Reference: *Biol. Bull.*, **150**: 118–127. (February, 1976)

LEAD UPTAKE IN TWO MARINE PHYTOPLANKTON ORGANISMS

MEINHARD SCHULZ-BALDES[1] AND RALPH A. LEWIN

Scripps Institution of Oceanography, La Jolla, California 92093

Hundreds of thousands of tons of lead are discharged annually into the earth's atmosphere in the exhaust gases of internal combustion engines fueled with leaded petroleum, *i.e.*, hydrocarbon mixtures supplemented with the antiknock agent lead tetraethyl. From the atmosphere the metal, largely as oxides and salts, is washed down by rain to the surface of the earth. The surface layer of the ocean, the euphotic zone, is thus particularly susceptible to lead input. The possibly hazardous effects of this element on phytoplankton have been studied for only a few species, mostly hardy laboratory organisms; they indicated a rather high tolerance to lead (Hessler, 1974; Malanchuk and Gruendling, 1973). However, even if lead at present environmental levels in the ocean is not appreciably toxic, its uptake by the phytoplankton, at the first level of the marine food chain, is of great ecological importance, since such organisms may not only play a role in the geochemical distribution of lead, but also, by serving as food, transport the lead to higher trophic levels. For example, lead-enriched algae can increase the lead content of a bivalve mollusc, *Mytilus edulis*, to the same extent as elevated lead concentrations in the surrounding medium (Schulz-Baldes, 1974).

In this paper we indicate the high potential of certain algal cells to take up lead, supplied at sub-toxic levels, quickly and completely from the medium.

Materials and Methods

Algal cultures

Axenic cultures of the diatom *Phaeodactylum tricornutum* (originally isolated by R. A. Lewin in Woods Hole, Massachusetts) and the green flagellate *Platymonas subcordiformis* (originally isolated by R. A. Lewin from Morro Bay, California) were grown in Erlenmeyer vessels at a constant temperature of 20° C. The cultures were continuously shaken on a reciprocating shaker (120 oscillations/min) and illuminated by "cool-white" fluorescent tubes (2000 lux).

The culture medium for *Phaeodactylum* consisted of filtered local sea water with additions of potassium nitrate (1 g/l), sodium glycerophosphate (0.1 g/l), and a trace-mineral solution containing Fe, etc. (Lewin and Lewin, 1967). The culture medium for *Platymonas* consisted of filtered local sea water with additions of sodium glutamate (1.7 g/l), sodium glycerophosphate (0.1 g/l) and the trace-mineral solution. For experiments on the uptake of lead, cells in the exponential phase were centrifuged from the enriched medium and resuspended in fresh, unenriched sea water which had been filtered through a 0.45 μm Millipore filter and then autoclaved. In this way, we attempted to minimize chelation of lead ions by the EDTA or glutamate present in the culture medium, to reduce interference by other cations, and to retard cell multiplication.

[1] Present address: Institut für Meeresforschung, Bremerhaven, West Germany.

Lead was administered from a stock solution containing 1 g Pb/l (as chloride, in 0.01 N HCl). After treatment with known concentrations of lead, some cell samples were extracted with the chelating agent EDTA (prepared as a stock solution containing 0.2 M) to determine how much of the adsorbed and/or absorbed Pb could be readily eluted. After EDTA treatment, in most experiments for one hour, the cells and the medium were analyzed in the same way as the cultures described below. During the experimental periods of 5–7 days the cell numbers, routinely determined with a Coulter Counter, increased by only about 30%.

Lead determinations

The contents of lead in the algae and the medium were determined by flameless atomic-absorption spectrophotometry, using a Perkin-Elmer model 403 with a heated graphite tube HGA-2000 and a deuterium background corrector. The algae were centrifuged from the medium, suspended in 0.1 N HCl, counted, transferred to a smooth graphite tube, and assayed for their lead content by the method of standard additions. The determination of lead in the medium was carried out after an extraction step using a solution of 2% ammonium pyrrolidine dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK), according to a modification of the micro-method of Kremling and Petersen (1974). The MIBK extract was measured in a grooved graphite tube.

In this paper the lead associated with the algae ("particulate" lead) is generally given as mg lead bound to the algal cells contained in one liter of medium, allowance being made for cell growth and biomass increase during the experiment. This value can then be directly compared with the lead concentration in the medium (soluble lead). However, in Figure 4 the lead content is expressed as μg per gram dry weight.

Dry weights per cell were estimated to be 2×10^{-11} g for *Phaeodactylum* and 6×10^{-11} g for *Platymonas*.

Results

The course of lead uptake by *Platymonas* and *Phaeodactylum* in batch assays exhibits two phases. In both species the first phase is a rapid increase in the lead associated with the algae. Although the first measurement in the experiments illustrated in Figures 1 and 2 was not carried out until one hour after the addition of lead, other experiments showed that the first phase takes only a few minutes. The second phase shows a pattern which differs for the two algal species. The lead content of *Platymonas*, after a slight decrease in the first day, steadily increases during the next 7 days, whereas after day 1 the lead concentration in the medium remains very low (Table I). The lead content of *Phaeodactylum* in the second phase increases until the second day, but then declines, and after day 2 there is a corresponding increase in the concentration of lead in the medium (Table II).

The sum of the measured amounts of soluble and "particulate" lead was generally less than the total amount of lead originally added to the medium: *e.g.*, in media initially containing 0.3 mg Pb/l, some 20% to 40% remained unaccounted for (see Fig. 3). The "missing" fraction had probably been adsorbed to the surface of the experimental vessel, from which it could later be desorbed, and subsequently taken up by the algae, as the concentration of soluble lead decreased.

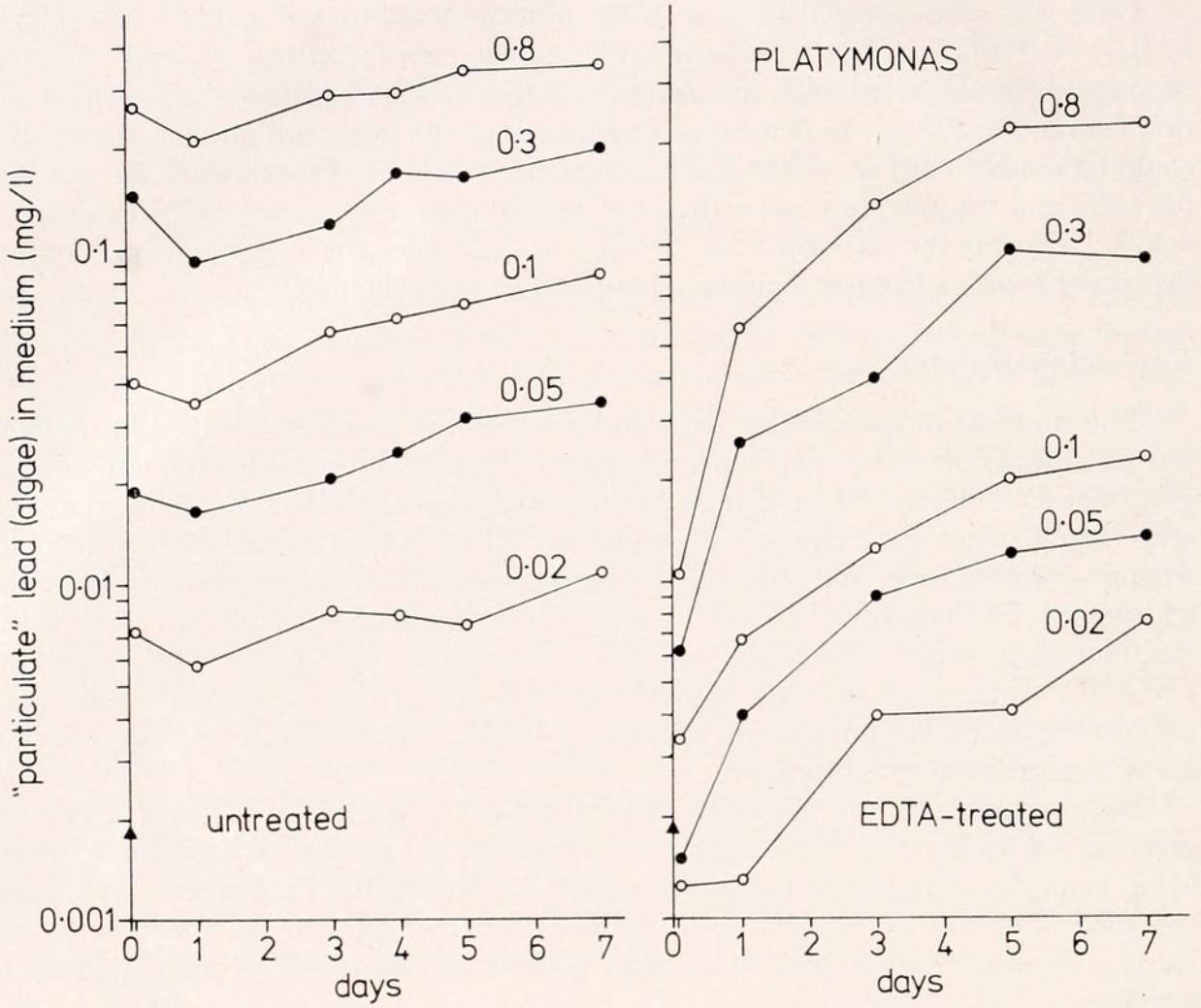

FIGURE 1. *Platymonas subcordiformis*: lead content (= "particulate" lead, expressed as mg Pb bound to the algal cells contained in one liter of medium) of cells suspended in a medium containing Pb (0.02–0.8 mg/l) and then untreated (left) or treated with 10^{-2} M EDTA for one hour (right), as a function of time. In all figures, the black triangles indicate the natural lead content of the cells. Cell numbers per ml rose from 3.2×10^6 (day 0) to 4.5×10^6 (day 7).

The uptake during the first phase can be described by a Freundlich adsorption isotherm $x/m = k \times c^n$, where x is the amount adsorbed, m, the mass of adsorbent, c, the concentration in solution, and k and n, the constants. The first equation can be rearranged to give a straight line in a log-log plot: $\log x/m = \log k + (n \times \log c)$.

TABLE I

Pb concentration determined in medium, Platymonas subcordiformis, *cultures.*

Day	Hour	Lead initially added to medium (μg/l)				
0	0	20	50	100	300	800
0	1	5	6	9	48	143
1		<4	<4	6	36	63
3		<4	<4	<4	21	32
5		5	<4	7	18	38
7		<4	<4	5	16	30

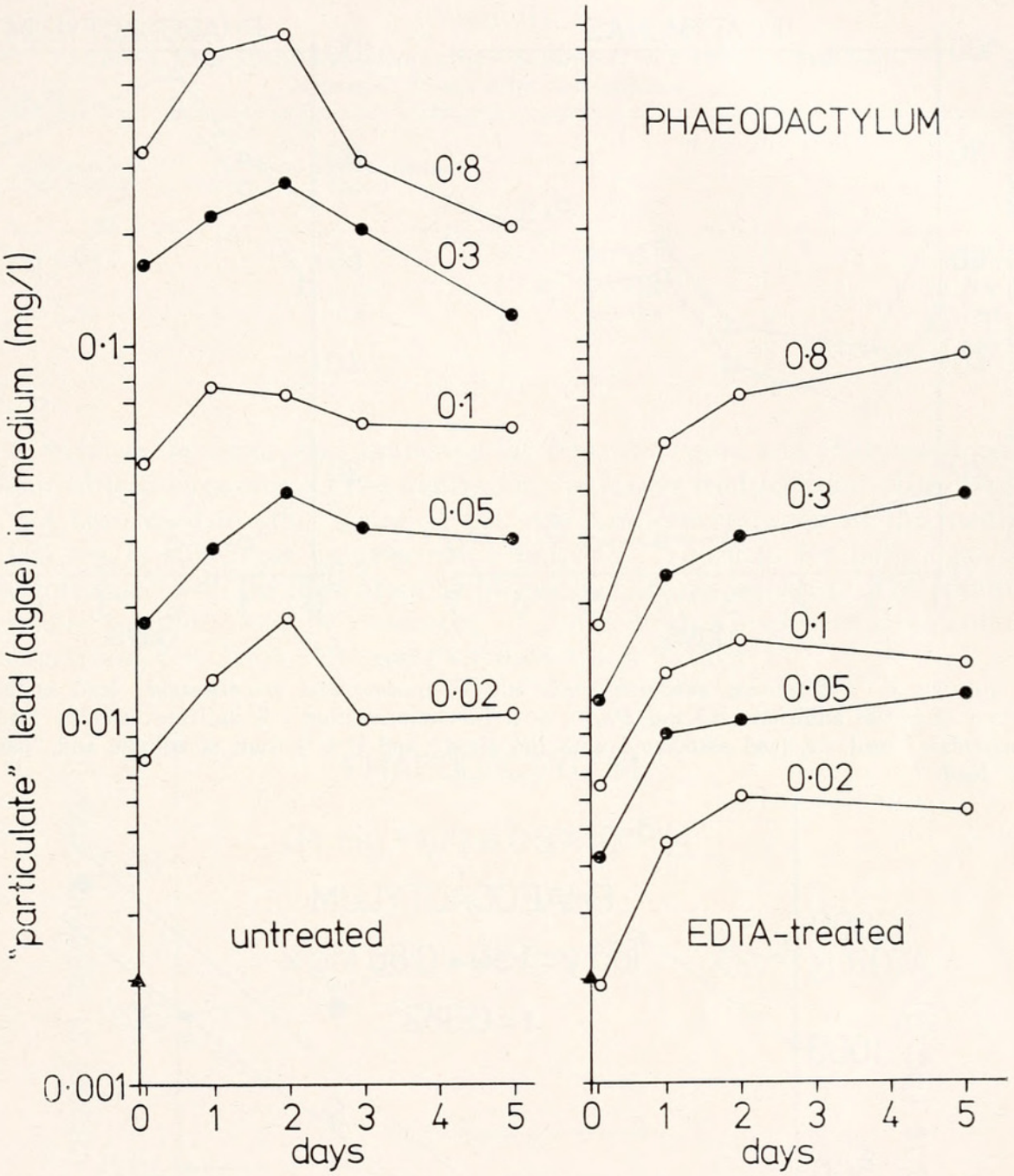

FIGURE 2. *Phaeodactylum tricornutum*: lead content of cells, as a function of time; see legend to Figure 1. Cell numbers per ml rose from 7×10^6 (day 0) to 10×10^6 (day 5).

TABLE II

Pb concentration determined in medium, Phaeodactylum tricornutum, *cultures.*

Day	Hour	Lead initially added to medium (μg/l)				
0	0	20	50	100	300	800
0	1	<4	6	20	47	194
1		<4	<4	7	18	85
2		<4	7	13	18	90
5		7	14	35	118	382

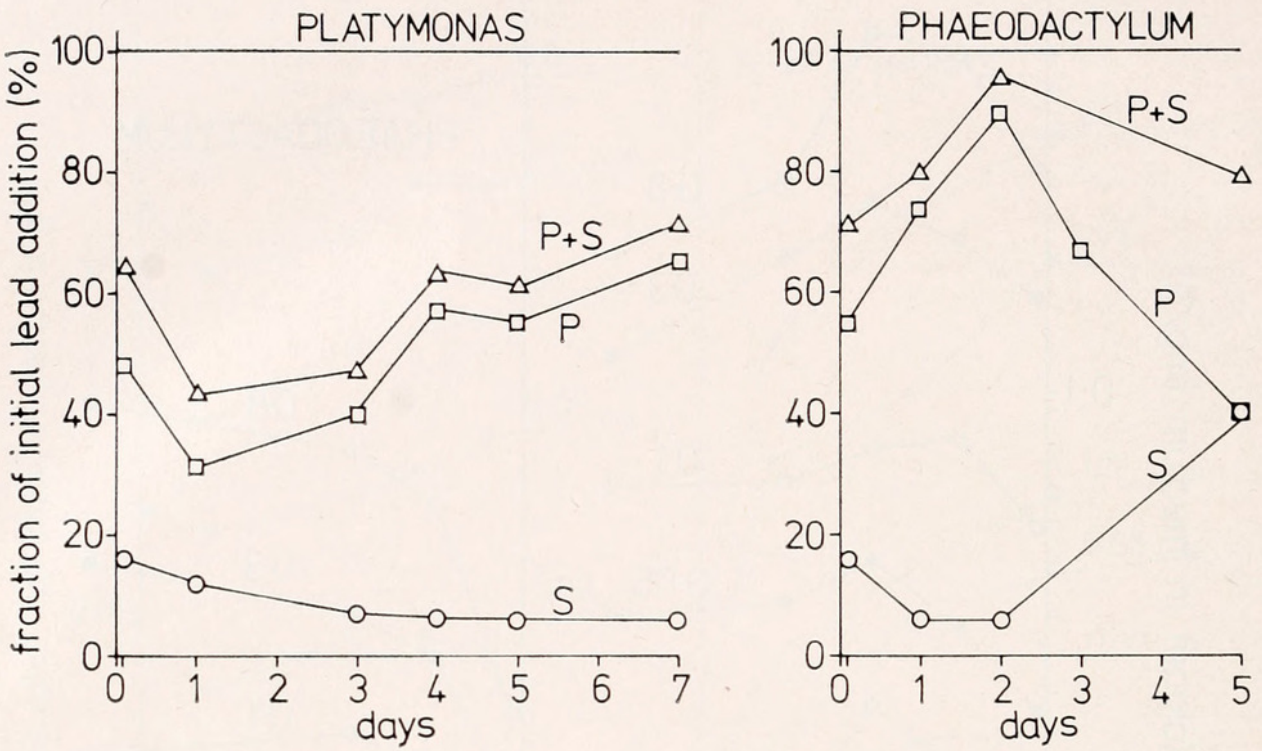

FIGURE 3. *Platymonas subcordiformis* and *Phaeodactylum tricornutum*: lead budget in percent of initial addition (0.3 mg Pb/l) as a function of time. S indicates soluble lead; P, "particulate" lead, *i.e.* lead associated with the algae; and P + S, sum of soluble and "particulate" lead.

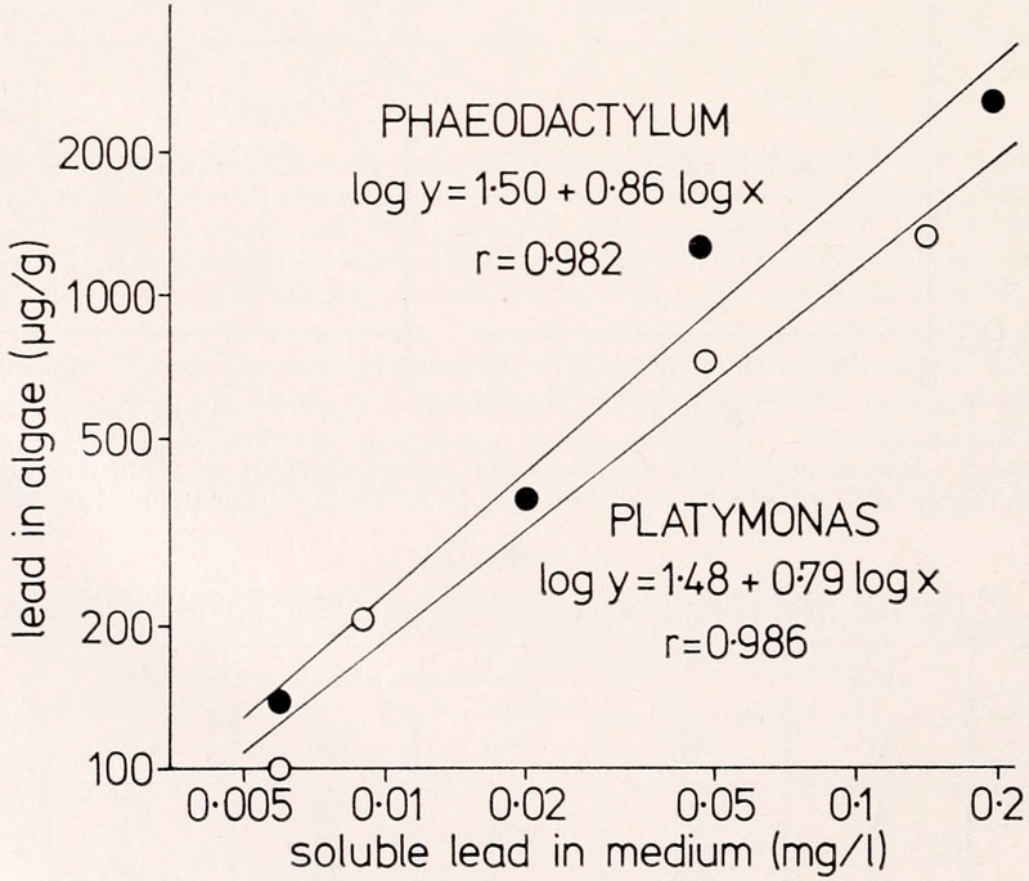

FIGURE 4. *Platymonas subcordiformis* and *Phaeodactylum tricornutum*: Pb adsorption isotherms after one hour of lead exposure.

TABLE III

Cells of Phaeodactylum tricornutum *"saturated" at the surface with lead, measured 10 min after lead addition.*

Cell number (10^9 cells/l)	Pb addition to medium (mg/l)	Pb adsorbed to cells	
		(mg/l)	(μg/g dry weight)
0.63	1	0.154	11,860
1.25	2	0.282	11,280
2.5	4	0.589	11,780
			Average: 11,640

Figure 4 shows the adsorption isotherms for *Phaeodactylum* and *Platymonas* after one hour of lead exposure. [The values for the lowest lead addition (0.02 mg/l) have not been used for this figure as the very low concentration in the medium (<0.004 mg/l) could not be determined exactly]. The data for both algae are highly correlated with the lines fitted by the method of least squares. The resulting logarithmic equations can be converted to the following exponential equations: *Phaeodactylum*, $y = 31.66\ x^{0.86}$; and *Platymonas*, $y = 30.25\ x^{0.79}$.

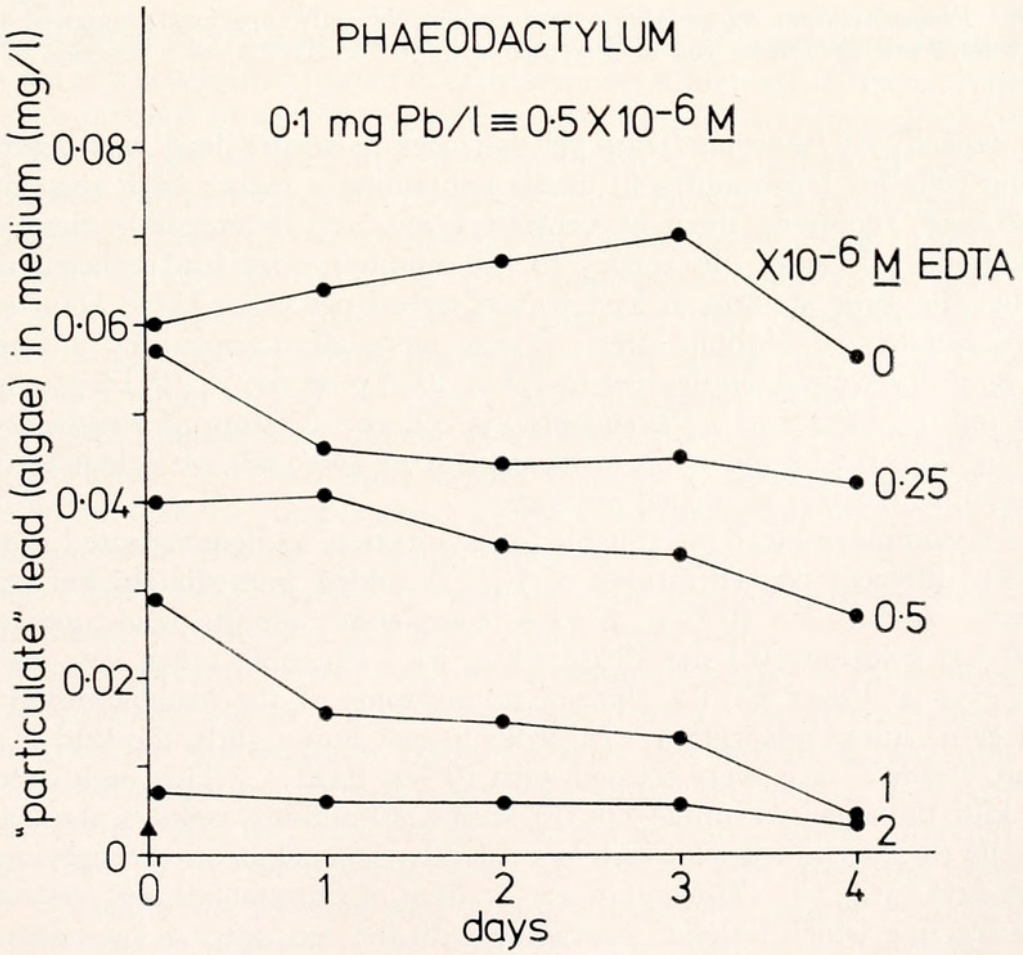

FIGURE 5. *Phaeodactylum tricornutum*: lead content of cells exposed to 0.1 mg Pb/l in the presence of 0 to 2×10^{-6} M EDTA, as a function of time.

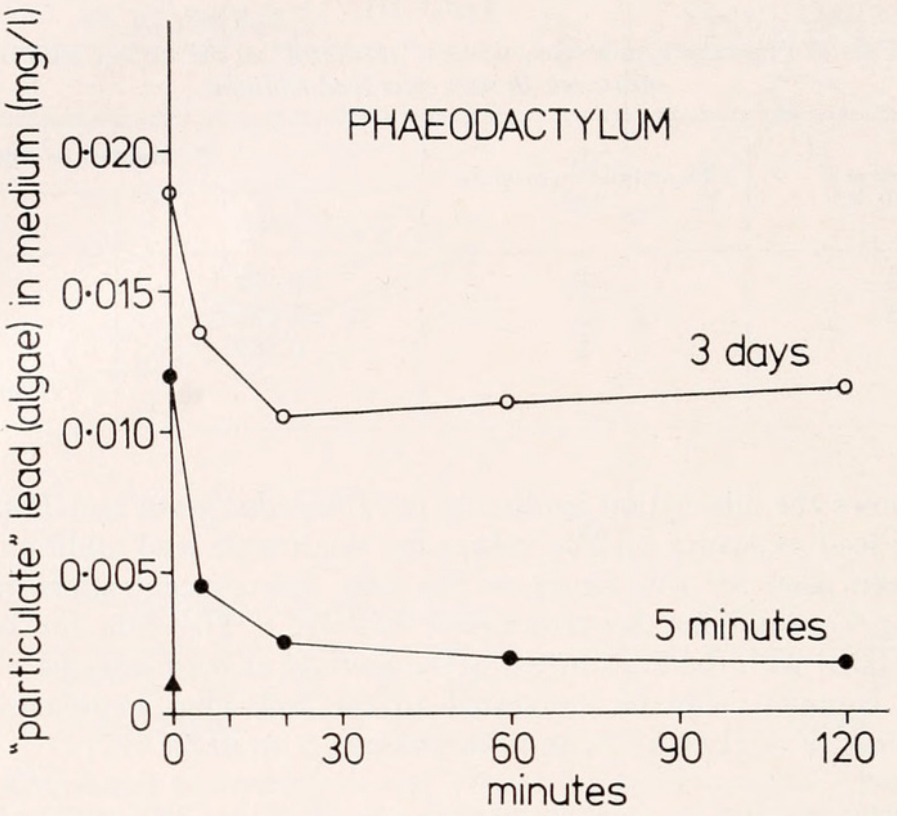

FIGURE 6. *Phaeodactylum tricornutum*: lead content of cells, previously exposed to 0.02 mg Pb/l for 5 min or 3 days, and then treated with 10^{-2} M EDTA, as a function of time.

The capacity of *Phaeodactylum* cell surfaces to adsorb lead was determined by supending cells for ten minutes in media containing a rather high concentration of dissolved lead, removing them by centrifugation, and determining the amounts of lead taken up. Despite differences in cell numbers and lead concentrations, we found that the same amount of lead was adsorbed per cell (Table III), suggesting a limited number of binding sites. These were all occupied by a lead load of 11,640 μg/g dry weight, equivalent to 2.3×10^{-13} g or 6.7×10^{8} Pb-atoms per cell. Considering the surface of a *Phaeodactylum* cell as a frustum of a right double cone 20 μm long and 6 to 3 μm wide, with an area of 440 μm^2, we calculate that about 1.5 atoms of lead can be absorbed per nm^2.

Only uncomplexed lead is available for adsorption, as demonstrated in an experiment with different concentrations of EDTA added immediately before the lead treatment. About 2×10^{-6} M EDTA completely inhibits lead uptake from a 0.5×10^{-6} M solution (0.1 mg Pb/l) (Fig. 5). Probably other ions are chelated, too, but even at lower EDTA concentrations some of the lead is eluted from the algae after its initial adsorption. In order to test how tightly the lead is bound on the algae, "leaded" cells were treated with 10^{-2} M EDTA. This high concentration (which kills the algae within 24 hours) within 20 minutes reduces the lead content of the cells to a certain level which is evidently dependent on the previous time of lead exposure (Fig. 6). The bound lead content of *Platymonas* and *Phaeodactylum*, *i.e.*, that fraction which remains associated with the cells after a one-hour treatment with 10^{-2} M EDTA, increases considerably with time (Figs. 1 and 2). In EDTA-washed cells of *Phaeodactylum* the lead content seems to be more or less constant

after day 2. By contrast, in *Platymonas* the lead loss after EDTA treatment is initially slightly higher than in *Phaeodactylum*, but later there is a distinct increase in bound lead which, after about seven days, seems to reach an equilibrium at a level much higher than in *Phaeodactylum*.

Discussion

When a lead solution is added to sea water the metal becomes distributed between the medium, the vessel surface, and (if enough is added) precipitates. At concentrations higher than 1 mg/l in sea water, lead tends to be precipitated as carbonate and phosphate. There is no loss by evaporation, as in the case of mercury (Davies, 1974). However, it is not known precisely in what chemical species the lead occurs in such solutions. Zirino and Yamamoto (1972) reported that in sea water there could be a variety of complexes: $PbCO_3^0$, $PbCl^+$, $PbCl_2^0$, Pb^{2+}, $PbCl_3^-$ and perhaps $PbOH^+$. Complexing and chelation by organic ligands in natural sea water are also to be expected, as pointed out in the case of copper by Davey, Morgan and Erickson (1973). In algal cultures, some of the lead is adsorbed or absorbed by the cells, and the fraction of chelated lead can be expected to increase if the cells liberate anionic colloids as extracellular metabolites. In fact, Hellebust (1965) reported that *Phaeodactylum* secretes up to 7% of the total assimilated carbon, some of it as polysaccharides liberated from cell surfaces, which could act as binding agents for lead.

The initial rapid increase in the lead content of the cells seems to be due almost entirely to the physicochemical process of adsorption to the cell surface. Afterwards the metal may proceed by active transport or by diffusion to other sites. Whether there is a true uptake of lead into the cytoplasm, or whether the lead is only bound to the cell wall or the plasma membrane, may have to be confirmed by microchemical methods similar to those employed by Ophus and Gulvåg (1974) in their studies of lead uptake by the moss *Rhytidiadelphus squarrosus*. By electron microscopy in connnection with X-ray microanalysis, they demonstrated the presence of lead and phosphorus within nuclear and chloroplast inclusions. The variation in the lead content of EDTA-treated cells also seems to indicate a change in the location of the bound lead during extended periods of exposure to solutions containing the metal. In both algal species there is a considerable increase, with time, in the bound lead, *i.e.*, that fraction which is not removable by EDTA, suggesting that the metal is moving, or being translocated, into the cells. In a similar way, Davies (1970, 1973) used this method to distinguish between iron or zinc absorbed on the exterior surface, and intracellular metal ions.

In another respect our results exhibit a striking similarity to those obtained by Davies (1973) on zinc uptake in *Phaeodactylum*. Loss of zinc from the cells begins about 19 hours after zinc addition, leading Davies to postulate a reduction in the number of zinc-binding sites (probably protein) within the cells as they go through their growth cycle. We, too, noted such a decline in lead content, although in our experiments the EDTA-treated cells show no loss of lead after the second day (Fig. 2). (Possibly the released lead is not readsorbed because it is complexed or chelated by organic material in the medium. The method we employed for the determination of lead in the medium involves the use of a very strong chelating agent, and thus does not distinguish between more weakly chelated and ionic lead.)

Measurements of lead bound on the algae and lead in the medium never added up to account for all of the lead originally added: in most cases only 60% to 80% could be found. One possible explanation for the observed "loss" of Pb was its adsorption on the walls of the glass vessels. To test this we assayed the dissolved lead in an Erlenmeyer flask containing filtered and autoclaved sea water to which lead (0.1 mg/l) had been added and which was continuously shaken and illuminated. After 7 days the lead concentration in solution had decreased by 23% probably due to adsorption on the glass. The adsorption characteristics of lead on borosilicate glass have been described in detail by Struempler (1973). Presumably when algal suspensions are assayed some of the cells and other organic matter also stick to glass, thus further enlarging the adsorptive surface and scavenging more lead. On the other hand, some of the previously adsorbed lead might be later released from the vessel surface during depletion of lead in the medium by algal activity, and then taken up by the cells. This may explain the increase in the lead content of the algae while the concentration of dissolved lead in the medium was at a very low level.

In spite of this somewhat unsatisfactory balancing of the lead budget in our experiments, we think we have demonstrated unequivocally that lead ions first are quickly and reversibly bound to the cell surfaces, and only later penetrate to deeper sites (from which they can be less readily dislodged with EDTA) where they may be expected to exert their main biological effects.

M. S.-B. acknowledges with thanks a research grant from the Deutsche Forschungsgemeinschaft, and R. A. L. a grant (No. GA—33230) from the National Science Foundation, U. S. A. We are also grateful to the Analytical Facilities, SIO, for use of the atomic adsorption spectrophotometer.

Summary

1. Uptake of lead by *Phaeodactylum tricornutum or Platymonas subcordiformis,* exposed to lead concentrations ranging from 0.02 to 0.8 mg/l, occurs in two phases.

2. The first phase, completed within minutes after addition of lead, can be described by a Freundlich adsorption isotherm. The number of binding sites per cell seems to be limited. Cells of *Phaeodactylum* become "saturated" when the lead burden reaches 11,640 μg/g (dry weight), equivalent to about 6.7×10^8 Pb atoms per cell.

3. In the second phase, the lead content of *Platymonas* cells continues to rise slowly, whereas that of *Phaeodactylum* declines after two or three days.

4. The addition of 2×10^{-6} M EDTA to a solution containing 0.5×10^{-6} M Pb completely inhibits the uptake of the metal by *Phaeodactylum* cells. When diatom cells, pre-treated with lead, are resuspended in a higher concentration of EDTA, 10^{-2} M, much of the adsorbed lead is eluted. The longer the pre-treatment period with lead, the less readily is the metal removed from the cells in this way.

5. Since in both species the content of bound lead, *i.e.*, the residual lead burden after EDTA extraction, increases with time, we suggest that during prolonged exposure to lead solutions the metal ions are first adsorbed to the cell surface and then translocated to within the cell wall, to the plasma membrane, and eventually to the cytoplasm.

LITERATURE CITED

DAVEY, E. W., M. J. MORGAN, AND S. J. ERICKSON, 1973. A biological measurement of the copper complexation capacity of seawater. *Limnol. Oceanogr.*, **18** : 993–997.

DAVIES, A. G., 1970. Iron, chelation and the growth of marine phytoplankton. I. Growth kinetics and chlorophyll production in cultures of the euryhaline flagellate *Dunaliella tertiolecta* under iron-limiting conditions. *J. Mar. Biol. Ass. U. K.*, **50** : 65–86.

DAVIES, A. G., 1973. The kinetics of and a preliminary model for the uptake of radio-zinc by *Phaeodactylum tricornutum* in culture. Pages 403–420 in *Radioactive contamination of the marine environment. Proc. symp. interaction of radioactive contaminants with constituents of the marine environment, I.A.E.A., Seattle, 10–14 July 1972.* Vienna: I.A.E.A.

DAVIES, A. G., 1974. The growth kinetics of *Isochrysis galbana* in cultures containing sublethal concentrations of mercuric chloride. *J. Mar. Biol. Ass. U. K.*, **54** : 157–169.

HELLEBUST, J. A., 1965. Excretion of some organic compounds by marine phytoplankton. *Limnol. Oceanogr.*, **10** : 192–206.

HESSLER, A., 1974. The effects of lead on algae. I. Effects of Pb on viability and motility of "*Platymonas subcordiformis*" (Chlorophyta: Volvocales). *Water, Air, Soil Poll.*, **3** : 371–385.

KREMLING, K., AND H. PETERSEN, 1974. APDC-MIBK extraction system for the determination of copper and iron in 1 cm^3 of sea water by flameless atomic-absorption spectrometry. *Anal. Chim. Acta*, **70** : 35–39.

LEWIN, J., AND R. A. LEWIN, 1967. Culture and nutrition of some apochlorotic diatoms of the genus *Nitzschia*. *J. Gen. Microbiol.*, **46** : 361–367.

MALANCHUK, J. C., AND G. K. GRUENDLING, 1973. Toxicity of lead nitrate to algae. *Water, Air, Soil Poll.*, **2** : 181–190.

OPHUS, E. M., AND B. M. GULLVÅG, 1974. Localization of lead within leaf cells of *Rhytidiadelphus squarrosus* (Hedw.) Warnst by means of transmission electron microscopy and X-ray microanalysis. *Cytobios*, **10** : 45–58.

SCHULZ-BALDES, M., 1974. Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edulis*. *Mar. Biol.*, **25** : 177–193.

STRUEMPLER, A. W., 1973. Adsorption characteristics of silver, lead, cadmium, zinc, and nickel on borosilicate glass, polyethylene, and polypropylene container surfaces. *Anal. Chem.*, **45** : 2251–2254.

ZIRINO, A., AND S. YAMAMOTO, 1972. A pH-dependent model for the chemical speciation of copper, zinc, cadmium, and lead in seawater. *Limnol. Oceanogr.*, **17** : 661–671.