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The effects of organolead compounds on a freshwater and a marine alga

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There are conflicting reports concerning the toxicity of tetraalkyllead (TAL) compounds to algae. A number of groups have found the TAL's to be comparable in toxicity with the trialkyllead compounds (R_3Pb^+), whereas in a recent report it is suggested that the TAL's themselves are completely non-toxic and any apparent toxicity is due to R_3Pb^+ breakdown products.

With the object of identifying the toxic agent, the effect of Et_4Pb (TEL) on two algal species was re-examined. Analyses were carried out during the course of the incubations to establish the nature and concentrations of organoleads present in both media and algae, and hence evaluate their relative contributions to total toxicity.

Algae were also cultured in the presence of Me_4Pb (TML), Me_3PbCl, Et_3PbCl, Bu_3PbCl and Et_2PbCl_2 to assess relationships between alkyl chain length and degree of substitution around the lead on algal activity. Additions of selenide and sulphide were made to the Et_3Pb^+ and Et_2Pb^{2+} systems to see if these environmentally abundant species reduced or enhanced organolead toxicity. Problems were encountered in the analysis of the heterogeneous TEL containing media. Regardless of the analytical problems, the results confirm the previous findings that TAL's are non-toxic to algae and it is the R_3Pb^+ breakdown products which are responsible for the apparent toxicity of the TAL's. The trialkylleads were the most toxic of the several alkyllead species studied, and within the trialkyl series toxicity increased with alkyl chain length. Neither selenide or sulphide had any significant ameliorative effect on alkyllead toxicity. It was found that the ionic organoleads were complexed on the TAL's and this complexing led to a number of unexpected results.

Keywords: Algae, alkyllead compounds, toxicity, alkyl chain length

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INTRODUCTION

There are conflicting reports in the literature concerning the toxicity of tetraalkyllead (TAL) compounds to algae. Marchetti[1] found that *Dunaliella tertiolecta* is completely unaffected by commercial tetraethyllead (TEL) at concentrations below 0.1 mg dm^{-3} but that 16 hours exposure to concentrations of 0.5 mg dm^{-3} completely inhibits photosynthesis. Kozyura and coworkers[2] (1961) found TEL to be toxic to the blue green algae *Cladophera* sp. and *Scenedesmus* sp. and Maddock and Taylor[3] (1977) observed complete inhibition of photosynthetic activity in the marine diatom *Phaeodactylum tricornutum* after 6 hours exposure to low concentrations of TEL (1.0–2.0 mg dm^{-3}). Maddock and Taylor found tetramethyllead (TML) to be about 10 times less toxic than TEL and the trialkyl derivatives (R_3Pb^+) to be generally less toxic than the TAL compounds. In mammals the trialkyl compounds have been found to be more toxic than the tetraalkyls[4,5] and it was concluded from these observations[3] that the reverse must be true for marine animals. In contrast Roderer[6] who carried out very detailed studies on the effects of TEL and its derivatives on *Poteriochromonas malhamensis*, a freshwater crysophyte, concluded that TEL itself is not toxic and that any observed toxicity is due to the action of triethyllead (Et_3Pb^+) which is formed by photolytic decomposition of TEL. The basis for Roderer's conclusion was that concentrations of TEL which in the dark produced only mild toxicity completely inhibited the growth mitosis and cytokinesis of the cells in illuminated cultures and since TEL is converted to Et_3Pb^+ rapidly in the light and slowly in the dark Et_3Pb^+ appeared to be the most likely toxic agent. Furthermore the absence of toxic effects in the dark also suggested that *P. malhamensis* is not capable of metabolising TEL to Et_3Pb^+.

Maddock and Taylor[3] used the marine alga *P. tricornutum* in their investigations and assessed toxicity by measuring ^{14}C uptake and hence photosynthetic activity over a 6 hour period. Over this short time span it would be expected that very little of the TEL would be converted to Et_3Pb^+ so they concluded that the toxicity was due to the TEL.

Roderer[6] used the freshwater algae *P. malhamensis* in his studies and toxicity was estimated from cell counts and changes in cell structures over several days. The possibility did exist that the conflicting results concerning TEL toxicity could arise from the different assessment techniques used by the various groups or because TEL toxicity was species-dependent although the latter explanation appeared exceedingly unlikely.

In an attempt to resolve the conflict we re-investigated the effect of TEL on *P. malhamensis* and *P. tricornutum* using the Roderer[6] and Maddock and Taylor[3] techniques respectively. The media and algae were analysed from time to time during the incubation period. We thought that, knowing which and approximately how much of each organolead species was present during exposure, it would then be possible to evaluate their relative contribution to the toxicity.

We also examined the effect of three trialkyllead compounds, and of Et_2PbCl_2 on the photosynthetic activity of *P. tricornutum*. It has been observed previously[3] that the triethyl compounds were more toxic than the trimethyl compounds to algae. We included the tributyl compound to determine if toxicity increased further with increasing chain length. Additions of selenide and sulphide were made to the triethyl and diethyl systems to see if these environmentally abundant species reduced organolead toxicity.

In addition *P. malhamensis* was incubated in the presence of triethyl-, trimethyllead chloride and pure TML. In all previous studies the anti-knock fluid TML–CB which contains dibromo and dichloroethane was used rather than pure TML.

MATERIAL AND METHODS

Reagents

Alkyllead compounds were kindly provided by the Associated Octel Company, Ellesmere Port. All other reagents were of analytical grade from a commercial supplier.

Analysis for tetraalkyllead

Tetraalkylleads were initially extracted from the sample by shaking with hexane. The TALs were then either analysed directly by GLC/MS or indirectly using the Hancock and Slater[7] procedure.

Analysis for alkyllead salts

Aqueous solutions of alkyllead salts were analysed by the method of Hancock and Slater[7] or by differential pulse anodic stripping voltametry (DPASV).[8] Using the former technique no attempt was made to differentiate between R_3Pb^+ and R_2Pb^{2+} species and the values given in Table 4 are total values for $R_3Pb+R_2Pb^{2+}$. Using the DPASV method, speciation of R_3Pb^+ and R_2Pb^{2+} is possible and separate values for R_3Pb^+ and R_2Pb^{2+} are given in Tables 1, 5 and 7.

Analysis of biological samples

The samples were initially homogenised with added inorganic lead in an iodide/chloride solution. This aids release of the organolead from the tissues. Sodium benzoate was then added to improve the extraction of dialkyllead, and finally EDTA to complex inorganic lead. The organic lead was extracted from the aqueous solution with toluene, the complexed inorganic lead being retained in the aqueous phase. After centrifuging, the toluene layer was separated, an aliquot of the toluene solution removed and analysed directly for TAL by GLC–MS. The remainder of the toluene solution was back extracted with dilute nitric acid, which isolates the alkyllead salts from TALs remaining in the toluene.[9] The alkyllead salts were determined either by the Hancock and Slater[7] procedure or by DPASV.[8]

Phaeodactylum tricornutum assay

The *P. tricornutum* were assayed using the procedure of Maddock and Taylor[3] which is based on a method originally described by Steeman-Neilsen[10] for the assessment of organic productivity in the sea. The photosynthetic uptake of ^{14}C from a known amount of labelled carbonate by a standardised number of algae is measured in AE50 medium[11] for a range of toxicants and compared with that of control cultures. The effect due to toxicant can be expressed as a percentage of the control activity.

Table 1 Concentrations[a] ($mg\,dm^{-3}$) of ethylleads in EVT medium dosed with TEL

Time (d)	TEL		Et_3Pb^+		Et_2Pb^{2+}	
	Light	Dark	Light	Dark	Light	Dark
0	0.29		0.002		<0.001	
1	0.44	—	0.05	—	<0.001	—
2	0.67	—	0.14	—	<0.001	—
3	0.967 ±0.806	1.100 ±0.930	1.590 ±1.500	0.082 ±0.041	<0.002 ±0.002	<0.001 ±0.001
5	0.43	0.23	0.77	0.09	<0.006	<0.001
6	0.19	—	0.96		<0.015	—
7	0.11	0.19	1.17	0.16	<0.17	<0.001

[a]The figures given in the table are average values. Most data were available for the 3 day systems and the standard deviations on the 3 day values give an indication of the wide variations in the analytical figures.

Poteriochromonas malhamensis assay

The method used was that described by Roderer[6] except that we used only cell titres in our assay whereas Roderer used cell titres, nuclear index and percentage polyploidy in analyses. The bioassay was simple. A known density of cells was incubated in EVT medium (Culture Centre for Algae and Protozoa, 1974)[12] in the presence of known concentrations of the organolead compounds for several days. The cell titres for dosed cultures were compared with those of control cultures.

RESULTS AND DISCUSSION

Effect of TALs on cultures of Poteriochromonas malhamensis

TAL compounds are very insoluble in water and only about $0.3\,mg\,dm^{-3}$ of TEL will dissolve in water according to the literature.[13] This amount of material would be rapidly lost from the aerated medium and in order to maintain the TEL in solution and study its effect on the algae over a period of time it is necessary to use emulsified suspensions and to keep a reservoir of TEL. Light and dark cultures of *P. malhamensis* were exposed to a suspension of $62.5\,\mu l$ TEL dm^{-3}. The cell count continued to increase in the dark culture whereas the illuminated culture showed a continuous decrease in growth. After 7 days there was a slight decrease in cell density in the dark culture but the effect was small compared with that of the illuminated culture (Figs. 1 and 2).

The media were analysed at intervals during the incubation period using DPASV and GLC–MS for the ionic and TAL compounds respectively. The use of DPASV made it possible to determine R_3Pb^+ and R_2Pb^{2+} derivatives directly. Analyses of aliquots taken separately from these heterogeneous media did not give reproducible results. Separate analyses on any one withdrawal gave the normal high reproducibility of the method[7,8] but analyses on separate withdrawals gave wide variations in the results (Tables 1, 2 and 7). One might not have

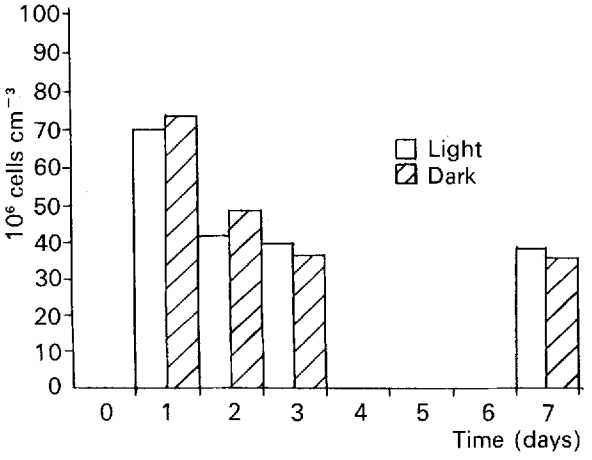

Figure 1 Typical growth of *P. malhamensis* in light and dark.

Table 2 Concentrations of ethylleads in algae (*P. malhamensis*) and media after 3 days exposure to 62.5 μl TEL dm^{-3}

	Medium			Algae			
Culture	TEL	Et_3Pb^+ (mg dm^{-3})	Et_2Pb^{2+}	Et_2Pb^+ (μg/10^6 cells)	Et_3Pb^+	Et_3Pb^- (% accumulation)	Et_2Pb^{2+}
1 light	1.80	3.23	<0.014	1.15	<0.09	0.44	32
2 light	0.19	0.28	<0.007	2.95	<0.18	13	33
3 light	0.91	1.26	<0.003	0.95	<0.06	0.95	26
4 dark	2.80	0.08	<0.002	0.17	<0.03	2.7	23
5 dark	2.00	0.06	<0.001	0.17	<0.06	3.5	75
6 dark	2.10	0.05	<0.002	0.49	<0.08	13	50
7 control	<0.001	<0.001	<0.001	<0.005	<0.001	—	—

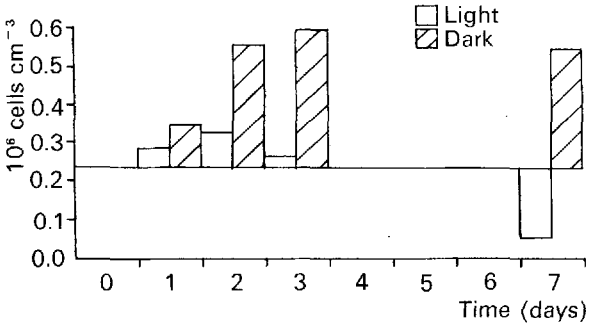

Figure 2 The effect of 62.5 μl TEL dm^{-3} on the growth of *P. malhamensis* in light and dark.

expected to obtain highly reproducible TEL analyses from these heterogeneous media, since when samples are withdrawn some suspended material and surface film may be removed as well as the measured amount of solution. This indeed may be why there is such wide variation[11,13] in the literature solubility values for TEL. Greater consistency would be expected in the analyses for the soluble Et_3Pb^+ and Et_2Pb^{2+} compounds. In fact considerable differences were observed even in the figures for these soluble ionic derivatives (Tables 1, 2 and 7.) The simplest explanation which would account for the wide variation in the analytical results for the ionic compounds is that much of this ionic material is adsorbed on the suspended TEL and in this form is not freely soluble in solution.

Taking the average values given in Table 1 it is observed that in both light and dark incubated cultures TEL concentrations tend to decrease with time. Since a reservoir of TEL remains on the bottom throughout the experiment, presumably aeration removes the suspended TEL and this is not replaced very rapidly from the reservoir. In the light incubations, highly toxic levels of Et_3Pb^+ are found even after one day and the levels continue to increase. As would be expected Et_3Pb^+ concentrations in the dark medium are much lower than in the illuminated medium. Apparently the Et_3Pb^+ produced by decomposition of TEL shows less toxicity than Et_3Pb^+ added directly to the medium. The light incubated cultures to which TEL has been added over the 4 day period show toxic effects comparable to cultures grown in the presence of about 0.1 mg dm^{-3} of Et_3Pb^+ but in fact analyses indicate that concentrations of Et_3Pb^+ in the TEL are on average much greater than this. The cultures incubated with TEL in the dark show growth similar to that of the controls whereas analyses indicate that these cultures contain on average about 0.1 mg dm^{-3} of Et_3Pb^+, and such concentrations of Et_3Pb^+ when added directly generally produce a considerable reduction in growth (cf. Figs 2 and 4). One could account for these anomalous toxicities in the same way as for the variations in the analytical figures for Et_3Pb^+ and Et_2Pb^+ in the presence of TEL, that is that the ionic Et_3Pb^+ is adsorbed on the TEL and is less toxic in this complexed form than in the free form.

TML showed similar effects to TEL (Fig. 3). Organolead analyses on the TML solutions (Table 3) were much more reproducible than for TEL. This may be because TML is more soluble than TEL and certainly it appears to be less oily and to spread less well. The illuminated cultures were inhibited to significantly greater degree than dark grown cultures, but the difference in growth for the light and dark cultures was smaller for TML than for TEL. This difference can be ex-

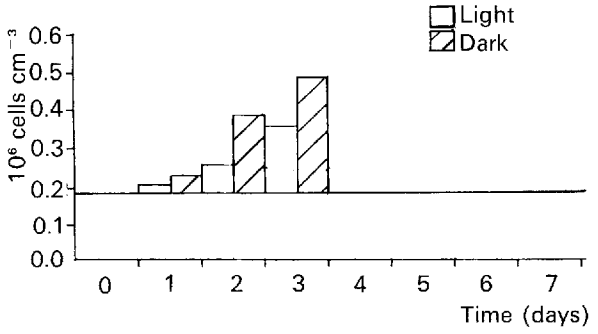

Figure 3 The effect of 50 μl TML dm^{-3} on the growth of *P. malhamensis* in light and dark.

plained by the lower stability of TML in the dark. After 3 days incubation, the mean $[Et_3Pb^+]$ in illuminated cultures was about $20 \times [Et_3Pb^+]$ in dark cultures but after a similar period the mean $[Me_3Pb^+]$ in illuminated cultures was only about $2 \times [Me_3Pb^+]$ in dark cultures. There is still a considerable difference in the inhibition in the light and dark systems suggesting that the toxicity is due to Me_3Pb^+ and that TML in its pure form, like TEL, is non-toxic.[6]

The insolubility of TALs in water and the use of suspensions rather than solutions introduced problems into the analysis of the algae just as it did in the analyses of the media. The TALs are denser than water and in consequence sedimented out with the algae on centrifugation. Washing with an organic solvent was impossible since it would have an adverse effect on the cells and one could not be certain that all the TALs would be removed by washing with water. Therefore it was impossible to measure the uptake of the TALs themselves by the algae. However, the adsorbed R_3Pb^+ and R_2Pb^{2+} species could be removed by washing with water and uptake of these ionic species by algae exposed to TALs could be measured (Tables 2 and 3).

After incubation, concentrations of Et_3Pb^+ and Et_2Pb^{2+} are higher in illuminated cells than dark cells (Table 2), as would be expected, since TEL breaks down more rapidly in the light than dark. Algal accumulation expressed in $\mu g/10^6$ cells is greater in the light grown than in the dark grown cultures. Percentage accumulations vary widely:

$$\text{Percentage accumulation} = \frac{[\text{organolead } (\mu g)] \text{ in } 10^6 \text{ cells} \times 10^2}{\text{total organolead } (\mu g) \text{ in medium}}$$

On average, percentage accumulations tend to be higher for the dark than the light systems.

Uptake of Et_3Pb^+ is greater for dark grown cells exposed to TEL suspensions than to Et_3Pb^+ solutions (cf. Tables 2 and 4) although on average lower concentrations of Et_3Pb^+ are present in the TEL media. The cells from the TEL dark incubations contained concentrations of Et_3Pb^+ two orders of magnitude greater than those from Et_3Pb^+ exposed incubations, which suggests that the lipid soluble TAL is transporting the ionic organoleads across the cell boundary. Since the dark-grown cultures appear to be almost unaffected by exposure to TEL (Fig. 2) it would appear that within the cell the ionic organoleads still remain closely associated with the TEL and in this complexed form are not freely available to the algae.

With TML as with TEL higher concentrations of Me_3Pb^+ and Me_2Pb^{2+} were found in light than in dark grown cells. Percentage accumula-

Table 3 Concentrations of methylleads in algae (*P. malhamensis*) and media after 3 days exposure to 62.6 μl TML dm^{-3}

	Medium			Algae			
Culture	TML	Me_3Pb^+ (mg dm^{-3})	Me_2Pb^{2+}	Me_3Pb^+ ($\mu g\ 10^3/10^6$ cells)	Me_2Pb^{2+}	Me_3Pb^+ (% accumulation)	Me_2Pb^{2+}
1 light	3.00	0.30	0.01	30.0	<0.18	0.1	0.02
2 light	2.62	0.28	<0.002	7.8	<0.19	0.04	0.15
3 light	2.54	0.27	<0.002	26.0	1.67	0.12	1.3
4 dark	2.21	0.10	<0.002	19.0	<0.19	0.24	0.17
5 dark	2.41	0.14	<0.001	13.0	1.13	0.12	1.0
6 dark	2.25	0.12	<0.002	38.3	<0.30	0.39	0.25
7 control	<0.001	<0.001	<0.001	<0.001	<0.001	—	—

Table 4 Concentrations of ethylleads in algae (*P. malhamensis*) amd media after 3 days exposure to Et_3PbCl (0.1 mg dm^{-3})

	Medium		Algae			
Culture	Et_3Pb^+ (mg dm^{-3})	Et_2Pb^{2+}	Et_3Pb^+ (μg $10^3/10^6$ cells)	Et_2Pb^{2+}	Et_3Pb^+ (% accumulation)	Et_2Pb^{2+}
1 light	0.086	<0.002	1.6	<0.6	0.02	0.4
2 light	0.074	<0.002	1.5	<0.5	0.03	0.5
3 light	0.076	<0.001	2.1	<0.1	0.03	0.5
4 dark	0.08	<0.00	1.0	<2.1	0.02	1.3
5 dark	0.10	<0.00	2.2	<2.3	0.03	1.4
6 dark	0.09	<0.002	1.9	<2.2	0.03	1.4
7 control	<0.001	<0.001	<0.01	<0.01	—	—

Table 5 Concentrations of methylleads in algae (*P. malhamensis*) and media after 3 days exposure to Me_3PbCl (0.1 mg dm^{-3})

	Medium		Algae			
Culture	Me_3Pb^+ (mg dm^{-3})	Me_2Pb^{2+}	Me_3Pb^+ (μg $10^3/10^6$ cells)	Me_2Pb^{2+}	Me_3Pb^+ (% accumulation)	Me_2Pb^{2-}
1 light	0.11	<0.003	1.8	<0.2	0.02	0.08
2 light	0.09	<0.002	1.3	<0.2	0.02	0.16
3 light	0.10	<0.002	1.6	<02	0.02	0.17
dark	0.11	<0.002	1.3	<0.2	0.02	0.16
dark	0.11	<0.002	1.2	<0.1	0.02	0.08
control	<0.001	<0.001	<0.1	<0.001	—	—

tions were rather more consistent in this system and as with TEL tended to be rather higher for the dark than for the light grown cultures. Again, as with the ethyl system, uptake of Me_3Pb^+ was greater for dark grown cells exposed to TML suspensions than to Me_3Pb^+ solutions (cf. Tables 3 and 5) although the average concentration of Me_3Pb^+ is similar in both media.

Effect of R_3Pb^+ derivatives on cultures of Poteriochromonas malhamensis

Cultures were grown in solutions containing 0.1 mg dm^{-3} of the trialkyllead salts. This represented roughly the concentrations of R_3Pb^+ produced in the dark TAL incubations and was chosen so that one might get an assessment of the contribution of the R_3Pb^+ salt to the TAL toxicity. Both Me_3Pb^+ and Et_3Pb^+ (Figs 4 and 5) depressed the growth of the cultures. The Et_3Pb^+ was markedly more toxic than Me_3Pb^+. *P. malhamensis* was cultured in both light and dark to assess the effect of light on toxicity. Trialkyllead salts undergo photolytic decomposition to give the much less toxic dialkyl and tetraalkylleads (Eqn. 1):

$$2R_3Pb^+ \rightarrow R_2Pb^2 + R_4Pb. \qquad [1]$$

It was thought that during the incubation period sufficient photolysis might occur to produce a reduction in toxicity. There was, however, no evidence that such a light induced detoxification occurred for either compound. Analyses of the media indicated that there was little change in trialkyllead concentration throughout the incubation period (Tables 4 and 5). Although trialkyllead salts are known to undergo the above disproportionation, no TALs were detected in solutions or cells, only R_3Pb^+ and R_2Pb^{2+}. Uptake by the cells and percentage accumulations of Et_3Pb^+ and Me_3Pb^+ are very similar and illumination does not greatly influence uptake. Data for the dialkylleads are more varied. Concentration factors for Me_2Pb^{2+} are very similar in both light and dark media. Uptake of

Et_2Pb^{2+} occurs generally to a greater extent than for Me_2Pb^{2+} and the percentage accumulation of Et_2Pb^{2+} in dark cultures is rather more than twice that for illuminated cultures. It is noticeable that percentage accumulation of both dialkyllead compounds is generally an order of magnitude greater than that of the corresponding trialkyllead compound. This could be due to preferential dialkyllead uptake or to metabolism within the cell. The greater accumulation in the dark supports the metabolic pathway, since one might not expect illumination to affect uptake. However, it would promote the breakdown of the dialkylleads to inorganic lead. This must be considered as a conjecture rather than as a conclusion since it must be pointed out that the dialkyllead results are less accurate than the trialkyllead because of the very low concentrations of dialkyl being determined. Aqueous concentrations of both dialkyls were calculated as 'less than' values by the polarograph computer. This means that the peaks obtained from the DPASV were so low in comparison to the standard addition peak that the concentrations could only be calculated to an accuracy of less than a maximum value. Thus percentage accumulations of dialkylleads must be regarded as rough estimates and emphasis should be placed on differences rather than actual figures.

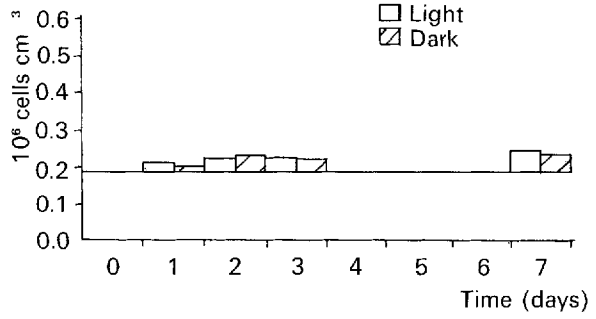

Figure 4 The effect of 0.1 mg dm^{-3} Et_3Pb^- on the growth of *P. malhamensis* in light and dark.

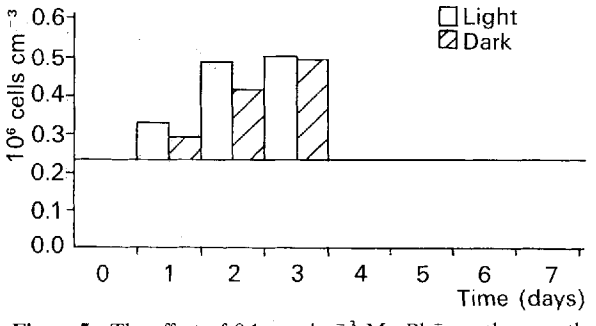

Figure 5 The effect of 0.1 mg dm^{-3} Me_3Pb^- on the growth of *P. malhamensis* in light and dark.

The effect of TEL on cultures of Phaeodactylum tricornutum

The uptake of ^{14}C by *P. tricornutum* was determined over a 6 hour period and used as a measure of photosynthetic activity. Algae exposed to suspensions of 62.5 μl TEL dm^{-1} and 125 μl TEL dm^{-3} showed 36 and 29% respectively of the normal photosynthetic activity.

It was not possible to analyse the active medium, so parallel incubations were set up under the same conditions except that ^{14}C was omitted from the AE50 medium. Samples were removed hourly and analysed. It did not at first seem likely that much decomposition of the TEL would occur within the short time period of these incubations but, in fact, relatively high concentrations of Et_3Pb^+ were found after 20 minutes, and the concentrations increased with time (Table 6). It may be that the strong illumination and cations present in the artificial seawater promote this rapid breakdown of the TEL. The organolead analysis in the heterogeneous AE50 media are, like the analyses in the EVT media (cf. Tables 1 and 6), not highly reproducible. But considering average values it would appear that even within the short time period of the incubation, sufficient Et_3Pb^+ is produced to account for all the observed toxicity (Fig. 6) and these results suggest that TEL is non-toxic to *P. tricornutum* just as it is to *P. malhamensis*.[6]

Uptake of the ethylleads from TEL suspension by *P. tricornutum* tends to be lower than for *P. malhamensis* (cf. Tables 2 and 7) but again there is some indication that accumulations are greater for dark than for light cultures.

The effect of trialkylleads on cultures of phaeodactylum tricornutum

As observed previously the photosynthetic activity of *Phaeodactylum* decreases with increasing trialkyllead concentration. The relationship is linear over most of the concentration range but there is a levelling off at higher concentrations. Maddock and Taylor[3] found that the triethyl compound was more effective than the trimethyl in inhibiting photosynthetic activity and we have found that this trend continues with increasing chain length; the tributyl compound is about an

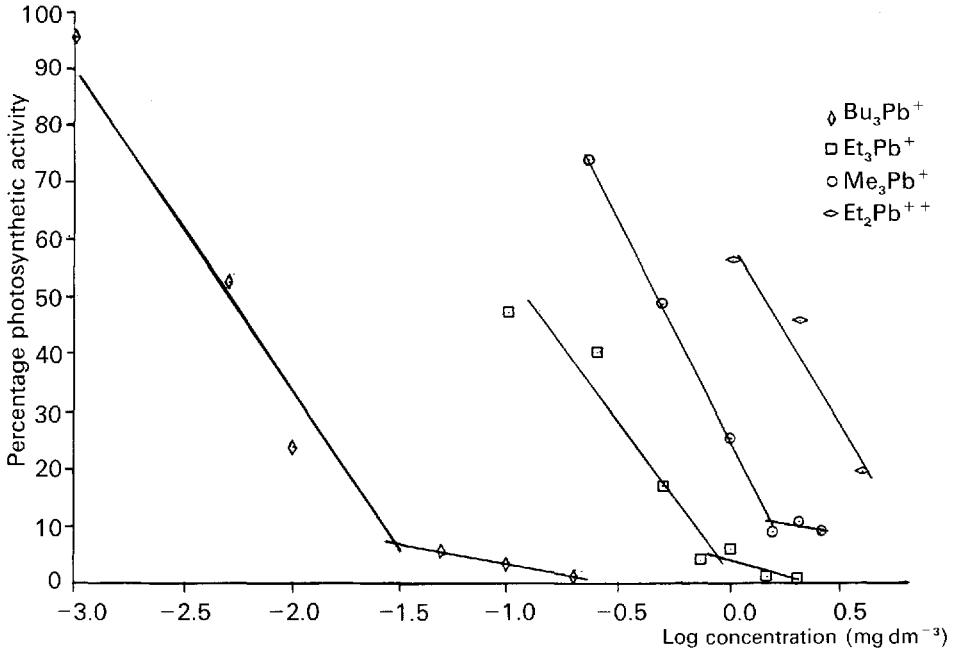

Figure 6 Effect of alkyllead compounds on the photosynthetic activity of *P. tricornutum*.

Table 6 Concentrations (mg dm^{-3}) of ethylleads in AE50 medium dosed with TEL

Time (h)	Run 1		Run 2	
	TEL	Et_3Pb^+	TEL	Et_3Pb^+
0.5	0.88	0.25	2.50	2.20
1	0.25	0.76	0.23	0.23
2	0.25	1.00	0.22	0.23
3	0.23	0.50	0.14	0.25
4	0.23	0.52	0.18	0.35
5	0.25	0.96	0.18	0.35
6	0.25	1.02	0.10	0.25

order of magnitude more toxic than the triethyl derivative (Fig. 6).

There are many reports in the literature on the ameliorative effect of selenium on metal toxicity. It has been shown to afford some protection to various species against the effects of mercury,[15,16] cadmium, arsenic and other heavy metal compounds.[17] There are however no reports in the literature on how selenium affects the activity of organometallic compounds. Using the ^{14}C incubation technique, cultures of the diatom were exposed to Et_3Pb^+ and Et_2Pb^{2+} and various concentrations of selenite (added as Na_2SeO_3). It was found that ^{14}C uptake was unaffected by

Table 7 Concentrations of ethylleads in algae (*P. tricornutum*) and media after 3 days exposure to 62.5 μl TEL dm^{-3}

Culture	Medium			Algae			
	TEL	Et_3Pb^+ (mg dm^{-3})	Et_2Pb^{2+}	Et_3Pb^+ (μg/10^6 cells)	Et_2Pb^{2+}	Et_3Pb^+ (% accumulation)	Et_2Pb^{2+}
1 light	9.30	2.08	<0.019	0.65	0.07	0.39	4.60
2 light	0.91	1.64	<0.016	0.49	0.01	0.37	0.78
3 dark	0.33	0.21	<0.002	0.19	0.07	1.12	43.8
4 control	<0.001	<0.002	<0.001	<0.001	<0.001	—	—

concentrations of selenium up to 5 $\mu g\ cm^{-3}$, but that 10 $\mu g\ cm^{-3}$ reduced uptake by about 15% (Table 8). The inhibition of photosynthesis by higher levels of Se is not unexpected since Se itself is known to be toxic above certain critical concentrations. Since it has been suggested that selenium acts by diverting the heavy metal to less critical tissue areas[18] which is obviously impossible in a unicellular organism, it may be that different effects will be observed in higher systems.

Sulphide which is abundant in anoxic systems has been shown to catalyse the conversion[19] of R_3Pb^+ to TALs (Eqn. 2):

$$2R_3Pb^+ \xrightarrow{S^{2-}} R_4Pb + R_2Pb^{2+} \qquad [2]$$

It was thought that sulphide might reduce the toxicity of R_3Pb^+ to *Phaeodactylum* by converting it to less toxic forms. Addition of sulphide did produce a slight reduction in the toxicity of R_3Pb^+ but the effect was not significant (Table 8).

Table 8 Effect of selenium and sulphur on the toxicity of ethyllead compounds to *P. tricornutum*

Compound	Ethyllead ($mg\ dm^{-3}$)	Na_2SeO_3 or Na_2S ($mg\ dm^{-3}$)	Photosynthetic activity (% normal)
	1.0	0.00	48
Et_3PbCl	0.25	1.0[a]	46
	0.25	10.0[a]	39
	2.00	0.0	45
Et_2PbCl_2	2.00	5.0[a]	47
	4.00	0.0	19
	4.00	10.0[a]	20
	0.00	1.0[a]	99
—	0.00	5.0[a]	101
	0.00	10.0[a]	85
	0.25	0.125[b]	48
Et_3PbCl	0.25	1.00[b]	52
	0.25	10.00[b]	28
	0.00	1.00	101
—	0.00	10.00	70

[a]Na_2SeO_3, [b]Na_2S.

CONCLUSION

Problems were encountered in the analysis of the heterogeneous TEL containing media. Separate analyses on any one withdrawal gives high reproducibility, but analyses on separate withdrawals gives wide variations in the results for TEL, Et_3Pb^+ and Et_2Pb^{2+}. The variation in TEL values can be attributed to contamination of the solution sample with suspended material and surface film. The simplest explanation for the variations in the values for the ionic compounds is that much of the ionic material is adsorbed on the TEL and in this complexed form is not freely soluble in solution. Organolead analyses on TML media are much more consistent than for TEL media, probably because of the greater solubility of TML in water.

Regardless of the analytical problems, the results confirm the previous findings[6] that TML and TEL are non-toxic to algae and it is the P_3Pb^+ breakdown products which are responsible for the apparent toxicity of TALs.

Complexing of the ionic organoleads with TALs leads to a number of unexpected results. It is found that dark grown TAL cultures show growth comparable to the controls even though the culture media and cells contain highly toxic concentrations of R_3Pb^+. This suggests that the complexed ionic organoleads are not freely available to the biological system and that these organoleads are much less toxic in the complexed than in the free form. Since concentrations of R_3Pb^+ in TAL exposed cells are several orders of magnitude higher than those found in cells exposed directly to R_3Pb^+ solutions, it would appear that complexing with lipophilic TALs also provides a means for transporting ionic organoleads across cell boundaries.

For the several alkyllead species studied, the trialkylleads were the most toxic and within the trialkyl series toxicity increased with alkyl chain length. Neither selenide nor sulphide had any significant ameliorative effect on alkyllead toxicity.

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