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METABOLISM OF TRIALKYLLEAD AND DIALKYLLEAD COMPOUNDS BY A FRESHWATER ALGA, ANKISTRODESMUS FALCATUS

P.T.S. Wong, Y.K. Chau, J.L. Yaromich
and O. Kramar

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Environmental Contaminants Division
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

• Environment Canada

EXECUTIVE SUMMARY

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P.T.S. Wong, Y.K. Chau, J.L. Yaromich and O. Kramar

Great Lakes Fisheries Research Branch and National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, L7R 4A6

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The results suggest that algae can play an important role in the cycling of organic lead compounds in aquatic ecosystems.

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SOMMAIRE ADMINISTRATIF

Métabolisme des composés trialcoyle et dialcoyle de plomb par une algue d'eau douce : Ankistrodesmus falcatus

P.T.S. Wong, Y.K. Chau, J.L. Yaromich et O. Kramar

Direction de la recherche sur les pêches dans les Grands Lacs et Institut national de recherche sur les eaux, Centre canadien des eaux intérieures, Burlington (Ontario) L7R 4A6

On a découvert qu'une algue d'eau douce, Ankistrodesmus Falcatus, mise en présence de composés de trialcoyle et de dialcoyle de plomb et de plomb (II) accumulait ces composés par des facteurs de concentration de 100, 2 000 et 20 000 respectivement. On a fait incuber l'algue dans du plomb triméthyle et diméthyle pendant une longue période (28 jours), et on s'est aperçu que l'organisme pouvait métaboliser ces composés. Pour le plomb triméthyle, les réactions métaboliques suivent un processus de déalcoylation entraînant la formation de plomb diméthyle et de plomb (II). On a découvert que l'algue accumulait non seulement le plomb diméthyle, mais produisait aussi des quantités importantes de plomb triméthyle et de plomb (II). Ni la dismutation ni la photodécomposition ne peuvent expliquer la grande quantité de composés produits.

Ces résultats suggèrent que l'algue en question peut jouer un rôle important dans le cycle des composés du plomb organique dans les écosystèmes marins.

METABOLISM OF TRIALKYLLEAD AND DIALKYLLEAD COMPOUNDS BY A FRESHWATER ALGA, *Ankistrodesmus falcatus*.

P. T. S. Wong
Department of Fisheries and Oceans
Great Lakes Fisheries Research Branch
Canada Centre for Inland Waters
Burlington, Ontario
L7R 4A6

Y. K. Chau
Department of Environment
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario
L7R 4A6

J. L. Yaromich and O. Kramar
Department of Fisheries and Oceans
Great Lakes Fisheries Research Branch
Canada Centre for Inland Waters
Burlington, Ontario
L7R 4A6

Wong, P.T.S., Y.K. Chau, J. L. Yaromich and O. Kramar. Metabolism of trialkyllead and dialkyllead compounds by a freshwater alga, *Ankistrodesmus falcatus*. Can. J. Fish. Aquat. Sci. 00: 000-000.

A freshwater green alga, *Ankistrodesmus falcatus*, exposed to solutions of trialkyllead, dialkyllead and inorganic lead (II) compounds (1 mg/L) for 24 h was found to accumulate these compounds with concentration factors of about 100, 2000 and 20000 respectively. Incubation of this alga with trimethyllead and dimethyllead species over a long period (28 d) revealed the ability of the organism to metabolize these compounds. The metabolic processes for

trimethyllead followed a dealkylation sequence with the formation of dimethyllead and lead (II) compounds. In the case of dimethyllead, the alga was found not only to accumulate dimethyllead, but also contain significant amounts of trimethyllead and lead (II) compounds. Neither chemical disproportionation reactions nor photo-decomposition could account for the quanitities produced.

Organolead compounds in the environment have caused much concern because of their inherent toxicity and wide-spread occurrence (Grandjean and Nielsen, 1979; Chau and Wong, 1984). There are two types of organolead compounds of environmental concern. One type is the tetraalkyllead (R_4Pb) which is volatile and insoluble in water. This includes tetraethyllead (Et_4Pb) and tetramethyllead (Me_4Pb) which are used as antiknock additives in gasoline. These compounds may be degraded in the aquatic environment into trialkyllead (R_3Pb^+), dialkyllead (R_2Pb^{2+}) and finally to inorganic lead (Pb^{2+}) species. There are no known monoalkyllead (RPb^{3+}) compounds due to their extreme instability. Tetraalkyllead compounds have been detected in water, fish, algae and other organisms from freshwater and marine environment (Sirota and Uthe, 1977; Chau et al., 1985). The second type is ionic species such as R_3Pb^+ and R_2Pb^{2+} which are used as biocides. These compounds are hydrophilic and are present in solution as salts. Their occurrence in the aquatic environment has also been reported (Chau et al., 1984 and 1985).

Accumulation of R_4Pb has been studied in fish (Maddock and Taylor, 1980; Wong et al., 1981) and in algae (Silverberg et al., 1977). Little information is available on the accumulation of R_3Pb^+ and R_2Pb^{2+} by

aquatic organisms . In this report, the ability of a freshwater green alga, Ankistrodesmus falcatus, to metabolize dimethyllead (Me_2Pb^{2+}) and trimethyllead (Me_3Pb^{+}) compounds is presented.

MATERIALS AND METHODS

Trimethyllead acetate and triethyllead acetate (Et_3Pb^{+}) were obtained from Alfa Chemicals (Danvers, MA). Dimethyllead chloride (Me_2Pb^{2+}) and diethyllead chloride (Et_2Pb^{2+}) were gifts from Associate Octel Co. (S. Wirral, Great Britain). Tetramethylammonium hydroxide (TMAH) was from Fisher Chemicals; sodium diethyldithiocarbamate (NaDDTC) from Baker; n-butyl Grignard reagent in tetrahydrofuran from Alfa Co. All other reagents and solvents were commercially available in high purity grade.

Algal culture--The axenic culture of Ankistrodesmus falcatus var. acicularis (Ontario Ministry of the Environment, Rexdale, Ontario) was grown in 1 L of Bold medium (Nichols and Bold, 1965)) at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light (5000 lx) and 6 h of darkness. When the culture reached the logarithmic phase of growth in about 7 d, it was used as inoculum.

Accumulation of R_3Pb^{+}, R_2Pb^{2+} and Pb^{2+} by alga in 24 h--The algal culture was inoculated into six 250-mL erlenmeyer flasks, each containing 200 mL of Bristol medium to give a cell biomass of 7×10^5 cells/mL (62 µg dry wt/mL). A concentrated stock solution of $Pb(NO_3)_2$, Et_3Pb^{+}, Me_3Pb^{+}, Et_2Pb^{2+} or Me_2Pb^{2+} prepared in double-distilled water was added to the flask to give an initial concentration

of 1 mg/L as Pb in the medium. The sixth flask without Pb addition was used to determine the Pb contamination from the culture and medium. The flasks were incubated in a shaker under a constant light intensity of 5000 lx. After 24 h of incubation, the culture was separated from the medium by centrifugation at 16000 x g for 20 min. The pellet was resuspended in 1 mL of the fresh medium. The cell suspension and 100 mL of the supernate were used for Pb analysis.

Accumulation and degradation of Me_3Pb^+ and Me_2Pb^{2+} by alga in 28 d--Four 10-L glass bottles, each containing 5 L of the medium, were sterilized by autoclaving. Upon cooling, two bottles were inoculated aseptically with 40 mL of the algal culture to give the biomass of 1.2×10^5 cells/mL (11 μg dry wt/mL). The other two bottles were not inoculated with the culture and were used to determine the process of chemical degradation and photodegradation. A concentrated stock solution of Me_3Pb^+ or Me_2Pb^{2+} was added to each of the two bottles (with and without the culture) to give an initial concentration of 1 mg/L in the medium. The culture and medium were mixed continuously with a magnetic stirrer and incubated under a constant light intensity of 5000 lx at 20°C. One L of the medium was removed from each of the four bottles immediately after the addition of the alkyllead compounds and after 7, 14, 21 and 28 d of incubation.

Determination of alkyllead and Pb species in medium--One hundred mL of medium was extracted with 5 mL of aqueous 0.5M NaDDTC, 5 g of NaCl and 5 mL of benzene for 30 min (Chau et al., 1985). The benzene phase was carefully evaporated in a rotary evaporator to 1 mL in a 15-mL centrifuge tube to which 0.2 mL of n-butyl Grignard reagent was added. The mixture was gently mixed

for 1 min and washed with 2 mL of 0.5M H_2SO_4. The organic phase was dried in anhydrous Na_2SO_4. Appropriate amounts (10-20 μL) were injected into the gas chromatography-atomic absorption spectrometry (GC-AAS) system for analyses (Chau et al., 1983). Detection limit for Pb in the medium was 50 ng/L.

Determination of alkyllead and Pb species in alga--The algal culture was digested with 5 mL of 20% TMAH in a water bath at 60°C for 2 h. After cooling, the mixture was neutralized with 50% HCl to pH 6-8 and extracted with 3 mL of 0.5M NaDDTC, 2 g of NaCl and 3 mL of benzene for 1 h. The mixture was centrifuged and 1 mL of the benzene phase was transferred to a glass-stoppered vial for butylation with 0.2 mL of n-butyl Grignard reagent. The mixture was washed with 2 mL of 0.5M H_2SO_4 and the benzene phase was dried in anhydrous Na_2SO_4. Aliquotes (10-20 μL) were injected to the GC-AAS system for Pb determination. Detection limit for Pb in alga was 150 ng/g.

Measurement of algal biomass--Algal biomass was enumerated under a microscope with a Petroff-Hausser counting chamber (Frobisher, 1968) and was then related to a previously constructed dry weight curve. All cell count data were based on the average of three separate counts of each sample.

RESULTS AND DISCUSSION

Recovery of ionic alkyllead and Pb species from medium and alga--To determine the quantitative recovery of the alkyllead and inorganic Pb compounds from medium and alga, known concentrations of these compounds were

added into the medium and algal pellet, incubated for one hour and then extracted with benzene (see Materials and Methods). With this technique, there was almost complete recovery (90-100%) of the Pb compounds from the medium and alga (Table 1). Birnie and Hodges (1981) used a combination of solvent extraction and a differential puse anodic stripping voltammetric technique for the isolation and determination of ionic alkyllead species in marine fauna tissues. Their recovery of Et_2, Et_3 and Me_3Pb^+ from the tissues was only 60-90% while the recovery of Me_2Pb^{2+} was poor (10-40%). Since the technique for speciation of ionic alkyllead was not available until recently (Birnie and Hodges, 1981; Chau et al., 1984), more data on ionic alkyllead in the aquatic system should be available in the near future.

Accumulation of alkyllead and Pb species by alga--A. falcatus was exposed to various alkyllead compounds at 1 mg/L, a level below toxicity (Wong et al., unpublished data) for 24 h. The short incubation time was used to lessen the possible biotic and/or abiotic degredation of the compounds. The results (Table 2) indicate that the alga was able to accumulate inorganic Pb the most with a concentration factor of 20135, followed by R_2Pb^{2+} (with concentration factors between 1937 and 2786) and R_3Pb^+ (98 and 170). There was no significant relation between the length of the alkyl chain and accumulation when R was either methyl or ethyl group. The abiotic degradation of the ionic alkyllead species was negligible, less than 5% in 24 h. Accumulation of inorganic Pb by algae may be by surface absorption or by absorption into specific cellular components (Schulz-Baldes and Lewin, 1976; Wong et al., 1978). The value of 6242 μg of inorganic Pb/g wet wt of cells from this experiment was considerably less than 11640 μg/g in two marine

phytoplankton (Schulz-Baldes and Lewin, 1976). The differences may be due to the variation in algal species and culture media used in the experiments. Data on the accumulation of ionic alkyllead compounds by other algae are not available for comparison. In other organisms, Maddock and Taylor (1980) observed very high concentration factors of R_4Pb in shrimp, mussels, and plaice and about one or two orders of magnitude lower concentration factors for the corresponding R_3Pb derivatives. The accumulation by alga was not included in this study. R_4Pb has been shown to penetrate the cell membrane and be deposited in the cytoplasm of algae and animals (Silverberg et al., 1977; Grandjean and Nielsen, 1979).

Uptake and degradation of dimethyllead and trimethyllead--In the experiment on the accumulation of alkyllead and inorganic Pb compounds by the alga (Table 2), the supernate was found to contain other alkyllead compounds at levels higher than could be explained by abiotic degradation. Since no information is available on the degradation of ionic alkyllead compounds by algae, a study was carried out to determine the possibility of degradation of Me_2Pb^{2+} and Me_3Pb^{+} compounds by A. falcatus. The alga was incubated with 1 mg/L of the lead compounds for a period up to 4 wks. At various time intervals, the concentrations of the lead compounds in the cells and in the medium were analysed. An identical set without the algal inoculum was also analysed for abiotic degradation. The results show that Me_2Pb and Me_3Pb in the medium alone (no algae) were quite stable (less than 20% decomposition) under light over 4 wks incubation (Figure 1). However, traces of Me_3Pb^{+} and Pb^{2+} were detected in Me_2Pb^{2+} solution while Me_2Pb^{2+} and Pb^{2+} in Me_3Pb^{+} solution. It is known that R_3Pb^{+} may be disproportionated

to R_2Pb^{2+} and R_4Pb while R_2Pb^{2+} may be disproportionated to R_3Pb^{+} and Pb^{2+} (Chau et al., 1984). Jarvie et al. (1981) also reported that Me_2Pb^{2+} and Me_3Pb^{+} decomposed very slowly in the presence of light with 4-10% loss in 30 d incubation.

In the presence of A. falcatus, the decreases of the alkyllead compounds were much more rapid (Figure 1). An estimated loss of 50% occurred in about 15 d. The disappearance of the alkyllead compounds from the medium mirrored the appearance of these compounds in the cells (Figure 2). Me_2Pb^{2+} was taken up quite rapidly by the cells from the medium and accumulated to a concentration factor of 1.6×10^3. There was a lag period in the uptake of Me_3Pb^{+} and the concentration factor only reached 1.1×10^3. The initial rates of uptake of Me_2Pb^{2+} and Me_3Pb^{+} were estimated as 0.2 and 0.06 mg/g wet wt cells/d respectively. The same algal species was found to accumulate 8×10^3 of tributyltin species (Maguire et al., 1984). Marine algae were reported to concentrate tin by a factor of 2×10^3 over water (Seidel et al., 1980). Concurrent with the accumulation of Me_2Pb^{2+}, the algae also contained significant amounts of Me_3Pb^{+} and Pb^{2+} (Figure 2). Similarly, Me_2Pb^{2+} and Pb^{2+} were detected in the cells incubated with Me_3Pb^{+}. It seemed unlikely that the products in the cells could derive solely from the disproportionation of Me_3Pb^{+} and Me_2Pb^{2+} in the medium since the levels of these compounds were much higher than could be explained by the slow disproportionation. Dealkylation of R_3Pb^{+} and R_2Pb^{2+} in animals have been reported (Jensen, 1984). The mechanism for the dealkylation is believed to be through lipid peroxidation. Studies by Roderer (1984) suggested that algae could not metabolize R_4Pb to R_3Pb. However, no study

was available until now to indicate that algae could metabolize Me_3Pb^+ and Me_2Pb^{2+}. The results also suggest that algae can play an important role in the cycling of lead compounds in the aquatic ecosystems.

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Table 1: RECOVERY OF ALKYLLEAD AND LEAD (II) COMPOUNDS FROM MEDIUM AND ALGAE.

COMPOUNDS	% OF RECOVERY	
	MEDIUM	ALGAE
Pb^{2+}	91	95
Me_2Pb^{2+}	100	90
Me_3Pb^{+}	100	100
Et_2Pb^{2+}	90	95
Et_3Pb^{+}	92	100

Table 2: ACCUMULATION OF ALKYLLEAD AND LEAD (II) COMPOUNDS BY A. FALCATUS AFTER INCUBATING IN 1 mg/L OF THE LEAD COMPOUNDS FOR 24 HRS.

COMPOUNDS	CONC. OF LEAD COMPOUNDS IN SUPERNATE (mg/L)	CELLS (µg/g)	CONC. FACTORS*
Pb^{2+}	0.31	6242	20135
Me_2Pb^{2+}	0.71	1978	2786
Me_3Pb^{+}	1.02	100	98
Et_2Pb^{2+}	0.94	1821	1937
Et_3Pb^{+}	1.00	170	170

*Concentration factor = conc. in algae/conc. in supernate.

Fig. 1. Rate of biotic and abiotic degradation of Me_2Pb^{2+} and Me_3Pb^{+} compounds in 28 d.

Fig. 2. Accumulation and degradation of Me_2Pb^{2+} and Me_3Pb^{+} compounds in A. falcatus.

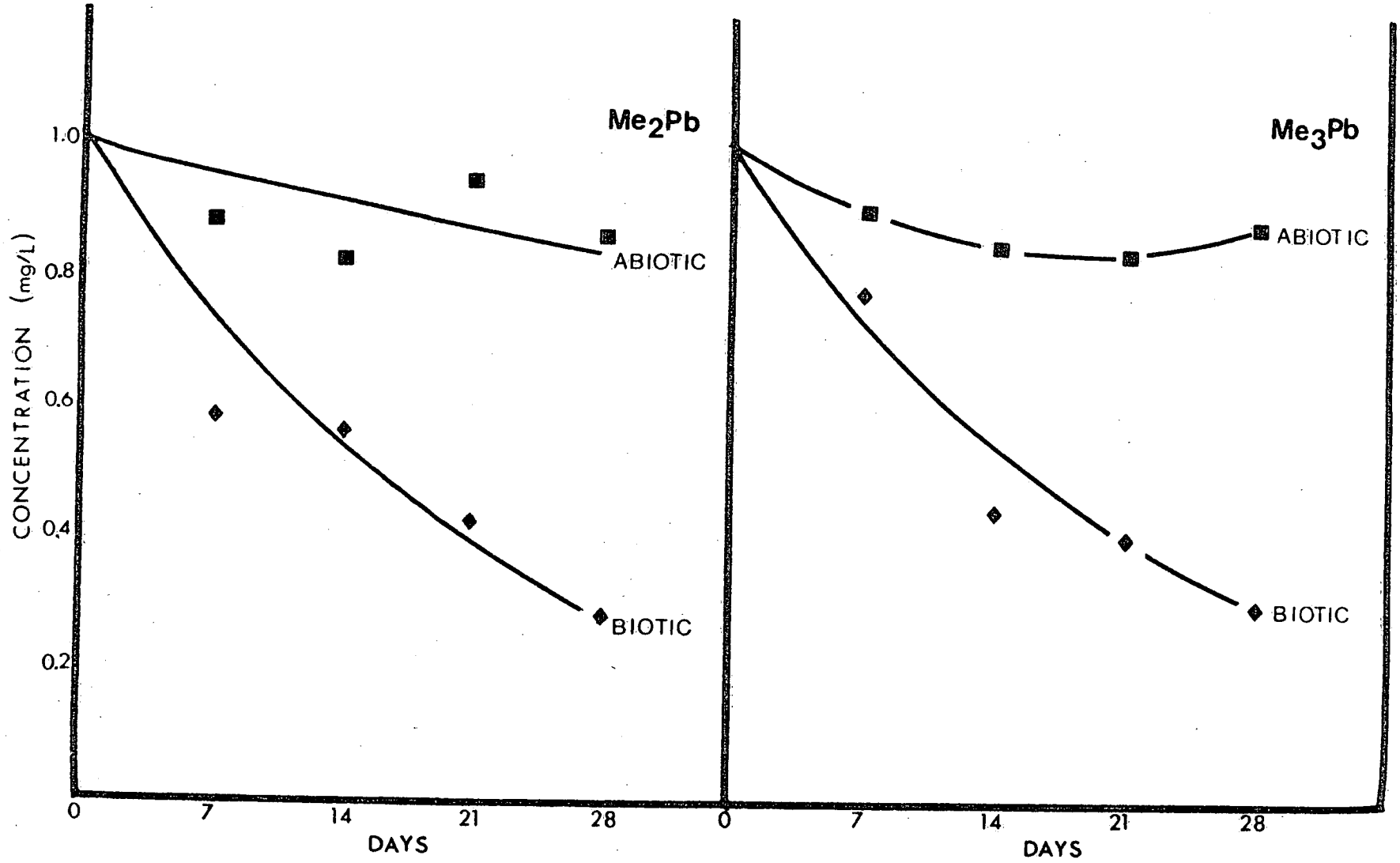
Me2Pb
Me3Pb
ABIOTIC
BIOTIC
ABIOTIC
BIOTIC
CONCENTRATION (mg/L)
1.0
0.8
0.6
0.4
0.2
0
7
14
21
28
DAYS
0
7
14
21
28
DAYS

FIG. 1

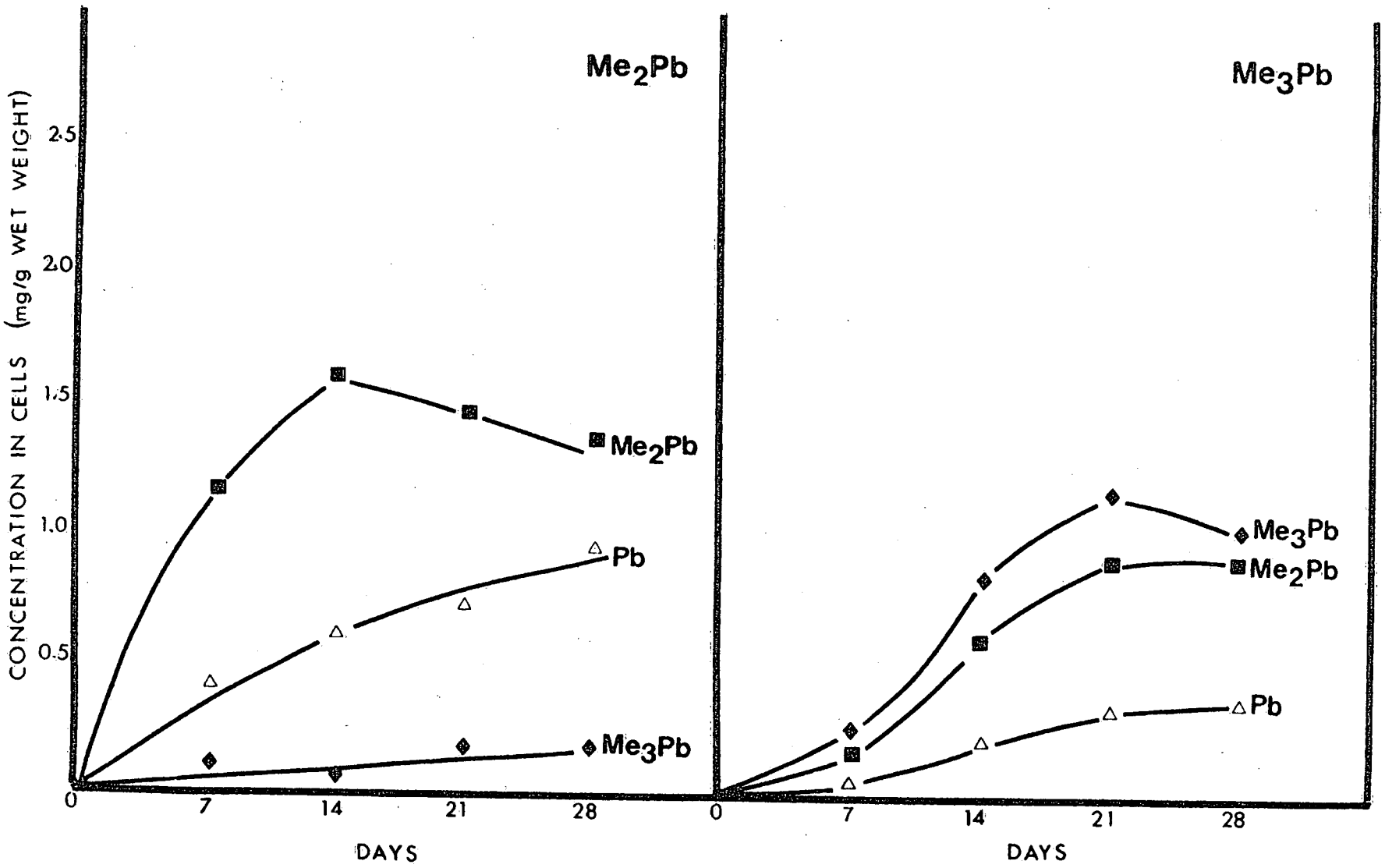
Me2Pb
Me3Pb
CONCENTRATION IN CELLS (mg/g WET WEIGHT)
2.5
2.0
1.5
1.0
0.5
0
7
14
21
28
DAYS
Me2Pb
Pb
Me3Pb
0
7
14
21
28
DAYS
Me3Pb
Me2Pb
Pb

FIG. 2