil and condensed on a cold finger, then removed by washing with nitric acid.

Filtration

Millipore filters of the cellulose ester type, 0.45-μm pore size, were used to separate algal cells and other particulates from suspensions, where indicated. In one experiment concerning a methanol extraction of filtered cells, a glass Millipore filter covered with MgCO₃ was used to retain the cell debris resulting from the extraction.

Centrifugation

The possibility of mercury adsorption onto filters, which could cause an error in the mercury content attributed to the residue collected, prompted the development of another method of collecting cells. This concerned the use of cultures tagged with radioactive mercury, which were centrifuged in glass tubes which fit directly in the radioactivity counter. After a 5-min centrifugation of a 10-ml aliquot, 5 ml were pipetted off the top and placed in another counting tube. Comparisons of the counts obtained with the two tubes provided a measure of the radioactivity associated with the cells, as follows:

Terms used:

Residue = counts from 5 ml remaining after pipetting upper 5 ml of solution

Supernatant = counts from upper 5 ml of solution

Suspension = counts from 5 ml of uncentrifuged suspension

Relationships between terms:

Residue - Supernatant = counts due to cells alone

$\frac{\text{Counts due to cells alone} \times 100}{\text{Supernatant} + \text{counts due to cells}} = \text{percentage of tagged mercury in cells}$

$(2 \times \text{Supernatant}) + \text{counts due to cells} = (2 \times \text{Suspension}).$

EXPERIMENTS CONDUCTED

Retention of Carrier-Free ^{197}Hg by Filter (No Cells Present)

This experiment demonstrates the significant effects of the culture medium on the quantity of carrier-free isotope retained by a filter. Three solutions were prepared from distilled (but not prefiltered) water and allowed to stand in 250-ml volumetric flasks for several hours before filtering; they were 3.5% NaCl and dilutions of it with culture medium (25% and 50%), all containing identical aliquots of carrier-free ^{197}Hg . No algal cells were present, the only particulates being those naturally occurring in the distilled water or in the reagents used for the preparation of the solutions.

The results shown in Table 1 indicate that the least activity retained by the filter occurred with the saline solution alone (25%), and the greatest with the 25% dilution with culture medium (85%), suggesting that the nutrients complex the mercury in such a way that it is retained by the filter. A lesser amount of activity (66%) is retained by the filter in the presence of the higher concentration of culture medium, probably because of the trace amounts of mercury in the culture reagents, thereby inducing the "hold-back carrier effect." This will be discussed in more detail later.

Retention of ^{197}Hg , Carrier-Free and in 0.1 mg Hg/l by Centrifugate (No Cells Present)

The previous experiment indicated that 85% of the carrier-free ^{197}Hg in the solution containing 25% culture medium had been retained by the filter, though no algal cells were

Table 1
Distribution of Hg^{197} (Carrier-Free) Between Filter and Filtrate
After Several Hours (No Algal Cells Present)

Solution	Counts per Minute				Percentage of Activity on Filter
	Filtrate	Filter	Total	Unfiltered Solution	
3.5% NaCl (100%)	1615	548	2163	2177	25
3.5% NaCl (75%)	399	1816	2215	2137	35
Culture medium (25%)					
3.5% NaCl (50%)	576	1416	1992	2207	66
Culture medium (50%)					

present. To differentiate between Hg adsorbed by the filter and that bound to the particulates, a similar experiment was conducted in which the particulates were separated by the centrifuge method. The same amount of ^{197}Hg was added to each of two solutions of 25% culture medium, one of which also contained 0.1 mg Hg/l. As before, no cells were added, the only particulates present being those from the distilled water from which the solutions were made or those occurring in the reagents.

After a half-hour, 10-ml aliquots were pipetted into centrifuge tubes and spun for 15 min, after which a 5-ml portion of the supernatant was pipetted off and transferred to another counting tube. The results (Table 2) indicate the same degree of retention by the particulates as was obtained with filtration, at least with the carrier-free solution, indicating that adsorption by the filter had been negligible and that the mercury had been retained by the particulates. In the presence of 0.1 mg Hg/l, the centrifugate had only 11% of the activity, once again a manifestation of the hold-back carrier effect. The exposure times in the two experiments were not identical (1/2 hr vs 2 hr), but the results seem unaffected by this.

Distribution of Carrier-Free ^{197}Hg Between *Phaeodactylum* Cells and Suspending Solution (Centrifuge Method)

Equal volumes of *Phaeodactylum* cells, previously washed to remove all nutrients, were placed in two solutions, 3.5% NaCl and a 25% culture medium of the same salinity. The solutions had been made from distilled water but had not been prefiltered to remove all particulates. Each solution was tagged with carrier-free ^{197}Hg , and 40-ml aliquots were transferred to screw-capped test tubes and rotated at a speed of 4 rpm for two days in front of a fluorescent lamp. Measurements of their cell volumes, by the fluorescence method, indicated that those in the culture medium had increased from the initial reading of 38 ppm to 71 ppm, while the nutrient-free cells showed a decline in fluorescence (the presumption here is that the cell volume might be unchanged, but that the fluorescence would diminish).

The mercury uptake by the growing cells was greater than by those not growing (96% vs 86%), as shown in Table 3. These measurements are based on the totals represented by the supernatants and cells; no total budget of the radioactivity added was made.

Table 2
Distribution of Hg^{197} Between Supernatant and Centrifugate as a
Function of Hg Concentration, With No Cells Present
(1/2-Hr Equilibration)

Solution 3.5% NaCl—75% Culture medium—25% Plus	Counts per Minute			Percentage of Activity in Centrifugate
	Supernatant	Residue	By Difference, Centrifugate	
Carrier-free Hg^{197}	249	2894	2649	$\frac{2649}{2804 + 249} = 84$
Hg^{197} in 0.1 mg Hg/l	1481	1858	377	$\frac{377}{1858 + 1481} = 11$

Table 3
Distribution of Carrier-Free Hg^{197} Between Supernatant and
Phaeodactylum Cells After Two Days Growth

Solution	Counts per Minute			Percentage of Activity in Cells*
	Supernatant	Residue	By Difference Cells	
3.5% NaCl (100%)	255	3337	3082	86
3.5% NaCl (75%) Culture medium (25%)	76	3646	3570	96

*The percentage of activity is based on the sum of the activities of the cells and the liquid at the time of counting; it does not represent the total radioactivity introduced, some of which is adsorbed onto the walls.

Distribution of Carrier-Free ^{197}Hg Between *Phaeodactylum* Cells (in Light and in Darkness) and Suspending Solutions (Filtration Method)

This experiment was an extension of the one just described, and included a more concentrated culture medium (50%); also, the effect of light and dark was investigated. Washed cells of equal volumes were added to 250-ml volumetric flasks containing the solutions indicated, and then six 40-ml aliquots were removed and placed in screw-capped test tubes to be illuminated. The remainder of the original suspension was left in the 250-ml volumetric flask and kept in darkness. After 24 hr the fluorescence of each suspension was determined, and a representative sample of each treatment was chosen for further study; a 5-ml portion was filtered through a 25-mm-diam Millipore filter directly into a 1.5- by 8.5-cm tube which was then placed in the radioactivity counter. The filter paper was washed with 25 ml of 3.5% NaCl solution and transferred to another tube for counting. Radioactivity determinations of the filtrates and filters (Table 4) indicated the following:

1. Illuminated cells suspended in nutrient-free solutions retained slightly more mercury than those kept in darkness.
2. The presence of culture medium, either 25% or 50%, resulted in 99% mercury up-take whether light or dark conditions prevailed.
3. Suspensions kept in the dark had a higher total count than their counterparts in the light, but this may have been the result of different surface/volume ratios of the vessels used; the suspensions in the dark were the 10-ml remainder in the 250-ml volumetric flasks which were not agitated, while those in the light were rotated constantly in 2.5- by 15-cm glass tubes.

Equilibration of ^{197}Hg with Mercury and Copper Already Bound in *Phaeodactylum*

A matter of ecological interest is the rate at which various aquatic organisms exchange heavy metals with the ambient medium. It was decided, therefore, to culture aliquots of *Phaeodactylum* in various concentrations of two heavy metals (Hg and Cu) to provide a range of metal concentrations within the organism, then to add carrier-free ^{197}Hg and determine the extent to which it was picked up by the cells. Comparisons were to be made with a control.

Table 4
Uptake of ^{197}Hg , Carrier-Free, by *Phaeodactylum*
After One Day's Growth

Solution	Light or Dark	Counts per Minute			Percentage of Activity on Filter
		Filtrate	Filter	Filter plus Filtrate	
3.5% NaCl (100%) Culture medium (0%)	Light	337	2663	3000	89
	Dark	776	2744	3520	78
3.5% NaCl (75%) Culture medium (25%)	Light	36	3174	3210	99
	Dark	53	4081	4134	99
3.5% NaCl (50%) Culture medium (50%)	Light	38	3068	3106	99
	Dark	88	3896	3984	99

For the initial growth of the organisms, a 25% culture medium was buffered with 0.01M Tris, filtered to remove all particulates, and then inoculated with washed *Phaeodactylum* cells; next, the resulting suspension was divided among 250-ml volumetric flasks to which were added, singly, equimolar amounts of nonradioactive copper and mercury (Cu = 0.01, 0.02, and 0.10 mg/l; Hg = 0.0427, 0.0854, and 0.427 mg/l). Forty-milliliter aliquots of each suspension were then placed in screw-capped test tubes and rotated before a fluorescent lamp for two days. Measurements of the fluorescence of the suspensions indicated (Fig. 1) that growth took place in the controls, in all the copper-treated cultures, and in the culture containing the lowest concentration of mercury. Such measurements are satisfactory in a qualitative sense, but they do not provide quantitative results because of the influence of heavy metal salts, and sometimes of the nutrients, on the fluorescence of the cells. There was no significant difference between the fluorescence of the 0.0427 mg Hg/l culture and the others, and these cultures probably contained the same numbers of cells within reasonable limits of error. Probably the number of cells in the two higher concentrations of mercury did not increase at all.

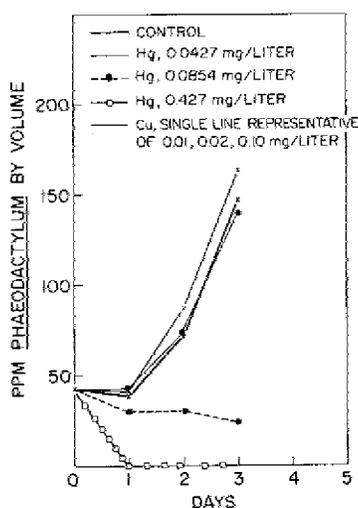


Fig. 1 — Growth of *Phaeodactylum* in equimolar concentrations of copper and mercury (25% culture medium buffered with 0.01 M Tris)

After the fluorescence measurements were made, a representative tube of each suspension was treated with 1.0 ml of a carrier-free ^{197}Hg solution (which had been adjusted to pH 7 by addition of alkali) and placed back in the light for 3 hr; after this equilibration period, 10-ml portions were centrifuged and analyzed for radioactivity by the method described previously as the centrifuge method; the remaining tubes were again placed in the light and rotated for another 5 days, when the centrifugation procedure and the radioactivity measurements were repeated. During the latter period the growth rate would be essentially zero because of nutrient deficiencies.

The radioactivity data (Table 5) indicate that at the end of a half-hour equilibration period the amount of ^{197}Hg in the controls was less than in the copper-treated cultures, and even less than in the culture containing 0.0427 mg Hg/l. The extent of the exchange of radioactive and nonradioactive mercury was not determined, but this would certainly be affected by the ambient mercury concentrations; therefore, lower ^{197}Hg concentrations in the cells should be expected with higher mercury concentrations in the medium. After 5 additional days under conditions of limiting growth, the radioactivity of the controls exceeded that of the copper-treated cultures but was considerably less than that in the 0.0427 mg Hg/l culture. Undoubtedly there were more cells in the controls than in the mercury- or copper-treated cultures at this point. No attempt was made to determine the masses of the cells because of the small samples involved. Fluorescence measurements made on the seventh day indicated that additional growth had taken place in all cultures except the one containing 0.427 mg Hg/l; the reliability of fluorescence as a measure of cell counts, however, is particularly suspect with cultures of this age because any significant amount of cell lysis could result in enhanced fluorescence.

Mercury Uptake by Cells as Determined by Tracer Method

In the preceding experiment in which ^{197}Hg was added to suspensions already growing in various concentrations of mercury, the radioactivity of the cells could not be interpreted as a measure of their mercury content unless complete equilibrium between the liquid and the cells had been established. This was not likely to be the case. It was decided, therefore, to determine the actual mercury uptake by first adding the ^{197}Hg to the carrier in an acid solution to assure equilibration throughout the liquid, and then, after the proper mercury adjustment, adding this solution to growing cultures and monitoring the increase in the radioactivity of the cells.

To do this, 1.0 ml of the strongly acid ^{197}Hg solution was mixed with appropriate volumes of a stock HgCl_2 solution, then neutralized with alkali and added to 250-ml volumes of the freshly inoculated solutions containing the 25% culture medium; six 40-ml aliquots were transferred to screw-capped test tubes from each flask. The same amount of radioactivity was provided each suspension, but the total concentrations were 0.0427, 0.0854, and 0.427 mg Hg/l as before. After 2 days' exposure to the light, the fluorescence values for the six replicates of each treatment were the same, which indicated that no growth inhibition had taken place, even with the highest mercury concentration. This is ascribed to the inordinately high concentration of nitrate, approximately 80 mg/l in the final solution, resulting from the procedure in which nitric acid was used to dissolve the ^{197}Hg deposited on the cold finger following the preparation of this isotope. (In the presence of such nutrient abundance, the algal cells have an enhanced resistance to the effects of a toxicant.)

Table 5
Equilibration of ^{197}Hg with Mercury and Copper in *Phaeodactylum*

Suspension	Counts per Minute*				Percentage of Hg in Cells	Total Counts ^{††}				Percentage of Hg in Cells
	Residue	Super-natant	By Difference Cells	Residue + Super-natant Total		Residue	Super-natant	By Difference Cells	Residue + Super-natant Total	
Cell-free controls	1537	1133	404	2670	15	ND	—	—	—	—
Control cells	2075	793	1282	2868	45	2013	1986	2040	97	97
Cu, 0.01 mg/l	2574	886	1688	3460	49	1450	1399	1501	93	93
Cu, 0.02 mg/l	2542	769	1773	3311	53	1250	1230	1270	97	97
Cu, 0.10 mg/l	2807	796	2011	3603	56	1570	1570	1570	100	100
Hg, 0.0427 mg/l	2731	660	2071	3391	61	2690	2637	2743	96	96
Hg, 0.0854 mg/l	1510	724	786	2234	35	1626	1406	1846	76	76
Hg, 0.427 mg/l	1426	1237	189	2663	7	869	503	1235	41	41

*After 2 days' growth and 30-min equilibration with ^{197}Hg .

†After 5 days' additional growth in presence of ^{197}Hg .

‡Longer counting time used, no correction made for decay.

§ Not determined.

Note: All counts contain correction for background levels.

A representative sample of each treatment was subjected to the centrifugation procedure, and the radioactivity attributable to the cells was determined. The multiplication of these counts by appropriate factors, representing the dilution of ^{197}Hg by the carrier, gave the mercury uptake figures shown in Table 6. For a tenfold increase in the mercury content of the culture medium, there was a twofold increase in the mercury content of the cells. Because there was no way to determine the mass of the cells accurately, the data can not be translated into parts-per-million of mercury in the cells.

Table 6
Uptake of Mercury by Cells Cultured in Various Concentrations of HgCl_2

Culture Medium Plus (mg Hg/l)	Counts per Minute				Percentage of ^{197}Hg in Cells	Total Hg in Cells From 10-ml Aliquot of Suspension*
	Residue	Super-natant	By Difference Cells	Residue Plus Super-natant		
0.427	8574	7349	1225	15,923	7.7	3.28×10^{-4} mg
0.0854	7752	3283	4469	11,035	40.5	2.40×10^{-4} mg
0.0427	7554	1839	5715	9,393	60.8	1.53×10^{-4} mg

*Assuming that 15,923 counts were present in each solution initially, i.e. no adsorption had taken place from the most concentrated solution.

An indication of the mercury lost to the walls of the container can be gleaned from these data. The same quantity of radioactivity was introduced into each suspension, viz 18,600 counts per 10 ml, but the total radioactivity accounted for in the cells and the liquid varied according to the total mercury used. Figure 2 is a plot of total counts obtained vs the mg Hg/l added initially to the cultures. The straight line across the top

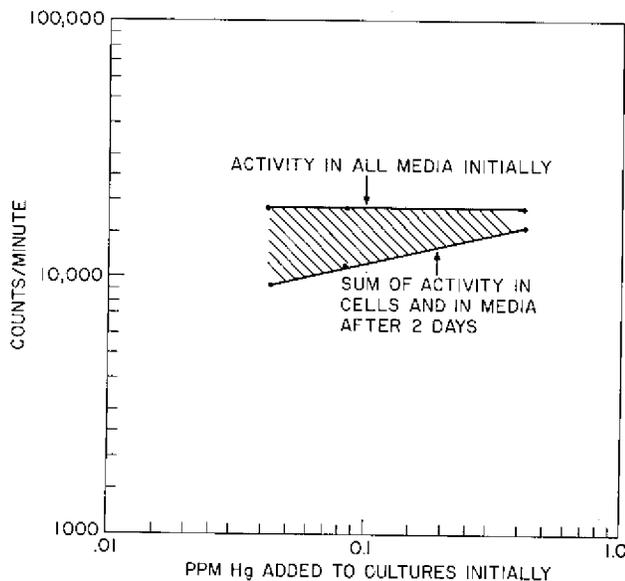


Fig. 2 — Distribution of mercury among culture medium, algal cells, and container surface as function of mercury concentration present initially

represents the 18,600 counts added to each culture; the bottom line denotes the combined counts from the cells and the supernatant for each suspension. All counts are corrected for background and for decay during the 2-day period. In percentage terms, the fractions nominally lost to the walls were 14%, 41%, and 50% for the solutions containing 0.427, 0.0854, and 0.0427 mg Hg/l.

Mercury Concentration in *Phaeodactylum* Cells (Gravimetric Method)

The previous experiments exploited the utility of ^{197}Hg as a tracer and provided considerable information on the distribution of mercury in the cells, the ambient solutions, and even the Millipore filters. In some instances the mass of mercury retained by the cells could also be calculated, but the absence of data on the masses of cells present ruled out the possibility of determining the concentration on a milligrams per kilogram basis. For this purpose it was necessary to culture large masses of cells, weigh them, and analyze them for mercury.

Two experiments were conducted to explore the effect of nutrient concentrations on the sorption of mercury. In one, a 10% concentration of the Guillard and Ryther medium in 3.5% NaCl was used; dilute alkali was added to raise the pH to 7.4 before the start of the experiment. In the other 0.01 M Tris was incorporated into a 25% concentration of the Guillard and Ryther medium in 3.5% NaCl to maintain the pH at approximately 8. In each experiment the solutions were passed through Millipore filters to remove all particulates before inoculation with washed *Phaeodactylum* cells. Ten-liter volumes, with concentrations of 0.01, 0.03, and 0.05 mg mercury/liter were used with the 10% culture medium, and 15-liter portions with concentrations of 0.01, 0.05, and 0.10 mg/liter were used with the 25% culture medium. Stirring was provided by Teflon-covered magnetic bars, the incident light intensity was 700 ft-c, and the temperature was 15-19°C. Growth in the 0.05 mg/liter suspension (25% culture medium) was retarded somewhat because of periodic problems with the stirring bar; as it migrated from the center of the flask, stirring would cease and as the cells settled they would be out of the lighted area.

The cells were harvested by repeated passages of the suspensions through a DeLaval separator. After most of the cells had been deposited on the inner cone of the separator they were rinsed with 3.5% NaCl into volumetric flasks from which aliquots were removed and filtered through weighed Millipore filters which were then dried in a desiccator. Corrections for the weight of salts deposited on the filter were made, in the case of the 10% culture medium, by subtracting the gain in weight of a filter beneath that used for the cells, and with the 25% culture medium by subtracting the weight gain of a filter treated with a cell-free solution of the same chemical composition. Following the dry weight determinations, the filters were digested in cold nitric acid and analyzed for mercury by atomic absorption spectroscopy. Duplicate determinations of the weights and mercury analyses were made and are summarized in Table 7 and represented graphically in Fig. 3.

Growth of these mercury-treated cultures was inhibited, but not stopped, by the presence of almost 2500 mg/kg. The higher concentration of mercury in the cells grown in the more dilute culture medium is partially the consequence of fewer cells being present. Figure 4 represents the growth of each culture and shows that after four days the controls in the 25% culture medium contained almost twice as many cells as those in the 10% medium. With both concentrations of culture medium the fraction of the mercury retained by the cells varied inversely with the concentration of mercury in the medium.

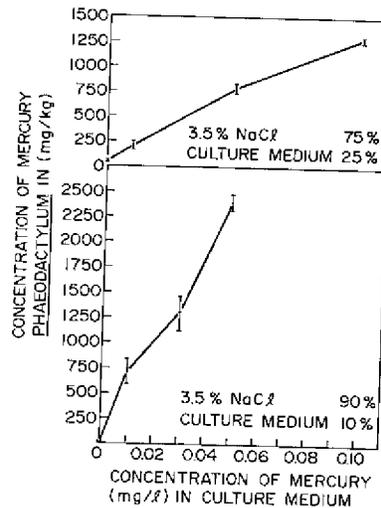
Table 7
Mercury Sorption by *Phaeodactylum* in Two Concentrations of Culture Medium and in Various Mercury Concentrations

Concentration of Guillard and Ryther Medium	Hg Content of Culture Medium (mg/l)	Total Yield of Cells (gm/l)	Cells Analyzed* (g)	Hg in Cells Analyzed (μg)	Conc. Hg in Cells (mg/kg)	Percentage of Hg Added Retained by Cells
25%	Control	0.0255	0.0279	1.3	47	Unknown†
			0.0279	1.6	57	
25%	0.01	0.0223	0.0289	7.3	253	41
			0.0289	5.0	173	
25%	0.05	0.0199	0.0259	19.1	737	29
			0.0259	21.7	838	
25%	0.10	0.0188	0.0241	30.3	1257	23
			0.0241	31.6	1311	
10%	Control	0.0113	0.0402	0.7	17	Unknown†
			0.0398	0.4	10	
10%	0.01	0.0086	0.0186	11	591	61
			0.0156	13	833	
10%	0.03	0.0079	0.0191	28	1466	34
			0.0204	23	1127	
10%	0.05	0.0062	0.0161	40	2484	30
			0.0147	34	2313	

*Identical aliquots of cell concentrate were used for the analyses of the cells grown in the 25% culture medium; weights were calculated from other aliquots. For the cells grown in the 10% culture medium, analyses were made of the weighed cells.

†The mercury content of the culture medium itself was unknown; contaminants of the salts used in the preparation of the medium were considered the source of the mercury.

Fig. 3 — Mercury sorption by *Phaeodactylum* after 4 days, as function of concentration of culture medium and of mercury added



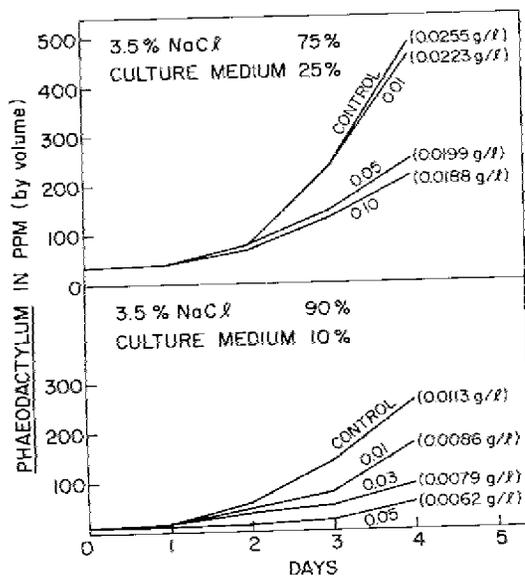


Fig. 4 — Growth of *Phaeodactylum* as a function of culture medium concentration and of mercury added. Numbers on lines denote mg Hg/l, those in brackets represent yield after 4 days.

Mercury Uptake by *Chaetoceros galvestonensis* (Gravimetric Method)

Chaetoceros galvestonensis was grown in a 25% culture medium buffered with 0.01 M Tris under the same conditions used with *Phaeodactylum*. Only two mercury concentrations were included in this study, 0.01 and 0.10 mg/l, and 5-l quantities were used instead of the 15-l volumes of the previous test. No growth took place in the suspension containing 0.10 mg Hg/l; consequently, there was no need to concentrate the cells by the DeLaval separator; instead, duplicate 1500-ml volumes were filtered and weighed. The control suspension and the one containing 0.01 mg Hg/l were concentrated by the separator and transferred ultimately to 250-ml volumetric flasks; aliquots of these dense suspensions were filtered, weighed, and analyzed.

The growth rate for *Chaetoceros* (Fig. 5) was considerably lower than that obtained with *Phaeodactylum*; despite a 50% higher concentration of cells as the inoculum, the volume of cells collected after four days was only half that of the *Phaeodactylum*. The mercury sorption, however, was considerably greater than that found with *Phaeodactylum*; the controls contained 145 mg/kg (Table 8) compared to the 52 found in *Phaeodactylum*, and the cells grown in 0.10 mg/l contained over 7400 mg Hg/kg compared to 1284 for *Phaeodactylum*. This avid scavenging of mercury undoubtedly accounted for the lack of growth in the 0.10 mg/l solution.

Mercury Content of *Phaeodactylum* Cells Grown in 100% Culture Medium

Previous experiments indicated that appreciable concentrations of mercury were present in both *Phaeodactylum* and *Chaetoceros* after four days of growth, even when no mercury was added to the culture medium. *Phaeodactylum* grown in 10% culture medium contained 14 mg Hg/kg, and in 25% culture medium the concentration was 52 mg/kg. The latter solution, however, contained 0.01 M Tris as a buffer and this may have contributed to the mercury contamination. To determine whether the nutrients in the culture medium were responsible for the mercury contamination, *Phaeodactylum* cells were grown in 100% culture medium which consisted only of the Guillard and Ryther medium made from Instant Ocean and the appropriate nutrients.

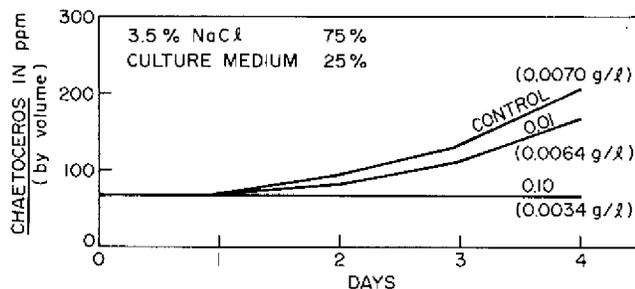


Fig. 5 — Growth of *Chaetoceros* in 25% culture medium. Numbers on lines denote mg Hg/l, those in brackets represent yield after 4 days.

Table 8
Mercury Sorption by *Chaetoceros galvestonensis*

Concentration of Guillard and Ryther Medium	Hg Content of Culture Medium (mg/l)	Total Yield of Cells (gm/l)	Cells Analyzed (g)	Hg in Cells Analyzed (μg)	Hg in Cells (mg/kg)	Percentage of Hg Added Retained by Cells	
25%	Control	0.0070	0.0275	1.5	54	Unknown	
			0.0076	1.8			237
25%	0.01	0.0064	0.0123	12.4	1008	70	
			0.0116	15.0			1293
			0.0078	7.6			974
25%	0.10	0.0034	0.0104	67	6442	25	
			0.0099	84			8485

Fifteen liters of 100% culture medium were inoculated and exposed to the same light intensity and temperature conditions as before. After four days a 5-l aliquot was harvested and the cells analyzed for mercury. As a sequel to this experiment the remaining 10l of suspension were treated with 0.10 mg Hg/l and illuminated for an additional day. The results shown in Table 9 may be summarized as follows:

1. The yield of cells in the 100% culture medium after four days was 0.0343 gm/l, compared to 0.0255 and 0.0113 for the 25% and 10% culture media, respectively. Differences in the numbers of cells used in the inocula would affect these yields, of course.
2. The mercury content of the controls in 100% culture medium, with no mercury added, was 116 mg/kg; after the additional day in the presence of 0.10 mg Hg/l the cells contained 538 mg/kg.

The results indicate that the nutrients added to the Instant Ocean to make the complete culture medium were primarily responsible for the mercury contamination of the cells. The matter has not been investigated further to determine which of the nutrients might be the principal source of the contaminant, but the subject is discussed later in this report.

Table 9
 Summation of Data on *Phaeodactylum* Grown in 100% Culture Medium for Four Days,
 then in 0.10 mg mercury/liter an Additional Day

Wt. Algae liter (g)	Total Weight Algae (g)	Wt. Sample for Hg Analysis (g)	Hg Found (μg)	$\frac{\text{mg Hg}}{\text{kg Algae}}$	Total Hg Bound in Cells (μg)
15 liters of suspension grown 4 days (no Hg added) gave the following					
0.0343	0.515	0.0681	$7.9 \pm .5$	116	60
10 liters of the above grown for 1 day in the presence of 0.10 mg mercury/liter					
0.0456	0.456	0.0905 0.0920	55 ± 4 43 ± 4	608 468 } 538 avg	245

Mercury Concentration in Pigments

Interest in the mercury concentration of the cell pigments was aroused by the repeated observation that the fluorescence of *Phaeodactylum* (and other) cells decreased in the presence of mercury salts; the effect was particularly pronounced when the suspending solution was devoid of nutrients. A search of the literature revealed little data pertinent to the subject, and nothing on the mercury content of the chloroplasts or pigments. The purpose of the study described here was to determine the effect of mercury (as HgCl_2) on the fluorescent intensity of *Phaeodactylum* cells, in the presence and in the absence of nutrients, and to determine the mercury content of intact cells and of their methanol extracts. In the extraction process other compounds would be removed with the pigments, and therefore mercury analyses of the extracts would be higher than the actual concentrations in the pigments.

Radioactive mercury was used as a tracer; ^{203}Hg was obtained from the New England Nuclear Corp. (as HgCl_2) in a 0.5M HCl solution. The solids content of this source was 2.7 mg in a total volume of 1.0 ml and the total activity was 25 mCi. This solution was diluted fivefold with water, and 2.0 ml of the resultant were mixed with 3.0 ml of a carrier solution containing 0.338 g HgCl_2/ℓ . The addition of 0.2 ml of this combination to 500 ml of cell suspension gave a calculated concentration of 0.12 mg Hg/ ℓ .

Cells from 4-day-old stock culture were centrifuged, washed three times to remove all nutrients, and made into a slurry; identical volumes of this slurry were then added to the appropriate solutions according to the plan shown in Fig. 6. The final cell suspensions contained approximately 40 ppm by volume, as determined by a comparison of the fluorescence of the control (in the dilute culture medium) with a calibration curve. Fluorescence measurements were made of all the suspensions immediately after their preparation, and after an additional 2 hr in room light. These data (Fig. 7) indicate the type of change often observed in the fluorescence of cell suspensions; from one experiment to another the shapes of the curves may vary appreciably, but the result is always that fluorescence decreases most markedly under the combined influences of nutrient deficiency and the presence of mercury.

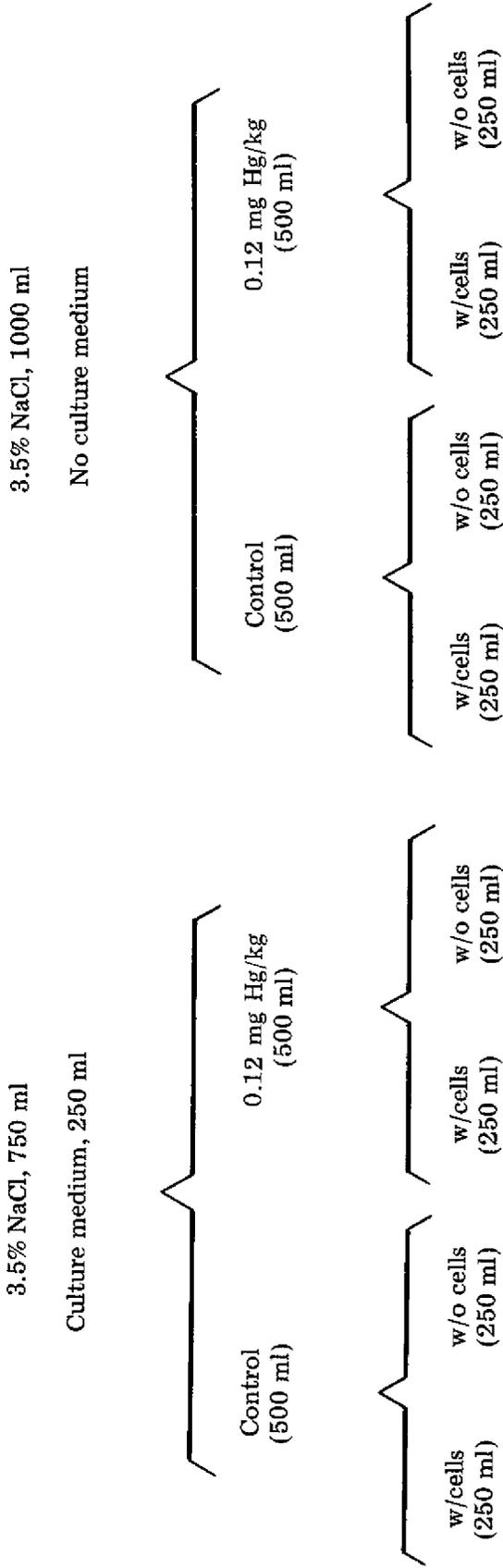


Fig. 6 --- Preparation of solutions and suspensions used in analysis of pigments for mercury

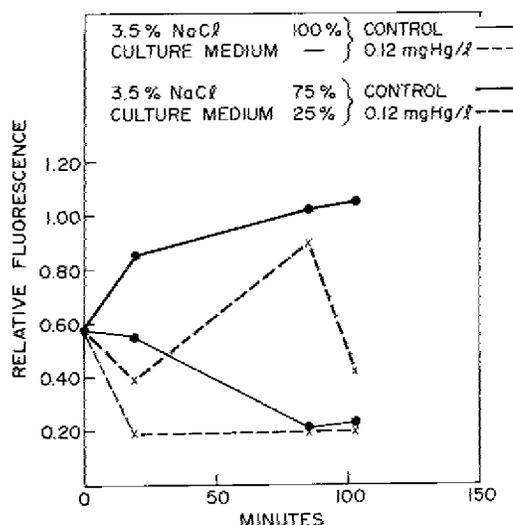


Fig. 7 — Fluorescence of *Phaeodactylum* as function of time in two concentrations of culture medium and of mercury

For the mercury uptake measurements made the following day, 100-ml aliquots of each suspension were passed through 0.45- μ m Millipore filters of the cellulose ester type, and the residues on the filter were washed with 100 ml of a 3.5% NaCl solution. Each filter was placed in a screw-capped test tube and extracted overnight with 10 ml of methanol, after which the resultant cell debris and partially dissolved filter were transferred to another filtration apparatus containing a glass fiber filter (Reeve Angel) which had been previously coated with $MgCO_3$. Additional washings with methanol provided a final extract of 25 ml volume.

Five-ml aliquots of the extracts were pipetted into 1.2- by 8.6-cm glass tubes, placed in the well of the counter, and counted for 10 min. Each glass fiber filter, containing the extracted cells and remnants of the cellulose ester filter, was also transferred to a glass tube and counted. However, the high activity levels associated with filters 3C and 7C (Table 10) necessitated a modification of the normal counting procedure; each tube was mounted an inch above the well and counted for 1 min, as was the tube containing filter 4C. A proportionality factor relating the counts obtained for 4C, in and out of the well, was used to multiply the counts for filters 3C and 7C so that direct comparisons could be made for all filters.

Calculations of the mercury content of the cells and extracts were made as follows:

1. Activity of cells = (filter containing Hg-treated cells) - (filter through which cell-free Hg solution was passed); e.g., 3C - 4C
2. Activity of extracts = (extract of filter containing Hg-treated cells) - (extract of filter through which cell-free Hg solution was passed)
3. Total activity of cells and extract = Sample 1 + Sample 2
4. Percentage of activity in extract = $\frac{\text{Sample 2}}{\text{Sample 1} + \text{Sample 2}} \times 100$

The radioactivity of the extracts was determined by counting a 5-ml aliquot from the total 25 ml used; therefore, after a correction for background had been made, this figure was multiplied by 5.

Table 10
Comparison of Radioactivities of Cells and CH₃OH Extracts

Preparation Sequence	Sample Number*	Time (min)	Radio-activity Counts†	Back-ground Counts	CPM Adjusted for Bkgd and Volumes			
3.5% NaCl—75% Culture medium 25% (1000 ml)	Control (500 ml)	W/cells (250 ml)	1 1C	10 ND‡	1113 ND	1000 ND	56 ND	
		W/O cells (250 ml)	2 2C	10 10	1014 4115	1000 1000	7 311	
	0.12 mg Hg/kg (500 ml)	W/cells (250 ml)	3 3C	10 1	142,300 3,937,400	1000 100	70,600 3,937,400	
		W/O cells (250 ml)	4 4C	10 10	6,900 471,100	1000 1000	2,950 47,000	
	3.5% NaCl—100% No culture medium (1000 ml)	Control (500 ml)	W/cells (250 ml)	5 5C	10 ND	1020 ND	1000 ND	10 ND
			W/O cells (250 ml)	6 6C	10 10	1033 2103	1000 1000	17 110
0.12 mg Hg/kg (500 ml)		W/cells (250 ml)	7 7C	10 1	141,300 3,691,800	1000 100	70,200 3,691,800	
		W/O cells (250 ml)	8 8C	10 10	1563 71,600	1000 1000	280 7,000	

*The number, by itself, refers to the alcoholic extract of the cells, The number followed by "C" refers to the filter cake containing extracted cells.

†For direct comparison, multiply the upper line of each couplet by 5.

‡Not determined.

Calculations made according to the above scheme show that the fractions of mercury extracted from the cells by methanol were 1.7% for the cells from the dilute culture medium and 1.9% for those from the NaCl solution; the difference between these may not be significant in view of the error inherent in the counting procedure necessitated by the extreme activities of the cells. Also these percentages represent upper limits of the mercury content of the pigments because of the simultaneous extraction of other compounds by methanol. Repeated extractions with methanol or the use of elevated temperatures may have removed more mercury, but the pigments, as determined visually, were completely removed by this procedure.

Data concerning the relative adsorption of mercury onto Millipore filters as a function of the solution containing it can be gleaned from Table 10. Note that filter 8C, used in the filtration of a cell-free solution of 3.5% NaCl, showed a count of 7000 which was considerably less than filter 4C used in filtering a cell-free solution of dilute culture medium; in this case the count was 47,000 or 6.7 times greater. The retention of the mercury by the filter in the presence of methanol differs also according to the solution

filtered. Only 4% of the adsorbed mercury was removed from filter 8C, while 6.3% was removed from filter 4C. Unfortunately there was no determination made of the total radioactivity of the solutions used, so the percentage adsorbed by the filter can not be calculated. The possible significance of these results is treated in the discussion section.

DISCUSSION

While this report concerns, in particular, the study of mercury as a toxicant to algae, some of the phenomena described are pertinent to other toxicants. In this discussion the chemical and physical aspects of algal assays will be treated first, and then the biological. The most important factors and the variables controlling them are listed below.

1. Concentration of toxicant
 - Rate of sorption by algae
 - Rate of adsorption onto container surfaces
2. Chemical species of toxicant
 - Complexation or dissociation
 - pH
3. Concentration of algal cells
 - Rate of growth
4. Concentration of nutrients
 - Rates of sorption by algae
 - Rates of adsorption onto container surfaces.

Chemical and Physical

Carrier-Free ^{197}Hg —The interpretation of any experiment involving a radioisotopic tracer depends on a knowledge of the chemical species of the tracer and of the nonradioactive isotopes of the same element. An advantage of carrier-free isotopes is that enough can be added to provide good tracer activity without affecting the biology of the system— ^{197}Hg is not a toxicant when used carrier free. A caution to be noted is that exchanges may take place between carrier-free isotopes and other isotopes of the same element which are not added to the system intentionally but happen to be present in unknown concentrations, often of a far greater magnitude.

Mercury is not a component of the culture medium by design, but it is present as shown by the significant quantities scavenged by *Phaeodactylum* and *Chaetoceros* controls (Tables 7-9); this is not surprising in view of the observation by Byrne et al. (11) that 100 ng/g is often present in chemical reagents of supposed ultrapure quality. Its presence is suggested also by the experiment summarized in Table 1 in which cell-free solutions spiked with ^{197}Hg were filtered; presumably, some particulates were retained by the filter, and the percent radioactivity was greater with the 25% culture medium than with the 50%. Identical amounts of radioactivity were added to each, and the data are interpreted to mean that more mercury must have been present in the 50% culture medium than in the 25%.

The least activity retained by the filter was with the 3.5% NaCl solution, for which two possible explanations are offered. The first is that this solution had a lower pH than those containing the culture media (5 vs 8 pH); Shimomura et al. (12) found that mercury was adsorbed to a greater extent at high pH than low, and while their study concerned plastic containers and the one reported here concerns a Millipore filter, there may still be a correlation. The second possibility is that chemical complexation of the mercury with components of the culture medium caused increased retention of the ^{197}Hg by the filter. This hypothesis is supported by the results in Table 10 in which sample 8C, representing the filter through which a cell-free solution containing 0.12 mg/l in 3.5% NaCl was passed, had only 71,600 counts compared to the 47,100 counts of sample 4C which had been used to filter a cell-free solution of 0.12 mg Hg/l in a dilute culture medium.

It may be happenstance that the same percentage of retention of carrier-free ^{197}Hg , in 25% culture medium, occurred with the filter in Table 1 and the centrifugate in Table 2. Both experiments concerned cell-free solutions made from distilled water, and the immediate interpretation is that there was no adsorption of mercury to the filter, that what was retained was particulate matter which had picked up mercury from solution. On the other hand, the data cited from Table 10 are convincing evidence that adsorption of mercury onto a Millipore filter does take place from dilute culture medium. Admittedly this was with a high concentration of mercury, 0.12 mg/l, compared to the carrier-free isotope data of Tables 1 and 2.

Adsorption and Loss of Mercury—In addition to the adsorption of mercury onto Millipore filters, another type of adsorption is pertinent to algal assays, i.e., the rapid rate of adsorption of mercury onto the walls of the culture vessel. Coyne and Collins (13) added a mercury solution of 0.05 mg/l to distilled water in 1-gal. polyethylene containers and made periodic analyses of the water; after 40 min the mercury content had dropped to 0.04 mg/l, and after 108 min it was only 0.032 mg/l, a decrease of 36% in less than 2 hr. Their attempts to prevent adsorption, by reducing the pH to 1 with acid, were successful only when nitric acid was used; after 5 days of storage in 1-gal. containers, distilled water solutions made up originally with 0.05 mg Hg/l and the required amounts of H_2SO_4 and HCl were analyzed and found to contain 0.011 and 0.001 mg/l, respectively, whereas the sample treated with HNO_3 had 0.049 mg/l in solution. Therefore pH alone is not the determinant in the adsorption process; perhaps the oxidant character of the nitrate is a necessary adjunct.

The shape of the culture vessel in algal assays is important because of its effect on the surface/volume (S/V) ratio. The rate of adsorption is proportional to the surface area, and the dependence of area on shape for a variety of containers is illustrated by the data in Table 11; the S/V ratio increases by an order of magnitude from a small vial to a 1-l Erlenmeyer flask. In tests with *Chlorella* (14), the growth obtained with a dilute culture medium was 100 times greater in a 1-l volumetric flask than in a small vial (3-day test), presumably because of the lower rate of nutrient adsorption to the walls. When various adsorbable components of a solution, toxicants or nutrients, are being removed at different rates, the growth obtained reflects a series of changing conditions which are difficult to resolve.

Table 11
Comparative Surface/Volume Ratios for Various Containers

Container	Surface/Volume Ratio (mm ² /mm ³)
Vial, 3 ml	0.50
Test tube, 50 ml	0.17
Erlenmeyer flask, 125 ml	0.086
Erlenmeyer flask, 250 ml	0.069
Erlenmeyer flask, 1000 ml	0.045
Sphere, 20 ft in diameter	0.01

The distribution of adsorbants between the glass and the algal cells depends, in part, on the relative surface areas of each. To demonstrate the importance of the size of the culture vessel, a calculation was made of the suspension densities required to provide the same surface areas of cells as of glass in various Erlenmeyer flasks. An assumption was made that each type of surface was smooth, which is obviously not the case, but this assumption would not affect the ratios sought. *Phaeodactylum tricorutum* was the subject of this comparison and its surface area was computed from the dimensions described by Lewin (15), i.e. an ovate structure 8 μm long by 3 μm wide. The glass and cell areas would be equal in the following combinations; a 1000-ml flask and a 21-ppm (by volume) suspension, a 500-ml flask and 26 ppm, and a 125-ml flask with a 45-ppm suspension. In this type of flask a reduction in flask volume by a factor of 8 would require a doubling of suspension density to provide the same surface area relationships in each.

Loss of mercury from a solution can also result from volatilization, as pointed out in numerous references cited by Coyne and Collins (13). Also comparisons by Corner and Rigler (16) of the mercury loss from plain seawater and that fortified with nutrients offer an insight into the problem. In the plain seawater only a small fraction of the mercury was adsorbed to the glass while the major loss was due to the release of a volatile component, and in the seawater containing nutrients a large amount of the mercury lost from solution was caused by adsorption to the glass with only a minor fraction volatilized. Volatilization was a very prominent factor in the results reported by Ben-Bassat et al. (8) on the growth of the freshwater alga *Chlamydomonas reinhardtii*. Constant aeration with 5% CO₂-in-air at 30 cc/min contributed to this loss; in 8 days the mercury content decreased from 0.20 mg/l to 0.12 mg/l.

Biological

Susceptibility to Mercury—In all the experiments reported here mercury was added as HgCl₂, which is essentially a covalent molecule that exists mainly as HgCl₄⁻ in seawater. Whether it is more, or less, toxic to algae than organic mercury compounds has not been established; in one experiment performed here to compare the toxicities of equimolar concentrations of HgCl₂ and (CH₃)₂Hg, the former was the more toxic. The literature concerning the relative toxicities of various mercury compounds to algae is sparse.

Boney (17) found that compounds of the N-alkyl mercuric chloride type were toxic to the red alga *Plumaris elegans*, and their toxicity increased with the number of carbons in the side chain. Corner and Sparrow (18) studied the toxicities of HgCl_2 , HgI_2 , and N-alkyl mercuric chlorides to larvae of the crustaceans *Artemia salina* and *Elminius modestus*. They found that all the mercury compounds were more toxic than HgCl_2 , that primary alkyl mercuric chloride was more toxic than the corresponding secondary compounds, and that toxicity increased with the homologous series. While some exceptions were noted, there was correlation between toxicity and lipid solubility which was the thesis expounded by Ferguson years before (19).

With all toxicants tested in this study there was an inverse relationship between nutrient concentration and growth inhibition by the toxicant. Many examples could be cited, typified by the relative growths of the controls and the 0.01 mg Hg/l cultures shown in Fig. 4; there was no significant inhibition in the 25% culture medium, but considerable inhibition was evident with the 10% culture medium. These data are not directly comparable because different cultures were used for the inocula and the concentrations of cells introduced initially were not the same.

This strong nutritional effect explains the sensitivity of natural phytoplankton populations to toxicants; Harriss et al. (20), for example, reported highly significant inhibitions of *Nitzschia delicatissima* and a freshwater phytoplankton population with concentrations of mercurials below 1 $\mu\text{g}/\ell$. In the nutrient-deficient conditions usually found in nature and in relatively low population densities, the concentration of a given toxicant required to produce a measurable inhibition would be considerably less than those used in the experiments reported here.

The matter of toxicant-nutrient relationships is but one component of the concept of a "limiting factor" which merits wider recognition than it generally receives. Verduin (21) discusses this fully and relates photosynthetic yield $Y = Y_{opt}(1-2^{-x})(1-2^{-y})(1-2^{-z})$ etc. where Y_{opt} is the yield obtainable if all factors are present at optimal intensity, and x , y , and z are the factors influencing the photosynthetic process. He cites an example of the increase in overall yield by slight changes in several factors; if three factors simultaneously have a relative intensity of 0.33, with all others in abundance, the yield will be $(0.20 \times 0.20 \times 0.20) = 0.008 Y_{opt}$, but if all three factors are increased to 0.50 the yield will be $(0.33 \times 0.33 \times 0.33) = 0.11 Y_{opt}$. Included among the factors would be all the chemicals constituting the culture medium, and such physical dimensions as temperature, light intensity, degree of cell shading, etc. A single factor can be of great importance; e.g., the addition of 80-mg/l nitrate rendered *Phaeodactylum* invulnerable to 0.427 mg Hg/l even though a concentration of only 0.1 mg Hg/l normally caused inhibition.

Mercury Content and Concentration Factor—Few data are available on the mercury content of algae, probably because of the difficulties in harvesting sufficient masses of organisms from natural environments for analyses. Hannerz (4) conducted experiments with freshwater algae in ponds, tanks, and aquaria, and used labeled phenylmercuric acetate, methylmercuric hydroxide, methoxyethylmercuric hydroxide, and mercuric chloride. After approximately a month the concentration factors for the algae (*Oedogonium*) ranged from 252 to 950, but the concentrations of mercury added were not the same for each compound, so direct comparisons would be difficult.

Klein and Goldberg (22) report mercury concentrations in coastal marine organisms to be several orders of magnitude greater than in comparable volumes of seawater. They

analyzed the soft parts of 81 marine organisms, most of which were epibenthic fauna, and the average mercury level on a dry-weight basis was 0.9 mg/kg. Vinogradov (23) reported that marine algae contain 0.03 mg/kg, dry weight. Raeder and Snekvik (24) reported that *Laminaria hyperborea* and *Fucus vesiculosus* contained 0.37 and 0.023 mg Hg/kg, respectively. In the Tay River estuary, in Scotland, Jones et al. (25) found variations according to the sampling area, the highest mercury concentrations occurring near the mouth of the Tay. Mercury concentrations in the algae ranged from 0.067 to 6.26 $\mu\text{g/g}$ wet weight. Chapman et al. (26) studied the concentrating capacity of aquatic organisms and concluded that the potential for mercury concentration from water by aquatic life is of the order of 1000 times for freshwater macrophytes and phytoplankton, 1000 times for fish, and 100,000 times for freshwater invertebrates.

The magnitude of mercury sorption by algae varies directly with the concentration of mercury in the medium, but the concentration factor (Hg conc. cells/Hg conc. soln.) is related inversely to the mercury concentration in the medium. For example, the following concentration factors were calculated from the data for the 10% culture medium in Table 7, with the assumption that the final concentration of mercury in the solution is the difference between the amount added to the solution and that sorbed by the cells (no adsorption to the walls); for the 0.05 mg Hg/l solution the concentration factor was 65,000 and for the 0.01 mg/l suspension the factor was 184,000. In natural waters containing much lower concentrations of mercury, the concentration factor for the algae would be higher.

The matter of active and passive uptake of mercury has not been explored extensively. Glooschenko (7) studied the mercury uptake by *Chaetoceros costatum* Pavillard with ^{203}Hg and found that dividing cells in light accumulated the isotope for a longer time than did nondividing cells, indicating the possibility of some active uptake of mercury. In this same study, formalin-killed cells accumulated more ^{203}Hg per cell than any other population, which indicates that in this case accumulation was not due to active processes but to surface adsorption, or to increased membrane permeability in the dead cells. Krauskopf (27) demonstrated that dead marine plankton adsorb up to 98% of the mercury in seawater.

Our study of the mercury content of pigments in *Phaeodactylum tricorutum* (28) confirms the postulate that mercury is bound predominantly to the surface. Less than 2% of the mercury in the cells was removed by methanol extraction; included among the extractants would be the pigments and other alcohol-soluble compounds, so the concentration in the pigments themselves would be even less. Under conditions promoting lysis of the cells, which would increase the availability of active binding sites, it is presumed that greater concentrations of mercury could be sorbed.

Fluorescence and Mercury Content of Pigments—Fluorescence measurements are made routinely to determine the concentrations of extracted pigments or of intact cells, yet they often can not be interpreted quantitatively. One reason is that the number of chloroplasts per cell changes according to light intensity during growth, and another is the great difference between the fluorescence intensities of various organisms. For example, a suspension containing 15 ppm by volume of *Chlorella sorokiniana* cells provides the same fluorescence intensity as 100-ppm *Phaeodactylum tricorutum*.

During many repetitions of the procedure developed here for algal assays, we had become acutely aware of the immediate decline in the fluorescence of freshly washed cells, particularly in the presence of small concentrations of mercury (29). When these

same concentrations of mercury were added to growing cultures, the decline in fluorescence was much slower even though the cell concentrations were approximately the same.

Changes in fluorescence intensity resulting from the addition of 0.12 mg Hg/l are shown in Fig. 7, and while the shapes of the curves may vary from one experiment to another, there is always a decline in fluorescence in the absence of nutrients and in the presence of mercury. Results from Table 10 indicate that the mercury content of each batch of treated cells was probably the same within the experimental error. The difference between 3,937,400 and 3,691,800 counts is only 6.2%, and the procedure used for measuring the radioactivity of such "hot" sources might be less reproducible than that. The mercury content of the methanol extracts was only 1.7% of that of the cells taken from the dilute culture medium, and 1.9% of those from the 3.5% NaCl solution, again perhaps not a significant difference. This leaves unanswered the question of whether the mercury in the pigments contributes to the decrease of their fluorescence.

It might well be that the decrease in pigment fluorescence is associated with a release of phosphate from the cell which, in turn, would result from alterations in membrane permeability caused by the presence of mercury. In yeast cells, mercury causes a generalized increase in permeability to cellular cations and anions (30); the nature of the cellular anions was not investigated extensively, but the authors suggest that a large proportion can be accounted for as inorganic and organic phosphates. A release of phosphate from the cell would also be expected when the cell is placed in a nutrient-free (therefore phosphate-free) solution; it would be reasonable to expect, therefore, that cells dispersed in a nutrient-free solution containing a slight amount of mercury would be particularly apt to lose their phosphate, thereby decreasing their potential for fluorescence.

Passow and Rothstein (30) were using mercury concentrations of 40 mg/l and higher, considerably higher than the 0.1 mg/l used in our studies, but their suspension densities were on the order of 60 mg/ml. The most concentrated cell suspension used in our studies, and this after 4 days' growth, was only 0.025 mg/ml; on an equal weight-of-cells basis we were using mercury loadings approximately six times greater than those in their studies.

There has been speculation concerning the relative degrees of penetration of mercury into intact organisms vs isolated chloroplasts. Webb (31) points out that intact organisms are less susceptible than isolated chloroplasts to the toxic effects of mercury and cites the limited effects of 100 mg Hg/l as p-chloromercuribenzoate (p-CMB) on the rate of CO₂ fixation by dahlia leaves (32) and by *Scenedesmus obliquus* cells. As evidence for the increased susceptibility of chloroplasts to mercury, Webb cites the 88% reduction in CO₂ fixation of spinach chloroplasts by 10 mg Hg/l (as p-CMB) and the inhibition of a dark reaction of Swiss chard chloroplasts by just 1.2 mg Hg/l (33). Direct comparisons of these threshold toxicities can be misleading, however, because of differences either in experimental techniques or in the time frames for which the percentages are calculated.

SUMMARY

The importance of mercury in the food chain has prompted this study of its effects on algae, the first step in the food chain. Three marine and one freshwater organisms have been included in this research, and a variety of chemical, physical, and biological parameters has been investigated.

Mercury concentrations used have ranged from carrier-free ^{197}Hg to 0.427 mg Hg/l. Sorption has been studied by radioactivity methods and by atomic absorption. Growth of cultures has been determined by changes in the magnitude of cell fluorescence, by changes in pH as a function of CO_2 absorption, and by gravimetric determinations of cell masses.

Some of the findings of this study are listed below.

1. The retention of Hg from solution by Millipore filters is promoted by the addition of culture medium to the solution.

2. A procedure has been devised which provides a convenient determination of the distribution of mercury among cells, solution, and walls of the container. Radioactive mercury is employed in this procedure, and the cells are separated from the test suspension by centrifugation.

3. The uptake of mercury by algae is appreciable, even when no mercury is added to the nutrient solution; this is the consequence of mercury impurities in even the best grade of reagent chemicals.

4. The susceptibility of algae to the effects of a toxicant depends directly on the concentration of the toxicant and inversely on the concentrations of nutrients available to the cells.

5. In a 4-day growth period *Phaeodactylum tricornutum* cells accumulated 2400 mg/kg in a 10% culture medium containing 0.05 mg Hg/l. The mercury concentration per cell was considerably less for cells grown in a more concentrated

6. Mercury uptake by *Chaetoceros galvestonensis* was greater than that for *Phaeodactylum tricornutum* under similar conditions; cells containing over 7400 mg Hg/kg had not grown.

7. The decrease of cell fluorescence by mercury is more pronounced when the suspending solution is devoid of nutrients than when they are present.

8. Less than 2% of the mercury sorbed by cells of *Phaeodactylum tricornutum* is in the pigments.

These findings, and others, have been discussed at length.

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