A thesis entitled

METAL METHYLATION IN ESTUARINE WATERS

presented by

ANDREW PAUL WALTON, B.Sc.

in part fulfilment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

COUNCIL FOR NATIONAL ACADEMIC AWARDS

Department of Environmental Sciences
Plymouth Polytechnic
Drake Circus, Plymouth PL4 8AA

Collaborating establishment
Marine Biological Association
Citadel Hill, Plymouth.

October 1986

Acknowledgements

I wish to express my sincerest gratitude to Professor L. Ebdon for his invaluable guidance and encouragement throughout the duration of this work. I would also like to thank Dr. G.E. Millward for his highly active role as second supervisor and Dr. M. Whitfield for his discussions and help with the environmental studies undertaken in this thesis. I am also grateful to the Natural Environment Research Council for the provision of a studentship and the Marine Biological Association the Collaborating institute.

I am indebited to the chemistry technical staff at Plymouth Polytechnic and the Marine Biological Association for their help with the organization of field work and laboratory experiments.

Finally I would like to thank Mrs. Sandra Kellaway for the typed presentation of this thesis.

Metal Methylation in Estuarine Waters

Andrew Paul Walton

Abstract

Metal methylation has been studied in estuarine waters by directly coupled GC-AAS/liquid nitrogen trapped hydride generation systems and determined accurately. Surveys were carried out in the estuaries of the River Tamar, Carnon and Beaulieu (S. England) over a seasonal cycle.

Methylated forms of Pb were only observed in rain, drain and estuarine water samples collected in the Tamar. In estuarine waters $(CH_3)_3Pb^+$ (<2-10 ng Pb l^{-1}) was the predominant methylated Pb species. Monomethylarsenic and dimethylarsenic species were observed in the water column in all 3 estuaries studied. In the Tamar, Beaulieu and Carnon concentrations of methylated As species were similar with concentrations ranging from <0.02-0.7 µg As l^{-1}. In Tamar porewaters concentrations of methylated As species ranged from <0.02-0.7 µg As l^{-1}. Methylated forms of Se ($Se(CH_3)_2$, 0.5 µg Se l^{-1}) were detected on an occasion in the Carnon.

Modelling studies on Pb methylation suggest that conversion of trimethyllead acetate to TML proceeds by a chemical sulphide-mediated pathway (maximum 2.8% conversion occurred for a biological sediment system). Methylation of inorganic Pb (II) salts was not reproducible (maximum conversion of 0.028% for $PbCl_2$) but when observed a biologically mediated methylation process was invoked. From laboratory modelling studies temporal variations in As speciation observed in the Tamar were attributed to seasonal contributions of methylated As species from 3 sources: (i) macro-algae; (ii) diatoms; (iii) porewaters. The predominance of dimethylarsenic species over monomethylarsenic species in summer reflects the importance of plankton sources. In winter a transition takes place where the predominance of methylated As species occur and concentrations of monomethylarsenic species exceeds that of dimethylarsenic species. This transition is attributed to the increased importance of the porewater source in winter.

The significance of metal methylation in the cycling of Pb, As and Se in estuarine waters appears to follow the order As>Se>Pb. The biomethylation of As being highly significant in the transportation of As in the environment.

CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 Overview

Since the Industrial Revolution of the late 18th century many of the small towns and villages located around the coastline of Britain have undergone expansion to a remarkable extent. This growth has been attributed to the development of port facilities and the attraction of industry, eager to reap the benefits of water transport and water supplies for industrial use. Coastal resorts have also become increasingly popular residential areas. This relocation of industry and human population has put an increased burden on U.K. estuaries in terms of pollution from industrial and urban discharge of waste materials. Anthropogenic perturbation of the natural balance of estuarine systems has been noticeable for nutrients, heavy metals (including radionuclides) and synthetic organic compounds such as polyclic aromatic hydrocarbons (Forstner and Wittmann, 1981).

Heavy metals are regarded with great concern since unlike many other pollutants they can be converted into extremely toxic species. In the estuarine environment inorganic Hg can be methylated to methylmercury (CH_3Hg^+), a form 100 times more toxic to humans than Hg(II) ions (Summers, 1978). The toxicity of metal pollutants has been clearly shown by the catastrophes relating to Hg (Forstner and Wittmann, 1981). Several incidents of Hg poisoning occurred in Japan between 1953 and 1973 (Minamata Bay, 1953; Niigata, 1964; Amakusu, 1973). Poisoning, resulting in death in some cases, was traced to the ingestion of fish and shellfish which had been contaminated with methylmercury. In the case of Minamata, methylmercury was discharged into the Bay in the effluent from a plastics (PVC) manufacturing plant, where it was taken up by the marine organisms present. Investigation of the methylation of Hg has shown that both biologically mediated and abiotic processes may account for the production of methylated forms of Hg from inorganic

Hg (Summers, 1978). Further studies have confirmed that methylation may occur for other heavy metals (Agnes et al., 1971), predictions have been made as to the presence or absence of certain methylated metals (Wood, 1974), however, as yet knowledge on the processes involved is limited (Craig, 1986). The work described in this thesis concentrates on 3 elements known to undergo environmental methylation, namely Pb, As and Se. The significance of the methylation process in the cycling of the elements in estuarine waters is assessed.

1.2 The Biogeochemical Cycling of Metals in Estuaries

From the results of biogeochemical studies (Forstner and Wittmann, 1981) the complex cycling of heavy metals in the estuarine environment has been strongly related to:

(1) Speciation - affected by pH, T, ionic strength and organic complexation;

(2) Adsorption/Desorption processes;

(3) Biological mediation.

The speciation of a metal refers to the individual physico-chemical forms of that metal which together make up its total concentration in a given sample (Florence, 1982). In the estuarine environment the speciation of a metal is directly related to the parameters, pH, temperature, ionic strength (Morris, 1977) and complexation with organic compounds (Stumm and Brauner, 1975) most importantly with soil derived organic compounds (Mantoura et al., 1978). In seawater($pH \geqslant 8$, ionic strength 0.7 M at 35‰) relatively low dissolved organic material is present, typically 0.5-1.5 mg C l^{-1} (Forstner and Wittmann, 1981). As a result the major metal species present are the simple inorganic complexes such as chloride, hydroxide, carbonate and sulphate (Florence, 1982). However, in freshwater ($pH \leqslant 7$, ionic strength 0.1 M at 5‰) much higher concentrations of dissolved organic material is present, typically 10-20 mg C l^{-1}

(Forstner and Wittmann, 1981) as a result higher concentrations of metal-organic complexes can occur. Both Cu and Hg are almost completely (>99.9%)complexed with humic material in freshwater (Mantoura et al., 1978). The abiotic methylation of Hg by humic and fulvic acids present in freshwater sediments has been reported (Nagase et al., 1984). It is possible to conclude from these results that metals follow the geochemistry of organic compounds.

The suspended particulate load in estuarine waters is mainly comprised of clay minerals, organic detritus and freshly precipitated Fe and Mn oxyhydroxides, all of which are capable of sorbing metal cations from solution and in turn releasing equivalent amounts of other cations into solution. The general mechanism which results in cationic exchange is based on the sorptive properties of negatively charged anionic sites e.g., SiOH-, $AlOH_2$- and AlOH- groups in clays, carboxyl and phenolic OH- groups in organic material and FeOH- groups in Fe oxyhydroxides. In the case of clays the cationic exchange capacity although large represents only a minor fraction of the total sorption capacity for metals of the suspended particulate matter in estuaries (Sayles and Mangelsdorf, 1979). The considerable scavenging capacity of Fe and Mn oxyhydroxides in estuarine waters is well documented (Gadde and Laitenen, 1974; Kinniburgh et al., 1976; Swallow et al., 1980). The adsorption of Cu, Mn and Zn on such surfaces has been found to be a function of, pH, activity of competing species and the ionic strength of the estuarine media (Millward and Moore, 1982). The affect of organic material on adsorption-desorption processes in natural waters is complex (Olausson and Cato, 1980). Rashid (1974) reported that Pb, Zn, Cu and Ni could be removed to a considerable extent during the flocculation of organic material. Organic materials are readily adsorbed onto Fe oxyhydroxides (Tipping, 1981) and clays (Perez Rodriguez et al., 1977). Such organic coatings result in a decrease in the sorption capacity of the Fe and clay particles.

Perturbations in the pH, temperature and ionic strength of estuarine waters have a profound affect on the adsorption-desorption reactions of cations with particulate material (Salomons, 1980). Such perturbations are most profound at the freshwater-seawater mixing zone (0-15‰) where various competing processes occur; the increase in pH favours the adsorption of metal cations by ion exchange, however, the increase in ionic strength lowers the activity of metal cations through inorganic complexation and as a result increases the competitive adsorption of major seawater cations (Ca^{2+}, Mg^{2+} etc.). It is unlikely therefore that direct adsorption is of prime importance with regard to heavy metals in the marine environment (Duinker, 1980).

Estuaries are, biologically speaking, fertile areas (Cloern et al., 1983) subsequently any biologically mediated processes involving metal interactions may exert a profound influence on the cycling of metals in the estuarine environment (Wangersky, 1986). Following the discovery that Hg was methylated in biologically active freshwater sediments (Jensen and Jernelöv, 1969) interest arose over the possible methylation of other heavy metals in the environment. The methylation of several heavy metals e.g., Hg, As, Sn and Ge is now known to occur in the environment (Craig, 1986). The process requires the presence of methyl donor compounds, many of which are biologically synthesized e.g., S-adenosyl-methionine (CH_3^+ donor) and methylcobalamin (CH_3^- donor). There has been considerable debate as to whether methylation processes are enzymatic or non-enzymatic. Enzymatic methylation involves the transfer of methyl groups to a metal within the cells of a living organism. This is a truly biological process and the term 'biomethylation' can legitimately be applied to such a process. Wood (1974) has proposed several enzymatic processes for the methylation of Hg and methylcobalamin was considered the natural methyl donor to Hg. Direct environmental

evidence for the enzymatic transfer of methyl groups to Hg is not available to date. Non-enzymatic methylation occurs outside of the cells of a living organism and involves the transfer of methyl groups to a metal from a natural product (metabolite) of an organism. The application of the term biomethylation, by many workers, to the non-enzymatic process is strictly speaking incorrect because the process is essentially non-biological. It is preferable to apply the term environmental methylation to encompass all the possible methylation processes and to use the term 'biomethylation' only when there is direct evidence of an enzymatic reaction.

The term environmental methylation may also be taken to include abiotic methylation processes, a number of which have been demonstrated and reported to involve chemical species such as partially or fully methylated metals and products of biological activity e.g. humic and fulvic compounds (Craig, 1986). Morton (1984) has reported that there are a total of 6 processes by which a methyl group may be transferred to a metal under environmental conditions. These are listed below.

1. Enzymatically;
2. Non-enzymatically;
3. Reactions involving a man-made methylating agent;
4. Disproportionation of a partially methylated species e.g., $(CH_3)_3Pb^+$;
5. Reduction of a one carbon fragment attached to a metal which results in the formation of a methyl group e.g., $-CH_2OH \longrightarrow -CH_3$;
6. Intramolecular rearrangement involving the transfer of a methyl group within a molecule e.g. Mercuric acetate $\longrightarrow$ methylmercury (under illuminated conditions).

The processes of environmental methylation have been shown to be effective in the formation of volatile compounds of As (Woolson and Kearney, 1973;

Cox and Alexander, 1973), Pb (Wong et al., 1975; Schmidt and Huber, 1976) and Se (Francis et al., 1974). In the case of Se evidence has been obtained that suggests methylation only occurs in the presence of certain microorganisms e.g., Aeromonas sp., Flavobacterium sp., and Pseudomonas sp., (Chau et al., 1976a). Ridley et al., (1977) has shown that the oxidation state of an element is important in its methylation by methylcobalamin such that the conditions for environmental methylation could be predicted from the reduction potentials of the metals involved. In studying the mechanisms and environmental significance of metal methylation it is necessary to employ sensitive analytical techniques, in many cases coupled approaches are required, which are capable of detecting and quantifying methylated species in a variety of environmental media. Sensitive analytical techniques of this type have only been available for the last decade as such limited information on the mechanisms and environmental significance of metal methylation is available.

1.3 Environmental Methylation of Heavy Metals

The methylation of heavy metals under environmental conditions has been known for over a century. Indeed, the first demonstration of the environmental methylation of a metalloid element, namely As (Challenger, 1945), originated from reports of numerous cases of 'arsenical poisoning' which occurred in Germany in 1815. More recently through direct environmental concern research into the environmental methylation of Hg (Jensen and Jernelöv, 1969) and Sn (Hallas et al., 1982), has been undertaken. It is the aim of this section to review the analytical methodology employed in the analysis of methylated metal species, to review the processes involved in methylation and demethylation and to review relevant environmental studies on metal methylation. The scope of this review

is limited to those elements of interest to this work namely, Pb, As and Se.

1.3.1 Lead

1.3.1.1 Analytical Approaches

Until recently much of the information on the concentration of Pb species in environmental samples has been considered unreliable due to poor control over the widespread contamination of such samples during handling and analysis. In the case of organolead species the simultaneous quantification of tetraalkyllead (TAL) and ionic alkyllead compounds has proven difficult and much of the data in the literature is contentious. The difficulties in developing sensitive instrumentation are compounded by the large variations in the chemical nature of organolead compounds. Tetraalkyllead compounds are relatively volatile, (tetramethyllead, vapour pressure 23.7 mm Hg at 20°C) and covalent compounds which are sensitive to light and heat. (Tetraethyllead, b.pt 200°C but decomposes below this temperature). The solubility of TAL compounds has been reported to be between 0.2-0.3 mg l^{-1} (Feldhake and Stevens, 1963). The properties of TAL compounds make them ideally suited to gas-chromatographic (GC) analysis. Trialkyllead and dialkyllead compounds are high melting point ionic compounds. Trialkyllead compounds are slightly volatile (Saunders, 1950). The compounds are soluble in water e.g., triethyllead chloride (Et_3PbCl) has a solubility of 20 g l^{-1} whilst diethyllead chloride (Et_2PbCl_2) has a solubility of 50 g l^{-1} (Markall, 1977). These properties make the compounds more suited to liquid-chromatographic analysis (LC). The analysis of TAL and ionic alkyllead compounds is further made difficult by the need to study both the atmospheric and dissolved Pb phases in the environment.

The speciation of individual organolead compounds in either atmospheric or dissolved samples is best achieved by means of chromatographic separation techniques. Both gas and liquid-chromatographic techniques have been applied to this task. In the case of GC alkyllead compounds in air (Cantuti and Cartoni, 1968) and petroleum samples (Soulages, 1968) have been speciated. A more attractive analytical approach has been the coupling of an atomic absorption spectrometer with a gas-liquid chromatograph, a technique first described by Kolb et al., (1966). In initial work the effluent from the GC was introduced to the detector through the nebulizer of the spectrometer and atomized in a conventional air-acetylene flame (Katou and Nakagawa, 1974; Chau et al., 1976b). Coker (1975) improved the detection limits of this system by passing the column effluent directly to the burner head itself. Some authors described the use of hydrogen flame atomization systems utilizing a heated furnace design (Chau et al., 1976c; Ebdon et al., 1982). Other workers have reported the interfacing of the GC directly to a graphite furnace (Segar, 1974; Robinson et al., 1975; Radziuk et al., 1979; De Jonghe et al., 1980). Coupled, gas chromatography-mass spectrometry, (GC-MS) (Laveskog, 1970; Craig and Rapsomanikis, 1985), and gas chromatography-microwave plasma emission spectrometry (GC-MPES) (Reamer et al., 1978; Quimby et al., 1978; Estes et al., 1981) systems have also been reported. After suitable derivatization methods have been employed e.g., butylation (Estes et al., 1982) propylation (Radojević et al., 1986) and ethylation (Rapsomanikis et al., 1986) individual TAL and ionic alkyllead compounds can be quantified using the coupled systems described above. Coupled high pressure liquid chromatography-atomic absorption spectrometry (HPLC-AAS) (Messman and Rains, 1981) and high pressure liquid chromatography-inductively coupled plasma emission spectrometry (HPLC-ICPES) have also been reported (Ibrahim et al., 1984).

Bearing in mind the desire to determine TAL and ionic alkyllead compound simultaneously in a given sample, the butylation procedure, followed by selective and sensitive quantification using the coupled GC-AAS system, appears to be the most satisfactory technique available to date. Other techniques including HPLC-ICPES and GC-MS although sensitive are not as selective and require longer analysis times.

1.3.1.2 Environmental Methylation and Demethylation Processes

The major organolead compounds present in the environment are the TAL compounds and their decomposition products the di- and trialkyllead compounds. Elevated levels of alkyllead compounds over other organolead compounds may be explained by (i) input of TAL species into the environment as a result of spillage and evaporation of petroleum and (ii) environmental methylation of Pb is potentially another source, however, its significance is a highly controversial subject of debate.

Several experiments with environmental media (natural sediments and waters) have been undertaken to study the environmental methylation of Pb. Since its first reported occurrence the biologically mediated methylation of Pb (Wong *et al.*, 1975) has generated much controversy. Wong *et al.*, (1975) reported that both inorganic Pb ($Pb(NO_3)_2$) and organic Pb (trimethyllead acetate) were methylated by a biologically mediated process. The biologically mediated methylation of inorganic Pb has been reported by other workers (Thompson and Crerar, 1980) and was confirmed by the use of abiotic (sterile) controls. More recently the alkylation of Pb in intertidal sediments using radioactively labelled $^{210}Pb(NO_3)_2$ has been studied (Hewitt, 1985), the work indicated that Pb alkylation was erratic with only some sediments generating volatile organolead compounds. Work in which no abiotic controls are used (Dumas *et al.*, 1977) cannot be considered to confirm the process as

having a biological nature. Certain workers (Reisinger et al., 1981; Jarvie et al., 1983) after exhaustive environmental analyses have been unable to obtain evidence for the biologically mediated methylation of Pb and have consequently suggested such a process does not occur in the environment.

Less attention has been paid to the abiotic or chemical processes by which Pb can be methylated. The workers Jarvie et al., (1975) have, however, proposed a chemical pathway by which the Pb(IV) compound trimethyllead acetate can be converted to tetramethyllead (TML). Jarvie et al., (1975) noted that the addition of triethyllead salts to sediments generated only tetraethyllead (TEL), this led the workers to suggest that a sulphide mediated disproportionation route was important (see Equation 1.1).

$$2R_3Pb^+ + S^{2-} \longrightarrow (R_3Pb)_2S$$
$$(R_3Pb)_2S \longrightarrow R_4Pb + R_2PbS$$

(Equation 1.1)

Craig (1980) later confirmed this work by incubating trimethyllead acetate solutions with lake sediments. In both sterilized and unsterilized sediment systems similar amounts of TML (mean value = 3.6%, 1.14×10^{-2} mmole) were evolved. This compared well to the 2.0% TML yield reported by Jarvie et al., (1975) in which unsterilized sediments were incubated with trimethyllead acetate. Craig (1980), concluded that it was not necessary to invoke a biologically mediated mechanism but that simple chemical disproportionation and dismutation could account for the conversion of trimethyllead acetate to TML. In further experiments with organolead compounds Reisinger et al., (1981) used labelled Pb and C compounds but was unable to detect any biologically mediated methylation products, however, the work did confirm the feasibility of a sulphide induced chemical pathway.

Studies into the conversion of inorganic Pb(II) salts to TML by methyl donors, including methylcobalamin and methyliodide have been reported. In the case of methylcobalamin, methylation is reported not to occur (Lewis et al., 1973; Taylor and Hannah, 1976). Reports on the conversion of Pb(II) salts to TML by methyliodide have been both positive (Whitmore, 1981) and negative (Harrison, personal communication, 1985). The methylation of organolead compounds to TML by methylcobalamin has been reported (Ridley et al., 1977), a dimethylcobalt (III) complex has also been used as the methyl donor compound (Rhode and Weber, 1984).

The production of TML from the reaction of Pb(II) salts with methyliodide as reported by Ahmad et al., (1980) is now generally considered to be incorrect. Careful studies on the abiotic conversion of Pb(II) salts to TML (Jarvie and Whitmore, 1981; Craig and Rapsomanikis, 1982; Snyder and Bentz, 1982) reported levels of partially methylated species but no TAL compounds. They concluded that the aluminium foil used in experiments undertaken by Ahmad et al., (1980) had reduced small amounts of Pb(II) present to Pb(0) which was then converted to TML by an oxidative addition reaction. The reaction of Pb(0) with carbocation donors is known and not considered controversial (Jarvie and Whitmore, 1981). Craig (1986) has recently proposed a mechanism for the reaction of Pb(0) and Pb(II) species with carbocation donor compounds (e.g. CH_3I). The mechanisms are described below.

In the case of Pb(0) initial oxidative addition by a carbocation donor (CH_3I) results in the formation of the monomethyl species CH_3Pb^+ which is highly unstable and quickly reacts to form the dimethyl species $(CH_3)_2Pb^{2+}$ (see Equations 1.2 and 1.3, respectively).

$$Pb(0) + CH_3I \longrightarrow CH_3PbI \qquad \text{(Equation 1.2)}$$

$$CH_3PbI + CH_3I \longrightarrow (CH_3)_2PbI_2 \qquad \text{(Equation 1.3)}$$

Next the $(CH_3)_2PbI_2$ decomposes and the resulting $(CH_3)_3PbI$ dismutates yielding TML (see Equations 1.4 and 1.5, respectively).

$$2(CH_3)_2PbI_2 \longrightarrow (CH_3)_3PbI + PbI_2 + CH_3I \qquad \text{(Equation 1.4)}$$

$$4(CH_3)_3PbI \rightleftharpoons 2(CH_3)_4Pb + 2(CH_3)_2PbI_2 \qquad \text{(Equation 1.5)}$$

In the case of Pb(II), Craig (1986) proposed that the reaction proceeds similarly, by oxidative addition followed by dismutation of CH_3Pb^{3+} (Equations 1.6 and 1.7, respectively).

$$PbX_2 + CH_3I \rightleftharpoons CH_3PbIX_2 \qquad \text{(Equation 1.6)}$$

$$2CH_3PbIX_2 \longrightarrow (CH_3)_2PbX_2 + PbI_2X_2 \qquad \text{(Equation 1.7)}$$

Craig (1986) explained that the monomethyllead product (CH_3PbIX_2) formed in Equation 1.6 may not be sufficiently stable to dismutate but may decompose to inorganic Pb, thus the proposed mechanism is not an entirely satisfactory explanation for the production of ($(CH_3)_2Pb^{2+}$) observed by Craig and Rapsomanikis (1985). The same authors also noted that the formation of TML from Pb(II) is not observed, this was explained by successive diminishing yields occurring for the reactions shown in Equations 1.4 to 1.7. The above mechanism although tentative has application in the environment since the carbocation donor, methyliodide, may be found at elevated levels in natural waters (Lovelock, 1975).

Demethylation of alkyllead compounds in the environment may occur either by abiotic or biologically mediated processes. In the atmosphere homogeneous gas phase reactions with the hydroxy radical (OH) and ozone (O_3) together with photolytic decomposition are considered to be the most important demethylation mechanisms (Nielsen et al., 1982; Harrison and Laxen, 1978a). Hewitt and Harrison (1985) have estimated the atmospheric

half-life of TML and TEL to be 10 h and 2 h, respectively for summer months and 34 h and 8 h respectively for winter months. These relatively short half-lives would suggest that TML and TEL compounds will not travel large distances in the atmospheric environment.

In natural waters TAL compounds would decompose *in situ* (Robinson *et al.*, 1979), the resultant decomposition product being Pb(II) salts (Noden, 1977). Both TML and TEL are relatively stable in the dark in aqueous solutions (Jarvie *et al.*, 1981). Only 2% of TEL being decomposed to the triethyllead form after a period of 77 days but 26% of TML was decomposed to the trimethyllead form after only 22 days in the dark. In direct sunlight rapid demethylation occurs with 99% of TEL decomposing to the triethyllead form after 15 days and 59% of TML decomposing to the trimethyllead form after 22 days. The trimethyllead and triethyllead decomposition products are relatively stable in aqueous solution (Craig, 1986), but further demethylation of such compounds occurs in the presence of bacteria. Bacterial demethylation of alkyllead compounds is well known although the mechanism is not fully understood. Huber *et al.*, (1978) reported that bacteria in sewage sludge could demethylate 50-60% of trimethyllead chloride to the Pb(II) salt in a period of 5 days.

1.3.1.3 The Environmental Chemistry of Lead

To date no definitive studies on the concentration of inorganic Pb in environmental samples are cited in the literature. However, several studies on the organolead concentration of environmental samples have been undertaken (see Tables 1.1-1.4). In a study of fish, water, vegetation, algae and sediments taken from various rivers in Ontario, (Canada), Chau *et al.*, (1980) reported that only fish samples contained TAL compounds above the detection limits (0.1 ng Pb g^{-1} for fish and

Table 1.1 Alkyllead compounds in the environment

Sample	Concentration of Pb compounds speciated ($ng\ g^{-1}$, wet wt.)	Reference
BIOLOGICAL SAMPLES		
Fresh and marine water: fish (various) (Ontario, Canada)	Tetraalkyllead species Ⓐ (0.2-9.3)	Chau et al., (1980)
Fresh and marine water: fish (various)	Alkyllead species Ⓑ (<0.1-8000)	Chau et al., (1984)
Macro-algae (various) (Ontario, Canada)	(<0.1-16,515)	
Estuarine Macoma (N.W. England)	Alkyllead species Ⓒ (<20-50)	Birnie and Hodges (1981)

Footnote

Ⓐ Tetraalkyllead species: individual identification of all 5 mixed TAL species

Ⓑ Alkyllead species: including identification of TAL and di- and trialkyllead compounds

Ⓒ Alkyllead species: Only trialkyllead species identified

Table 1.2 Alkyllead compounds in the environment

SEDIMENT SAMPLES		
Sample	Concentration of Pb compounds speciated ($ng\ g^{-1}$)	Reference
St. Lawrence River Sediment, (Canada)	Alkyllead species Ⓐ (22-1200, wet wt.)	Chau et al., (1984)
Drain sediments (Birmingham, England)	Tetraalkyllead Ⓑ species (50-96000, dry wt.)	Potter et al., (1977)

Table 1.3 Alkyllead compounds in the environment

WATER SAMPLES		
Sample	Concentration of Pb compound speciated	Reference
Rain water (Antwerp, Belgium)	Alkyllead species Ⓐ (<2-68 $ng\ l^{-1}$)	Chakraborti et al., (1984)
Estuarine water (River Mersey, U.K.)	Ionic alkyllead Ⓒ species (<9-100 μg Pb l^{-1})	Riley and Towner (1984)

Footnote

Ⓐ Alkyllead species: TML, TEL and di- and trialkyllead species.

Ⓑ TAL: TML and TEL only.

Ⓒ Ionic alkyllead species: di- and trialkyllead species only.

sediment, 0.5 ng Pb g^{-1} for water). The TAL concentration of various freshwater fish was found to vary between 0.2-9.3 ng Pb g^{-1} representing less than 10% of the total Pb (30-325 ng g^{-1}). The workers Chau et al., (1980) suggested that the presence of TAL compounds in fish in waters containing no detectable TAL compounds indicated that biologically mediated methylation of Pb may have occurred. Further detailed studies by Chau et al., (1984) confirmed the presence of tetraalkyllead and di- and trialkyllead compounds in fish samples collected from the St. Lawrence River (Maitland, Ontario) with alkyllead concentrations varying between $<$0.1-8000 ng Pb g^{-1}. In the same study Chau et al., (1984) reported concentrations of alkyllead species in macro-algae species, typically concentrations ranged from 0.1-16500 ng Pb g^{-1}, the major alkyllead compound present in both fish and macro-algae was TEL. The possibility that the organisms had been contaminated with alkyllead compounds as a result of contact with petroleum products was not discounted. Birnie and Hodges (1981) studied the alkyllead concentration of Macoma in estuaries in N. Wales (England) and reported that only trialkyllead compounds were present above the detection limits (10 ng Pb g^{-1}), typically trialkyllead concentrations of 10-50 ng Pb g^{-1} were observed. In the same study Birnie and Hodges (1981) reported that fish caught in the English Channel remote from anthropogenic sources of alkyllead compounds, did not contain levels of alkyllead compounds above the detection limit.

Chau et al., (1984) have reported data for the concentration of alkyllead compounds in sediments collected from the St. Lawrence River (Canada), typically concentrations ranged from $<$0.1-1200 ng Pb g^{-1}, the major Pb species present was TEL. Potter et al., (1977) have reported much higher concentrations of TAL compounds (50-96000 ng Pb g^{-1}) in sediments collected from road drains located near to automobile garages.

Table 1.4 Alkyllead compounds in the environment

Sample	Concentration of Pb compounds speciated ($ng\ m^{-3}$)	Reference
ATMOSPHERIC		
Copenhagen Air	Tetraalkyllead species Ⓐ	Nielsen et al., (1981)
Urban	(185-195)	
Residential	(5- 60)	
Rural	(0.5-2.5)	
Antwerp Air	Alkyllead species Ⓑ	De Jonghe et al., (1981)
Urban	(49-116)	
Rural	(0.3-3.9)	
Petrol station	(17-410)	
Baltimore (USA)	Alkyllead species Ⓑ	Reamer et al., (1978)
Tunnel air	(0.5- 64)	
London (UK)	Tetraalkyllead species Ⓒ	Birch et al., (1980)
Urban air	(16-200)	
Lancaster (UK)	Tetraalkyllead species Ⓒ	Harrison and Laxen (1978b)
Rural	(0.5-230)	
Hebrides (UK)	Tetraalkyllead species Ⓒ	Hewitt et al., (1984)
Rural	(1 - 8)	

Footnote

Ⓐ Tetraalkyllead species: TML, TEL detected as individual species

Ⓑ Alkyllead species: Individual identification of ionic and tetraalkyllead species

Ⓒ Tetraalkyllead species: Total tetraalkyllead i.e. (TML + TEL)

To date detectable concentrations of alkyllead compounds in estuarine waters have only been reported for the Mersey Estuary. The Mersey Estuary is of particular interest because of the close vicinity of a large TAL production plant. Riley and Towner (1984) have reported concentrations of alkyllead compounds as high as 100 μg Pb l^{-1}, the major species present being trialkyllead compounds.

The atmospheric environment may be regarded as a reservoir of TAL compounds from which substantial concentrations of TAL compounds can be returned to the estuarine environment following precipitation processes. Chakraborti et al., (1984) have reported alkyllead concentrations of $\lesssim$2-68 ng Pb l^{-1} for rainwater collected over a 2 week period at an urban site in Antwerp (Belgium). The concentration of alkyllead compounds in urban, rural and residential atmospheric samples in European countries (Nielsen et al., 1981) and N. American countries (Reamer et al., 1978) have been reported. However, because of the use of a wide variety of techniques, many of which are non-specific and cannot differentiate between individual alkyllead species, large apparent variations in the alkyllead content of atmospheric samples have been reported. Sampling problems associated with the form of organolead compounds in the atmosphere add to the wide variation in reported concentrations. The organolead compounds present may exist in 2 phases in the atmosphere (i) in the vapour phase or (ii) in association with atmospheric particulates.

In urban areas vapour phase atmospheric alkyllead concentrations of 10-200 ng Pb m^{-3} are typical (De Jonghe et al., 1981, Nielsen et al., 1981; Reamer et al., 1978; Birch et al., 1980). This normally represents 1-10% of the total Pb content of atmospheric samples. Close to petroleum stations alkyllead levels of up to 400 ng Pb m^{-3} have been reported (De Jonghe et al., 1981). Studies by Harrison and Laxen (1977)

have shown that organolead compounds associated with atmospheric particulates account for 0.2-1.2% of the total particulate Pb at an urban site and this represented <10% of the total organic Pb present.

At rural sites in N.W. England atmospheric alkyllead concentrations ranging between 0.2-230 ng Pb m^{-3} were measured (Harrison and Laxen, 1978b) which represented 1-33% of the total Pb sampled. Much lower TAL concentrations were measured in rural air sampled in Denmark, typical concentrations ranged between 0.5-2.5 ng Pb l^{-1} (Nielsen et al., 1981). Hewitt et al., (1984) have measured the atmospheric organolead concentration at sites in the Outer Hebrides and reported concentrations ranging between 1-8 ng Pb m^{-3}. The workers Hewitt et al., (1984) suggested that the high ratio of organic Pb to inorganic Pb measured at the remote Outer Hebrides sites (an average of 22% organic Pb to 88% inorganic Pb for 14 air samples) indicated the possible input of organolead compounds into the atmosphere via environmental methylation of Pb. The Outer Hebrides sites are not regarded as being truly remote from industrial inputs of TAL compounds and further studies in more remote locations are required to validate the hypothesis proposed by Hewitt et al., (1984).

1.3.2 Arsenic

1.3.2.1 Analytical Approaches

The determination of As at the low concentrations observed for most environmental samples requires a sensitive and precise method. Hydride generation meets these requirements. Due to the versatility of the method it is used as the basis of most environmental As measurements.

The determination of methylated As species in environmental samples has recently been made possible by the development of sensitive coupled

systems. The volatile species such as trimethylarsine have been determined by means of coupled gas chromatography-graphite furnace atomic absorption spectrometry (GC-GFAAS) (Parris et al., 1977). The determination of alkylarsenic species by GC-MS after derivatization using 3-methyl-catechol has also been reported (Fish et al., 1983). Since most alkylarsenic compounds are involatile many workers have reported an initial hydride generation derivatization method using sodium tetra-borohydrate to form volatile arsines, which are more easily separated by GC. Analysis by GC-AAS, after the initial formation of volatile arsines, has been employed in the identification of arsenite, arsenate, monomethylarsenic species and dimethylarsenic species. The arsines are collected in a liquid nitrogen trap and distilled to the detector with and without the use of a GC, (Wong et al., 1977; Andreae,1977; Howard and Arbab-Zavar, 1981). Gas chromatography - DC Arc atomic absorption spectrometry (Braman and Forebeck, 1973) and gas chromatography - microwave emission spectrometry (GC-MES) (Talmi and Bostick, 1975; Limentani and Uden, 1985) have been employed in a similar manner.

The involatile organoarsenic compounds, monomethylarsonic acid and dimethylarsinic acid have been identified using high pressure liquid chromatography-atomic absorption spectrometry (HPLC-AAS) (Brinckman et al., 1980; Woolson and Aharonson, 1980) and HPLC-ICPES (Morita et al., 1981). Again derivatization of such compounds to the volatile arsine form using sodium tetrahydroborate has proved popular. Low pressure (ion exchange) liquid chromatography - atomic absorption spectrometry (LC-AAS) of hydride generated arsines of monomethylarsonic and dimethylarsinic acid was reported by Ricci et al., (1981). Detection after hydride generation of the arsine form has also been reported for HPLC-AAS (Haswell et al., 1985) and HPLC-ICPES (Bushee and Krull, 1984).

The hydride derivatization technique only identifies species with respect to the number of methyl groups bound to As. As such it is therefore possible that species other than monomethylarsonic acid, dimethylarsinic acid and trimethylarsine oxide may also be reduced to the mono, di-, and trimethylarsine derivatives, respectively. To overcome any ambiguity in a given sample the borohydride reduction should be preceded by a liquid chromatographic separation technique (Dietz and Perez, 1976). Because of the possibility of generating methylarsines from any methylarsenic species many authors prefer the term 'monomethylarsenic', 'dimethylarsenic' and 'trimethylarsenic' species with reference to the simultaneous hydride generation determination of methylated arsenic compounds in environmental samples.

The most promising techniques for the determination of alkylarsenic compounds in environmental samples involves the coupling of liquid or gas-chromatographs to an AAS detector. These techniques offer high selectivity and sensitivity and relatively fast analysis times. The best sensitivity is achieved by GC separation with liquid nitrogen trapping facilities. The best selectivity is obtained when LC separation is employed. Separation by HPLC, involving a preconcentration step prior to AAS detection would therefore be the most ideal technique. GC-MS and HPLC-ICPES are less suited to the determination of alkylarsenic compounds because of the lower selectivity and longer analysis times involved.

1.3.2.2 Environmental Methylation and Demethylation Processes

Methylated forms of As may be present in the estuarine environment as a result of their use as herbicides (Fitzgerald, 1983) or through the environmental methylation of As. The methylation of As by various organisms in the estuarine environment involves several biotransformations. These are summarised thus:

- uptake of arsenate by lower organisms
- reduction and methylation
- assimilation into cellular structure and/or release into environment
- metabolism and excretion by higher organisms.

The biologically mediated methylation of As by freshwater microorganisms has been reported by Wong et al., (1977). Under aerobic conditions the bacteria Aeromonas sp., Flavobacterium sp. and Escherichia coli produced monomethylarsenic species, dimethylarsenic species as well as di- and trimethylarsine compounds, Wong et al., (1977). Under anaerobic conditions, however, methanogenic bacteria were only able to methylate As to the dimethylarsine species (McBride and Wolfe, 1971). From studies on the fungus Scopulariopsis brevicaulus Challenger (1945) suggested that the biologically mediated methylation of As involved the transfer of carbocations from S-adenosylmethionine (SAM) to the lone electron pair on As(III) (see Figure 1.1). Further studies by Cullen et al., (1984) suggests that this mechanism is applicable to most microorganisms present in freshwater environments.

In the marine environment a great variety of organoarsenic compounds are synthesized by the organisms present. In their need to take up phosphate nutrients from natural waters primary producers such as algae may encounter two serious problems since (1) arsenate is similar to phosphate and (2) the 2 ions may occur at equimolar concentrations in some seawaters. At best algal discrimination between phosphate and arsenate at equimolar concentrations is only by a factor of 2-10 (Benson and Nissen, 1982). In biologically productive waters, where phosphate is depleted, algae may therefore accumulate high levels of arsenate, leading to poisoning by uncoupling oxidative phosphorylation. As a defence against such poisoning cells either increase their selectivity to exclude arsenate

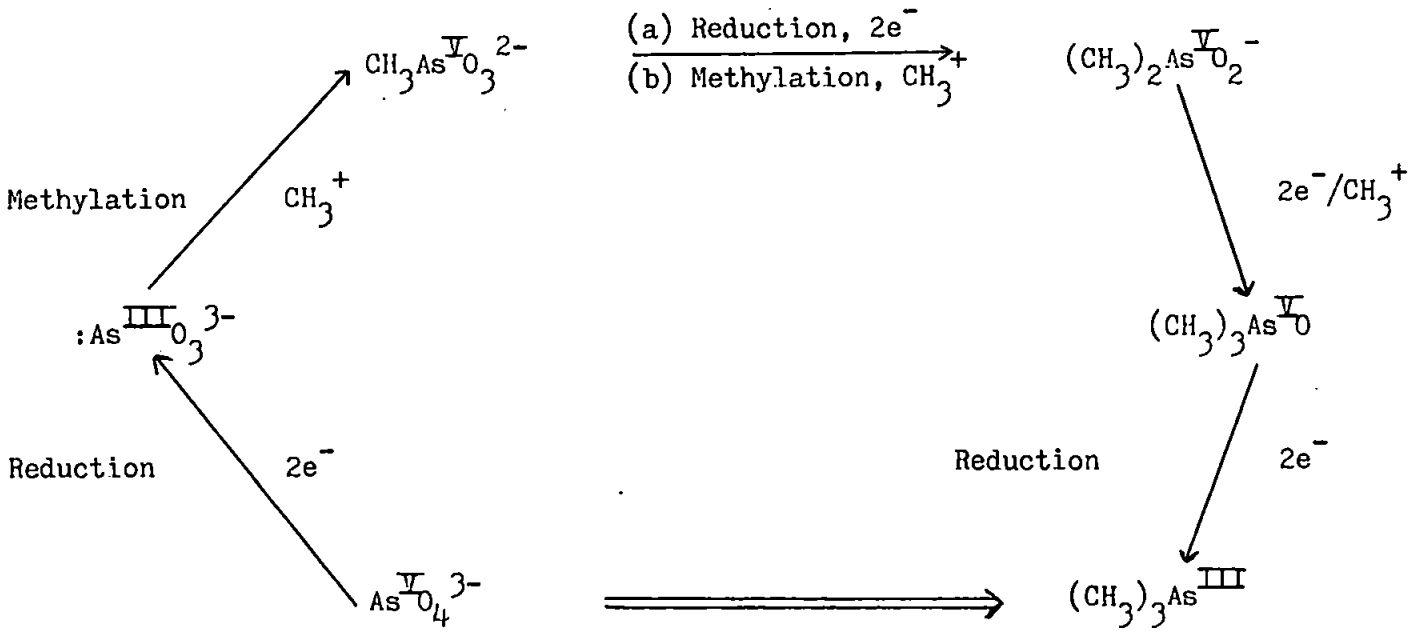


Figure 1.1 Pathway for the biomethylation of arsenic.

Modified after Challenger, 1945. (Cullen et al., 1984)

or rapidly metabolize the arsenate to methylated arsenic compounds and, or, more complex molecular compounds such as arsoniumphospholipds (Benson and Nissen, 1982). Several studies in recent years have been undertaken to identify the cellular metabolites of algae. Andreae and Klumpp (1979) have reported that up to 12 water and lipid soluble organoarsenic compounds are formed by algae. The elucidation of the structure of 2 As containing sugars present as the major As metabolites in the brown kelp Ecklonia radiata has been reported (Edmonds and Francesconi, 1981). Other work has shown that lipid derivatives of the glycosugars identified by Edmonds and Francesconi (1981) are also major As metabolites in all marine algae (Bensen and Nissen, 1982). Algal release of methylated As species into natural waters has been suggested to arise from the bacterial oxidation of such arsenolipids that are present in outer cell membranes, resulting in the formation of dimethylarsenic species.This may be the major pathway for methylated As compounds to enter the aquatic environment. The simplistic form of As methylation developed by Challenger (1945) has been developed by Edmonds et al., (1982) and a complex model for the formation of methylated As species found in higher organisms has been tentatively proposed.

Bacteria are known to be able to demethylate alkylarsenic species. Shariatpanahi et al., (1981) isolated 7 bacterial species from soils and sediments and upon incubation with monomethylarsonic acid 5 species, Achromobacter sp, Flavobacterium sp, Nocardia sp., Alicaligenes sp. and Pseudomonas sp., demethylated monomethylarsonic acid to arsenate and CO_2 at a rate of 3-5% over a 48 h period. The 2 other species Aeromonas sp. and Enterobacter sp. actually methylated monomethylarsonic acid to the trimethylarsine form.

1.3.2.3 The Environmental Chemistry of Arsenic

Much of the environmental data on As considers the estuarine environment

and most emphasis has been on inorganic As concentrations. Studies by Andreae et al., (1983) in the Tejo Estuary, (Portugal) (average inorganic As concentration 1-6 μg l^{-1}), Knox et al., (1984) in the Tamar Estuary (S.W. England) (average inorganic As concentration 1.5-6.5 μg l^{-1}) and Millward and Marsh (1986) in the River Carnon (S.W. England) (average inorganic As concentration 5-100 μg l^{-1}) indicate that As behaviour is non-conservative, the removal of As from solution by Fe particulates has been proposed (Langston, 1983). This is in contrast to studies on the estuarine chemistry of As in relatively unpolluted estuaries in South-East USA. Waslenchuk (1979) studied various South Eastern USA estuaries (average inorganic As concentration 0.05-0.6 μg l^{-1}) and found that As behaved conservatively such that their As concentration showed a linear relationship with salinity.

Recently several workers have reported the concentration of methylated As species in a variety of environmental samples (refer to Table 1.5 and 1.6). Johnson and Braman (1975) reported levels of methylated As species in various members of the pelagic Sagassum community. The authors employed a weak NaOH digest and reported that the relative distribution of inorganic and organic As species was similar in both the marine organisms tissues and the surface water surrounding them. In a further study, N. American rivers and coastal waters were sampled (Andreae, 1978). Various Phaeophyta and Rhodophyta species were digested in concentrated HCl, as a result only combined inorganic As(III) and As(V) data is reported (Table 1.5). The elevated levels of methylated As species in the Sargasso Sea (Johnson and Braman, 1975) compared to coastal USA waters (Andreae, 1978) are a reflection of the high biologically productivity occurring in the Sargasso Sea. Klumpp and Peterson (1979) reported elevated methylated As concentrations in macro-algae samples collected from the Restronguet Creek system of the

Table 1.5 Alkylarsenic compounds in the environment

Sample	Concentration of As compound speciated ($ng\ g^{-1}$ wet wt.)				Reference
	BIOLOGICAL SAMPLES				
Sargassum Weed: (various)	As(III) (0-890)	As(V) (1000-9700)	MMA (5-16)	DMA (16-100)	Johnson and Braman (1975)
Phaeophyta: (various)	inorganic As (2-17)		MMA (0.3-5)	DMA (1-10)	Andreae (1978)
Rhodophyta: Ligartina spinosa	(8.5)		(2.3)	(4.9)	Andreae (1978)
Macro-algae:	As(V)		MMA	DMA	Klumpp and Peterson (1979)
Ascophyllum sp.	(2500)		(2100)	(82000)	
F. Spiralis	(1200)		(1700)	(100,000)	

Footnote

Inorganic As refers to analyses of As(III) and As(V) together.

MMA refers to monomethylarsenic species

DMA refers to dimethylarsenic species

Table 1.6 Alkylarsenic compounds in the environment

Sample	Concentration of As compound speciated ($\mu g\ l^{-1}$)				Reference
WATER SAMPLES					
Estuarine surface water;	As(III)	As(V)	MMA	DMA	Braman and Forebeck (1973)
Tidal flat	(0.62)	(1.29)	(0.08)	(0.29)	
Causeway	(0.12)	(1.45)	(<0.02)	(0.20)	
Estuarine surface water;	As(III)	As(V)	MMA	DMA	Andreae (1977)
River water	(0.11)	(1.95)	(0.06)	(0.05)	
Sea water	(0.019)	(1.75)	(0.017)	(0.12)	
Oceanic surface water;	As(III)	As(V)	DMA		Waslenchuk (1978)
(various)	(<0.02-0.07)	(0.7-1.7)	(0.02-0.06)		
Estuarine surface water;	As(III)	As(V)	MMA	DMA	Howard et al., (1982)
marine water	(0.06-0.33)	(0.13-1.0)	(0.06-0.38)	(0.18-0.39)	
Porewater;	As(III)	As(V)	MMA	DMA	Haswell et al., (1985)
estuarine	(1-15)	(3-210)	(1-22)	(<1)	

Fal Estuary, S.W. England, typically, concentrations ranged from 1000-100,000 ng As g^{-1} (dry wt.) for various species when a $HNO_3/HClO_4$ digest was employed.

Braman and Forebeck (1973) have reported the concentration of methylated As species in N. American coastal waters, typically concentrations of <0.02-0.29 µg As l^{-1} for the methylated species were observed. Subsequent studies by Andreae (1977) and Waslenchuk (1978), also in N. American coastal waters, confirmed Braman and Forebeck's early work, the maximum reported methylated As concentration was 0.3 µg As l^{-1}. In studies of the Beaulieu Estuary (S. England) the workers Howard et al., (1982) and Howard et al.,(1984) reported that the highest levels of methylated species occurred during summer months when water temperatures exceeded 12°C. Typically concentrations of the methylated species ranged from 0.02-0.40 µg As l^{-1}, values slightly higher than those observed in N. American estuaries. The porewaters of estuarine and marine sediments have been studied for N. American estuaries (Andreae, 1979; Peterson and Carpenter, 1986), however, the authors were unable to detect methylated species above the detection limits (0.1 ng As l^{-1} and 50 ng As l^{-1}, respectively) of the techniques used. However, in pore-waters of estuarine soils collected from the Tamar Estuary (S.W. England), Haswell et al., (1985) detected levels of monomethylarsonic acid (1-22 µg As l^{-1}). In discussing porewater As concentrations the influx of macro-algae debris, a known source of methylated As species, as well as biologically mediated methylation of As by sediment microorganisms, must be considered.

Few studies on the concentration of methylated As species in estuarine sediments are readily available. Maher (1981) has reported concentrations

of monomethylarsenic species (700-5000 ng As g^{-1}) and dimethylarsenic species (300-400 ng As g^{-1}) using a HCl digestion procedure. To date no environmental atmospheric data is readily available.

1.3.3 Selenium

1.3.3.1 Analytical Approaches

Analysis of Se in environmental samples, particularly natural waters has proved to be an exacting task. The low concentration of Se in such samples necessitates an initial preconcentration step. In the case of inorganic Se speciation the extraction of samples into an organic solvent followed by GC using electron capture detectors has proven popular (Measures and Burton, 1978). In the case of methylated species of Se a similar technique was reported by Evans and Johnson (1966).

Most recently the combination of GC with AAS detection has proved to be an excellent technique for the detection of volatile alkylselenium compounds. The first report of such a coupling was by Chau et al., (1975) who utilized an electrically heated quartz furnace. Radziuk and Van Loon (1976) reported a similar design. Improvements in detection limits were reported when a GC was coupled directly to a graphite furnace atom cell (Parris et al., 1977; Jiang et al., 1983). Both GC-MS (Reamer and Zoller, 1980) and gas chromatography-microwave emission spectrometry GC-MES (Reamer 1978; Duebelbeis et al., 1986) have been employed in the determination of alkylselenium compounds. The coupled GC-GFAAS method is the most satisfactory method to date. This method is highly sensitive and selective and capable of determining a large number of samples in a short period of time. Both coupled GC-MS and GC-MES are sensitive methods but lack selectivity. These methods are also more time

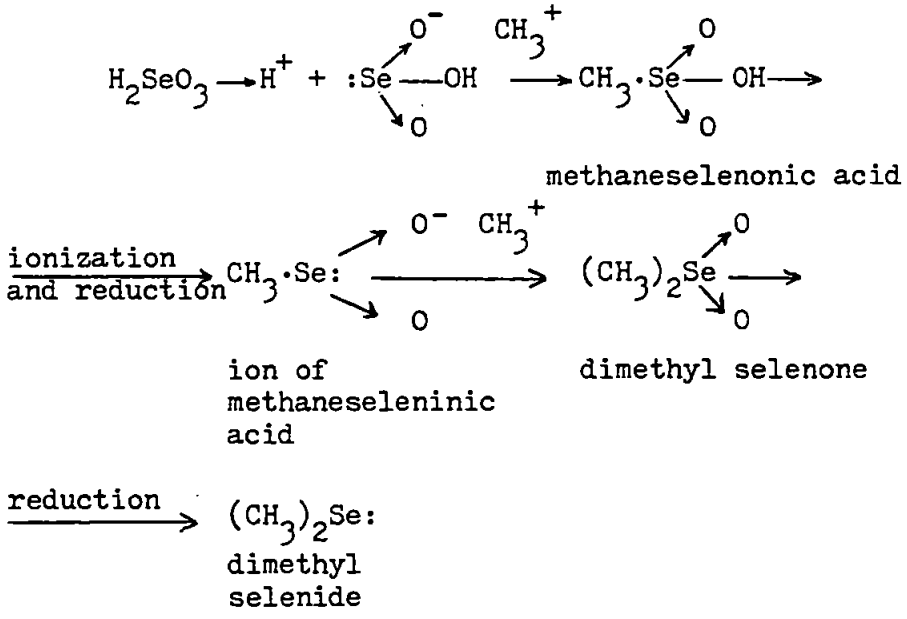


Figure 1.2 Mechanism proposed by Challenger (1951) for the methylation of selenium by fungi.

consuming than coupled GC-AAS methods.

1.3.3.2 Environmental Methylation and Demethylation Processes

Although the methylation of Se compounds under environmental conditions has been known since the early work of Challenger in the 1930s, little data is readily available for the environmental methylation of Se. Challenger (1951) proposed that methylation involved the transfer of a carbocation to Se (which was considered to be a nucleophile, see Figure 1.2). Recently an alternate mechanism for the methylation of Se by microorganisms has been proposed by Reamer and Zoller (1980), the mechanism is similar to that of Challenger in that carbocation methyl transfer is invoked. Little data is readily available for environmental demethylation of alkylselenium compounds.

1.3.3.3 The Environmental Chemistry of Selenium

Much of the literature on the environmental chemistry of Se concentrates on the speciation of inorganic forms of Se. Measures and Burton (1978) have studied the behaviour of dissolved inorganic Se in various rivers in S. England and have reported that Se(VI) (calculated as the difference between total Se and Se(IV)) was probably the major species present in the oxygenated waters sampled. Measures and Burton (1978) observed that total dissolved Se varied from 76-369 ng l^{-1} and Se(IV) from 2-31 ng l^{-1}, both total and Se(IV) species behaved conservatively. The workers Takayanagi and Wong (1985) studied the behaviour of dissolved Se in Chesapeake Bay (N. America) and reported similarly that total and Se(IV) behaved conservatively but in contrast to the work of Measures and Burton (1978) the species Se(IV) was the dominant species present.

Studies on the organic Se concentration of natural waters have been

undertaken (Takayanagi and Wong, 1985; Cutter, 1982). The concentration of dissolved organic Se compounds was found to increase sharply in the suboxic waters of the Orca Basin (Gulf of Mexico, N. America) and concentrations of 210 ng Se l^{-1} were observed in anoxic brines. In further studies on reducing water systems Cutter (1982), reported that dimethylselenide was below the detection limit (0.72 ng Se l^{-1}) in Saanich Inlet (Vancouver, W. Canada). Environmental atmospheric data on methylated Se compounds has been reported by Jiang et al., (1983). The concentration of dimethylselenide and dimethyldiselenide ($Se_2(CH_3)_2$) was found to vary between 0.15-0.85 ng Se m^{-3} in air samples collected above aquatic environments (Ostend, Belgium). The work by Jiang et al., (1983) is the only reported work on atmospheric concentrations of methylated Se compounds to date.

1.4 Estuarine Systems Studied

In the couse of this work 3 estuaries were studied, namely the Tamar Estuary (S.W. England), River Carnon and associated Restronguet Creek (S.W. England) and the Beaulieu Estuary (S. England). The estuaries were selected for their contrasts in hydrodynamic conditions, and biological and chemical composition.

1.4.1 The Tamar Estuary: Physical Setting and Hydrogeochemistry

The River Tamar is located in the South-West of England (see Figure 1.3) and is considered to be an excellent example of a drowned river valley or Ria. The general North-South axis of the Tamar Estuary extends from Gunnislake (the limit of tidal incursion) to Plymouth Sound (at the mouth),a distance of some 30 km. In the upper reaches the river flows through a steep sided valley whilst in the middle reaches the river meanders over a flat alluvial plain and is joined by 2 major

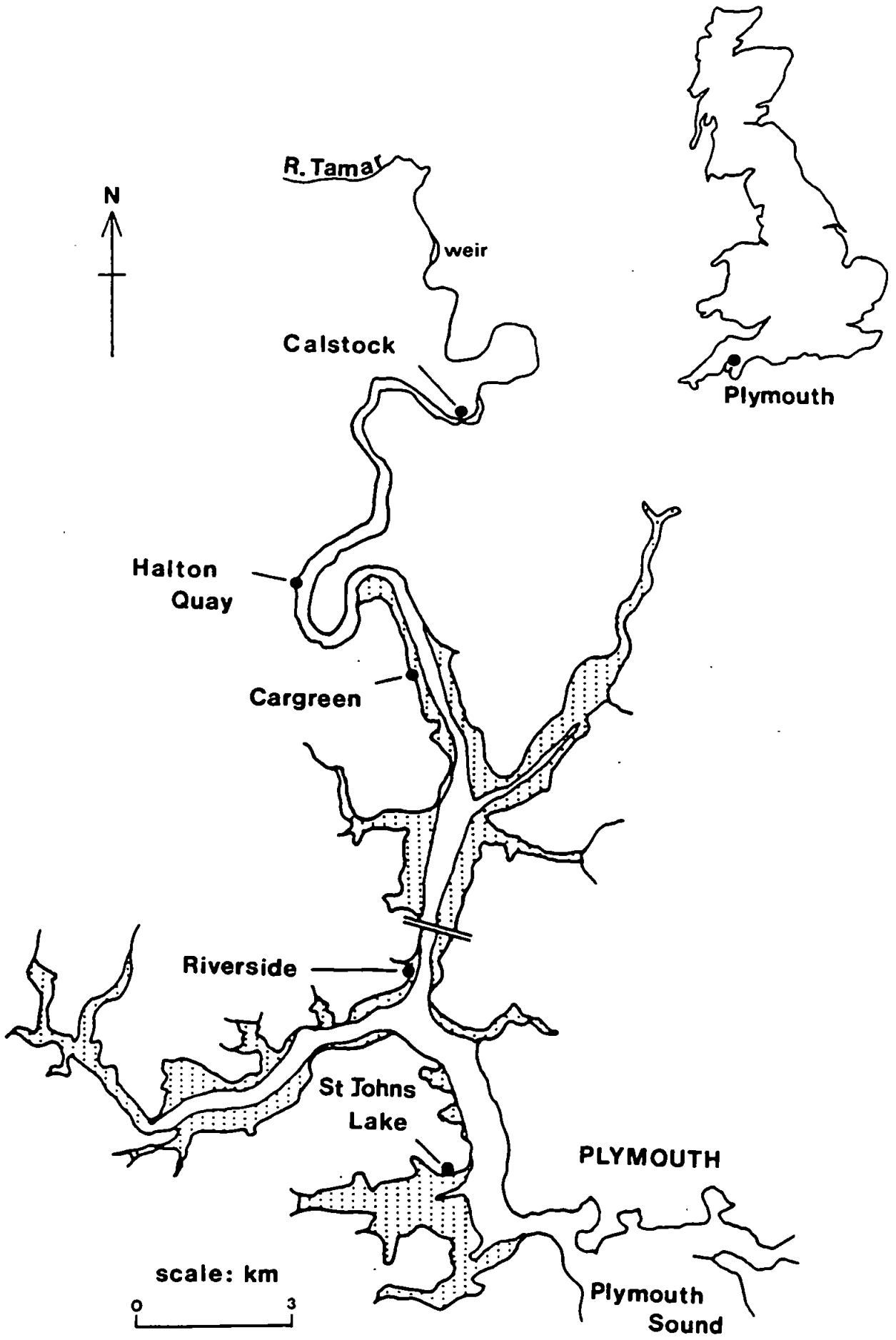


Figure 1.3 The geographical location of the Tamar Estuary; sediment and porewater sites are marked.

tributaries, namely the Lynher and Tavy. The Tamar, however, constitutes the major fresh water input to the estuary. In the middle (Cargreen) and lower regions (St. Johns' Lake) of the estuary, extensive mudflat areas are found. The river flow rate may vary considerably between winter and summer months, however, on average winter flow rates of 30-40 m^3 s^{-1} and summer flow rates of 5-10 m^3 s^{-1} are observed (Ackroyd, 1983). The tidal ranges near the river mouth (Devonport) are 5.5 m at springs and 1.5 m at neaps, towards the source these diminish to 1-2 m ranges. In the middle to upper region the estuary becomes narrow and the confinement of the tidal energy (especially on springs) often results in a well developed turbidity maximum (Morris et al., 1982). The turbidity maximum can significantly affect the biological activity by reducing the transmission of light into the column water. The flushing time of the estuary (t_1) can vary from 7 to 12 days (Uncles et al., 1985) and is controlled to a large extent by the river flow rate and tidal state. The retention of metal species within the estuarine system is important from the chemical reactivity point of view. The catchment area surrounding the Tamar Estuary has long been associated with the mining of metal ores (Dines, 1956) and was at one time producing half the worlds arsenic requirement. Although the majority of mining activity ceased in the early 20th century, drainage from adits and secondary inputs from spoil tips still contribute significant concentrations of dissolved and particulate metals to the Tamar Estuary. Morris et al., (1986) have reported data for dissolved and sediment metal concentrations: dissolved Fe (10-300 $\mu g\ l^{-1}$), Al (5-50 $\mu g\ l^{-1}$), Cu (1-12 $\mu g\ l^{-1}$), Cd (0.01-0.12 $\mu g\ l^{-1}$), Zn (2-12 $\mu g\ l^{-1}$) and Ni (0.5-2.5 $\mu g\ l^{-1}$); sediment (dry wt.), Fe (2.5-4.5%), Cu (100-500 $\mu g\ g^{-1}$), Zn (200-500 $\mu g\ g^{-1}$) and Mn (200-1600 $\mu g\ g^{-1}$). Knox et al., (1984) have reported data for dissolved concentrations of inorganic As(III) (range 0.15-1.0 $\mu g\ l^{-1}$) and inorganic As(V) (range 1.5-6.0) over a full estuarine survey (<0.5-35‰).

The concentration of Pb and As in Tamar Estuary sediments have been reported to range from 19-239 $\mu g\ g^{-1}$ and 25-236 $\mu g\ g^{-1}$ (dry wt.), respectively, (Bland et al., 1982). The average dissolved organic carbon content of the Tamar Estuary has been reported to be 6 mg C l^{-1} (Langston, 1983) and the same author reported pH values to range between 7 and 8 within the estuary.

1.4.2 The River Carnon and Restronguet Creek: Physical Setting and Hydrogeochemistry.

The River Carnon is a small river draining into Restronguet Creek in the Fal Estuary (S.W. England), Figure 1.4. The river flows over highly metalliferous Devonian rocks as does the Tamar. River flow rates are known to vary from 0.5-0.9 $m^3 s^{-1}$ in summer and 2.0-3.0 $m^3 s^{-1}$ in winter (Langston, 1983). Little data is available for the tidal ranges in the Restronguet Creek area, however, the estuary is observed to be stratified with little mixing in contrast to the Tamar Estuary. The high degree of stratification is a result of the strong attenuation of tidal energy throughout the broad and highly tributed Fal Estuary. The low tidal energy regime present gives rise to low resuspension of particulates, this in turn influences the chemical and biological nature of the estuary.

The River Carnon receives mine drainage from the active Wheal Jane and Mount Wellington mines, as a result strong metal enrichments occur in both surface waters and sediments. The average concentration of dissolved metals in the River Carnon has been reported by Langston (1983): dissolved Fe (8600 $\mu g\ l^{-1}$), Cu (500 $\mu g\ l^{-1}$), Zn (57,000 $\mu g\ l^{-1}$), Mn (3300 $\mu g\ l^{-1}$) and total inorganic As (233 $\mu g\ l^{-1}$), inorganic As(III) accounted for 40% of the total As present. Boyden et al., (1979) reported data on dissolved and sediment metal concentrations in Restronguet Creek: dissolved

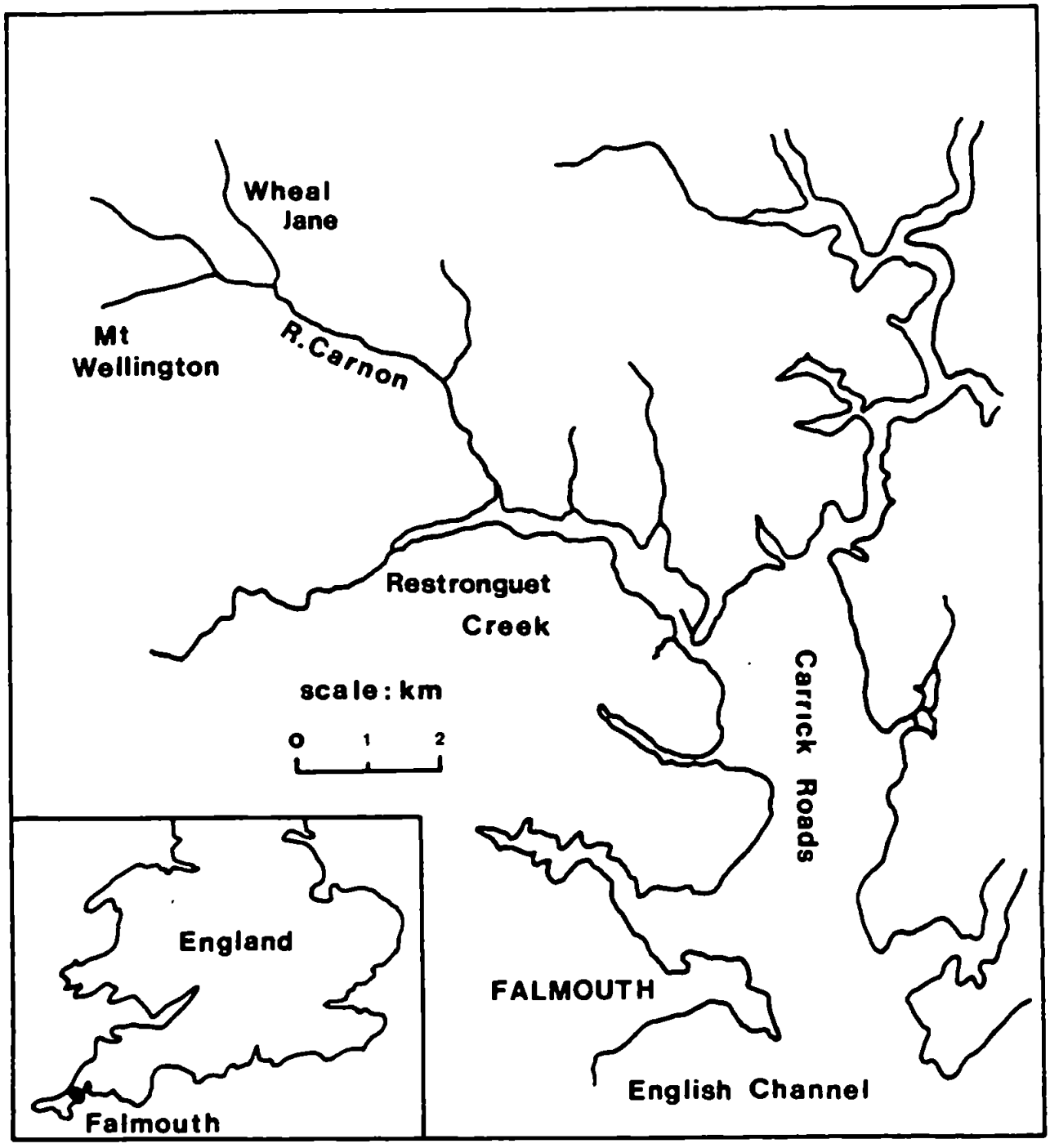


Figure 1.4 The geographical location of the River Carnon and associated Restronguet Creek.

Fe (9-60 $\mu g\ l^{-1}$), Cu (5-26 $\mu g\ l^{-1}$), Zn (17-300 $\mu g\ l^{-1}$) and Pb ($\lesssim$2-3 $\mu g\ l^{-1}$); sediment (dry wt.), Fe (44000-59000 $\mu g\ g^{-1}$), Cu (1350-2350 $\mu g\ g^{-1}$), Zn(1250-2300 $\mu g\ g^{-1}$) and Pb (210-350 $\mu g\ g^{-1}$), most metal concentrations were highest in winter months reflecting the increased weathering and transport of metals during these months. The metal enrichments observed in the Carnon system are probably related to the low pH 3-7 observed (Millward and Marsh, 1986). Data for dissolved organic carbon concentrations in the Carnon system is not readily available, however, it is to be expected to be similar to that of the Tamar since the 2 rivers flow over similar geological strata. The precipitation of dissolved organic compounds in the low pH freshwater region of the Carnon system suggests that such compounds would only weakly influence the behaviour of the metals in this estuarine system.

1.4.3 The Beaulieu Estuary: Physical Setting and Hydrogeochemistry

Situated in Hampshire (S. England), the River Beaulieu flows through marshes and heathlands of the New Forest and thus drains an organic-rich catchment area. The Beaulieu Estuary extends for some 6-7 kms, before its waters mix with open sea in the Solent (Southampton Waters), Figure 1.5. Although data is not readily available for river flow rates these would be expected to be lower than those for the River Carnon or River Tamar and would exhibit marked seasonal variations. In contrast to the Tamar and Carnon systems the tidal range in the Beaulieu shows a distinct double high-water maximum. Also due to the presence of a weir on the River Beaulieu the formation of a salt wedge does not occur, subsequently no turbidity maximum is observed and suspended particulate material is noticeably low.

High levels of metals are rarely observed in the estuary since no major industrial inputs occur in this area. Elevated concentrations of

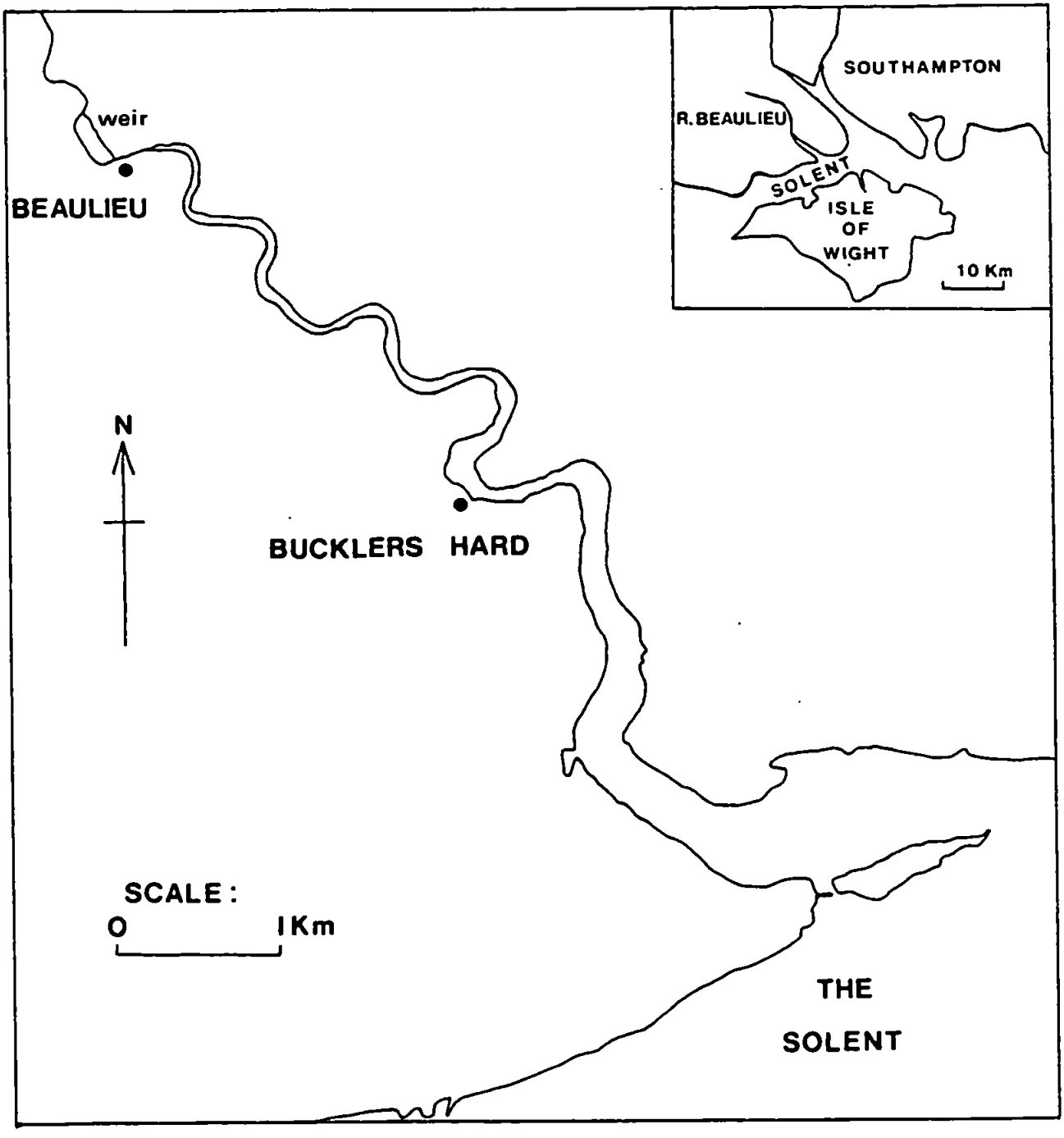


Figure 1.5 The geographical location of the Beaulieu Estuary

nutrients enter the estuary from the waters which drain the organic rich marshland of the New Forest (Hampshire). The high concentration of organic acids present and moderate pH values (6.0 pH fresh - 8.5 pH marine) afford the stabilization of metals in these waters. Thus the metal levels observed are generally uniformly distributed in the estuary and exhibit typical conservative chemical characteristics (Moore et al., 1979). Holliday and Liss (1976) have reported data for dissolved metal concentrations for full estuarine surveys ($<$0.5-30‰); dissolved Fe (10-450 $\mu g\ l^{-1}$), Zn (5-65 $\mu g\ l^{-1}$) and Mn (15-130 $\mu g\ l^{-1}$). Howard et al., (1984) have exploited the high productivity of the Beaulieu Estuary to study As methylation. Howard et al., (1984) reported that inorganic As(V) was the dominant As species (0.1-1.0 $\mu g\ l^{-1}$) during the winter months when water temperatures were below 12°C, however, during summer months ($T > 12°C$) a significant portion of dissolved As existed in the reduced As(III) form (0.1-0.33 $\mu g\ l^{-1}$) and the monomethylarsenic (0.1-0.32 $\mu g\ l^{-1}$) and dimethylarsenic (0.2-0.45 $\mu g\ l^{-1}$) forms. Measures and Burton (1978) have reported dissolved Se surface water concentrations (73-85 $ng\ l^{-1}$) for the Beaulieu Estuary. Data on the metal concentration of sediments and dissolved organic carbon concentration in the Beaulieu are not readily available.

1.5 Aims of Present Work

The main aim of this project is to investigate the significance of metal methylation in estuarine systems. Within this objective simulation of metal methylation was undertaken in an attempt to understand field observations observed in estuarine waters. In analysing the data the ultimate aim was to develop ideas on the pathways for the elements in estuaries. The mechanistic interpretation would then enable predictions of the cycling of metal species to be undertaken. The approaches adopted to achieve these aims are itemised below:

1. To carry out sediment surveys to identify sites in the Tamar Estuary having elevated metal levels which would be potential sites of methylation activity. The chemical analyses of sediments were selected to distinguish between total available and 'biologically available' (non-detrital) metal.

2. To carry out additional surveys over seasonal cycles to investigate major sources of methylated species in the estuarine environment. To include analysis of porewaters, column waters and macro-algae samples. To compare and contrast the results in the Tamar with those from the Beaulieu and River Carnon and associated Restronguet Creek.

3. Modelling of the estuarine system was to be undertaken by performing time dependent experiments under carefully controlled conditions similar to those found in the estuarine environment.

4. To provide a conceptual model of the estuarine biogeochemical cycles of the elements, As, Pb and Se.

CHAPTER TWO

EXPERIMENTAL METHODS

2.1 Reagents and General Analytical Operations

All chemicals were of analytical reagent grade unless otherwise stated. The suppliers of reagents and standard compounds are listed below.

The various acids used in this work (nitric, hydrochloric, sulphuric and acetic acid, Analar and Aristar grade) were obtained from BDH Chemicals (Poole, Dorset, U.K.). Nutrient Broth I, yeast extract and D-glucose (GPR grade) were obtained from Oxoid Chemicals (Basingstoke, Hants, U.K.). n-butylmagnesium chloride (1.9 M in THF) was obtained from Ventron Alpha Products (Coventry, U.K.). Sodium tetrahydroborate (99%+ pure) was obtained from Aldrich Chemicals (Gillingham, Dorset, U.K.). All other chemical reagents were supplied by BDH Chemicals.

Inorganic Pb standards (Analar grade) were obtained from BDH Chemicals. Tetraalkyllead and ionic alkyllead chloride standard compounds (99% pure) were obtained from the Associated Octel Company (S. Wirral, England). Trimethyllead acetate (99% pure) was obtained from Ventron Alpha Products (U.K.).

Inorganic As standards (Analar grade) were obtained from BDH Chemicals. Monomethylarsonic acid (disodium salt, 99.9% pure) and dimethylarsinic acid (sodium salt, 99.9% pure) were obtained from Dr. K.J. Irgolic, Texas A and M University, USA.

Inorganic Se standards (Analar grade) were obtained from BDH Chemicals. Alkylselenium standards (99% pure) were obtained from Lancaster Synthesis (St. Leonards Gate, Lancaster, U.K.).

All glassware and polythene containers were cleaned by soaking overnight in 10% (v/v) nitric acid (Analar grade) followed by rinsing in deionized doubly distilled water and drying.

Stock Pb, As and Se standard solutions were prepared at 1000 mg l^{-1} levels from analytical reagent grade chemicals (unless otherwise stated). Low concentration standards (<10 mg l^{-1}) were prepared on a daily basis by dilution in an appropriate solvent.

2.2 Analytical Methods

The analytical methodology employed in this work uses techniques which are applicable to both the two main areas of investigation. The first area of investigation involved the analysis of fauna, sediment, porewater and column water samples collected on various estuarine surveys. The investigation included both elemental analysis (sections 2.2.1 - 2.2.2) and also speciation analysis (sections 2.2.3 - 2.2.4). The second area of investigation was concerned with analysis of laboratory model systems designed to determine rate data for the formation and decomposition of methylated organolead and organoarsenic compounds (section 2.4).

2.2.1 Flame Atomic Absorption Spectrometry (FAAS)

Acidified aqueous samples or sediment extracts were aspirated directly into an atomic absorption spectrometer (SP9, Pye Unicam, Cambridge).

An air/acetylene flame was used initially for the analysis of Pb and As. Detection was achieved by monitoring the appropriate wavelength using a hollow cathode lamp as the spectral source. Where applicable deuterium arc background correction was used. Standard operating conditions were used throughout (Pye Unicam SP9 AAS Users Manual 1979).

2.2.2 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Sediment extracts, fauna extracts and acidified water samples were analysed for 'total' As, Pb and Se using a graphite furnace atomic absorption spectrometer (SP9, Pye Unicam). Determination of Pb was achieved by direct injection (25 µl, Pipettman injector, Gilson, France) of acidified samples into the graphite cuvette. For the determination of As and Se samples were matrix modified by stabilising all solutions with 1000 mg Ni l^{-1} (as nitrate) prior to injection into the cuvette. Detection was again achieved by monitoring the appropriate wavelength and using a hollow cathode lamp as the light source. Deuterium arc background correction was always applied. The optimisation of the graphite furnace operating conditions was achieved by the construction of both ash and atomization plots whilst using acidified standards of the individual elements. The optimum operating conditions for As, Pb and Se are shown in Table 2.1.

2.2.3 Gas Chromatography - Atomic Absorption Spectrometry (GC-AAS)

Column water, porewater and sediment were analysed for organolead and organoselenium compounds using a sensitive coupled GC-AAS system (Ebdon *et al.*, 1982), Figure 2.1.

The system consists of a gas chromatograph (series 104, Pye Unicam) and an atomic absorption spectrometer (SP9, Pye Unicam). The two instruments were interfaced by a glass lined stainless steel tube (0.70 mm I.D. x 1/16" O.D. Phase Separations, Queensferry, Clwyd) connecting the column outlet of the gas chromatograph to the atom cell of the atomic absorption spectrometer. Effluent from the gas chromatograph passes along the transfer line to a T-piece connection. At this point an auxiliary hydrogen flow is introduced, producing a small hydrogen flame capable

Table 2.1 Instrumental parameters for the electrothermal analysis of As, Se and Pb.

Arsenic: Wavelength: 193.7 nm
Lamp: HCL, 8.0 mA
Bandpass: 1 nm

Furnace conditions:

Dry:	110°C for 10 s, ramp-rate 5°C s^{-1}
Ash:	1400°C for 5 s, ramp-rate 10°C s^{-1}
Atomization:	2300°C for 5 s, maximum ramp-rate.

Selenium: Wavelength: 196.0 nm
Lamp: HCL, 5 mA
Bandpass: 1.0 nm

Furnace conditions:

Dry:	105°C for 5 s, ramp-rate 2°C s^{-1}
Ash:	850°C for 5 s, ramp-rate 10°C s^{-1}
Atomization:	2150°C for 5 s, maximum ramp-rate.

Lead: Wavelength: 283.3 nm
Lamp: HCL, 6 mA
Bandpass: 0.5 nm

Furnace conditions:

Dry:	120°C for 10 s, ramp-rate 5°C s^{-1}
Ash:	400°C for 5 s, ramp-rate 10°C s^{-1}
Atomization:	1800°C for 5 s, maximum ramp-rate.

* maximum ramp-rate > 2000°C s^{-1}

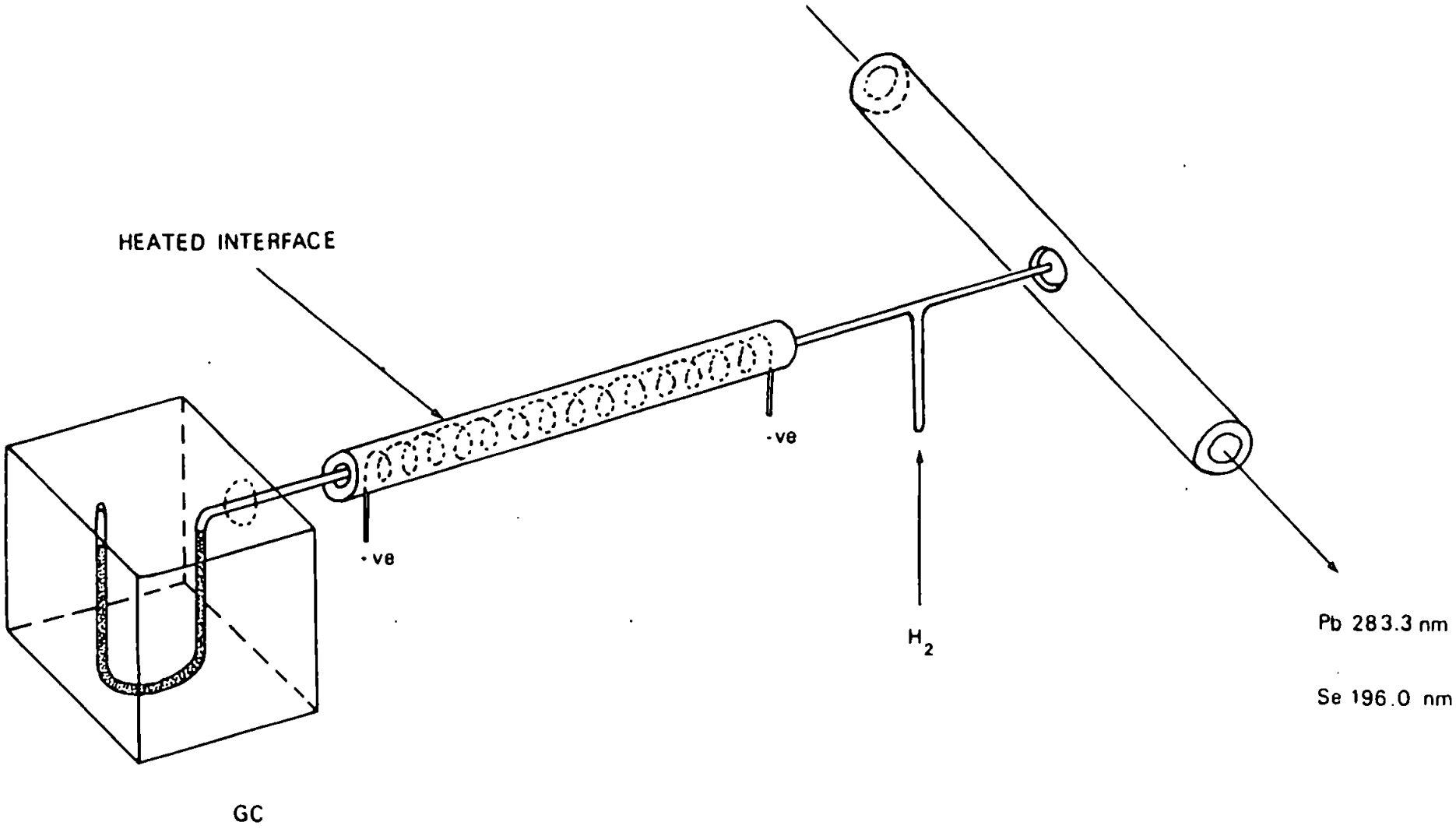


Figure 2.1 Coupled gas chromatography-atomic absorption system

of atomizing the effluent. The atoms are swept into and detained by the atom cell which is mounted in the light path of the spectrometer. The atom cell consisted of a ceramic tube, constructed from re-crystallised alumina (6 mm I.D. x 10 mm O.D. x 110 mm, Thermal Syndicate Limited, Wallsend, Tyne and Wear) and was heated by a conventional air/acetylene flame to prevent adsorption or condensation of analyte atoms in the tube. For the same reason the interface line was heated electrothermally using a design similar to Quimby et al., (1978). The organometallic species to be determined were separated chromatographically by means of a glass column (1.5 m x 4 mm I.D.) packed with 10% OV-101 on chromosorb W (80-100 mesh). Liquid samples of sediment and water extracts were injected directly onto the column using a microlitre syringe (Scientific Glass Engineering, Melbourne, Australia). Detection of Pb or Se was achieved by monitoring the appropriate wavelength and using a hollow cathode lamp as the spectral source. The output from the spectrometer was processed by an electronic integrator (Model 3390A, Hewlett Packard, Pennsylvania, U.S.A.) which reported information on peak height and peak area in addition to retention times.

The extraction procedures used for the analysis of alkyllead and Se compounds are described in detail in sections 2.3.1.2 and 2.3.2.2. The optimal conditions identified for the analysis of alkyllead compounds are in good agreement with Chakraborti et al., (1984), Table 2.2. Similarly the conditions derived for the determination of alkylselenium compounds are similar to those reported by Chau et al., (1975), Table 2.3. Detection limits achieved and precision found for alkyllead and alkylselenium compounds are shown in Tables 2.4 and 2.5 respectively. In the case of Pb compounds the detection limits (14-240 pg, Pb) and precision (1.3-24.2%, relative) achieved compare favourably with the detection limits (40-100 pg, Pb) and precision (overall precision 10%) quoted by Chakraborti et al., (1984). For Se compounds the detection

Table 2.2 Instrumental parameters for the GC-AAS analysis of inorganic and alkyllead compounds.

GC		AAS
Injector temperature	150°C	Lamp HCL(Pb) 6 mA
Column temperature	50° $\xrightarrow{10°C\ min^{-1}}$ 250°C	Wavelength 283.3 nm
Interface line	140°C	Bandpass 0.5 nm
N_2 carrier gas	40 ml min^{-1}	Air flow 4.5 l min^{-1}
H_2 auxiliary gas	105 ml min^{-1}	Acetylene flow 0.6 l min^{-1}
		Deuterium arc background correction applied

Table 2.3 Instrumental parameters for the GC-AAS analysis of alkylselenium compounds.

GC		AAS
Injector temperature	220°C	Lamp HCL(Se) 5.0 mA
Column temperature	50° $\xrightarrow{49°C\ min^{-1}}$ 120°C	Wavelength 196.0 nm
Transfer line	120°C	Bandpass 1.0 nm
N_2 carrier flow	60 ml min^{-1}	Air flow 4.5 l min^{-1}
H_2 auxiliary flow	100 ml min^{-1}	Acetylene 1.0 l min^{-1}
		Deuterium arc background correction applied

Table 2.4 Analytical characteristics for the GC-AAS determination of inorganic and organic lead compounds.

Analyte	Retention Time (mins)	Detection Limit (pg) Ⓐ	Precision, Ⓑ Relative Standard Deviation (%)
$PbMe_4$	2.5	14	1.3
$PbEt_4$	10.6	14	1.3
$PbMe_3Bu$	8.6	65	6.7
$PbEt_3Bu$	13.9	105	10.4
$PbEt_2Bu_2$	16.8	140	13.8
$PbBu_4$	20.3	240	24.2

Ⓐ Detection Limit: that quantity of the analyte which gives rise to a reading equal to twice the standard deviation of 10 determinations at the 500 pg Pb level (injection volume of Pb standard was 1 µl).

Ⓑ Precision: measured as the percentage relative standard deviation of the signal response arising from 10 measurements of a 1 µl injection of a 500 pg μl^{-1} Pb standard.

Table 2.5 Analytical characteristics for the GC-AAS determination of alkylselenium compounds.

Analyte	Retention Time (mins)	Detection Limit (A) (ng)	Precision, (B) Relative Standard Deviation (%)
$Se(CH_3)_2$	1.3	0.14	3.5
$Se_2(CH_3)_2$	3.7	0.15	3.8

(A) Detection Limit: that quantity of the analyte which gives rise to a reading equal to twice the standard deviation of 10 determinations at the 2.0 ng Se level (injection volume of Se standard was 1 µl).

(B) Precision: measured as the percentage relative standard deviation of the signal response arising from 10 measurements of a 1 µl injection of a 2 ng μl^{-1} Se standard.

limits (0.14-0.15 ng, Se) and precision (3.5-3.8%, relative) achieved also compare favourably with the detection limits (0.1 ng, Se) and precision (overall precision 10%) quoted by Chau et al., (1975).

2.2.4 Coupled Liquid Nitrogen Trapped Hydride Generation/GC-AAS

Natural water samples and macro-algae extracts were analysed for both inorganic and organic As compounds by means of a coupled liquid nitrogen trapped hydride generation/GC-AAS system. Gaseous covalent hydrides were generated from sodium arsenite, sodium arsenate, monomethylarsonic acid (disodium salt) and dimethylarsinic acid (sodium salt) the hydrides were then collected in a liquid nitrogen cooled trap as described by Howard and Arbab-Zavar (1981). The trap was then connected to the gas chromatograph via a switching valve and heated. The separated hydrides were then detected by GC-AAS as described by Ebdon et al., (1982).

Using the coupled hydride generation liquid nitrogen trapping system described above the determination of As was achieved in two stages (Figure 2.2). In the first stage aqueous samples were introduced at 2.5 ml min^{-1} by a peristaltic pump. In the analysis of sodium arsenite (As(III)), sodium arsenate (As(V)), monomethylarsonic acid (disodium salt) and dimethylarsinic acid (sodium salt), hydrochloric acid (1 M) and sodium tetrahydroborate (4%, m/v) were pumped sequentially with sample also at 2.5 ml min^{-1} via a mixing coil (14 loops) into a gas-liquid separator. The liberated arsines were dried with sodium hydroxide pellets and swept by nitrogen to the cryogenic trap for collection onto glass beads (40 mesh) held at -196°C. Further speciation of sodium arsenite (As(III)) and sodium arsenate (As(V)) was achieved by exploiting the pH dependence for reduction of As compounds by sodium tetraborohydrate (Aggett and Aspell, 1976). Buffering the hydride generation system to pH 5 with sodium acetate-acetic acid buffer allows the production

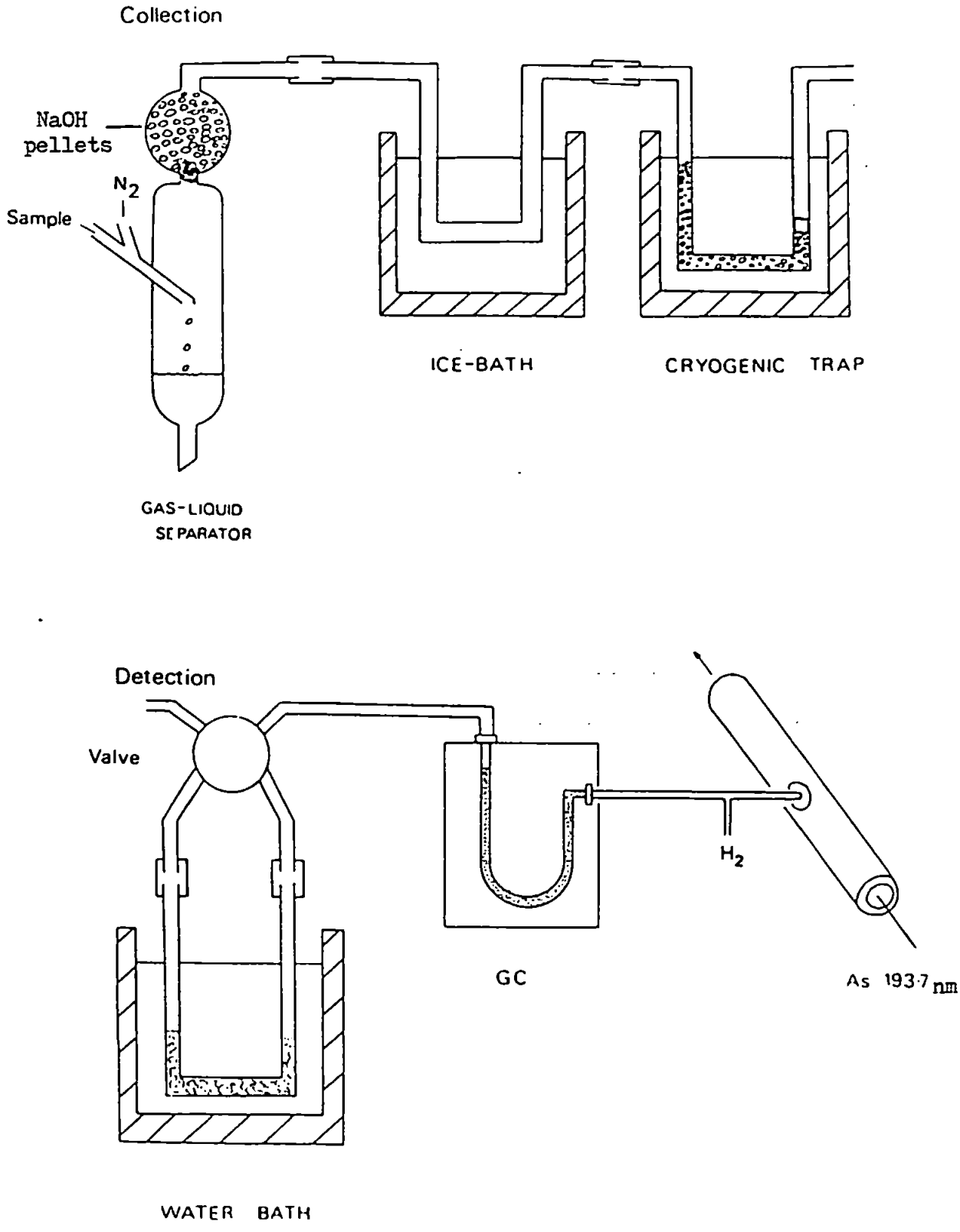


Figure 2.2 Determination of arsenic using the coupled hydride generation liquid nitrogen trapping system

of arsine solely from sodium arsenite(As(III)). By performing the analysis at two pH values it was therefore possible to determine sodium arsenite(As(III)) and 'total' inorganic As and subsequently sodium arsenate(As(V)) by difference.

In the second stage of analysis the cryogenic trap is connected to the GC-AAS system via a 4-way valve as described by Chau et al., (1976c) The trap was heated (hot water bath, 80°C) and the desorbed arsines were flushed into the GC-AAS system by a flow of nitrogen of 30 ml min^{-1}. Atomization was achieved using a small hydrogen flame and the atoms detained in a ceramic tube heated by a conventional flame. Detection of As species was achieved by monitoring the appropriate wavelength and using a hollow cathode lamp as the spectral source. The output from the spectrometer was processed by an electronic integrator (model 3390A, Hewlett Packard) which reported information on peak height and peak area in addition to retention times.

The instrumental conditions used and analytical characteristics obtained for the analysis of As are given in Tables 2.6 and 2.7 respectively. The optimal GC-AAS conditions employed are similar to those reported by Wong et al., (1977). The optimal hydride generation conditions used were also found to be similar to those quoted by Howard and Arbab-Zavar (1981). The detection limits (0.16-0.46 ng, As) and precision (0.8-2.3%, relative) achieved compare favourably with the detection limits (0.25 ng, As) and precision (3.1-7.3%, relative) quoted by Howard and Arbab-Zavar (1981).

2.3 Environmental Studies

This section reports the sampling and preparation procedures adopted throughout the estuarine surveys, the collection of natural waters,

Table 2.6 Optimal GC-AAS/Hydride generation parameters for the determination of arsenic compounds.

GC-AAS		HYDRIDE GENERATION	
Column Packing:	10% OV101 on chromosorb W(80-100)	Reagents: (flow rate 2.5 ml min^{-1})	
Temperatures:			HCl (1.0 M)
			$NaBH_4$ (4.0% m/v)
Column	30°C isothermal		Buffer, pH 5.0 (sodium acetate/ acetic acid)
Injector	50°C		
Interface	60°C		
Gas flow rates:		Drying agent:	
			NaOH pellets/water bath
N_2	30 ml min^{-1}		
H_2	100 ml min^{-1}	Trapping system:	
Air	4.0 l min^{-1}	Packing:	glass beads (40 mesh)
C_2H_2	0.6 l min^{-1}		
Light source:	As (HCL) 8 mA	Trapping period:	5 mins
Wavelength:	193.7 nm	Purge flow (N_2):	300 ml min^{-1}
Bandpass:	0.5 nm		
Deuterium arc background correction applied			

Table 2.7 Analytical characteristics for the GC-AAS determination of arsenic compounds.

Analyte	Retention Time (mins)	Detection Limit (ng) (A)	Precision, Relative Standard Deviation (%) (B)
Sodium arsenite (pH 5)	0.60	0.46	2.3
Sodium arsenate:	0.60	0.20	1.0
Monomethylarsonic acid:	0.90	0.16	0.8
Dimethylarsinic acid:	1.50	0.21	1.1

(A) Detection Limit: that quantity of the analyte which gives rise to a reading equal to twice the standard deviation of 10 determinations at the 10 ng As level (injection volume of As standard was 1 ml).

(B) Precision: measured as the percentage relative standard deviation of the signal response arising from 10 measurements of a 1 ml injection of a 10 ng ml^{-1} As standard.

sediments and fauna for modelling experiments and the subsequent analyses performed on these samples. It also deals with the studies carried out on the water, sediment and fauna extraction procedures employed in this work.

2.3.1 Water Samples

Water samples for the estuarine surveys were collected from the River Carnon and associated Restronguet Creek and Tamar and Beaulieu Estuaries (see Figures 1.3-1.5).

2.3.1.1 Water Sampling

Surface waters were collected from a rubber inflatable dingy, the actual sites being located geographically or by using salinity as an indicator of tidal incursion. Samples were filtered (0.45 μm millipore cellulose acetate filters) *in situ* into polythene bottles, containing sufficient hydrochloric acid to bring the pH of the sample to $<$pH 1.0, for the analysis of inorganic arsenic, monomethylarsonic acid, dimethylarsinic acid and 'total' Pb and Se. High acid concentrations (pH$<$1.0) were used to preserve transported samples and this has been reported not to alter markedly ($<$5%) the original As speciation present (Andreae, 1979). Duplicate water samples were also collected for the solvent extraction analysis of organolead and Se compounds, these were not acidified but transported in a cool box at 4°C. In addition the surface water temperature and salinity of all samples was measured *in situ* using a precalibrated MC5 Mk.II T/S bridge (Kent Industrial Measurements, Surrey), pH was also measured using a portable digital pH meter (DC66, WPA Scientific Instrumentation, Saffron Waldon, Essex).

Unacidified samples were solvent extracted immediately upon return to

the laboratory and analysed within 8 h. Acidified samples were stored at 4°C in the dark before analysis. Water samples collected for use in laboratory modelling experiments were stored in acid washed polythene containers and upon return to the laboratory stored in the dark at 4°C.

Porewater samples were collected at specific sites in the Tamar Estuary (see Figure 2.3) using *in situ* dialysis. The cellulose acetate dialysis bags (Scientific Instrument Centre, London) were contained in perspex holders (Knox *et al.*, 1981). Each dialysis bag, containing 20 ml of deoxygenated deionized doubly distilled water, was buried into the surface sediment layer at low tide and left for a period of 7 days (Benes and Steinnes, 1974). The samples were returned to the laboratory with a small amount of sediment surrounding the holder, which minimised atmospheric oxidation of the sample during retrieval. The analysis of interstitial water samples was performed immediately within 4 h, and employed techniques identical to those used for the analysis of surface waters.

2.3.1.2 Water Analysis

Aqueous samples were analysed for 'total' metal levels as described in section 2.2.2. Similarly samples were analysed for As concentration and speciation as described in section 2.2.4. The determination of organolead and organoselenium compounds required an initial selective extraction procedure before samples (filtered, 0.45 μm) could be analysed by means of the GC-AAS system described in section 2.2.3, 2 particular extraction procedures were employed for the determination of organolead and organoselenium compounds in water. The first allows simultaneous extraction of both Pb and Se species and was amenable to preconcentration techniques. The second procedure was developed at a later date and involved derivatization of analyte to a butyl-derivative. This latter

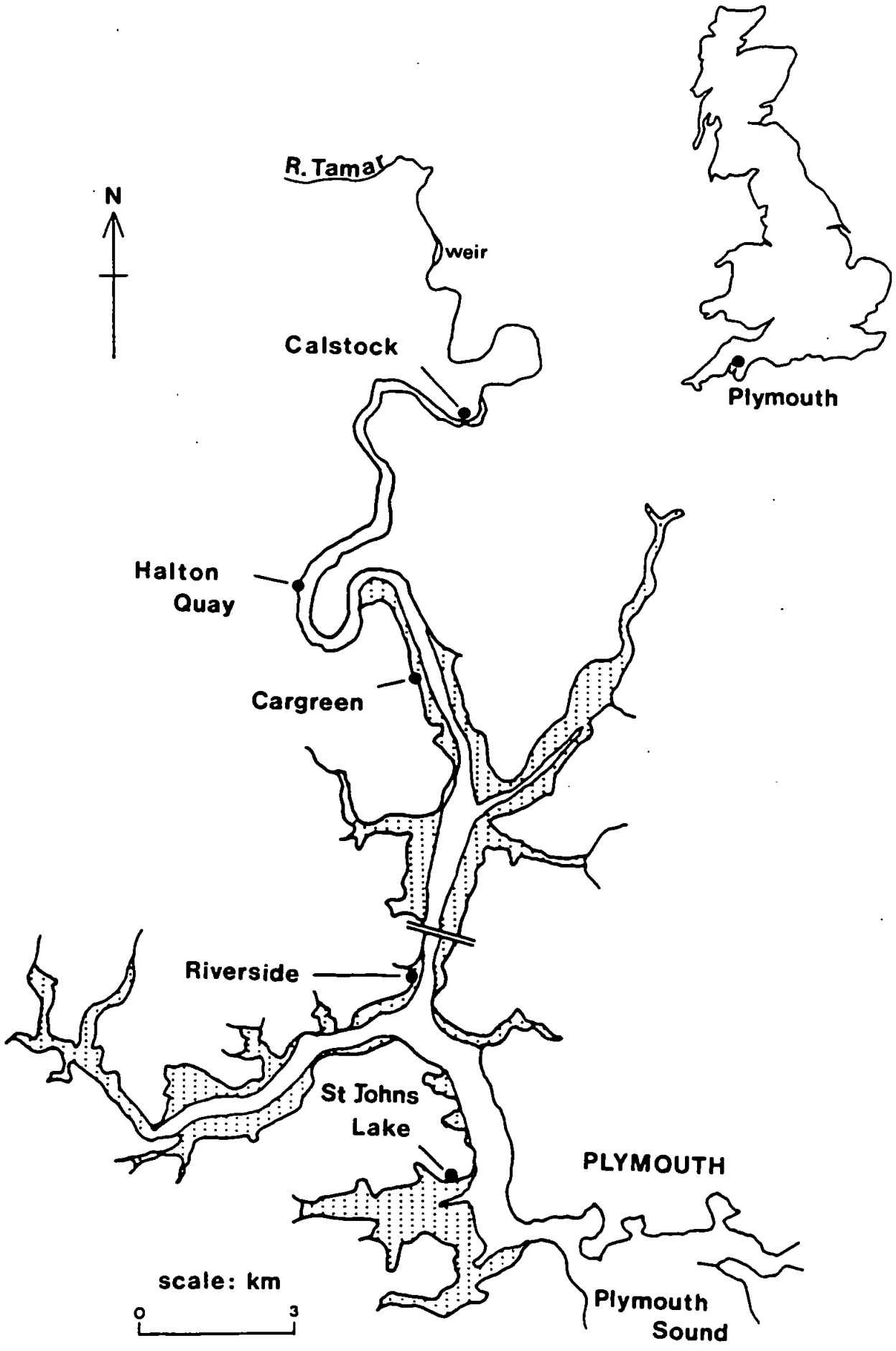


Figure 2.3 Porewater sampling sites in the Tamar Estuary

procedure was only used for Pb determination.

2.3.1.2.1 Simultaneous Extraction of Organolead and Organoselenium Compounds in Estuarine Waters

The procedure adopted was a minor modification of a method reported by Chau et al., (1979). Estuarine water (1 litre) was placed in a 1 litre separating funnel to which pentane (10.0 ml, Analar) and sodium chloride (10 g, GPR) were added. The mixture was shaken vigorously for 5 minutes using a mechanical shaker, then left to stand for a further 5 minutes to allow phase separation to occur. An aliquot of the pentane phase (0.5-1.0 ml) was pipetted into a small vial, sealed, and kept at $4^{o}C$ in the dark until required for analysis. Suitable aliquots of the sample extract (1-10 µl) were injected directly into the GC-AAS system (section 2.2.3). Aliquots (1-10 µl) of Se and Pb standard compounds (prepared in n-pentane) were injected directly into the GC-AAS system using a glass syringe. Calibration curves were obtained for each of the alkyllead and alkylselenium standard compounds from which the concentration of compounds in the sample extract could be calculated.

The precision of the method was evaluated by determining 10 replicate samples from 1 litre of seawater enriched with known concentrations of organolead and Se standards. The spiked samples were allowed to equilibrate in the sealed separating funnel for approximately 30 minutes in the dark and then processed as described in the procedure above. In spiked samples equilibrated in open flasks poor recovery ($<15\%$) was achieved for tetramethyllead (TML) which was attributed to the high volatility of the compound. As a result the separating funnel was kept sealed during extractions. The relative standard deviation (%) for the species studied is shown in Tables 2.8 and 2.9. Recovery was assessed by adding different levels of alkyllead and Se compounds to 1 litre of seawater which had been previously extracted with n-pentane

Table 2.8 Precision of the extraction method employed in the determination of alkyllead compounds in natural water samples.

Analyte:	Pb Added:Ⓐ (μg)	Pb Concentration:Ⓑ (μg l^{-1})	Precision,Ⓒ Relative Standard Deviation (%)
$PbMe_4$	0.21	0.18	9.0
$PbEt_4$	0.24	0.23	5.0

Ⓐ Spike (μg/Pb) added to seawater (1 litre), extracted into pentane (10 ml) using sodium chloride (10 g).

Ⓑ Average Pb concentration of 10 replicate 1 litre spiked seawater samples.

Ⓒ Precision: measured as percentage relative standard deviation of the signal response arising from 10 replicate samples of stated Pb concentration (see Ⓑ). Volume of pentane extract injected for analysis was 10 μl.

Table 2.9 Precision of the extraction method employed in the determination of alkylselenium compounds in natural waters.

Analyte:	Se added: (A) (μg)	Se concentration: (B) (μg l^{-1})	Precision, (C) Relative Standard Deviation (%)
$Se(CH_3)_2$	10.0	8.7	6.5
$Se_2(CH_3)_2$	10.0	9.6	6.1

(A) Spike (μg/Se) added to seawater (1 litre), extracted into pentane (10 ml) using sodium chloride (10 g).

(B) Average Se concentration of 10 replicate 1 litre spiked seawater samples.

(C) Precision: measured as percentage relative standard deviation of the signal response arising from 10 replicate samples of stated Se concentration (see (B)). Volume of pentane extract injected was 10 μl.

to remove any of its original alkyllead or Se content. The recoveries of alkyllead and Se compounds are shown in Tables 2.10 and 2.11 Recoveries were within the range 88 to 108% and this was deemed acceptable with precision 1 to 4% relative.

Initial work on the extraction of natural waters with pentane yielded no detectable levels of alkyllead or Se compounds. A further preconcentration step was required and a micro-Kuderna Danish apparatus (Radojevic et al., 1986) was employed. Other methods of preconcentration have been reported (Hill, 1985) including rotary evaporation or nitrogen bubbling of samples, however, all were reported to cause significant loss of volatile species such as tetramethyllead.

Using the Kuderna Danish apparatus, preconcentration was achieved by transferring pentane extracts into the lower reservoir of the apparatus and gently refluxing the sample in a steam bath maintained at a temperature of 15-25°C above the boiling point of the solvent employed. The preconcentration factors achieved and concentration efficiencies obtained are given in Tables 2.12 and 2.13. The micro-Kuderna Danish apparatus worked well for the preconcentration of alkyllead compounds yielding concentration efficiencies (95.5 to 96.8%) and precision (5.7 to 4.7%, relative) at the x 10 concentration factor. At greater concentration factors the efficiency and precision markedly decreased, such that at a concentration factor of x 20 concentration efficiencies (32.4 to 64.1%) and precision (18.8 to 12.0%, relative) were observed. The micro-Kuderna Danish apparatus generally gave poorer extraction efficiencies for alkylselenium compounds than alkyllead compounds although the precision in both cases was similar. At the highest concentration factor (x 10) concentration efficiencies of (13.5 to 26.7%) with precision (14.5 to 9.6%, relative) were reported for alkylselenium compounds.

Table 2.10 Recovery of alkyllead compounds from spiked seawater samples.Ⓐ

Analyte	Pb added Ⓐ (μg)	Recovery, Ⓑ %	Pb added Ⓐ (μg)	Recovery, Ⓑ %
$PbMe_4$	0.42	90 (3)	0.84	90 (1)
$PbEt_4$	0.48	100 (2)	0.96	100 (1)

Ⓐ Spike (μg/Pb) added to seawater (1 litre) and extracted into pentane (10 mls) using sodium chloride (10 g).

Ⓑ Recovery: average of 10 replicate samples at quoted concentration. Figures in parentheses refer to the percentage relative deviation of the 10 replicate samples at the concentration stated (see Ⓐ).

Table 2.11 Recovery of alkylselenium compounds from spiked seawater samples.Ⓐ

Analyte	Se added Ⓐ (μg)	Recovery Ⓑ, %	Se added Ⓐ (μg)	Recovery Ⓑ, %
$Se(CH_3)_2$	2.8	93 (4)	10	88 (2)
$Se_2(CH_3)_2$	2.8	103 (2)	10	108 (1)

Ⓐ Spike (μg/Se) added to seawater (1 litre) and recovered into pentane (10 ml) using sodium chloride (10 g).

Ⓑ Recovery: Average of 10 replicate samples at quoted concentration. Figures in parentheses refer to the percentage relative standard deviation of the 10 replicate samples at the concentration stated (see Ⓐ).

Table 2.12 Preconcentration of alkyllead compounds using Kuderna Danish apparatus (Pentane extracts).

Analyte	Concentration Factor Ⓐ	Concentration Efficiency (%) Ⓑ
$PbMe_4$	x 10	95.5 (5.7)
	x 20	32.4 (18.8)
$PbEt_4$	x 10	96.8 (4.7)
	x 20	64.1 (12.0)

Ⓐ Concentration factor refers to the reduction in volume of the sample concentrated. For $PbMe_4$ and $PbEt_4$ the initial volume was 5.0 ml of a 50 ng Pb ml^{-1} pentane standard. A concentration factor of x 10 involved a reduction in volume from 5.0 ml to 0.5 ml and a concentration factor x 20 involved a reduction in volume from 5.0 ml to 0.25 ml.

Ⓑ Concentration efficiency refers to the percentage of analyte retained during evaporation of solvent, i.e., 100% concentration efficiency involved no loss of analyte. Figures in parentheses refer to percentage relative standard deviation of 6 replicate samples of stated concentration (see Ⓐ).

Table 2.13 Preconcentration of alkylselenium compounds using Kuderna Danish apparatus (Pentane extracts).

Analyte	Concentration Factor Ⓐ	Concentration Efficiency (%) Ⓑ
$Se(CH_3)_2$	x 5	18.6 (7.8)
	x 10	13.5 (14.5)
$Se_2(CH_3)_2$	x 5	36.9 (4.1)
	x 10	26.7 (9.6)

Ⓐ Concentration factor refers to the reduction in volume of the sample concentrated. For $Se(CH_3)_2$ and $Se_2(CH_3)_2$ the initial volume was 5.0 ml of 0.7 µg Se ml^{-1} pentane standard. A concentration factor of x 5 involved a reduction in volume from 5.0 ml to 1.0 ml.

Ⓑ Concentration efficiency refers to the percentage of analyte retained during evaporation of solvent i.e., 100% concentration efficiency involved no loss of analyte. Figures in parentheses refer to percentage relative standard deviation of 6 replicate samples of stated concentration (see Ⓐ).

2.3.1.2.2 Determination of Inorganic Lead and Ionic Alkyllead Compounds in Estuarine Waters

The procedure adopted for the determination of inorganic and ionic alkyllead compounds is that reported by Chakraborti et al., (1984). The procedure is outlined below.

Estuarine water (1 litre) was transferred to a 1 litre separating funnel to which citric acid (4.0 g, Analar) and ethylenediaminetetraacetic acid (EDTA) (1.0 g, Analar) were added after which concentrated ammonium hydroxide (Analar) was added to obtain pH 9.0. Next, sodium diethyl-dithiocarbamate (2.0 ml, 0.25 M NaDDTC aqueous solution, Analar) and pentane (10.0 ml, Analar) were added and the mixture shaken for 2 minutes. After phase separation, the lower (aqueous) phase was run into one flask whilst the pentane phase was collected in another Erlenmeyer flask (50 ml). In the original funnel, the aqueous solution was extracted a second time with a fresh portion of pentane (10 ml). The extracts were combined and then carefully rotary evaporated under vacuum at 20°C until dry. The dried extracts were treated with n-butylmagnesium chloride Grignard reagent (1.0 ml, 1.9 M in tetrahydro-furan) and the mixture gently swirled around the flask for 1 minute. Then, nonane (250 µl, Analar) and sulphuric acid (10.0 ml, 0.5 M, Analar) were added. The mixture was shaken for a further 1 minute and left for 2 minutes to allow phase separation. The nonane phase was then transferred to a clean flask and washed with water (10.0 ml, deionized doubly distilled) to remove traces of tetrahydrofuran in the sample extract. The nonane phase was then transferred to a small vial using a Pasteur pipette. Extracts were stored in the dark at 4°C prior to injection into the GC-AAS system described in section 2.1.3. In order to obtain data on inorganic Pb the above procedure was modified such that the addition of citric acid and EDTA was omitted and the pH of water samples unchanged.

The method was calibrated by processing a blank and standards prepared in seawater which had been previously stripped of Pb content by extraction with EDTA/citric acid and NaDDTC. Suitable aliquots of the standards (1-10 µl) in nonane were injected directly into the GC-AAS system. Calibration curves were obtained for the species present from which the concentration of compounds in sample extracts could be calculated.

The precision of the method was evaluated by determining 10 replicate samples from 1 litre of seawater enriched with known concentrations of alkyllead and Pb(II) species. The percentage relative standard deviation for the species is given in Table 2.14. Recovery of the Pb compounds was assessed by adding different levels of alkyllead and Pb(II) to 1 litre of seawater which had been pre-extracted of Pb content with EDTA/citric acid and NaDDTC. The recoveries achieved are shown in Table 2.15. The recoveries (80 to 100%) and precision (3 to 12%, relative) achieved compare favourably with the recoveries (90 to 108%) and (precision 6 to 10%, relative) quoted by Chakraborti et al., (1984).

2.3.2 Sediment Samples

Sediment samples were collected from the chosen porewater sites located in the Tamar Estuary (Figure 2.3).

2.3.2.1 Sediment Sampling

Surface sediment samples were collected using an acid washed 'Teflon' spatula at low tide and stored in sealed acid washed polythene containers. Immediately upon return to the laboratory sub-samples were washed with deionized doubly distilled water to remove any water soluble salts and then freeze dried for 48 h prior to analysis. This procedure is reported to result in negligible losses of metals (De Groot and Zschuppe, 1981).

Table 2.14 Precision of Butylation method in the extraction of inorganic and alkyllead compounds from seawater.

Analyte	Pb added Ⓐ (μg)	Pb Concentration Ⓑ ($\mu g\ l^{-1}$)	Precision, Ⓒ Relative Standard Deviation (%)
$PbMe_3Bu$	0.20	0.19	6.5
$PbEt_3Bu$	0.20	0.17	7.8
$PbEt_2Bu_2$	0.20	0.17	8.9
$PbBu_4$	0.20	0.16	11.5

Ⓐ Spike (μg/Pb) added to seawater (1 litre)

Ⓑ Average Pb concentration of 10 replicate 1 litre spiked seawater samples.

Ⓒ Precision: measured as percentage relative standard deviation of the signal arising from 10 replicate samples of stated Pb concentration (see Ⓑ). Volume of nonane extract injected was 5 μl.

Table 2.15 Recovery of alkyllead and inorganic lead from seawater.

Pb added Ⓐ (μg)	Recovery (%) Ⓑ			
	Me_3PbBu	Et_3PbBu	Et_2PbBu_2	$PbBu_4$
0.20	95 (7)	85 (8)	85 (9)	80 (12)
0.40	100 (3)	96 (5)	95 (4)	97 (4)

Ⓐ Spike (μg/Pb) added to seawater (1 litre)

Ⓑ Recovery: average of 10 replicate samples at quoted concentration. Figures in parentheses refer to the percentage relative standard deviation of the 10 replicate samples at the concentration stated (see Ⓐ).

2.3.2.2 Sediment Analysis

Sediments collected from the Tamar Estuary were subjected to a series of analyses in order to establish the bulk properties of the sediment. The procedures employed are described below.

2.3.2.2.1 Sediment Extracts

An aqua-regia leach of each sediment was prepared as follows.

Sediment (1-2 g dry wt.) was placed in a PTFE digestion bomb (Loring and Rantala, 1977), to which concentrated nitric acid (3.0 ml, Analar) and concentrated hydrochloric acid (9.0 ml, Analar) were added. The bomb was sealed and heated in an oven maintained at 110°C for 4 h. After cooling the mixture was filtered (Whatman No. 1 filter) into a 50 ml volumetric flask, and made up to volume with nitric acid (5% v/v, Analar).

An 'available' (non-detrital) leach of each sediment was undertaken as follows.

Sediment (5-10 g, dry wt.) was placed in an acid-washed polythene centrifuge tube to which aqueous acetic acid (50.0 ml, 25% v/v, Analar) was added. The tube was sealed and shaken mechanically for 12 h at room temperature (Loring and Nota, 1973). The resultant leachate was filtered (0.45 μm cellulose acetate filters) into a 100 ml volumetric flask and made up to volume using aqueous acetic acid (25% v/v, Analar).

Sediment extracts were analysed directly, after appropriate dilutions using deionized doubly distilled water, for Pb (section 2.2.1) and As and Se (section 2.2.2). To establish the validity of the sediment

extraction procedure 3 replicate analyses were carried out on 2 certified reference sediments, B C S S-1 and M E S S -1 (N.R.C. Marine Sediment Reference Materials, N.P.C. Montreal, Ottawa, Canada). Identical procedures to those described above were followed and the results are given in Tables 2.16 and 2.17.

An aqua-regia leach of standard sediments showed good agreement with the certified value for As and acceptable precision for As, low results for Pb and poorer precision (Table 2.16). Unfortunately no certified value is quoted for Se. The results obtained for Pb were low, especially for sample B.C.S.S.-1. This was considered to be due to the presence of Pb in such sediments being associated with the silica lattice structure which would not be attacked by an aqua-regia leach. Similar results have been obtained by Haswell (1983). In realizing the low results for Pb and bearing in mind that true 'total' metal levels are less appropriate to this work than biologically available metal levels, the consistency of results for the standard sediments was considered sufficient to validate the extraction procedure employed.

An acetic acid leach of the standard sediments was also carried out and the results shown in Table 2.17. Generally the precision obtained was poorer than for the aqua-regia leach. Since no certified 'available' figures are available a comparison of results obtained by several authors using the same extraction procedure on Tamar sediments is given (Table 2.18). In comparison the results for Tamar sediments were reasonable especially when one considers the range of sites sampled. The only notable discrepancies present are those for Pb. The results obtained are low compared to Luoma and Bryan (1981) but high compared to Haswell (1983). No other data was available for Se in Tamar sediments. It should be noted that the acetic acid digestions of the reference materials gave

Table 2.16 Analysis of N.R.C. Standard Sediments by HCl/HNO_3 (Aqua-Regia) leach.

Element	Replicate Analysis Ⓐ ($\mu g\ g^{-1}$)	Certified Value ($\mu g\ g^{-1}$)
As BCSS-1	11.2 ± (0.6)	11.1 ± (1.4)
MESS-1	10.4 ± (0.8)	10.6 ± (1.2)
Pb BCSS-1	15.8 ± (1.2)	22.7 ± (3.4)
MESS-1	29.1 ± (1.9)	34.0 ± (6.1)
Se BCSS-1	0.27 ± (0.09)	Not certified
MESS-1	0.39 ± (0.08)	Not certified

Ⓐ Average of 3 replicate analyses. Figures in parentheses refer to the range in sample concentrations found.

Table 2.17 Analysis of N.R.C. Standard Sediments by an Acetic Acid leach.

Element	Replicate Analysis (A)	% of Aqua-Regia leach
As BCSS-1	0.89 ± (0.06)	7.9
MESS-1	1.01 ± (0.13)	9.7
Pb BCSS-1	3.38 ± (0.26)	21.4
MESS-1	5.24 ± (0.91)	18.0
Se BCSS-1	0.12 ± (0.07)	44.6
MESS-1	0.28 ± (0.10)	56.1

(A) Average of 3 replicate analyses. Figures in parentheses refer to the range in sample concentrations found.

Table 2.18 A comparison of acetic acid leaches on different Tamar (A) sediments by various workers.

Element	Trace metal concentrations found, $\mu g\ g^{-1}$				
	This work	Luoma and Bryan (1981)	Haswell (B) (1983)	Ackroyd (1983)	Marsh (1983)
As	4 - 10	-	0.5 - 5	-	1 - 4
Pb	10 - 40	60 - 100	1 - 5	5 - 40	5 - 35
Se	0.1 - 0.3	-	-	-	-

(A) The range of sites sampled extends from a fresh water site at Gunnislake Weir (Cornwall) to a marine site at St. Johns' Lake (Cornwall).

(B) Mainly soil samples at Gunnislake Weir site (Cornwall).

low metal extraction yields compared to the Tamar results. This discrepancy is considered by Loring (1976) to be due to the different sediment mineralogy in the Tamar Estuary compared to the Miramichi Estuary (MESS-1 Standard) and Baie des Chaleurs (BCSS-1 Standard).

To summarise, the use of an aqua-regia leach procedure was endorsed, by comparison with standard sediments, as providing an acceptable representation of the total 'labile' metal fraction. The acetic acid leach procedure was also established as a generally good indication of non-detrital ('available') metal content of sediments, being no less consistent than other leaching solutions reported in the literature (De Groot and Zschuppe, 1981).

A pentane extraction of each sediment was also undertaken to establish the presence of any alkyllead and alkylselenium compounds adsorbed onto particle surfaces. The method employed is that reported by Chau et al., (1979) and is described below.

Unwashed sediment (20.0 g, wet wt.) was placed in a 50.0 ml centrifuge tube, to which pentane (5.0 ml) was added. The tube was sealed and centrifuged for 5 minutes at 2000 x g. After leaving the mixture to stand for 5 minutes an aliquot of the pentane phase (approx. 1 ml) was pipetted into a small vial and sealed until analysed. Suitable aliquots of the sample extract (1-10 µl) were injected directly into the GC-AAS system (Section 2.2.3). Aliquots (1-10 µl) of Se and Pb standard compounds (prepared in n-pentane) were injected directly into the GC-AAS system. Calibration curves were obtained for each of the organolead and Se standards, from which the concentration of compounds in the sample extract could be calculated.

The recoveries of alkyllead and alkylselenium compounds from samples was

assessed by spiking pre-extracted sediment (20.0 g wet wt.), with known concentrations of alkyllead and Se standards. The spiked samples were allowed to equilibrate for approximately 30 minutes in sealed containers and then processed as described in the procedure above. The recoveries of organolead and Se compounds added to sediment samples are summarised in Table 2.19. The recoveries achieved compare favourably with those quoted by Chau et al., (1979), and range from 88 to 102% with acceptable precision.

2.3.2.2.2 Organic Carbon

The organic carbon content of sediment samples was accurately determined using an elemental analyzer (Model 1106, Carlo Erba, Milano). The instrument employed was capable of determining C, N and H by gas chromatographic measurement of the combustion products CO_2, N_2 and H_2O respectively. The determination of organic carbon was achieved as follows:- the sediment sample (0.1-8 mg, dry wt.) was placed into a small tin container. The container was dropped at preset times into a vertical quartz tube, heated at 1050°C, through which a constant flow of helium was maintained. As each sample was introduced into the quartz tube the helium flow was temporarily enriched with pure oxygen. At this stage flash combustion of the sample takes place, primed by the oxidation of the tin sample container. Quantitative combustion was achieved by passing the gases evolved over Cr_2O_3. The mixture of the combustion gases produced is then transferred to a reduction reactor filled with elemental copper at 650°C allowing the removal of excess oxygen. Finally the gaseous mixture is introduced into the chromatographic column (Poropak Q5, packing material) which is heated isothermally at 80°C. The individual components are eluted in the order N_2, CO_2 and H_2O. Individual components were measured by a thermal conductivity detector. The response signal was processed by an elemental analyzer

Table 2.19 Recovery of alkyllead and alkylselenium compounds from sediment.

Analyte	Amount added (A) (μg)	Recovery (%) (B)
Me_4Pb	0.42	95.6 (6.5)
Et_4Pb	0.48	102 (5)
$Se(CH_3)_2$	2.8	88 (10)
$Se_2(CH_3)_2$	2.8	101 (5)

(A) Spike (μg/Pb or Se) added to sediment (20.0 g)

(B) Recovery: average of 10 replicate samples at the quoted concentration. Figures in parentheses refer to percentage relative standard deviation of the 10 replicate samples at the stated concentration (see (A)).

processor (Model 2000 c, Carlo Erba). The instrument was calibrated by a standard compound, namely acetanilide, (Elemental Micro-Analysis Ltd., Beaworthy, Devon). The elemental composition of the standard compound was quoted as:- C = 71.09%, N = 10.36%, H = 6.71% and O = 11.84%. The reproducibility of the technique is quoted as $\pm$ 0.2% for the determination of carbon over the range 0.1-8.0 mg.

2.3.2.2.3 Percentage Moisture Content

Sediment (10.0 g wet wt.) was placed in an open porcelain crucible and inserted into an oven maintained at 60°C for approximately 48 h, until a constant weight reading was obtained, (Butler and McManus, 1979). Samples were maintained dry in a sodium sulphate dessicator. The percentage moisture content of the sediment was calculated from the weight loss on drying, and expressed as a percentage of wet weight.

2.3.2.2.4 Dissolved Sulphide Content of Sediments

Sediment samples obtained from the Tamar Estuary were analysed for sulphide levels using an electrode method developed by Green and Schnitker (1974). The use of an antioxidant buffer (SAOB) allows potentiometric titrations of dissolved sulphide, providing cadmium nitrate is used as the titrant. Sulphide antioxidant buffer (SAOB) was prepared by dissolving potassium hydroxide (560 g, Analar) in 800 ml of distilled water, allowing the solution to cool and adding ascorbic acid (17.6 g, Analar). The final volume of the solution was 1 litre. Cadmium nitrate (0.001 mol. l^{-1}, Analar) was used as the titrant. A sulphide ion-selective electrode (Model 94-16, Orion Research Inc., Massachusetts, USA) and a double junction reference electrode (Model 90-02, Orion Research Inc.) were used in the analysis. The outer chamber of the reference electrode was filled with KNO_3 solution (10% v/v) and a digital voltmeter was used to monitor the potential of the electrode, (Model 701A, Orion

Research Inc.). The experimental method is described below.

Sediment (1-20 g, wet wt.) was weighed into a 250 ml beaker. SAOB (50 ml) and distilled water (50 ml) were added to the beaker and the sample magnetically stirred continuously. The sulphide and reference electrode were placed in the solution which was then titrated with cadmium nitrate solution. The analysis of sulphide was performed on fresh (1 h old) sediment. The change in electrode potential upon addition of titrant to form cadmium sulphide was recorded and from this data a titration curve was drawn. The end point of the reaction was identified from the point of inflexion in the titration curve. Sulphide concentrations of sediment samples were calculated knowing that 1 mole of sulphide ions react with 1 mole of cadmium ions to form cadmium sulphide. The sulphide concentration (dry wt. of sediment) is calculated from Equation 2.1. No sulphide was detected in a (SAOB) solution blank.

$$\text{Sulphide concentration } (\text{mg g}^{-1}\text{ dry wt.}) = \frac{\text{Titre}}{1000} \times 0.001 \times \frac{1}{\text{Wet wt. (g) sample}} \times \frac{100}{\%\text{ dry wt. of sample}} \times 32{,}000$$

(Equation 2.1)

The precision of the method was evaluated by determining the sulphide concentration of 6 replicate sub-samples obtained from a single sediment sample. The results are presented below.

Sample	Sulphide concentration mg g^{-1}
1	0.26
2	0.27
3	0.25
4	0.26
5	0.25
6	0.26

Average = 0.26

Standard Deviation (σ_{n-1}) = 0.008

Relative Standard Deviation = 2.9%

The precision (2.9% relative) achieved compared favourably with that quoted by Green and Schnitker (1974). As a result the method was deemed satisfactory for the determination of water soluble sulphide concentrations in natural sediments.

2.3.3 Fauna Samples

Macro-algae samples were collected on routine estuarine surveys in the Tamar Estuary. Initial samples were taken from the area around St. Johns' Lake, however, at a later date, samples were also collected from the chosen porewater sites (Figure 2.3).

2.3.3.1 Fauna Sampling

Samples were collected from land during low tide, washed several times in their native estuarine water to remove any debris, and stored in sealed polythene containers for transportation to the laboratory.

2.3.3.2 Fauna Analysis

Macro-algae samples collected were subjected to a series of extraction procedures in order to establish the total and speciated forms of As present. The procedures employed are described below.

A 'total' digest of each algal sample was prepared by refluxing an air dried finely chopped (mortar and pestle ground) sub-sample (0.5-1.0 g) with concentrated nitric acid (10.0 ml, Aristar) at 70°C for 24 h. The resultant digest was washed into a volumetric flask (100.0 ml) and made up to volume with deionized doubly distilled water.

A leach of each algal sample was further prepared by refluxing an air dried finely chopped (mortar and pestle ground) sub-sample (0.5-1.0 g)

with concentrated hydrochloric acid (10.0 ml, Aristar) at 70°C for 24 h. The resultant leachate was filtered (Whatman GF/A, glass fibre filters) into a volumetric flask (100.0 ml) and made up to volume with deionized doubly distilled water.

Macro-algae extracts were analysed directly, after appropriate dilutions with deionized doubly distilled water, for 'total' As (section 2.2.2) and speciated forms of As (section 2.2.4). No reference materials were available for the speciation of organoarsenic compounds in macro-algal samples. In order to establish the validity of the 'total' nitric acid extraction procedure, triplicate analyses were performed on NBS No. 1571 certified orchard leaves (N.B.S. Washington, 25 D.C., U.S.A.). An identical procedure was followed to that described above. Analysis of three replicate NBS orchard leaves samples gave an average value of 7.91 $\mu g\ g^{-1}$ with a standard deviation of 0.46 $\mu g\ g^{-1}$. NBS orchard leaves were certified to contain $10 \pm 2\ \mu g\ g^{-1}$ As. Given the overlap of 95% confidence limits this result was considered sufficient to validate the 'total' As digestion procedure employed. Recovery of organoarsenic compounds was assessed by spiking macro-algae with known concentrations of As standards and processing samples as described previously. The recoveries obtained are shown in Tables 2.20 and 2.21. The results confirm that complete recovery of added As can be achieved within experimental error and that no loss occurred due to decomposition or rearrangement of As species present.

2.4 Laboratory Modelling Studies

This section describes the procedures employed in the investigation of the kinetics of methylation and demethylation of various forms of Pb and As under simulated environmental conditions.

Table 2.20 Hydride generation recovery of added arsenic from a selected macro-algal species (Fucus Serratus) using an hydrochloric acid leach.

Arsenic (µg)								
Added Ⓐ			Found Ⓑ			Recovery (%)		
As^5	MMA	DMA	As^5	MMA	DMA	As^5	MMA	DMA
0	0	0	0.06	0.12	7.06	-	-	-
			(28)	(32)	(14.1)			
1.0	1.0	1.0	1.06	1.10	8.10	103	86	102
			(2.1)	(4.0)	(2.3)	(23)	(24)	(5.6)

Ⓐ 1.0 g (dry weight) sample of Fucus Serratus spiked with 1.0 µg As (0.1 ml x 10 mg As l^{-1} as arsenic standards, sodium arsenate (As^5), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)).

Ⓑ Average of 5 replicate analyses. Figures in parentheses refer to percentage relative standard deviation of 5 replicate samples of stated concentration (see Ⓐ).

Table 2.21 Hydride generation recovery of added arsenic from a selected macro-algal species (Fucus Serratus) using a nitric acid digest.

Arsenic (µg)								
Added (A)			Found (B)			Recovery (%)		
As^5	MMA	DMA	As^5	MMA	DMA	As^5	MMA	DMA
0	0	0	0.29	0.50	8.64	-	-	-
			(28.0)	(23.0)	(15.6)			
1.0	1.0	1.0	1.30	1.47	10.00	105	94	104
			(6.7)	(8.7)	(5.5)	(28)	(27)	(6.0)

(A) 1.0 g (dry weight) sample of Fucus Serratus spiked with 1.0 µg As (0.1 ml x 10 mg As l^{-1} as arsenic standards, sodium arsenate (As^5), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)).

(B) Average of 5 replicate analyses is reported for all data. Figures in parentheses refer to the percentage relative standard deviation of 5 replicate samples of stated concentration (see (A)).

2.4.1 Laboratory Models

Time dependence studies were carried out using an established modelling procedure (Chau and Wong, 1978).

The production and decomposition of methylated Pb species, observed in headspace gases was studied using the GC-AAS system developed (section 2.2.3). Whilst the formation of methylated As compounds, observed in aqueous solution, was studied using the coupled liquid nitrogen trapped hydride generation GC-AAS system, (section 2.2.4). Models were run under carefully controlled conditions of pH, T^{o}C, light, added nutrient and added metals.

2.4.2 Modelling Reagents

Seawater solutions were prepared using filtered (0.45 μm) seawater collected from the area off Plymouth Sound, Devon. Estuarine water solutions were prepared in a similar manner to seawater samples and were collected from Calstock (Cornwall) and St. Johns' Lake (Cornwall) during routine survey work. All model solutions were kept at 4^{o}C in the dark in polythene containers prior to use. Estuarine sediments were collected from Calstock (Cornwall) and St. Johns' Lake (Torpoint, Cornwall) immediately prior to their use in modelling experiments and returned to the laboratory in sealed flasks. Nutrients were added to experimental flasks to promote the growth of microorganisms and bacteria. The nutrients chosen included nutrient broth I, yeast extract and D-glucose. Sterile estuarine waters, sediments, nutrients, chemical reagents were obtained by 3 separate autoclaving exposures of 30 minutes each and were tested for sterility by streaking on nutrient agar and soil extract agar samples (Craig, 1980).

2.4.3 Laboratory Modelling Procedures

Although some similarities exist between the modelling procedures adopted for Pb and As studies, considerable differences occur such that the procedure for each are best described separately.

2.4.3.1 Lead Modelling Procedure

In this work the methylation of inorganic Pb compounds (lead (II) chloride, lead (II) nitrate and lead (II) acetate) and trimethyllead acetate was examined. The methylation of Pb compounds was studied both in biological and abiotic systems. The systems are described below.

(a) Biological System:

In each study Tamar sediment (10.0 g wet wt.) and Tamar water (50.0 ml, filtered) were placed in a 150 ml Erlenmeyer flask. Nutrient broth I (0.5%), D-glucose (0.1%) and yeast extract (0.1%) were added to promote microbial growth. The Pb compounds to be tested were added to the flasks, the flasks were then capped with subaseals and incubated at 15 or 30°C ± 1°C for 1 to 4 weeks. Initial experiments involved incubations in normal light and in the dark. In later experiments the rate of production of TML was investigated when solutions were amended with sodium sulphide (1000 μg Na_2S l^{-1}). Experiments were performed in duplicate.

(b) Abiotic System

In each study distilled water (50.0 ml) was autoclaved whilst in a 150 ml Erlenmeyer flask. The pH of the solution was amended with either nitric acid or sodium hydroxide. In selected experiments china clay (SPS, supreme, ECC International, St. Austell, Cornwall) was added as an abiotic substrate, this was performed to assess the role of particulates in the

methylation reaction. Again, the Pb compounds to be tested were added to flasks, after being sterilized by filtration, and the flasks sealed and incubated at $30^{\circ}C \pm 1^{\circ}C$ for 1-4 weeks. As with the biotic studies sodium sulphide was added to selected flasks, and the rate of methylation followed in both light and dark conditions. All experiments were repeated in duplicate.

Headspace gases were analysed at various intervals, over a period of 4 weeks, using a 1 ml gas tight syringe (S.G.E. Melbourne, Australia). The loss of 'total' Pb from solution was measured in model systems running concurrently with a biotic modelling experiment. In such experiments an aliquot of solution was removed from the flask and filtered (0.45 μm cellulose acetate filters) before analysis by FAAS (Section 2.2.1).

Finally, the quantum flux of visible light (400-700 nm range) was measured both inside the constant temperature room and inside the reaction flask itself. A measurement of the quantum flux was considered important since the rate of conversion of various forms of Pb to TML could be strongly influenced by the amount of light present. The quantum flux in the constant temperature room was 3.05×10^{15} quanta s^{-1} cm^{-2} compared to 3.02×10^{15} quanta s^{-1} cm^{-2} inside the flask. The negligible reduction in the quantum flux inside the flask was due to the filtering of visible light by the glass walls of the reaction flask. This reduction was considered to be insignificant ($<1\%$).

2.4.3.2 Arsenic Modelling Procedure

The investigation into the methylation of various forms of As included analysis of several biological systems. The individual systems studied are described below and the procedures adopted for each are explained below.

2.4.3.2.1 Simulation of Environmental Behaviour of Arsenic

In this work the methylation of arsenic trichloride (As(III)) and sodium arsenate (As(V)) by microorganisms and bacteria present in natural waters and sediment was examined. The basic regimes studied included a freshwater regime (water and sediment samples collected from Calstock, Cornwall) and a marine regime (water and sediment samples collected from St. Johns' Lake, Cornwall). The exact procedure adopted in this study was as follows.

In each study Tamar sediment (25.0 g, wet wt.) and Tamar water (150 ml, filtered) were placed in a 250 ml Erlenmeyer flask. Nutrient broth I (0.1%), D-glucose (0.03%) and yeast extract (0.03%) were added to promote microbial growth. The As compounds to be tested namely arsenic trichloride and predominantly sodium arsenate were added aseptically to the flasks. The level of added As compounds was varied from 0-25 μg As l^{-1}. An appropriate abiotic control was run concurrently with biotic studies. The flasks were capped with an aseptic air filter and allowed to equilibrate with the atmosphere in constant temperature rooms maintained at 5°C and 15°C respectively. The experiments involved incubation of inoculums in 'normal' light (quantum flux, 3.05 x 10^{15} quanta s^{-1} cm^{-2} in the 400-700 nm visible range). Filtered aqueous sub-samples from each flask were analysed for their As content and speciation, over a period of 1000 h, by means of the coupled liquid nitrogen trapped hydride generation/GC-AAS system (section 2.2.4).

Sub-samples of inoculum medium were also analysed colourimetrically for reactive phosphate using the Molybdenum blue method (Murphy and Riley, 1962), modified by including an initial reduction procedure (Pett, 1933). The modified procedure was necessary because sodium arsenate, present at elevated levels (0-25 μg As l^{-1}) in inoculum medium, was reported to cause an interference in the 'Molybdenum blue' determination of reactive

phosphate (Pett, 1933). The initial reduction step removes this interference by reducing sodium arsenate ions to the arsenite form which does not cause an interference (Pett, 1933). After reduction the reactive phosphate concentration of the sample was analysed exactly as described by Murphy and Riley (1962). The method is described below.

The inoculum sample (5.0 ml, 0.45 μm filtered) was accurately transferred to a volumetric flask (50.0 ml) and made up to volume with distilled water. The diluted sample was then transferred to a conical flask (100 ml) to which sulphuric acid (1.0 ml, 1 M H_2SO_4, Analar) and sodium metabisulfite (0.4 g, GPR) were added. The sample was then heated for a period of 1 h in a water-bath maintained at 50°C (± 1°C). Next, mixed reagent (5.0 ml) was added to the warmed sample and the mixture shaken for 1 minute. After a further 5 minutes the absorbance of the solution was measured in a 1 cm quartz cell at a wavelength of 885 nm, using a spectrophotometer (SP500, Pye Unicam). The instrument was calibrated by processing dilute phosphate standards exactly as described above. A reagent blank was also processed in the same manner and all phosphate levels quoted are blank corrected. The precision of the method was evaluated by determining the reactive phosphate concentration of 6 replicate seawater samples. The results are shown below.

Sample	Reactive Phosphate Concentration, μg at P l^{-1}
1	0.17
2	0.16
3	0.17
4	0.15
5	0.17
6	0.16
	Average = 0.16
	Standard Deviation (σ_{n-1}) = 0.008
	Relative Standard Deviation = 5.1%

The precision (5.1% relative) achieved compared favourably with that reported by Pett (1933) and Murphy and Riley (1962).

A comparison of phosphate concentrations, in sodium arsenate spiked seawater, obtained using the modified (Pett, 1933) and unmodified method (Murphy and Riley, 1962) is given in Table 2.22. From Table 2.22, the modified and unmodified methods gave identical phosphate values for unspiked seawater samples. The relatively low arsenate concentration of seawater, 1-1.5 $\mu g\ l^{-1}$ (Sanders and Windom, 1980), apparently does not interfer in the 'Molybdenum blue' determination of reactive phosphate. This is confirmed by Murphy and Riley (1962). However, unless modified, the presence of higher concentrations of sodium arsenate (0-60 μg As l^{-1}) can give rise to a positive interference. When samples containing 10-60 μg As l^{-1} (as sodium arsenate) are reduced using the modified method (Pett,1933) this interference is removed completely. The ability of the method to correct for the presence of 60 μg As l^{-1} (as sodium arsenate) was deemed sufficient to validate the modified (Pett,1933) 'Molybdenum blue' method (Murphy and Riley, 1962) employed.

2.4.3.2.2 Skeletonema Costatum Modelling Procedure

In this work the methylation of sodium arsenate by a pure marine phytoplanktonic species Skeletonema costatum was examined. Axenic stock cultures of Skeletonema costatum were obtained from batch cultures grown at the Marine Biological Association (Plymouth) and were grown in a filtered seawater medium which was collected beyond the continental shelf, filtered (0.45 μm) to remove particulate material and sterilized by autoclaving. Nutrients, 20 μM nitrate and silicate were added to promote cellular growth. The inoculum of cells from stock cultures was designed to give an initial cell density of approximately 10^6 cells l^{-1}. The cultures were placed in 10 litre glass flasks and kept in a constant temperature room maintained at $15^{\circ}C \pm 1^{\circ}C$ at a light flux of 3.05×10^{15} quanta s^{-1} cm^{-2} (400-700 nm visible light) and a photoperiod of 16 h light

Table 2.22 Comparison of reactive phosphate concentrations, in sodium arsenate spiked seawater, determined using the modified (Pett,1933) and unmodified method (Murphy and Riley, 1962).

Sample	Reactive phosphate concentration, µg at P l^{-1}	
	Unmodified Method (Murphy and Riley, 1962)	Modified Method (Pett, 1933)
Seawater (0.45 µm filtered)	: 0.07 ± (0.01)	: 0.07 ± (0.01)
Seawater + 10 µg As L^{-1} (as sodium arsenate)	: 0.29 ± (0.04)	: 0.08 ± (0.01)
Seawater + 20 µg As L^{-1} (as sodium arsenate)	: 0.41 ± (0.07)	: 0.08 ± (0.02)
Seawater + 60 µg As L^{-1} (as sodium arsenate)	: 0.98 ± (0.11)	: 0.07 ± (0.01)

(A) Average of 2 replicate samples quoted. Figures in parentheses refer to the range in results obtained.

with 8 h dark. Sodium arsenate (10 μg As(V) l^{-1}, level) was added aseptically to the culture which was run until the culture had reached stationary phase. An abiotic control flask was run concurrently with the biological study. Sub-samples were removed periodically for cell counts, chlorophyll A measurements (by *in vivo* fluorescence measurements, Sanders and Windom, 1980), reactive phosphate level (Pett, 1933) and As concentration and speciation. The As concentration and speciation of *Skeletonema costatum* cells was determined by the method described by Sanders and Windom (1980). Approximately 5 mg of cells (dry wt.) were digested in concentrated nitric acid (5 ml, Aristar) under low heat (85°C). The final volume of the digest solution was 5.0 ml. Digests were analysed as described in Section 2.2.4.

2.4.3.2.3 Algal Modelling Procedures

In this work the release of methylated and inorganic forms of As by the macro-algae *Ascophyllum nodosum* was investigated.

The release of organic and inorganic species of As was examined at 5°C and 15°C. The macro-algal species *Ascophyllum nodosum* was collected immediately prior to the study. Approximately 180 g of macro-algae was allowed to grow in a filtered seawater medium which was collected from beyond the continental shelf. The macro-algae was placed in a 20 litre polythene tank containing 10.0 litre of seawater and was gently aerated for the period of study (7 days). No nutrients were added to promote growth. The aquaria were kept in constant temperature rooms maintained at 15°C ± 1°C and 5°C ± 0.1°C at a light flux of 3.05×10^{15} quanta s^{-1} cm^{-2} (400-700 nm visible light region) and a photoperiod of 16 h light and 8 h dark. Sub-samples were taken periodically for As concentration and speciation studies (Section 2.2.4).

CHAPTER THREE

RESULTS AND DISCUSSION OF LEAD STUDIES

3.1 The Distribution of Lead Species in Estuarine Environments

This chapter reports the results of both the environmental survey work and laboratory modelling studies on Pb methylation. If environmental methylation of Pb occurs in the estuarine environment it is logical to assume that the highest levels of methylated Pb species would be detected in areas of high biological productivity with detectable Pb contents. The Tamar, Beaulieu and Carnon systems meet these requirements, however, at present reliable information on the speciation of Pb in these 3 estuaries is limited. This is mainly attributed to the fact that during collection and analysis contamination or modification of samples can occur. Although this possibility can here be ruled out since great care was taken during this study to ensure that samples were analysed in a relatively short time after collection. During the period June 1985 to June 1986 a series of surveys was carried out to determine the concentration of inorganic and methylated Pb species in the waters and sediments of the 3 estuaries referred to above. A summary of the work undertaken is given in Table 3.1.

3.1.1 Tamar Estuary

The results of the Tamar Estuary Pb survey work are reported in Tables 3.2-3.5 and Figures 3.2-3.3 and are discussed below.

In an attempt to identify the sources of alkyllead species in the estuarine environment sites close to and removed from land derived petroleum inputs were sampled. Rainwater, drainwater and surface water samples collected at sites close to petroleum inputs were found to contain detectable levels of alkyllead species. During rainfall TAL species and their decomposition products, the trialkyllead and dialkyllead species, are washed out of the atmosphere (Chakraborti *et al.*, 1984). A

Table 3.1 Summary of estuarine survey work on lead methylation

Samples Collected	Estuarine Systems Studied		
	TAMAR	CARNON	BEAULIEU
Rain and drainwater:	Alkyllead and inorganic Pb(II) species detected (Table 3.2)	Not collected	Not collected
Surface waters :	Alkyllead species detected (Table 3.3 and Figure 3.1). Inorganic Pb(II) results given in Figures 3.2 and 3.3, 30/7/85 and 25/6/86.	Only inorganic Pb(II) species detected, 4/6/85 (Table 3.6)	Only inorganic Pb(II) species detected, 18/6/85 (Table 3.7)
Porewaters :	Only inorganic Pb(II) species detected (Table 3.4)	Not collected	Not collected
Sediments :	Only inorganic Pb(II) species detected (Table 3.5)	Not collected	Not collected

rainwater sample collected at a site 3 km east of the mouth of the Tamar Estuary (see Table 3.2) contained detectable concentrations of the tri- and diethyllead species (range 28-107 ng Pb l^{-1}), tri- and dimethyllead species were below the detection limit. Chakraborti et al., (1984) using an identical method of analysis detected tri- and diethyllead species in the range 9-68 ng Pb l^{-1} for samples collected in urban areas of Antwerp (Belgium). Chakraborti et al., (1984) did not report concentrations for TAL species. In this thesis TAL species were below the detection limit (1.4 ng Pb l^{-1}, after x 10 preconcentration using the Kuderna Danish apparatus) in rainwater samples. The TAL species are volatile and unlikely to remain in solution (Rohbock et al., 1980) as such they are unlikely to be detected in rainwater. The absence of detectable concentrations of the TML decomposition products, the tri- and dimethyllead species, in rainwater may be accounted for by the greater thermal and photolytic stability of TML compared to TEL (Rohbock et al., 1980). Harrison and Laxen (1978a) have estimated that TEL decomposes at a 3-4 fold greater rate than TML in the atmosphere, typical decomposition rates of 16-29% h^{-1} for TML and 67-93% h^{-1} for TEL occur for summer days.

The addition of petroleum products to drainwater and specific estuarine water samples was evident from the presence of a thin hydrocarbon film on the waters surface. Drainwater contained detectable levels of TEL (a known additive to petroleum) and other partially methylated Pb species (see Table 3.2). The concentration of dissolved alkyllead species ranged from 40-478 ng Pb l^{-1}. The lower levels of tri- and dimethyllead species compared to tri- and diethyllead in drainwater reflect the greater volatility and photolytic stability of TML compared to TEL, as was the case for a rainwater sample. In estuarine surface waters at sites where land derived petroleum inputs occurred trace levels of

Table 3.2 Determination of alkyllead and inorganic lead (II) species in rain and drainwater samples (filtered 0.45 μm)

Sample	Lead concentration (A) (ng l^{-1})						
	$(CH_3)_4Pb$:	$(C_2H_5)_4Pb$:	$(CH_3)_3Pb^+$:	$(C_2H_5)_3Pb^+$:	$(CH_3)_2Pb^{2+}$:	$(C_2H_5)_2Pb^{2+}$:	Pb^{2+}
Rainwater (B)	<1.4	<1.4	<2	28	<2	107	66.4×10^3
Drainwater (C)	<1.4	87	215	235	40	478	44.2×10^3

(A) Tetraalkyllead species determined as in Section 2.3.1.2.1 (detection limit was 1.4 ng Pb l^{-1} after x 10 preconcentration step). Ionic alkyllead species determined by the butylation method (refer to section 2.3.1.2.2).

(B) Rainwater sample collected using a rainwater gauge (sited at roof height (15 m) on top of the meteorological office at Plymouth Polytechnic; Sample site was 3 km east of the mouth of the Tamar Estuary) over the period 1/3/85-12/3/85.

(C) Drainwater sample collected from a city centre road drain (Drake Circus, 3 km east of mouth of the Tamar Estuary) at 10.15 GMT on 15/3/86. Collection took place after a period of 2 h of heavy rainfall.

l methylated Pb species were detected. Samples collected under the Tamar Bridge and at Mayflower Marina (see Table 3.3) contained the trimethyllead species (5 and 10 ng Pb l^{-1}, respectively). The relatively low levels of methylated species found in estuarine surface waters compared to rainwater and drainwater may be accounted for by the dilution of such species during estuarine mixing. The absence of detectable levels of other involatile partially methylated Pb species in the filtered water (0.45 μm) samples collected may be attributed to their more rapid photolytic decomposition as discussed above or adsorption onto estuarine particulates. Jarvie et al., (1981) also reported that trialkyllead species are decomposed in sunlight and that over a 15 day period 4% of trimethyllead and 99% of triethyllead species were decomposed directly to inorganic Pb in aqueous solutions. The same authors Jarvie et al., (1981) also reported that adsorption onto particulates (silica substrate) was more rapid for the triethyllead species than trimethyllead species, however, no rate data was reported. The above data, although limited, indicates that land derived petroleum inputs are a significant source of estuarine alkyllead species.

In a further series of surveys sites removed from land derived petroleum inputs were sampled in an attempt to identify levels of methylated Pb species which occur as a result of environmental methylation processes. During 2 surveys of mid-channel surface water sites (30/7/85 and 25/6/86) alkyllead species were always below the detection limit. The levels of inorganic Pb(II) recorded are shown in Figures 3.2 and 3.3. These profiles show that mid-channel levels of dissolved inorganic Pb(II) are relatively low, in many cases only double those found in the Beaulieu Estuary (refer to section 3.1.3). It is likely that the processes responsible for the removal of Fe, Mn, and Cu,namely scavenging by suspended particulate matter is important to the removal of Pb from

Table 3.3 Determination of alkyllead and inorganic lead (II) species in the surface waters of the Tamar Estuary (filtered 0.45 μm)

Site Number Ⓐ	Lead concentration Ⓑ (ng l^{-1})				
	$(CH_3)_3Pb^+$:	$(C_2H_5)_3Pb^+$:	$(CH_3)_2Pb^{2+}$:	$(C_2H_5)_2Pb^{2+}$:	Pb^{2+}
1 (Calstock)	<2	<3	<2	<3	520
2 (Warren Point)	<2	<3	<2	<3	1000
3 (Ernesettle Pier)	<2	<3	<2	<3	1620
4 (Tamar Bridge)	5	<3	<2	<3	400
5 (Combe Bay)	<2	<3	<2	<3	880
6 (Henn Point)	<2	<3	<2	<3	600
7 (Chremyl Shoals)	<2	<3	<2	<3	1050
8 (Mayflower Marina)	10	<3	<2	<3	6300

Ⓐ Refer to Figure 3.1 for sites sampled in the Tamar Estuary. Samples collected on 20/3/85, survey period extended from 1300-1530 h (GMT). Low water at 11.32 h (GMT)

Ⓑ Tetraalkyllead species were below the detection limit (1.4 ng Pb l^{-1}), ionic alkyllead species were determined by the butylation method (Section 2.3.1.2.2)

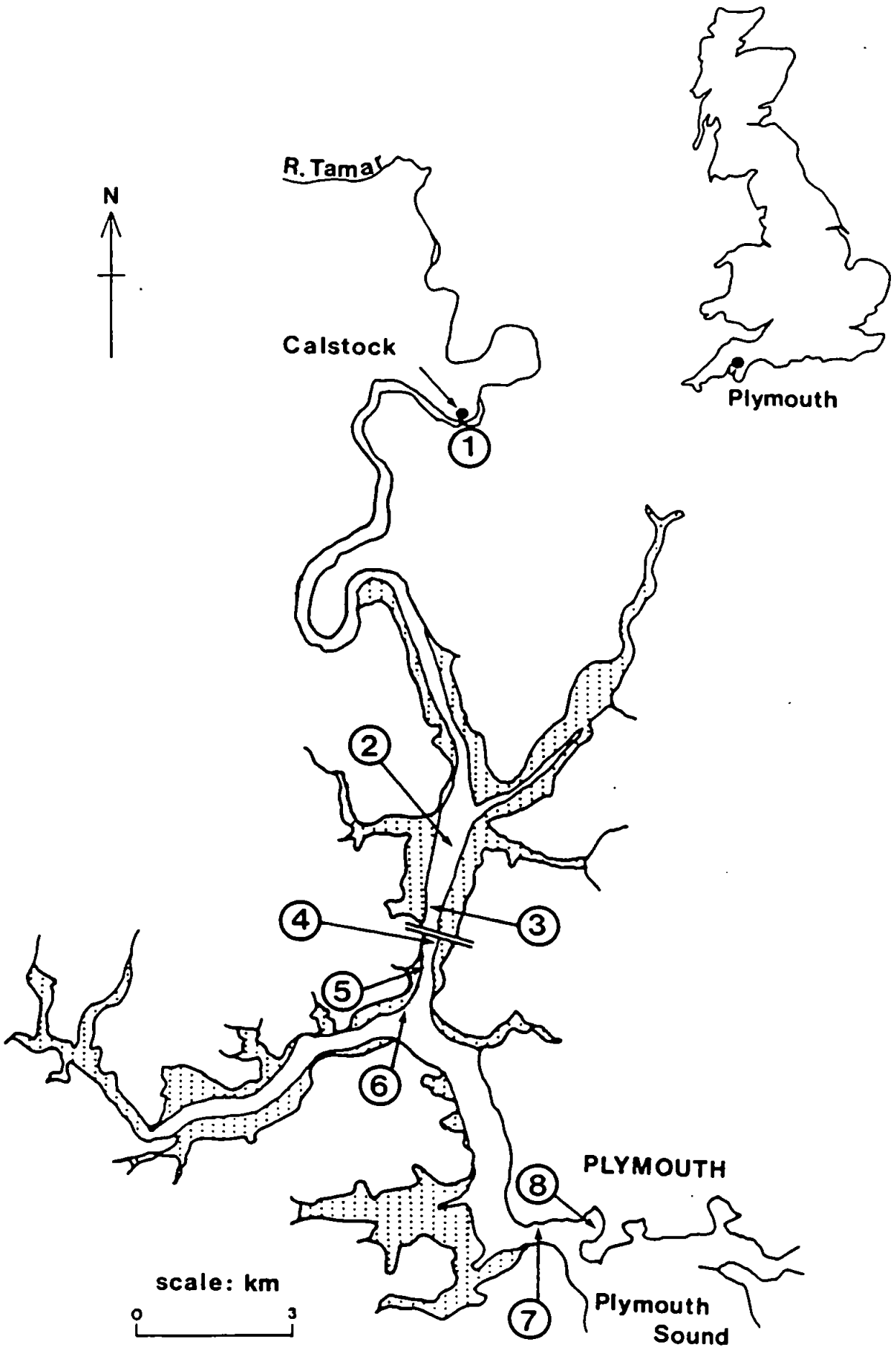


Figure 3.1 The Tamar Estuary: surface water sampling sites; Inorganic and alkyllead survey studies.

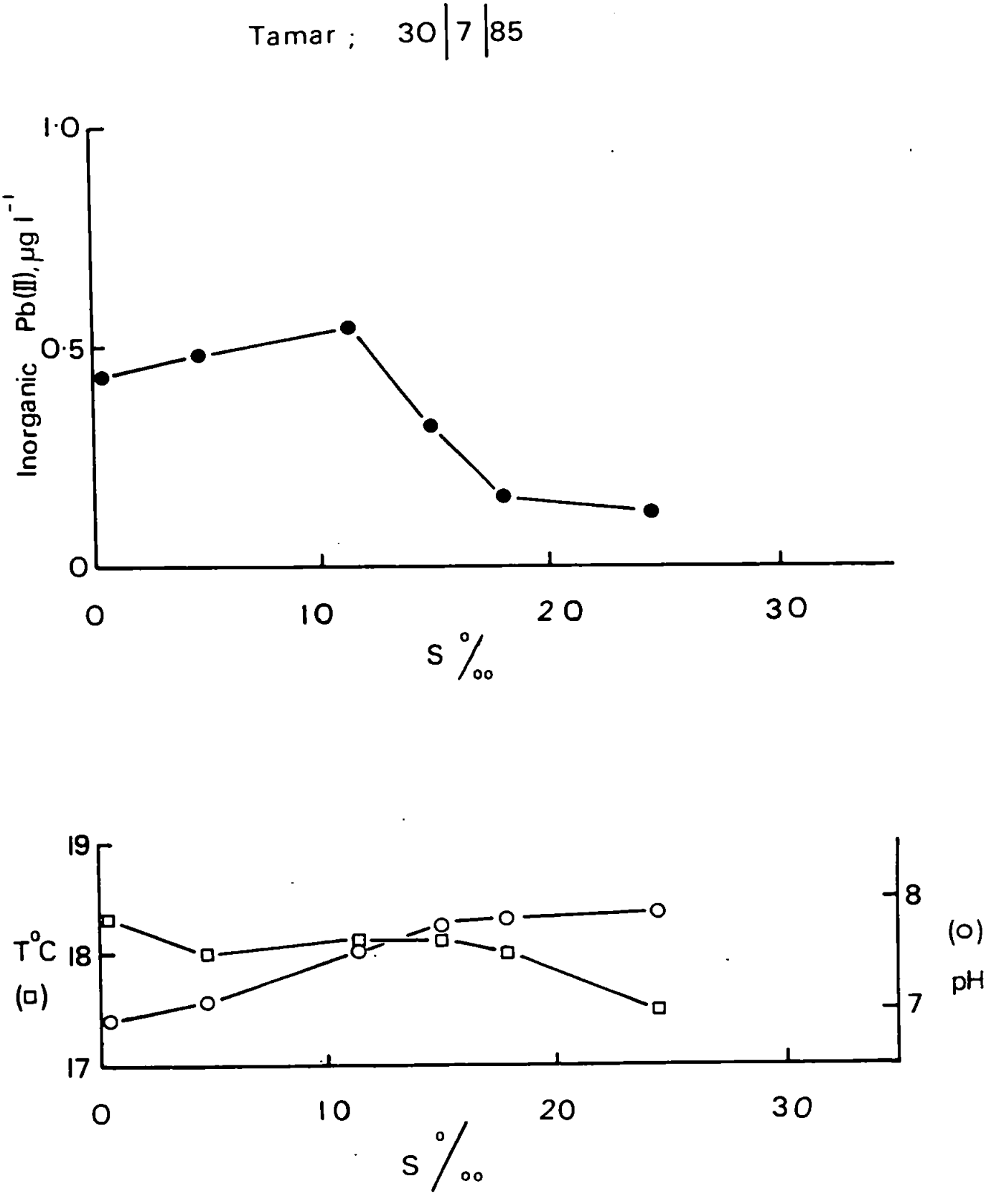


Figure 3.2 Dissolved inorganic lead (II) concentrations in Tamar surface waters (30/7/85)

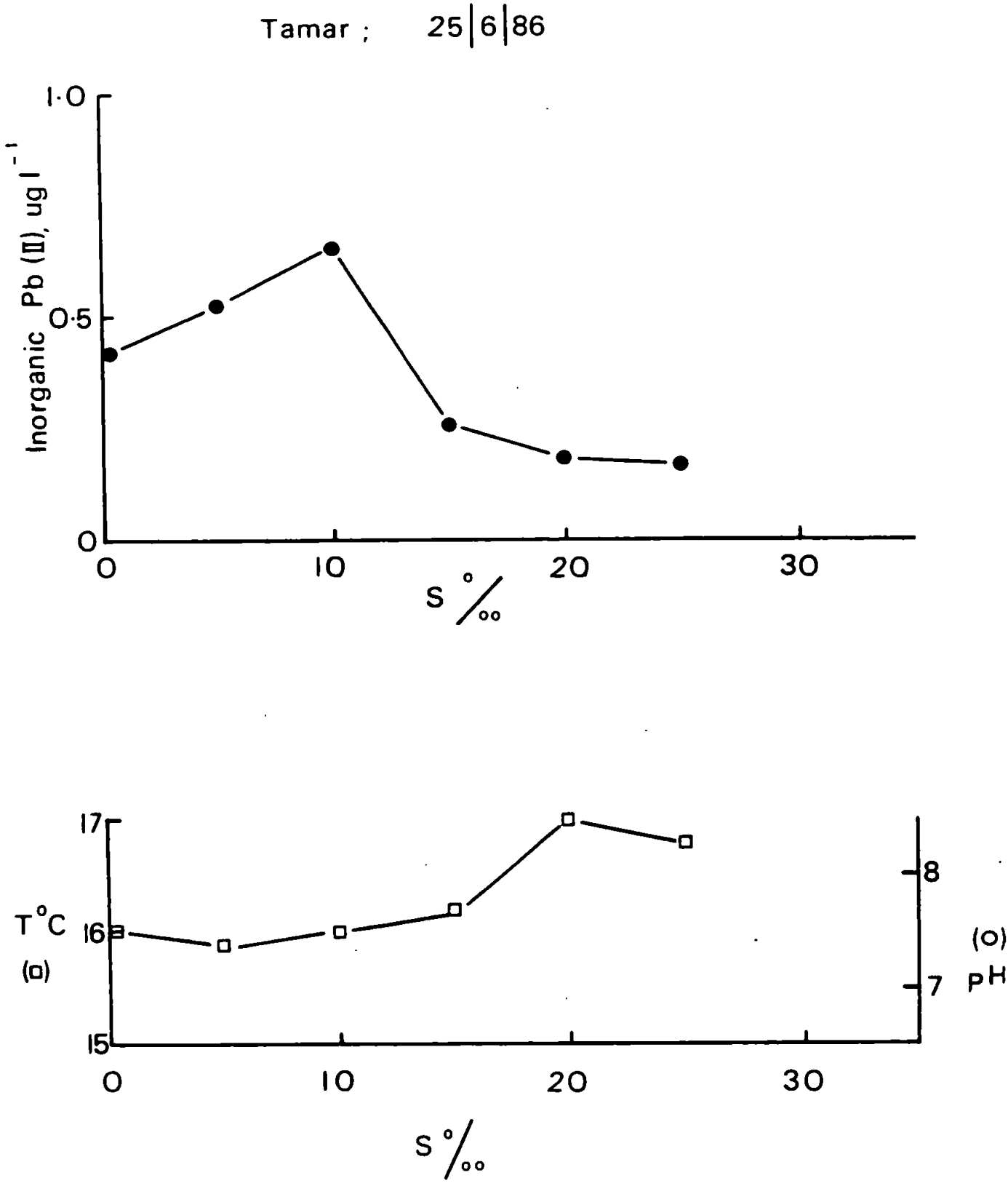


Figure 3.3 Dissolved inorganic lead (II) concentrations in Tamar surface waters (25/6/86)

these waters. The profiles of dissolved inorganic Pb(II) show a pronounced broad maxima over the salinity range (1-12‰), this has been attributed to the release of metal from cationic exchange sites on suspended particulate material and influxes from porewaters during periods of estuarine mixing (Morris et al., 1986). The dilution of freshwater with seawater causes a dramatic decrease in dissolved Pb(II) concentrations at salinities >12‰.

The inability to detect methylated Pb species in mid-channel surface water samples collected during periods of peak biological activity may be attributed to the fact that (i) such species are produced below the detection limits of the methods employed or (ii) the rate of decomposition of such species is greater than their rate of formation. Unfortunately direct data on the rate of formation of methylated Pb species in estuarine waters is not readily available. However, recently Thompson and Crerar (1980) have reported a maximum atmospheric production of 0.026% of TML after a period of 672 h in an environmental system spiked with dissolved inorganic Pb(II) salts. In this thesis work maximum atmospheric productions of TML of 0.005-0.028% (based on Pb added to system, 500 µg) after a period of 400 h (see Section 3.2) were observed for Tamar Estuary systems. Since TML is volatile, once formed in sediments of the environmental systems it would evaporate directly into the gaseous phase. It can therefore be assumed that the maximum production of TML in the dissolved phase would not exceed 0.028% and that the period for maximum production would similarly not exceed 400 h. From these assumptions a tentative overall maximum rate of production of TML of 0.028% per 400 h can be assigned to estuarine systems. This data can be applied to the Tamar Estuary environment and the production of TML can be estimated. Assuming a maximum production of TML of 0.028% from inorganic Pb(II) species and using a

maximum mid-channel dissolved inorganic Pb(II) concentration of 500 ng l^{-1} the maximum concentration of TML produced in the water column would be 0.13 ng l^{-1} (as Pb). This concentration of TML would not be detected by the methods employed in this thesis. Also if the maximum overall rate of production of TML is 0.028% per 400 h this is much slower than the overall rate of decomposition of 59% per 500 h reported for TML in aqueous illuminated solutions (Jarvie et al., 1981) hence TML once formed would rapidly be decomposed. Similarly the decomposition of other methylated species may occur at a faster rate than their production and thus negate their detection by current methods. At present, rate data for the formation of partially methylated Pb species is not readily available.

It has been suggested that methylation may play a greater role in intertidal mudflat areas than in the water column (Harrison and Laxen, 1978b). The Tamar Estuary has extensive mudflat areas in its middle and lower reaches. To assess the significance of Pb methylation in such areas porewaters and sediments were analysed for their methylated Pb content. Sediment and porewater samples were collected from the same sites and in all samples alkyllead species were below the detection limit. In porewater samples Pb(II) species were present at levels significantly greater than those in surface water samples (Table 3.4) which proves the hypothesis that Pb is removed from the water column on Fe phases or particulates or precipitates. From Table 3.5 total Pb levels in Tamar sediments ranged from 100-200 μg g^{-1} (dry wt.) these figures agree with other work in the Tamar by Luoma and Bryan (1981); Bland et al., (1982); Ackroyd (1983); Haswell (1985). An acetic acid leach of Tamar sediment gave Pb concentrations ranging from 10-40 μg g^{-1}. The acetic acid leach extraction method yields information on the concentration of biologically available metals (Luoma and Bryan, 1981). Such data on Pb would be important in assessing the amount available

Table 3.4 Determination of inorganic lead (II) species in porewater samples collected from sites in the Tamar Estuary (17/3/86-24/3/86)

Site (A)	Distance from Calstock (km)	Inorganic Pb(II) concentration (B) (ng l^{-1})
Calstock	0	18350 (16300-20400)
Halton Quay	7.0	24250 (18200-30300)
Cargreen	13.5	8950 (14700-23200)
Riverside	18.5	9650 (6100-13200)
St. Johns' Lake	22.5	15450 (12300-18600)

(A) Porewater and sediment samples collected from the same sites

(B) Determination of inorganic Pb(II) was achieved using the butylation method. Values quoted are the average of 2 replicate samples. Figures in parentheses refer to the range in sample concentration. Tetraalkyllead species were below the detection limit (1.4 ng Pb l^{-1}) in all porewater samples collected.

Table 3.5 Bulk characteristics of sediment samples collected from specific sites in the Tamar Estuary (see previous Table 3.4)

Site	Distance from Calstock (km)	Moisture (A) Content (%)	Organic (B) Carbon (%)	Sulphide (C) ($mg\ g^{-1}$)	Total (D) Pb($\mu g\ g^{-1}$)	'Available' (E) Pb($\mu g\ g^{-1}$)
Calstock	0	65.2 (61.9-68.4)	4.5 (4.3-4.6)	0.78 (0.71-0.84)	166.4 (159.6-173.2)	40.7 (39.7-41.6)
Halton Quay	7.0	66.7 (63.6-69.8)	4.1 (4.0-4.2)	1.60 (1.5 -1.7)	161.8 (159.3-164.3)	25.4 (21.4-29.3)
Cargreen	13.5	59.6 (62.3-56.9)	3.8 (3.6-4.0)	2.20 (2.1 -2.3)	196.5 (189.6-203.4)	36.2 (34.6-37.8)
Riverside	18.5	45.6 (41.2-49.8)	3.2 (2.9-3.5)	0.30 (0.1 -0.5)	179.6 (167.9-191.2)	23.4 (19.4-27.3)
St. Johns' Lake	22.5	65.2 (63.0-67.4)	4.3 (4.1-4.5)	2.60 (2.5 -2.7)	106.3 (102.9-109.6)	10.2 (6.8-13.5)

(A) Refer to Section 2.3.2.2.3

(B) Refer to Section 2.3.2.2.2

(C) Potentiometric titration method (Section 2.3.2.2.4)

(D) Aqua regia digestion method (Section 2.3.2.2.1)

(E) Acetic acid leach method (Section 2.3.2.2.1)

All data quoted are the average of 2 replicate measurements. Figures in parentheses refer to the range in sample concentration. Sediment samples collected on 17/3/86.

for biologically mediated methylation of Pb in sediments. Sediments were found to have organic carbon contents ranging from 3.2-4.5%, associated with inputs of terrestial material to the estuary. Sediments were also found to contain relatively high sulphide levels which ranged from 0.3-2.6 mg g^{-1} of sulphide, these values compared well with values of <0.1-2.0 mg g^{-1} quoted for Tamar sediments by Moreton (1984).

The inability to detect methylated Pb species in the metal/organic rich porewater environment, where bacterial action is high, may be attributable to (i) if methylation proceeds it occurs at levels below 1.4-2 ng Pb l^{-1} i.e., the detection for the methods employed or (ii) as appears to be the case for column waters the rate of decomposition may exceed the rate of formation. Decomposition by photolysis would not be significant in the darkened porewater environment and also no detectable levels of alkyllead species were lost by sorption onto sediments. Hence only volatilization and decomposition by bacteria may be important in depleting porewaters of their alkyllead contents.

3.1.2 River Carnon and Associated Restronguet Creek

The results of the River Carnon and associated Restronguet Creek Pb survey work are reported in Table 3.6 and discussed below.

In a single survey (4/6/85) a total of 8 surface water samples (mid-channel) were collected over the salinity range 1-25‰, alkyllead species were below the detection limit in all 8 samples. The total inorganic Pb concentration of 3 of the 8 samples was determined by GFAAS and found to range from 10500-14500 for low salinity waters (see Table 3.6)

Table 3.6 Total inorganic Pb concentration of River Carnon surface waters (0.45 μm filtered)

Salinity (‰)	pH	T°C	Pb concentration (ng l^{-1})
1.1	6.0	16.3	14500
1.3	6.0	16.5	14000
2.1	6.3	16.4	10500

Due to logistical constraints on time and the physical handling of sampling equipment estuarine sediment and porewater samples were not collected for analysis.

The highly metal polluted Carnon system was considered to be an area where Pb methylation might occur and be significant in the cycling of Pb in the estuarine environment. From Table 3.6 levels of total inorganic Pb (average 13000 ng l^{-1}) are significantly higher than the corresponding inorganic Pb(II) levels observed in the low salinity region of the Tamar Estuary (average 500 ng l^{-1}, see Section 3.1.1). Thornton et al., (1975) reported levels of total Pb of 8-200 μg l^{-1} (mean on 5 samples, 85 μg l^{-1}) for sites in the upper reaches of the River Carnon, in the area of Mount Wellington and Wheal Jane mines. Boyden et al., (1979) also reported levels of total Pb of 2000-3000 ng l^{-1} for sites in Restronguet Creek and the total Pb concentration was not found to vary significantly over a seasonal cycle.

The total inorganic Pb concentrations reported in this thesis were lower than those reported by Thornton et al., (1975). This would be expected since in this thesis work sites in the lower reaches of the

River Carnon, 3-5 km from the Mount Wellington and Wheal Jane mines were sampled. Further dilution with seawater and loss of Pb onto particulate phases may explain the lower levels of Pb recorded by Boyden et al., (1979) in Restronguet Creek. Natural waters containing similarly high levels of inorganic Pb i.e., River Rheidol, N. Wales, England with concentrations of inorganic Pb exceeding 300 $\mu g\ l^{-1}$ have been studied (Jarvie et al., 1983) and as with the River Carnon no methylated Pb species were detected. The workers Jarvie et al., (1983) reported that microorganisms were active in mine waters containing upto 10 mg l^{-1} of Pb and that the microorganisms present did not detoxify their environment of Pb by methylating the element. It is possible that microorganisms in the waters of the Carnon system can methylate Pb, if the estimated maximum conversion of inorganic Pb to TML of 0.028% is applied to the maximum dissolved Pb concentration of 14500 ng l^{-1} (Table 3.6) then 3.7 ng Pb l^{-1} as TML would be produced in the water column. Such a concentration would be detectable if no loss due to evaporation or photolytic decomposition occurred. Since significant losses due to photolysis (Jarvie et al., 1981) would occur this may explain the inability to detect alkyllead species in the waters of the River Carnon and associated Restronguet Creek.

3.1.3 Beaulieu Estuary

The results of the Pb survey work undertaken in the Beaulieu Estuary are reported in Table 3.7 and are discussed below.

In a single survey (18/6/85) a total of 7 surface water samples (mid-channel) were collected over the salinity range 1-25‰, alkyllead species were below the detection limit in all samples. The total inorganic Pb concentration of 4 of the 7 samples was determined by GFAAS and found to range from 300-400 ng l^{-1} for low salinity waters (see Table 3.7).

Table 3.7 Total inorganic Pb concentration of Beaulieu Estuary surface water samples (0.45 μm, filtered)

Salinity (‰)	pH	T°C	Lead Concentration (ng l^{-1})
0.8	6.9	12.4	400
1.7	7.1	12.6	400
1.8	7.1	13.2	350
2.5	7.0	13.6	300

Due to logistical constraints on time and the physical handling of sampling equipment estuarine sediment and porewater samples were not collected for analysis.

The highly productive, organic-rich Beaulieu Estuary is an area where the methylation of As is known to occur (Howard et al., 1984). Since conditions appeared favourable for As methylation in this estuary it was considered that they may also be favourable for Pb methylation. From Table 3.7 total inorganic Pb (average on 4 samples = 360 ng l^{-1}) levels are relatively low compared to the Carnon system. Information on the speciation of Pb in the Beaulieu system is not readily available. Previous studies on the metal content of the Beaulieu are restricted to Fe, Mn, Zn (Holliday and Liss, 1976), Se (Measures and Burton, 1978) and As (Howard et al., 1984). It is widely accepted that Fe behaves non-conservatively, removal of Fe from solution being related to the formation of insoluble hydrous oxides (Aston and Chester, 1973). However, both Mn and Zn behaviour is apparently conservative such that no major physical or biological removal processes occur for these elements (Holliday and Liss, 1976). Biological effects on the behaviour of As appear significant (Howard et al., 1984; see also this study for As work).

Since biological processes appear important in the Beaulieu Estuary the known biologically mediated methylation of Pb (Wong et al., 1975) might take place in this estuary. If a maximum conversion of 0.028% of inorganic Pb to TML occurred in the water column the TML concentration produced (maximum 0.1 ng Pb l^{-1} as TML for a 400 ng Pb l^{-1} water column concentration) would be below the detection limit of the methods employed. If produced in the sediments of this estuary methylated species would also be rapidly lost to the atmosphere or decompose in situ as discussed previously in Section 3.1.1..

In conclusion the inability to detect methylated species of Pb at sites removed from land derived petroleum inputs is not conclusive evidence for the absence of environmental methylation processes. The work undertaken in this thesis, however, indicates that 10-100 fold lower detection limits than those currently available would be needed to detect such species in the aquatic environment. Further because of the known volatility of certain species, atmospheric sampling would be necessary for a full understanding of the environmental methylation of Pb.

3.2 Laboratory Modelling Studies on Lead Methylation

In this section laboratory kinetic modelling experiments were undertaken to evaluate the rate of formation and decomposition of TML under environmental conditions. Headspace gas evolution of TML was examined. In an attempt to mimic environmental conditions models were run under strictly controlled conditions of pH (7.0 $\pm$ 0.3), light 3.02 x 10^{15} quanta s^{-1} cm^{-2} and temperature (15-30°C $\pm$ 1°C). Nutrients were added to promote microbial growth and the approach adopted by Chau and Wong, (1978) was followed in which a 0.5% solution of nutrient broth (I) was employed. Sulphide was only added to flasks in experiments where the

effect of added sulphide was to be examined. The addition of various forms of Pb, at concentrations 2-10 mg l^{-1}, although elevated compared to environmental porewater levels (9-25 $\mu g\ l^{-1}$, see Table 3.4), was required for the identification of the trace levels of TML evolved in headspace gases. The concentration of amended Pb was not found to inhibit microbial growth. Previous workers (Wong et al., 1975; Jarvie et al., 1975; Schmidt and Huber, 1976; Thompson and Crerar, 1980) studying the production of TML failed to adopt conditions that mimic the environment, as a result the data obtained is less applicable to the environment than work undertaken in this thesis.

3.2.1 Studies on Trimethyllead Acetate

The Pb(IV) compound trimethyllead acetate ($(CH_3)_3PbOCOCH_3$) is of environmental interest since it is feasible that $(CH_3)_3Pb^+$ is an intermediate in the methylation of Pb(II), the more environmentally significant species. However, $(CH_3)_3PbOAc$ itself has not been detected in the environment. Reports on the conversion of $(CH_3)_3PbOAc$ to TML published in the literature have suggested biological mediated processes to be important (Wong et al., 1975; Schmidt and Huber, 1976) and chemical processes to be important (Jarvie et al., 1975). More recently experiments have been carried out to differentiate between the possible biologically mediated or chemical routes for $(CH_3)_3Pb^+$ methylation (Craig, 1980). This latter work suggests a chemical process adequately explains the methylation mechanism. In the following work studies by Craig (1980) are repeated and expanded upon using Tamar sediments. In this way a knowledge of the chemical and physical processes occurring under environmental conditions may be gained. A summary of the experiments on the conversion of $(CH_3)_3PbOAc$ to TML is given in Tables 3.8-3.9.

The importance of the chemical process in the conversion of $(CH_3)_3PbOAc$ to TML was assessed in a series of abiotic (reactants autoclaved) experiments

Table 3.8 Conditions for abiotic experiments: the evolution of TML from trimethyllead acetate

Experiment	Description of System	Remarks
Exp. 1	Distilled water system : (autoclaved)	
	Conditions: (a) Light (visible range quantum flux 3.02×10^{15} q s^{-1} cm^{-2})	see Figure 3.4
	(b) Dark (silver foil covered flask) pH = 7.0 ± 0.2 T°C = 30 ± 1°C	
	Reaction Media: Glass flask volume = 150 ml; Distilled water = 50 ml; Headspace gas volume = 145 ml	
	Concentration of Spike: 99 µg of Pb as trimethyllead acetate (0.1 ml injection of 990 mg Pb l^{-1} standard solution)	
Exp. 2	Distilled water system with added sodium sulphide (Na_2S) : (autoclaved)	
	Conditions: As in Exp. 1(a)	see Figure 3.6
	Reaction Media: As in Exp. 1	
	Spiking level: As in Exp. 1 with addition of 1000 µg Na_2S l^{-1}	
Exp. 3	Sediment systems : (autoclaved)	
	(a) SPS China Clay System:	see Figure 3.7
	Conditions: As in Exp. 1(a)	
	Reaction Media: As in Exp. 1 with addition of 10 g ± 0.1 g SPS clay	
	Spiking level: As in Exp. 1	
	(b) Tamar Sediment system:	
	Identical system to Exp. 4(a) except autoclaved	

Table 3.9 Conditions for biological experiments: the evolution of TML from trimethyllead acetate

Experiment	Description of System	Remarks
Exp. 4	Tamar sediment system: (freshwater Calstock regime)	
	Conditions (a) Light (visible range see Exp. 1(a) for flux) (b) Dark (see Exp. 1(b)) pH = 7.0 ± 0.3 T°C = 30 ± 1°C	see Figure 3.9
	Reaction Media: Flask volume = 150 ml Tamar water = 50 ml Tamar sediment= 10 ± 0.1 g Nutrients = 0.5% Nutrient Broth 0.1% Yeast Extract 0.1% D-glucose Headspace gas volume = 145 ml	
	Concentration of Spike: As in Exp. 1	
Exp. 5	Tamar sediment system with added sodium sulphide (Na_2S):	
	Conditions: As in Exp. 4(a)	see Figure 3.10
	Reaction Media: As in Exp. 4(a)	
	Spiking Level: As in Exp. 4(a) with addition of 1000 μg Na_2S l^{-1}	
Exp. 6	Distilled water system: (Non autoclaved)	see Figure 3.6
	Conditions: As in Exp. 1(a)	
	Reaction Media: As in Exp. 1	
	Spiking Level: As in Exp. 1	

(refer to Table 3.8). After a period of 40 h TML production in the headspace gases of a distilled water system (see Figure 3.4) had reached a maximum. For an illuminated system the maximum yield represented a 0.25% conversion of the Pb originally added to the solution, in dark experiments the conversion was 0.32%. The conversion yields were found to be much greater than those reported by Craig (1980), who spiked water samples at a much higher concentration of Pb ($40\ g\ l^{-1}$, $(CH_3)_3PbOAc$). However, no data for the temperature or light flux in such experiments were reported and so a direct comparison cannot be made. In this present work TML was below the detection limit in flasks containing distilled water only, hence in agreement with work by Craig (1980) the compound $(CH_3)_3PbOAc$ can dismutate or disproportionate to TML in solution. Kinetic analysis of the data for the abiotic conversion of $(CH_3)_3PbOAc$ to TML revealed that the reaction was exponential growth and shown in subsequent analysis to be first order with respect to TML concentration (Figure 3.5). The forward rate constant (k_1) for the dark reaction was $0.064\ h^{-1}$ whereas in the light the rate constant decreased to $0.054\ h^{-1}$. The half life of the reactions were calculated to be 10.38 h and 13.08 h respectively. Since light and dark rate constants are different there may be a reversible or consecutive reaction occurring in the illuminated system which are absent in the dark system. The known photolytic instability of TML (Harrison and Laxen, 1978a; Jarvie et al., 1981) would suggest that once produced TML would slowly decompose back to $(CH_3)_3Pb^+$ in the presence of light. The dark and light reactions are considered in Equations 3.1 and 3.2 respectively.

Dark : $(CH_3)_3Pb^+ \xrightarrow[(\text{fast } t_{\frac{1}{2}}\ 10\ h)]{k_1 = 0.06\ h^{-1}} TML$

Equation 3.1

Light : $(CH_3)_3Pb^+ \underset{}{\overset{k_1 = 0.06\ h^{-1}}{\rightleftharpoons}} TML$

overall $k_1 = 0.05\ h^{-1}$

($t_{\frac{1}{2}} = 13$ h)

Equation 3.2

Although the $(CH_3)_3Pb^+$ species has not been identified to date in natural waters the above data suggests if formed it would be rapidly converted to the TML form both in the upper euphotic layer and at depth. The half-lives estimated for the formation of TML (10-13 h) appear similar to those estimated by various workers for the decomposition of TML. Hewitt and Harrison (1985) estimated atmospheric TML half-lives of 10 h for summer months and 34 h for winter months. Under laboratory controlled dark conditions TML half-lives of 320 h have been estimated (Harrison and Laxen, 1978a). It is possible to conclude from this work that if Pb is environmentally methylated to TML, decomposition to $(CH_3)_3Pb^+$ will limit its transportation over large distances (>500 km) resulting in a relatively localised distribution close to the source.

The significance of sulphide ions in the conversion of $(CH_3)_3PbOAc$ to TML in 'abiotic' systems has been demonstrated (Jarvie et al., 1975; Thompson and Crerar, 1980). However, the reaction conditions appear varied and the parameters pH, light and temperature were poorly controlled. The concentration of added Pb and sulphide is also at the milligram level rather than microgram level, the latter being more relevant to environmental conditions. In the work in this thesis stringent controls on pH, light and temperature were undertaken, and levels of added Pb and sulphide were in the microgram range (refer to Table 3.8).

In an abiotic system comprising of distilled water (50 ml), $(CH_3)_3PbOAc$ (100 µg) and sodium sulphide (1000 µg l^{-1}) the evolution of TML compared

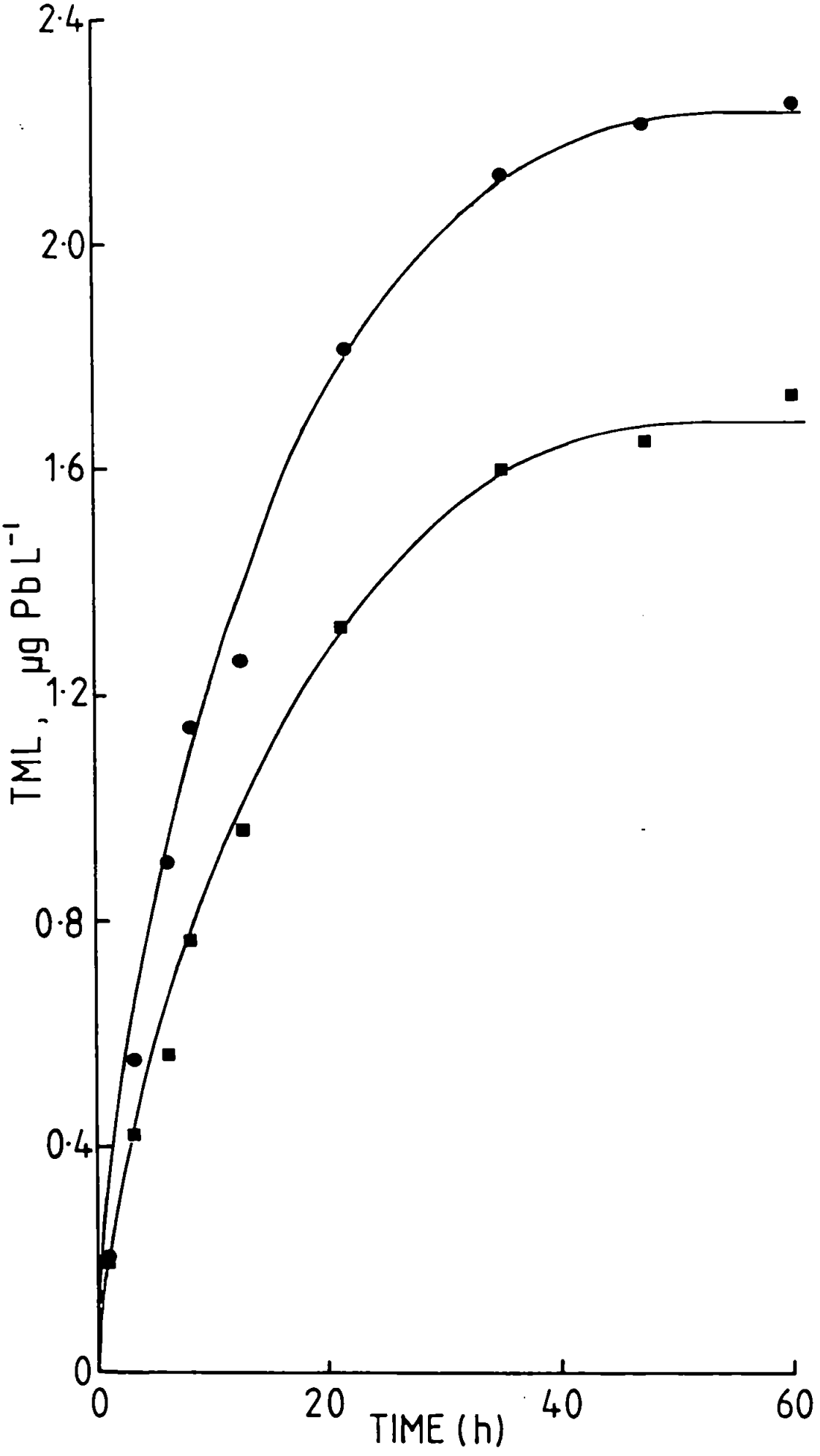


Figure 3.4 Abiotic conversion of $(CH_3)_3PbOAc$ to TML. Light (visible range) and dark conditions.
(■) light experiment
(●) dark experiment

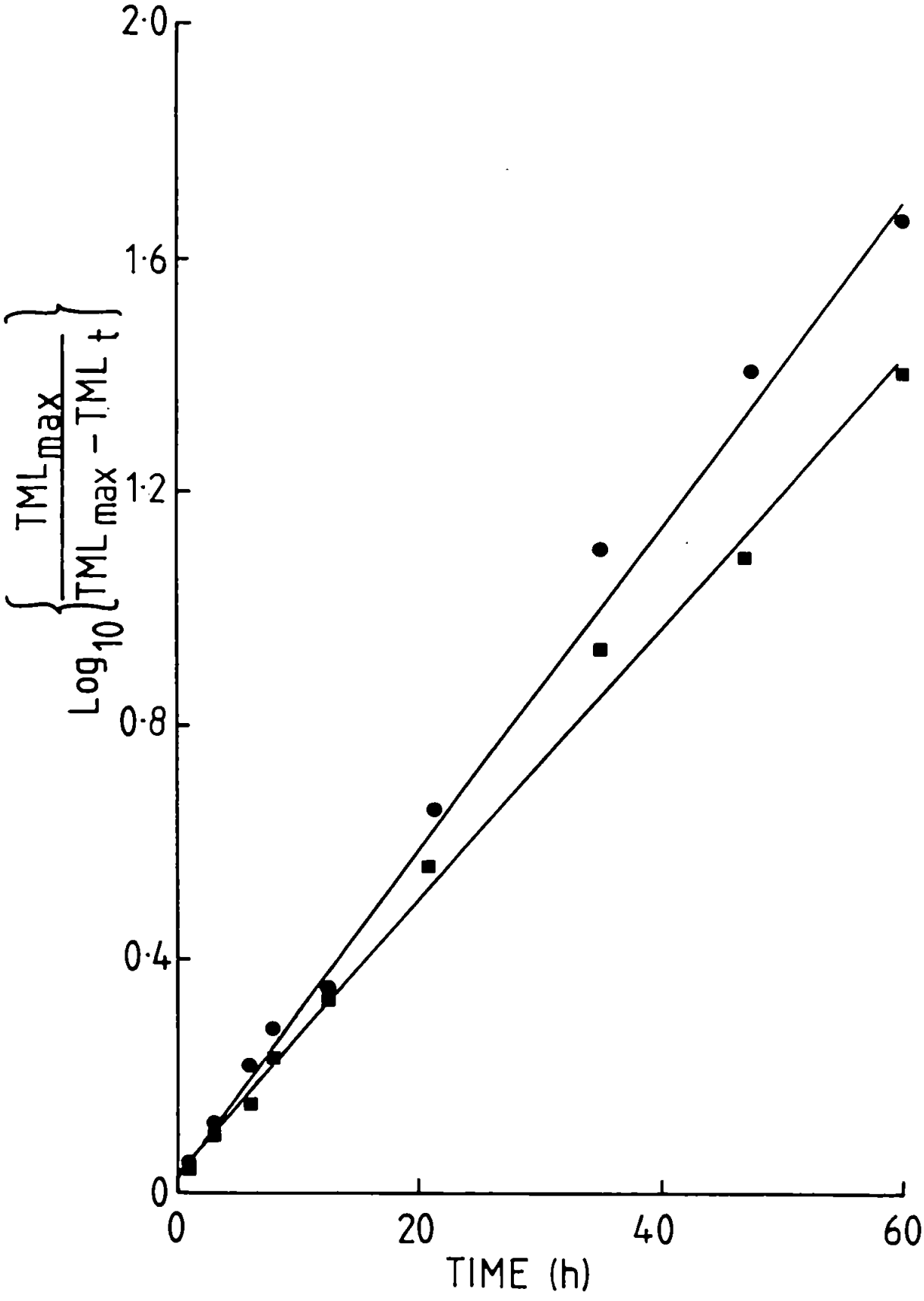


Figure 3.5 First order kinetic plot for the abiotic conversion of $(CH_3)_3PbOAc$ to TML. Light (■) and dark (●) reactions shown.

to an identical system with no added sulphide was found to be markedly enhanced. The maximum yield was 1.6% (based on Pb) for an illuminated reaction (Figure 3.6) a 4 fold increase over the system containing no added sulphide (see Figure 3.4). Direct comparison of this work with that of other workers is made difficult by the fact that reaction conditions are not compatible. However, the most compatible to the work in this thesis is the work of Jarvie et al., (1975) who reported a 2.0% yield (based on Pb) of TML when $(CH_3)_3PbOAc$ (75 mg l^{-1}, in 200 ml distilled water) was incubated in the dark with sodium sulphide (15 mg). In the present work the evolution of TML was studied over a period of 250 h (see Figure 3.6). A complex 2 stage growth curve for TML was observed. In the first stage (0-20 h) rapid evolution of TML was observed, at 20 h 40% of the total TML evolved was present in the reaction flask. In the second stage a slower evolution of TML took place, with an equilibrium level of TML being achieved after 200 h.

The production of TML from $(CH_3)_3PbOAc$ in the presence of sulphide ions is suggested to occur by a simple chemical, sulphide-mediated,disproportionation reaction as in Equation 3.3 (Jarvie et al., 1975).

$$2(R_3Pb^+) + S^{2-} \longrightarrow (R_3Pb)_2S \longrightarrow R_4Pb + R_2PbS$$

Equation 3.3

The first stage of rapid evolution of TML observed may be explained by $(CH_3)_3Pb^+$ reacting rapidly with available S^{2-} ion, surface catalysis on glass reaction vessel walls may be important here (Craig and Rapsomanikis, 1985). The slower rate of production occurring after a period of 20 h suggests that a competing reaction takes place whereby the TML produced is decomposed, most likely to $(CH_3)_3Pb^+$ (Jarvie et al., 1981). The conversion of $(CH_3)_3PbOAc$ to TML in this present work reached equilibrium

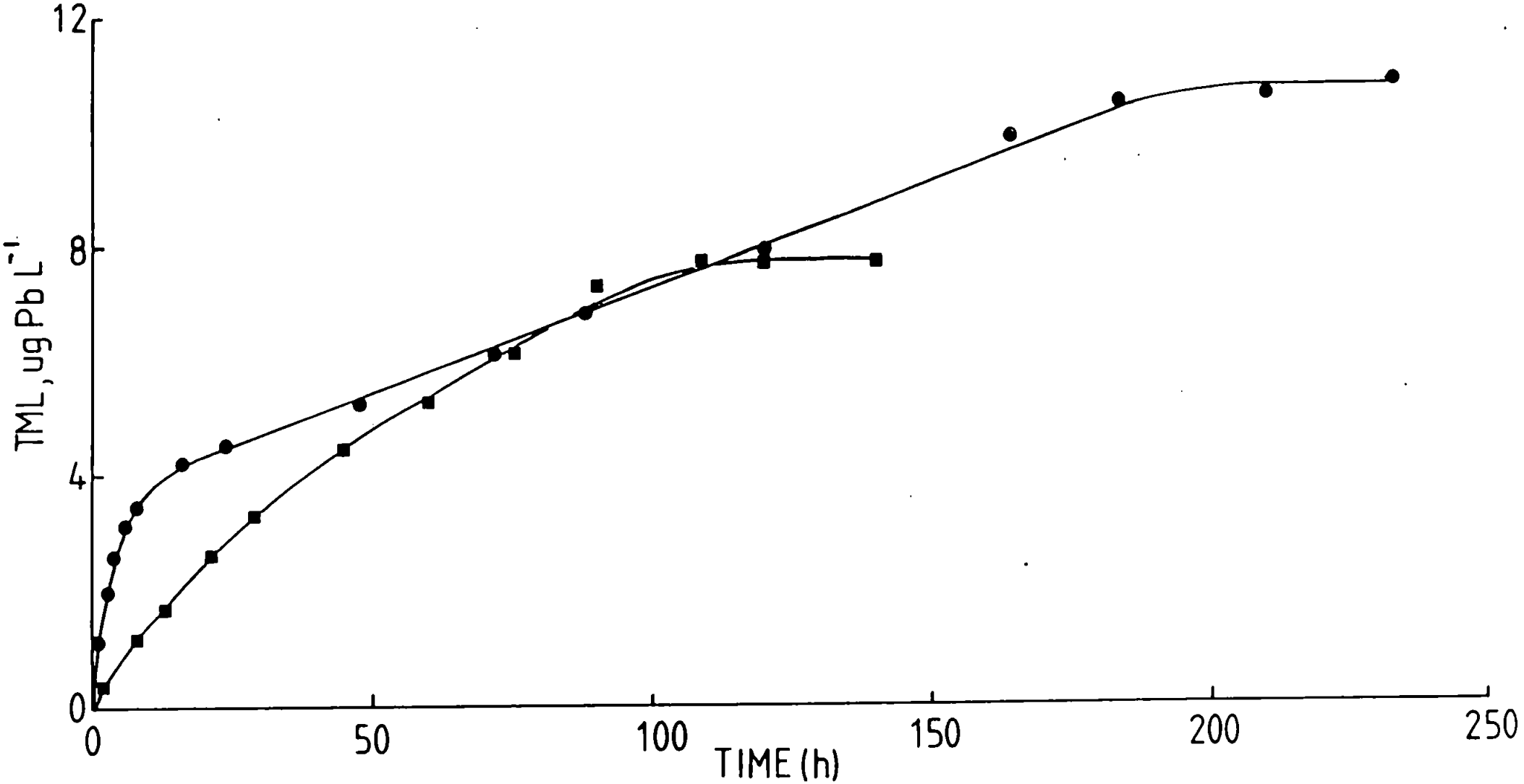


Figure 3.6 Evolution of TML in distilled water systems - Autoclaved and non-autoclaved
(■) illuminated distilled water system (non-autoclaved)
(●) illuminated abiotic water system containing 1000 µg Na_2S l^{-1}

after 200 h. This work confirms that $(CH_3)_3PbOAc$ can be converted to TML by a chemical pathway. However, the data obtained did not lend itself to simple first or second order kinetic interpretation.

In a further set of abiotic experiments the significance of sediment substrates was examined. The sediments were extracted as in (Section 2.3.2.2) and TML was found to be below the detection limit in all sediment samples. The sediments were autoclaved thus killing any microorganisms present. In the China Clay and Tamar sediment systems (Figure 3.7) maximum Pb yields of 0.06% and 0.19% were observed respectively. The higher level of TML evolved in Tamar sediment systems may reflect the presence of water soluble sulphide (0.26 mg g^{-1} sulphide present in sediment before autoclaving takes place) in the reaction flask. The TML concentration in the 2 systems was observed to reach equilibrium in a 20-30 h period, 5-10 h faster than for systems containing no substrate (refer to Figure 3.4). In the estuarine environment sediment particulates may act as catalytic surfaces hence the sediment substrate in these systems may account for the faster rates observed. In 2 separate experiments the uptake of $(CH_3)_3PbOAc$ onto particulates was examined (see Figure 3.8). In the first, uptake onto biologically active sediment was examined. Rapid initial uptake followed by a period of slow removal from solution was observed over a period of 250 h. In such a system the elevated levels of organic carbon (4%) and leachable Fe (40 mg/l^{-1}) material present act as adsorption sites for $(CH_3)_3PbOAc$. In the second system uptake onto abiotic China Clay particulates was examined. In this system no detectable uptake of Pb was observed. The carbon content of China Clay (0.024%) was much lower than Tamar sediments. The latter China Clay sediment system is more applicable to the abiotic series of experiments described above since China Clay is an abiotic

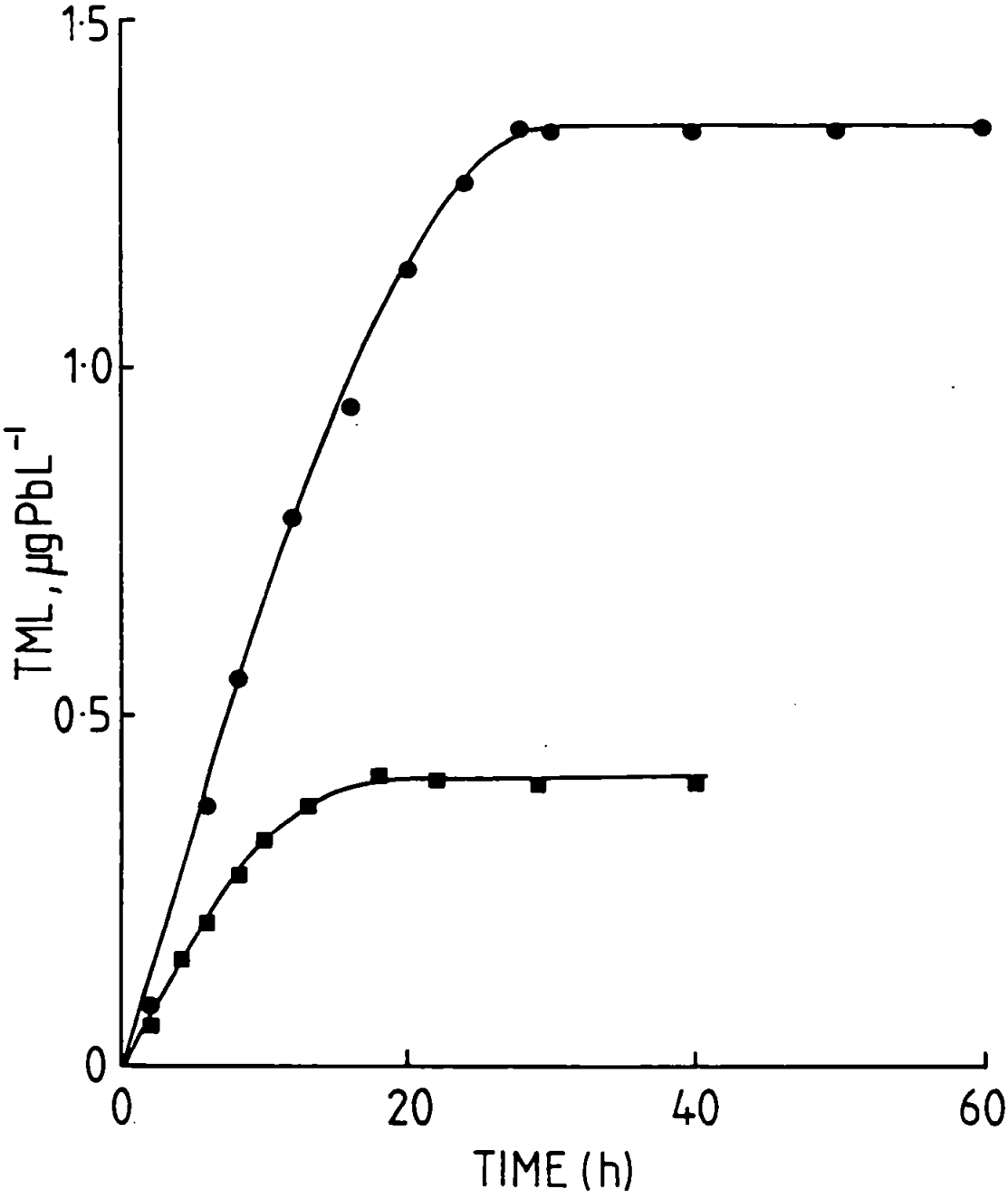


Figure 3.7 Evolution of TML from abiotic sediment/water systems spiked with $(CH_3)_3PbOAc$

(■) Illuminated autoclaved China Clay system

(●) Illuminated autoclaved biological system

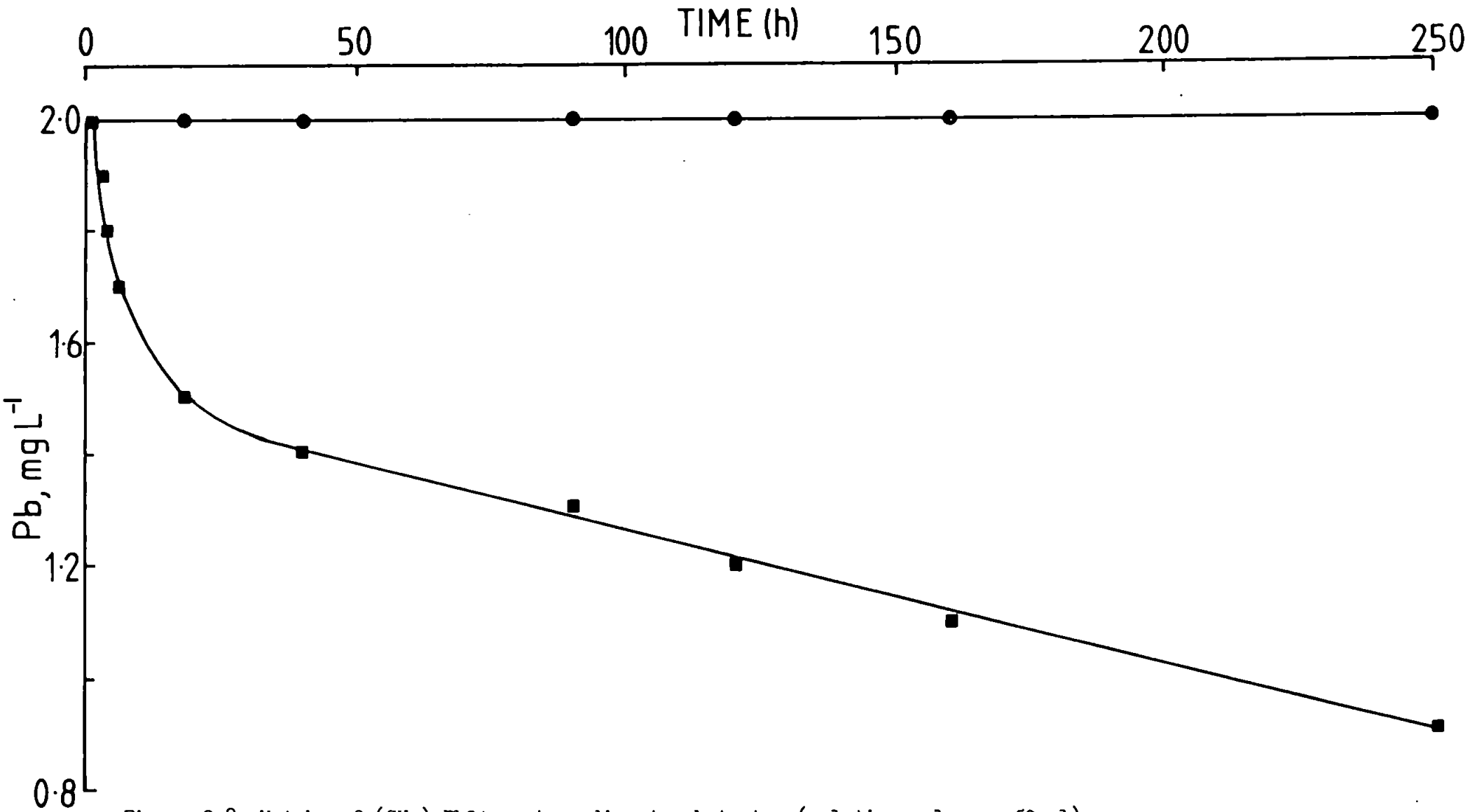


Figure 3.8 Uptake of $(CH_3)_3PbOAc$ onto sediment substrates (solution volume = 50 ml).
(■) Uptake onto freshwater (Calstock) sediment (10 g), illuminated biotic system
(●) Uptake onto SPS China Clay (10 g), illuminated abiotic system

substrate whereas microorganisms were present in the Tamar sediment system. Since no detectable sorption of $(CH_3)_3PbOAc$ onto an abiotic substrate (China Clay) was observed it is unlikely that surface catalysis by substrate material in abiotic systems will effect the rate of TML production. Surface catalysed reactions may, however, be important in biologically active systems. The faster rate of production of TML in abiotic substrate systems (Figure 3.7) compared to non-substrate systems (Figure 3.4) may alternatively be explained in terms of the light transmitted through the 2 systems. In substrate systems less light is transmitted through solutions as a result of suspended particulates being present hence less photolytic decomposition of TML produced will occur. In non-substrate systems greater light penetration occurs such that a competing decomposition reaction may occur resulting in a slower rate of production as observed between light and dark abiotic experiments (refer to Figure 3.4 and 3.5). The profiles obtained from these experiments did not fit any of the known irreversible integrated rate equations (Swinborne, 1971).

The possible biologically mediated pathway in the evolution of TML from $(CH_3)_3PbOAc$ was also examined. In an initial illuminated experiment the evolution of TML in distilled water (non-autoclaved) spiked with $(CH_3)_3PbOAc$ was examined (see Figure 3.6) and a maximum TML yield of 1.18% (based on Pb) was reached after a period of 100 h. Compared to the equivalent abiotic system (Figure 3.4) this represents a 3-4 fold greater TML yield. The slower rates of production of TML from $(CH_3)_3PbOAc$ in non-sterile sediments compared to sterile sediments has been suggested to reflect the bacterial mediated production of sulphide (Thompson and Crerar, 1980). A similar explanation maybe attributed to the case above, although to a much lesser extent because of the lower levels of bacterium present in distilled water. Again it is difficult

to compare the results of this work to those of other workers because of the incompatibility of reaction conditions. Similar work by Craig (1980) using abiotic samples in which $(CH_3)_3PbOAc$ (40 g l^{-1}, 5 ml distilled water) was incubated (light regime and temperature not stated) gave a TML yield of 1.8 x 10^{-3}% after 7 days. Work by Jarvie et al., (1975) in which $(CH_3)_3PbOAc$ (75 mg l^{-1}, distilled water non-autoclaved) was incubated in the dark gave a TML yield of 3.3 x 10^{-3}%, which was constant over a period of 2 weeks. Both experiments, using different concentrations of Pb and using abiotic and biotic conditions, gave similar yields of TML. In the work in this thesis yields were a factor of 1000 higher, the fact that experiments were performed at 30°C ± 1°C and pH 7.0 ± 0.2 may account for this, previous workers did not state the data for these parameters.

In further experiments biologically active sediment systems were examined (refer to Table 3.9). The sediment used in these experiments was found to contain aerobic microorganisms of the Genus bacillus and Pseudomonas, anaerobic species were also present but not identified. The flushing of flasks with N_2 ensured that conditions were anaerobic hence only anaerobic microorganisms should exert a biological effect in such systems. The freshwater sediment used (Calstock site, collected October 1984) was characterized with respect to its bulk properties (organic carbon content 4.8%; water soluble sulphide content = 0.26 mg g^{-1}; total inorganic Pb = 157.5 µg g^{-1}; biologically available Pb = 32.9 µg g^{-1}). Under both light and dark conditions detectable concentrations of TML were evolved from sediments spiked with $(CH_3)_3PbOAc$ (see Figure 3.9). In both cases the rate of production of TML was similar, the reactions reaching equilibrium after 120-160 h. The maximum TML yields were 1.75% in the dark and 2.8% in the light. The variation in duplicate

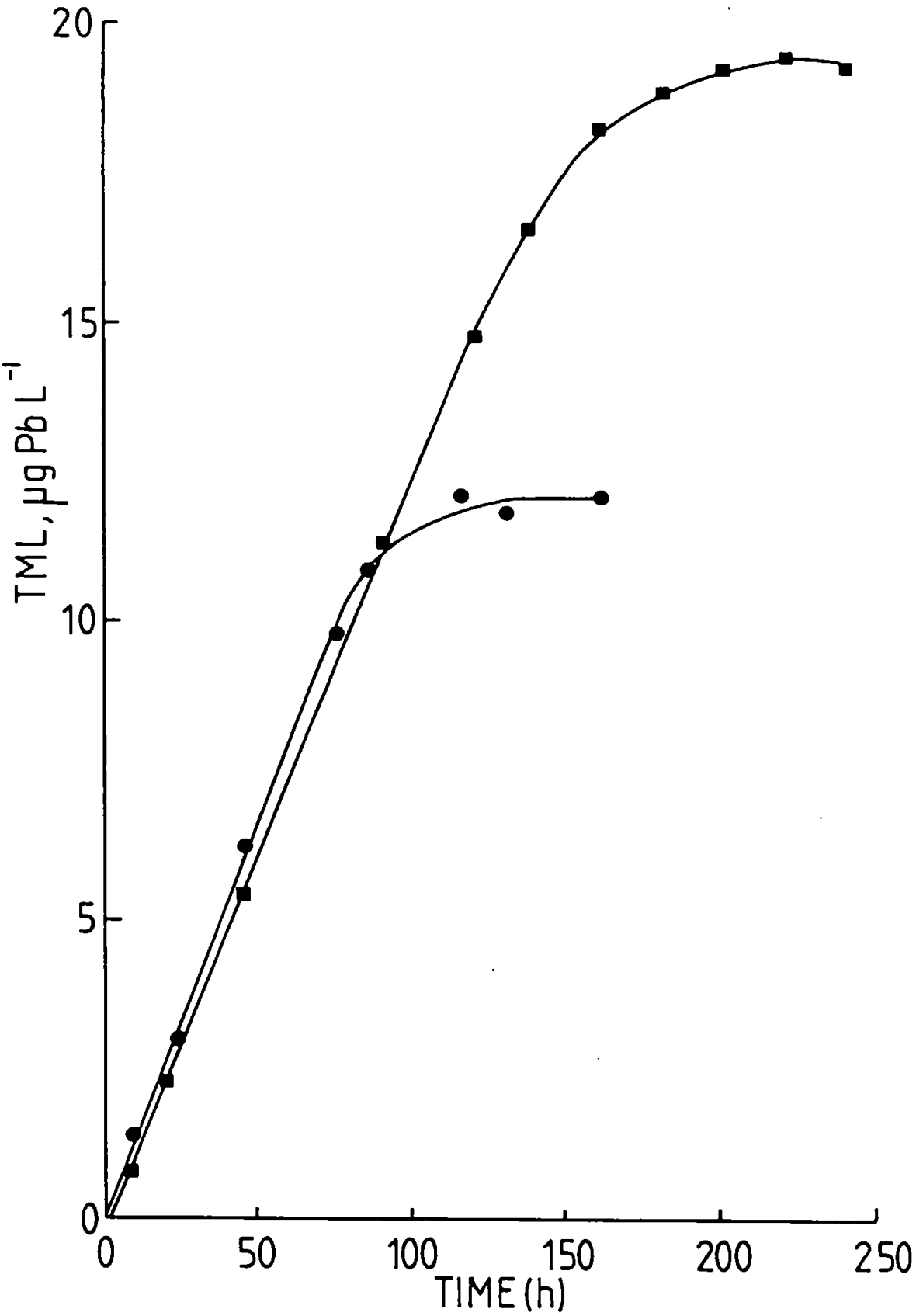


Figure 3.9 Evolution of TML from $(CH_3)_3PbOAc$ spiked biologically active sediments
(■) Illuminated biotic system
(●) Dark biotic system

flasks was ± 10%. Hence a significant variation between light and dark regimes exists. It is possible that under illuminated conditions the level of hydrogen sulphide produced by anaerobic microorganism will be higher than under dark conditions. Under increased levels of sulphide greater levels of TML would be expected hence the variation in the light and dark regime may be accounted for.

The first evidence for a biologically mediated pathway being important was given by Wong et al., (1975). The workers reported that TML was evolved from Ontario lake sediment when incubated with $(CH_3)_3PbOAc$ and added nutrients. The maximum yield, 6% TML (as Pb) was observed after 2 weeks of incubation, however, in autoclaved sediments no detectable concentration of TML was produced. The authors therefore suggested a biological origin for TML. The work by Jarvie et al., (1975) confirmed that a chemical sulphide-mediated mechanism for the conversion of $(CH_3)_3PbOAc$ to TML in active anaerobic sediments was possible. In dark experiments a yield of TML of 2% was reported. The workers explained that the inability of the Wong et al., (1975) group to detect TML in autoclaved sediment was a result of the loss of available sulphide from such systems. During autoclaving sulphide may be volatilized from solution and also any bacteria capable of regenerating sulphide levels would be killed. The fact that triethyllead salts added to sediment systems produced TEL only, lended support to the sulphide mediated pathway. In studies on aquarium microorganisms Schmidt and Huber (1976) reaffirmed the importance of the biological methylation process. Work by Baker et al., (1981) suggested that the biologically mediated pathway was the dominant reaction and accounted for 50-76% of the total TML produced from $(CH_3)_3PbOAc$. In this work a 0-0.009% yield of TML (as Pb) was reported when acidic lake sediments were incubated with $(CH_3)_3PbOAc$ and nutrients at pH ranges 3.5-7.5 and temperatures of $20^{\circ}C \pm 1^{\circ}C$. The yield of TML was found to increase progressively with pH. It is clear

that the importance attached to the biological and chemical components is contentious. Much of the work reported in this thesis confirms the work of Jarvie et al., (1975) and Craig (1980) although even here disagreement in absolute yields of TML are noticeable.

In an experiment to stress the importance of the sulphide, 'chemical', disproportionation/dismutation mechanism sodium sulphide (1000 $\mu g\ l^{-1}$) was added to a biologically active sediment system. The sulphide was added aseptically and hence the increased evolution of TML amounting to a 4.9% conversion (based on Pb) can only be attributed to the addition of sulphide ions (see Figure 3.10). The chemical sulphide-mediated disproportionation reaction is therefore considered most important and in agreement with Craig (1980) a biological mechanism for this reaction need not be invoked.

3.2.2 Studies on the Methylation of Inorganic Lead (II) Salts

The conversion of Pb(II) salts to TML has direct relevance to the cycling of Pb in the environment. From the estuarine studies reported in Section 3.1, 300-14500 ng l^{-1} of inorganic Pb(II) may be found dissolved in natural waters. In the following series of experiments the conversion of Pb(II) to TML was examined in biologically active sediment systems. The sediment, light, pH nutrients and temperature employed were identical to those used in the work with $(CH_3)_3PbOAc$ (Section 3.2.1, refer to Table 3.9). However, in this section smaller flasks (gas volume 105 ml) and high concentrations of Pb(II) salts (500 μg Pb as $Pb(NO_3)_2$, $Pb(OAc)_2$ and $PbCl_2$) were used to afford detection of TML in headspace gases. In sterile controls of the sediment, water, and added nutrients and Pb(II) salts no detectable levels of TML were observed. In biologically active sediment systems TML was produced from $Pb(NO_3)$, $PbCl_2$ and $Pb(OAc)_2$ (Figures 3.11-3.13). In all cases

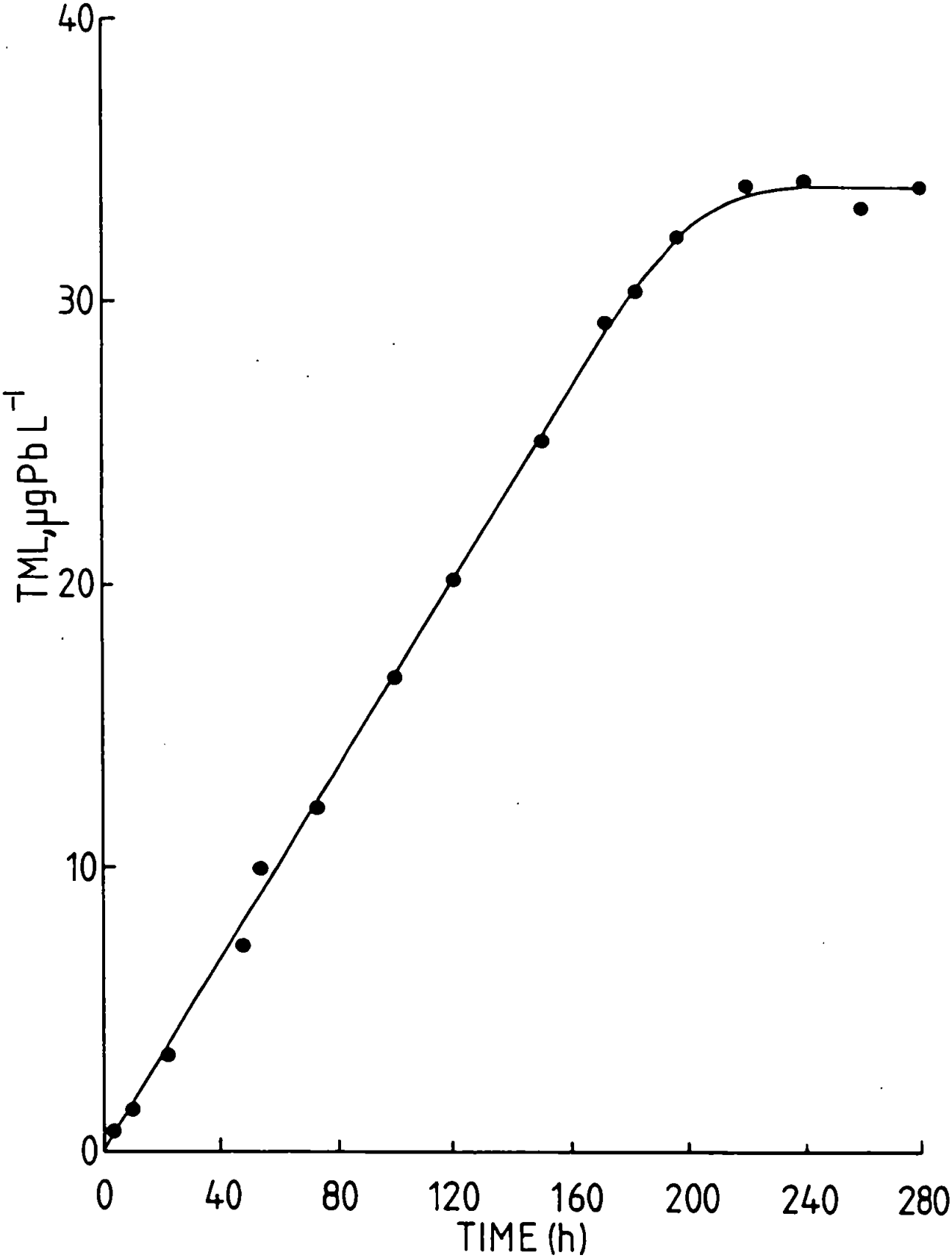


Figure 3.10 Evolution of TML from $(CH_3)_3PbOAc$ in biologically active sediments spiked with 1000 µg Na_2S l^{-1}.
(●) Illuminated biotic system spiked with 1000 µg Na_2S l^{-1}

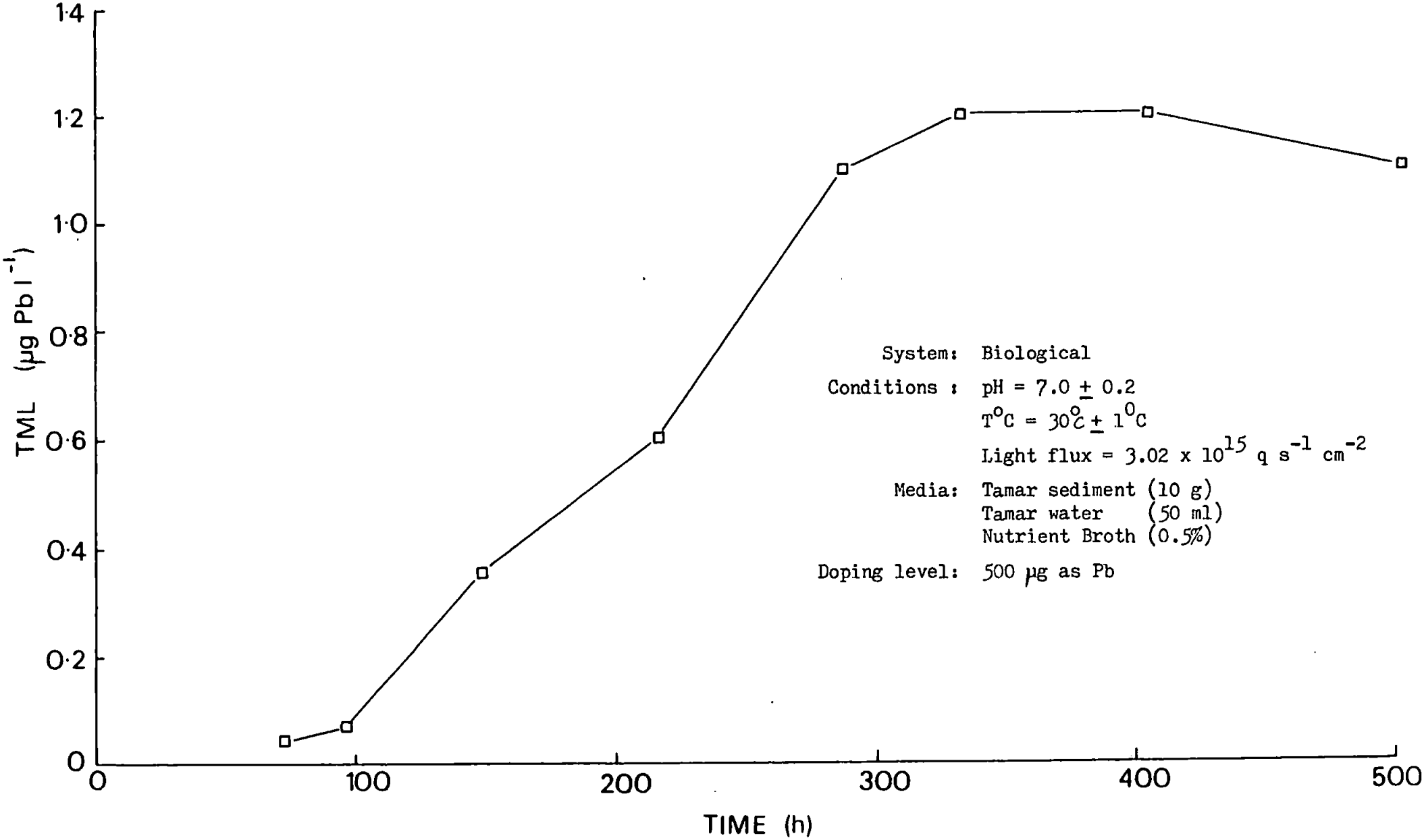


Figure 3.11 Evolution of TML from sediment spiked with Lead(II) nitrate

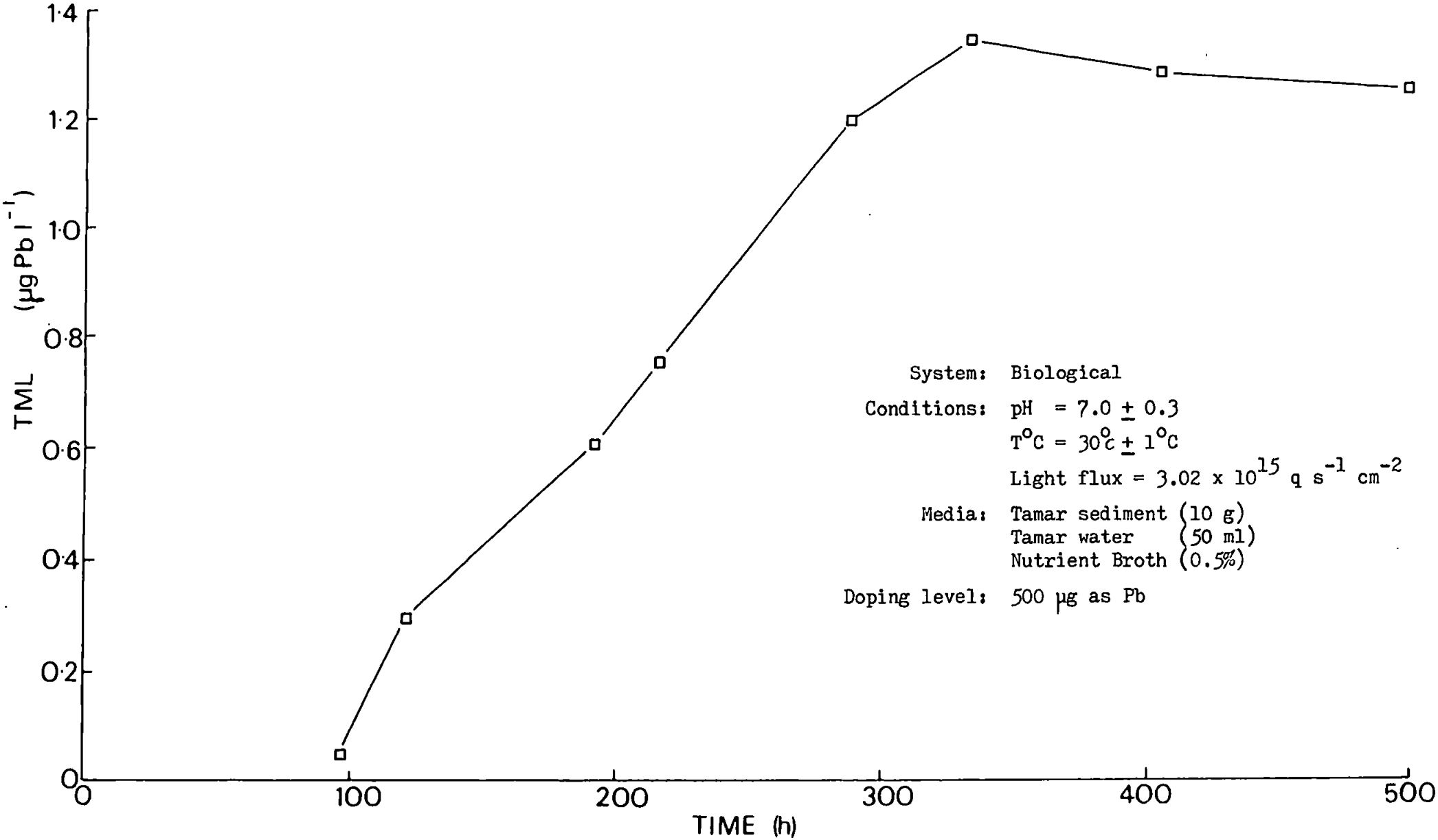


Figure 3.12 Evolution of TML from sediment spiked with Lead(II) chloride

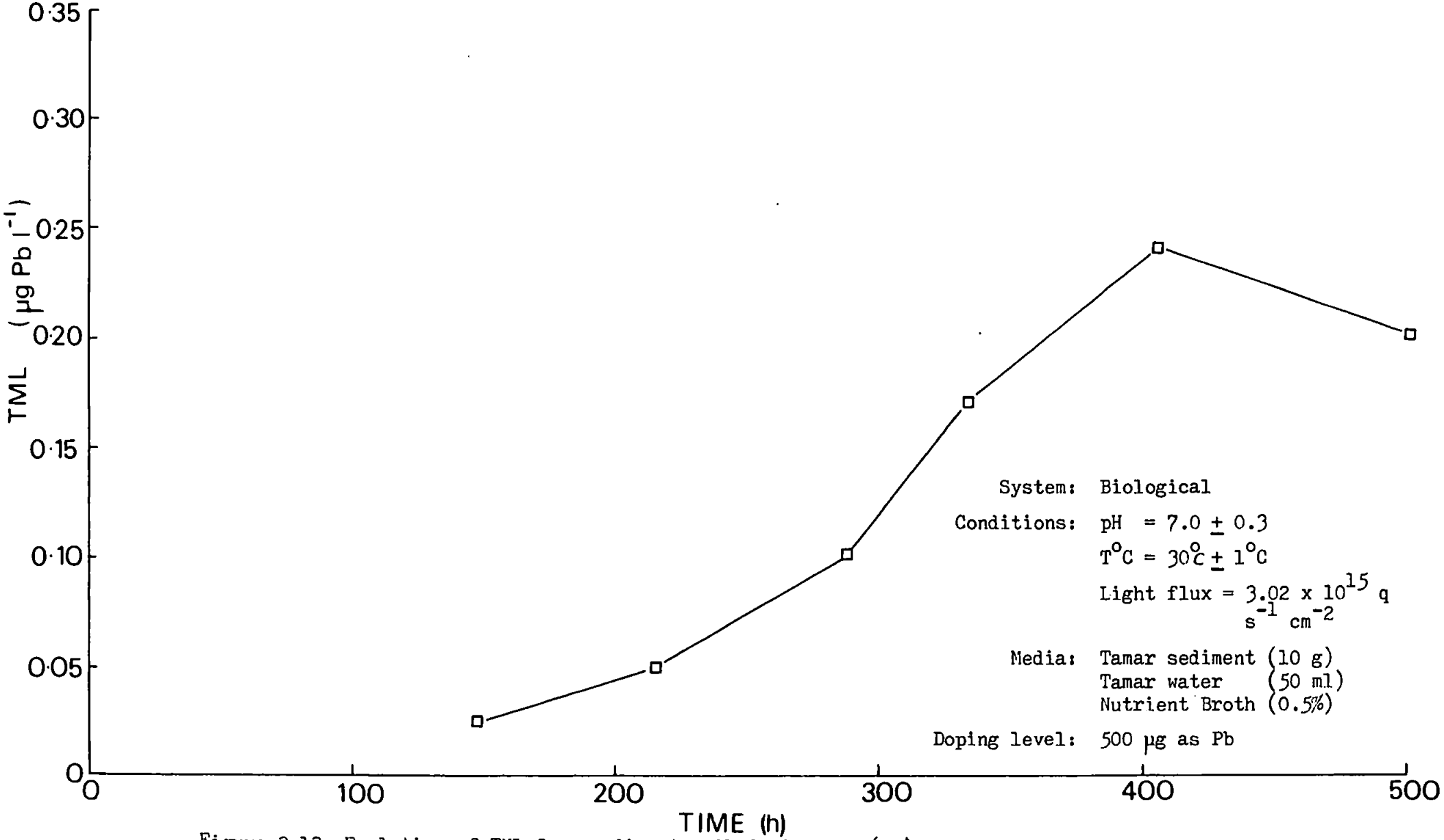


Figure 3.13 Evolution of TML from sediment spiked with Lead(II) acetate

production of TML followed after an initial delay period lasting 80-150 h. The production of TML reached equilibrium after a period of 300-400 h and was found to be stable over this period of time. The yield of TML was 0.005% ($Pb(OAc)_2$), 0.026% ($Pb(NO_3)_2$) and 0.028% $PbCl_2$ (based on Pb), these represent yields 100 fold less than the equivalent $(CH_3)_3PbOAc$ systems. The much smaller yields of TML from Pb(II) salts compared to Pb(IV) ($(CH_3)_3PbOAc$) salts may be due to 2 factors (Thompson and Crerar, 1980). First it is well known that the oxidation of Pb(II) to Pb(IV), the observed oxidation state for TML, is thermodynamically difficult and it has been suggested that the reaction may occur by an initial oxidative addition of a carbocation (CH_3^+) (Huber et al., 1978; Craig and Rapsomanikis, 1985) followed by dismutation of $(CH_3)Pb^{3+}$ to form partially and possibly fully methylated species. Secondly the production of sulphide by various anaerobic bacteria in the system may react with any 'available' Pb to produce PbS which will be unreactive.

Work by Schmidt and Huber (1976) on aquarium microorganism cultures reported the production of TML from $Pb(OAc)_2$, incubation was at 30°C. The incubation temperature of 30°C was chosen for this work since during summer periods surface sediments may approach this temperature. In agreement with Schmidt and Huber's work it was found that TML was generated from $Pb(OAc)_2$. Interestingly the production of TML from $Pb(OAc)_2$ was lower by a factor of 4-5 than for $Pb(NO_3)_2$ or $PbCl_2$. It has been suggested that the anion associated with the Pb may play a part in the production of TML (Thompson and Crerar, 1980), it is possible that Pb(II) is stabilized in such a lower oxidation state by the anion present. The effect of various anions present in the reaction medium may also be important. Recently the production of TML from marine sediments incubated at 15°C with nutrients and added Pb (5 mg l^{-1} as

$Pb(NO_3)_2$) was reported (Thompson and Crerar, 1980). The workers quoted a yield of TML of 0.026% after a period of 600 h, this is almost identical to the yield and period of study reported in this thesis, when a freshwater site was examined. Similar bulk properties of sediments were reported. The author in agreement with the work done in this thesis stated that the presence of added Pb appeared to be the governing factor in the production of TML.

To confirm that the conversion of Pb(II) salts to TML was a biological process and to understand more fully the conditions required for the conversion, a further series of experiments were performed, at a later date. In these experiments temperature was examined (15-30°C) and the effect of various sediments (marine and estuarine) was examined. In all of the experiments production of TML was below the detection limit (14 ng l^{-1} as Pb) and work to repeat the initial experiments also failed. In the first series of experiments on Pb(II) salts sediment was collected in October 1984 (end of summer, see Section 3.2.1 for bulk characteristic of sediment) whilst in the second series of experiments sediment was collected in March 1986 (typical winter month, see Table 3.5 for bulk characteristics of sediment). It is possible that different microorganisms were present in the sediments collected at these times. This may account for the failure to detect TML in the latter series of experiments. The work reported here emphasises the general lack of reproducibility of Pb methylation observations, though in this work strict control of reaction conditions was adhered to. The maximum conversion of inorganic Pb(II) to TML reported in this thesis work was 0.028%. If a 0.028% conversion of Pb to TML were to occur in the mudflat areas of estuaries a significant concentration of Pb may be mobilized from sediments. However, further atmospheric studies in mudflat areas which are truly remote from land derived petroleum

inputs are required to confirm this hypothesis.

In conclusion to this section a summary of the modelling approach employed is detailed and emphasises the difference between the studies on Pb and As undertaken. Common areas of study for Pb and As included:

1. identical experimental conditions;
2. identical kinetic analysis approach;
3. identical mechanistic processes considered.

The latter common area involved consideration of both physical and biochemical mechanisms occurring within the reaction flasks. The physical mechanisms are determined by the nature of the experiment and the physical properties of the chemicals involved, as such they are related to (a)diffusion in the sediment and gas phases, (b) solubility in the water phase. The chemical and biochemical mechanisms available are (a) adsorption onto active surfaces e.g., glassware or particulate matter, (b) radical reactions including photochemical sources of ($CH_3\cdot$) radicals and biological sources. The essential difference between Pb and As studies is that for Pb a biological (enzymatic) process need not be invoked to explain the formation of methylated species whereas for As such a process is invoked.

CHAPTER FOUR

RESULTS AND DISCUSSION OF ARSENIC STUDIES

4.1 The Distribution of Arsenic Species in Estuarine Environments

This chapter reports the results of both the environmental survey work and laboratory modelling studies on As methylation. The same basic experimental approach outlined in the previous Chapter on Pb was employed in the environmental studies on As. Emphasis was placed on maintaining sample integrity by carrying out sample analysis in a relatively short time after collection. During the period July 1985 - June 1986 a series of surveys was undertaken to determine the concentration of inorganic and methylated As species in the fauna, waters and sediments of the Tamar, Carnon and Beaulieu Estuaries. Due to logistical constraints on time and physical handling of sampling equipment only column water samples were collected from the Beaulieu and Carnon systems.

4.1.1 Water Column Studies of Dissolved Arsenic

The results of water column studies of dissolved As in the Tamar, Carnon and Beaulieu estuarine systems are reported below. The physical setting and hydrogeochemistry of the 3 estuaries have been described in detail in previous Chapters.

4.1.1.1 Tamar Estuary

The speciation of inorganic As (arsenite (As(III)) and arsenate (As(V))) in water samples was found to be a time consuming process and the data obtained provided information that was less appropriate to the present study than information on the speciation of organoarsenic compounds. For these reasons speciation of inorganic As into As(III) and As(V) was not undertaken during full estuarine surveys.

Dissolved inorganic and methylated As species were detected in surface

water samples, over the salinity range 0.5-30‰ during winter months (water temperature $\leqslant 10^{o}C$) and summer months (water temperature $\geqslant 15^{o}C$), refer to Figures 4.1 to 4.8. The dissolved inorganic As concentration (arsenite and arsenate combined) observed ranged from 0.1-7.0 $\mu g\ l^{-1}$ compared with 3.7-5.3 $\mu g\ l^{-1}$ ('total' As) reported by Knox et al., (1984) for the same estuary. In oxygenated waters arsenate (As(V)) is the stable state with the predominant dissolved form being $HAsO_4^{2-}$ (Lowenthal et al., 1977). In early experiments in this thesis work on speciation of arsenate and arsenite in surface water samples (samples collected from River Carnon 4th June, 1985, 0.45 μm filtered), arsenate accounted for 50-80% of the total dissolved inorganic As. Langston (1983) reported that arsenate accounted for 97% of the 'total' As concentration at marine sites (salinity >15‰) in the Tamar. The same author (Langston, 1983) also reported that at freshwater sites, in the Tamar, close to abandoned mines arsenite may account for 30% of the 'total' As concentration. A strong seasonal variation in dissolved inorganic As concentrations was observed. In summer months average dissolved inorganic As concentrations ranged from 0.1 to 4.0 $\mu g\ l^{-1}$, whilst in winter months concentrations ranged from 0.4-7.0 $\mu g\ l^{-1}$. The higher concentration of dissolved inorganic As during winter months reflects the increased weathering and transportation of As during periods of high rainfall. This situation is similar to that reported by Boyden et al., (1979) for the River Carnon (Falmouth, S.W. England).

In general higher dissolved inorganic As levels were observed in the low salinity regions of the estuary close to metalliferous waste inputs (maximum concentrations 7.0 $\mu g\ l^{-1}$). The dilution of freshwater with seawater reduced the concentration of dissolved inorganic As such that the concentration at marine sites seldom exceeded 1.0 $\mu g\ l^{-1}$. Examination of the estuarine profiles obtained in the present study reveal that a general broad As maxima occurs in the Tamar Estuary and this is

Key

(●) inorganic As

(■) MMA species

(▲) DMA species

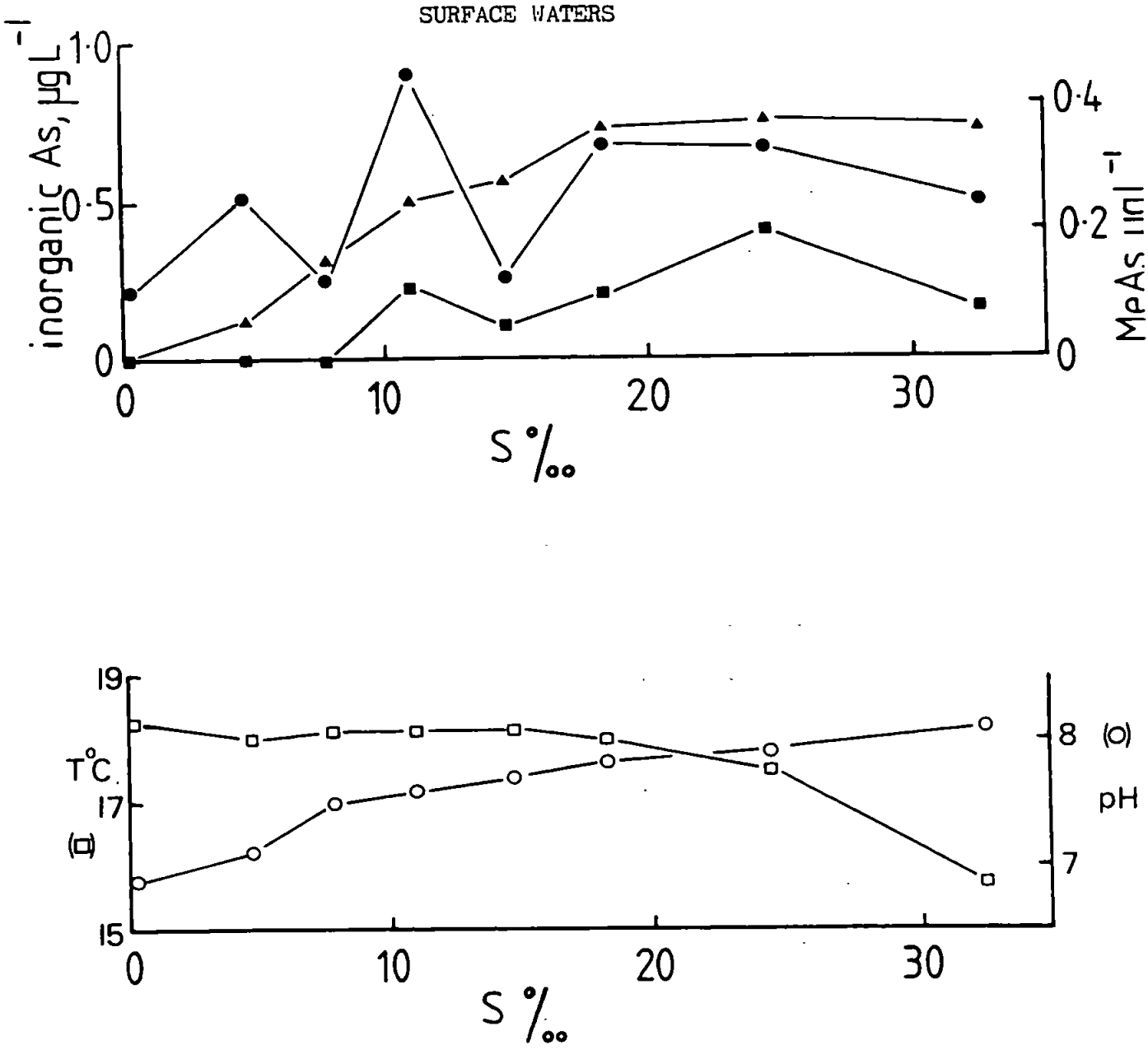


Figure 4.1 Tamar Estuary survey 30th July 1985.

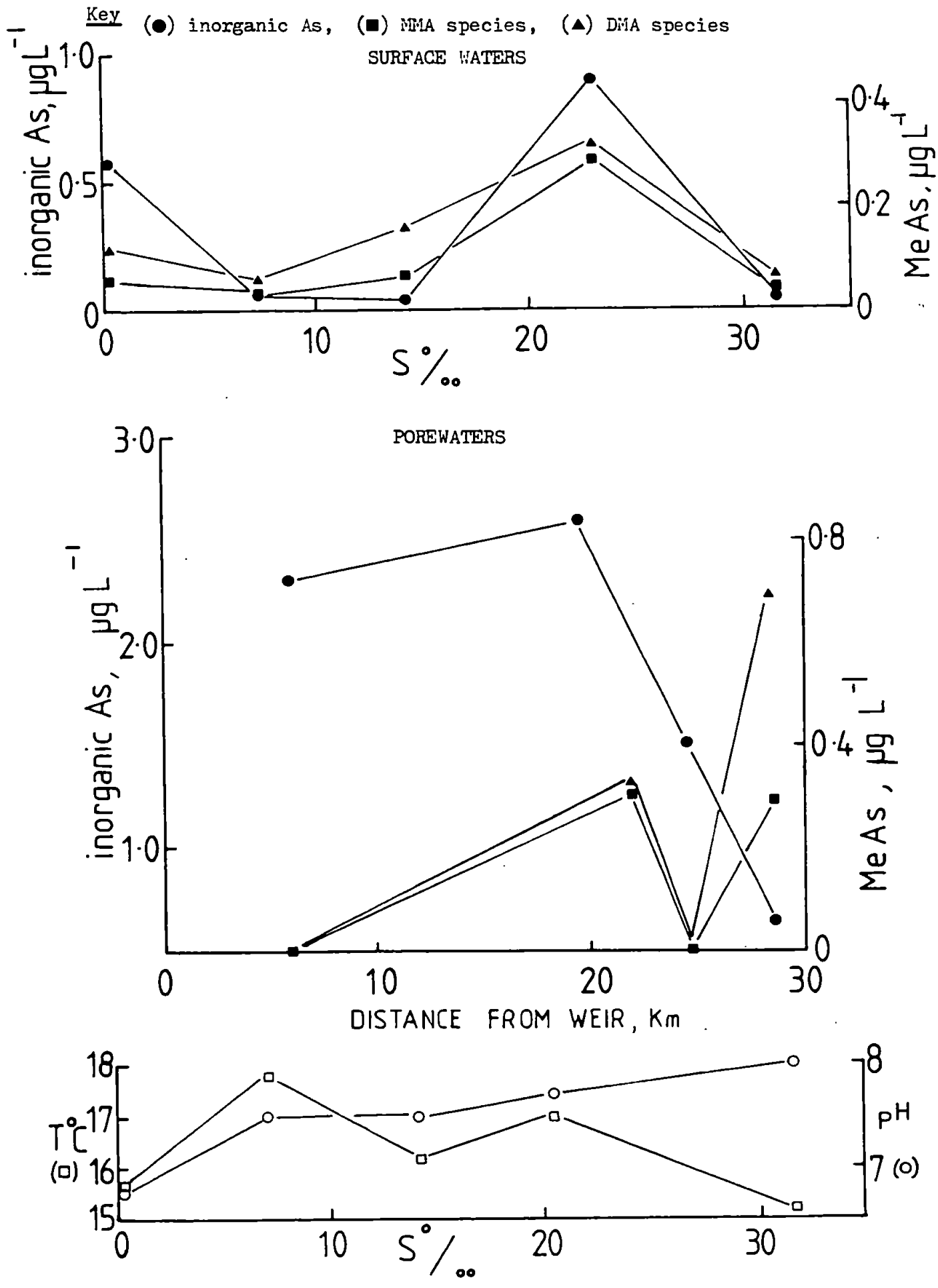


Figure 4.2 Tamar Estuary survey 6th August 1985

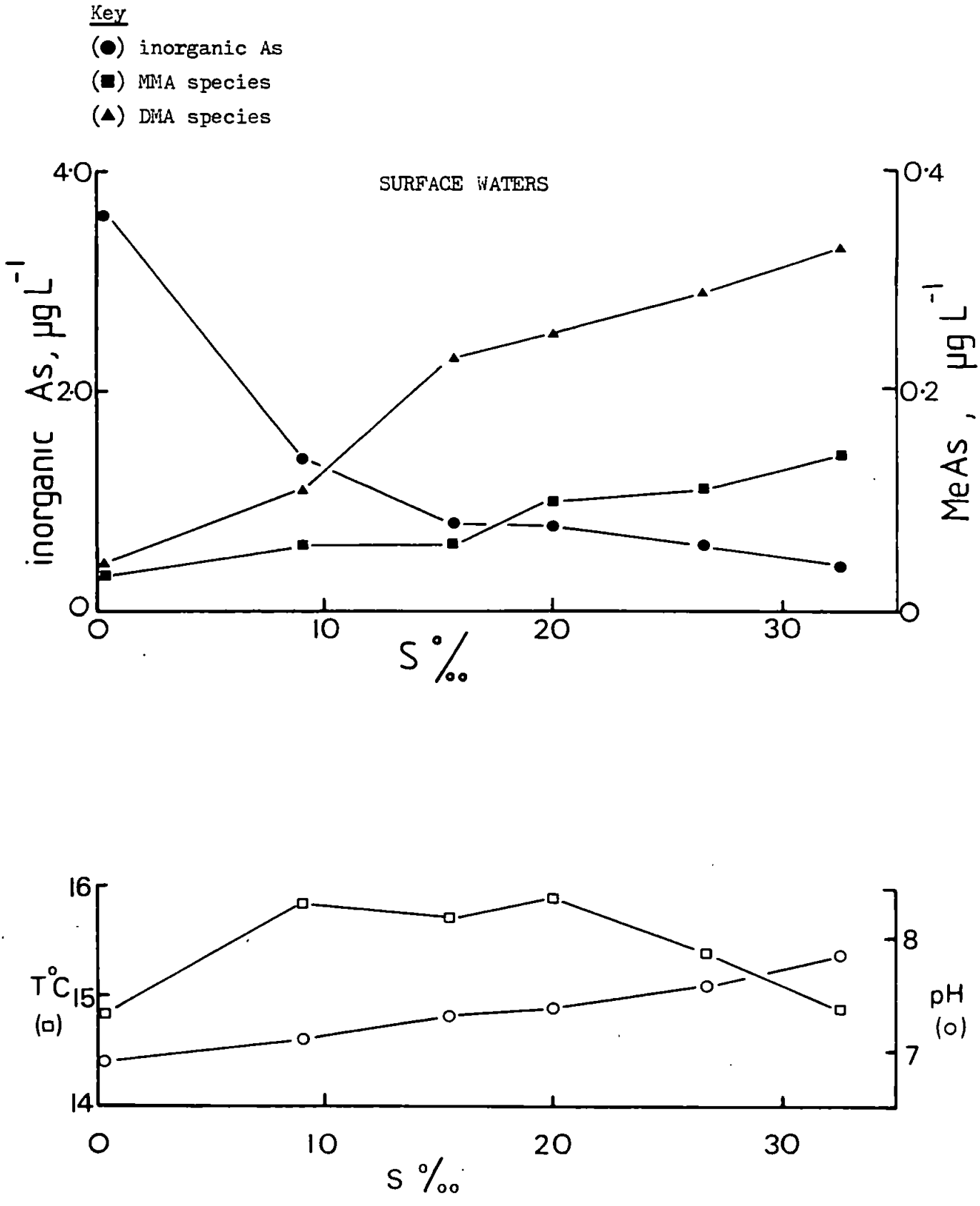


Figure 4.3 Tamar Estuary survey 21st August, 1985

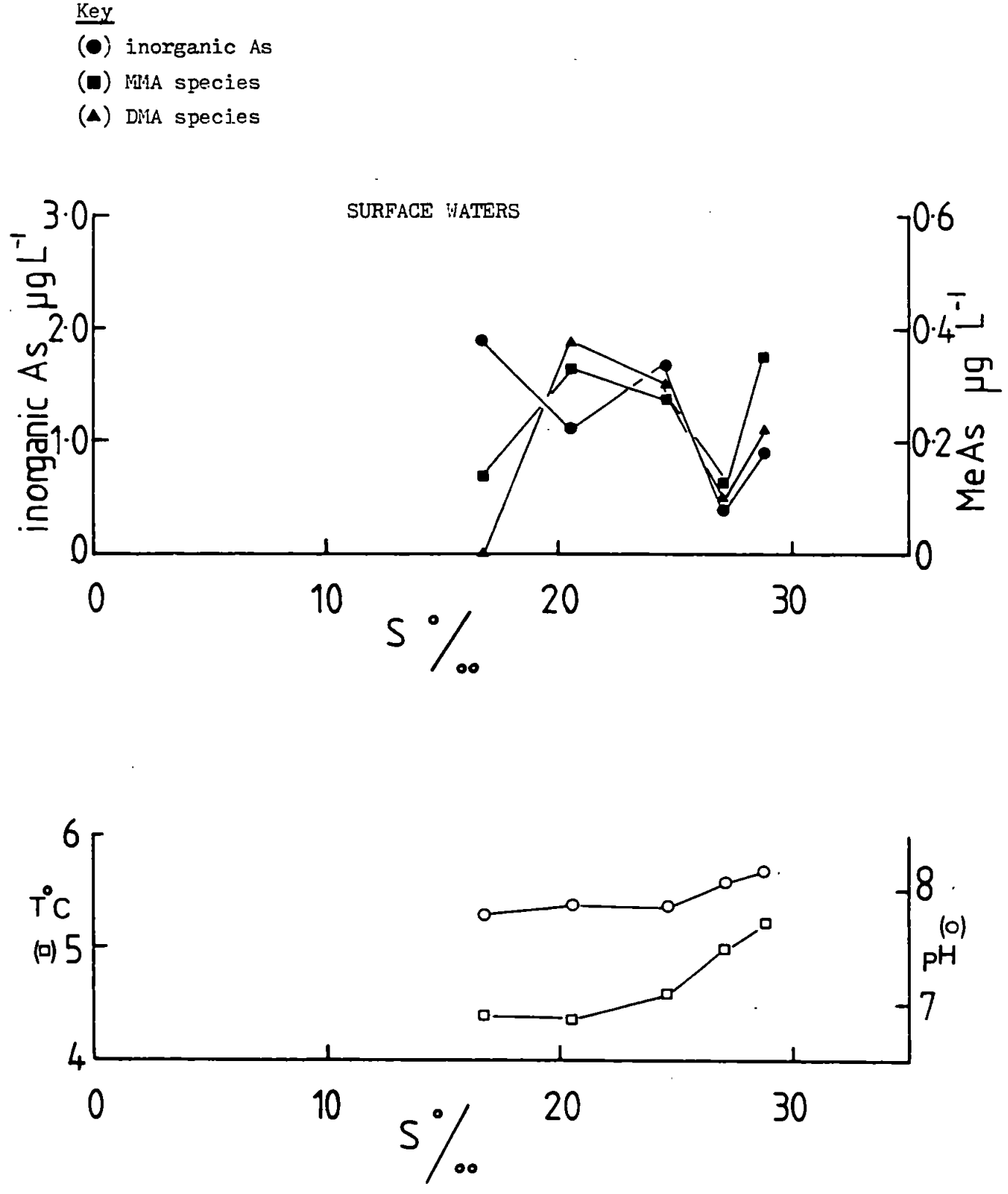


Figure 4.4 Tamar Estuary survey 12th February 1986

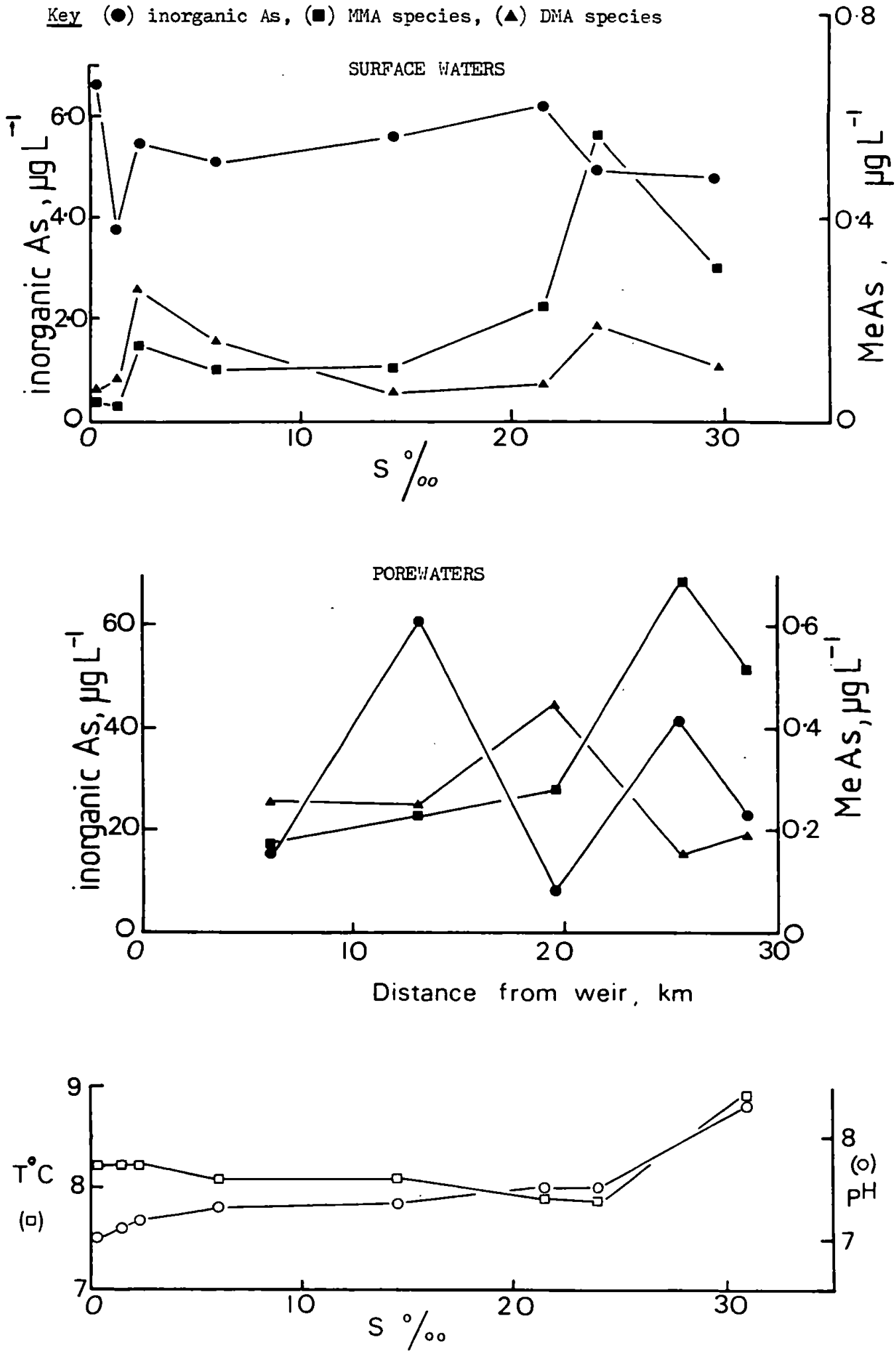


Figure 4.5 Tamar Estuary survey 24th March 1986

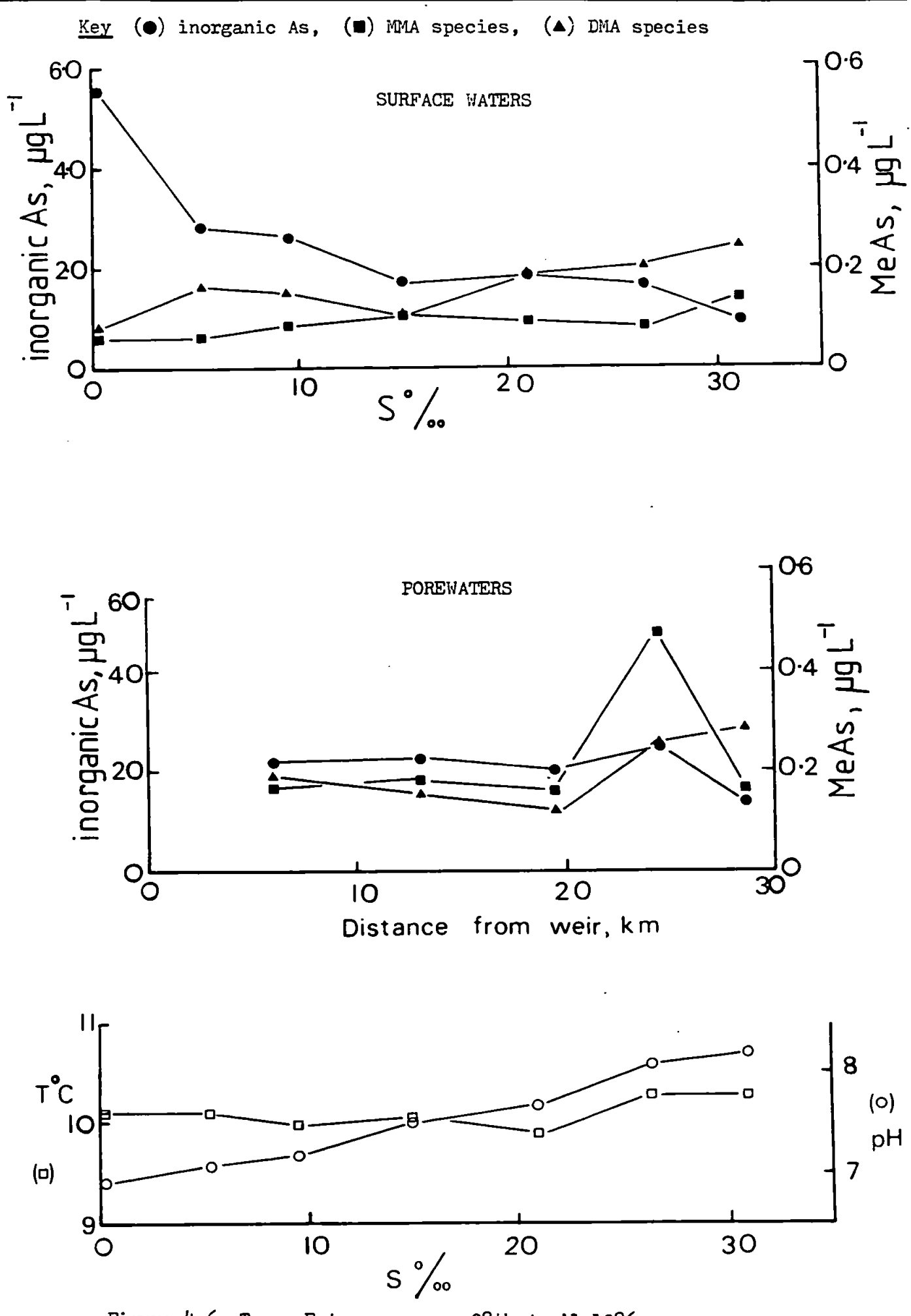


Figure 4.6 Tamar Estuary survey 28th April 1986

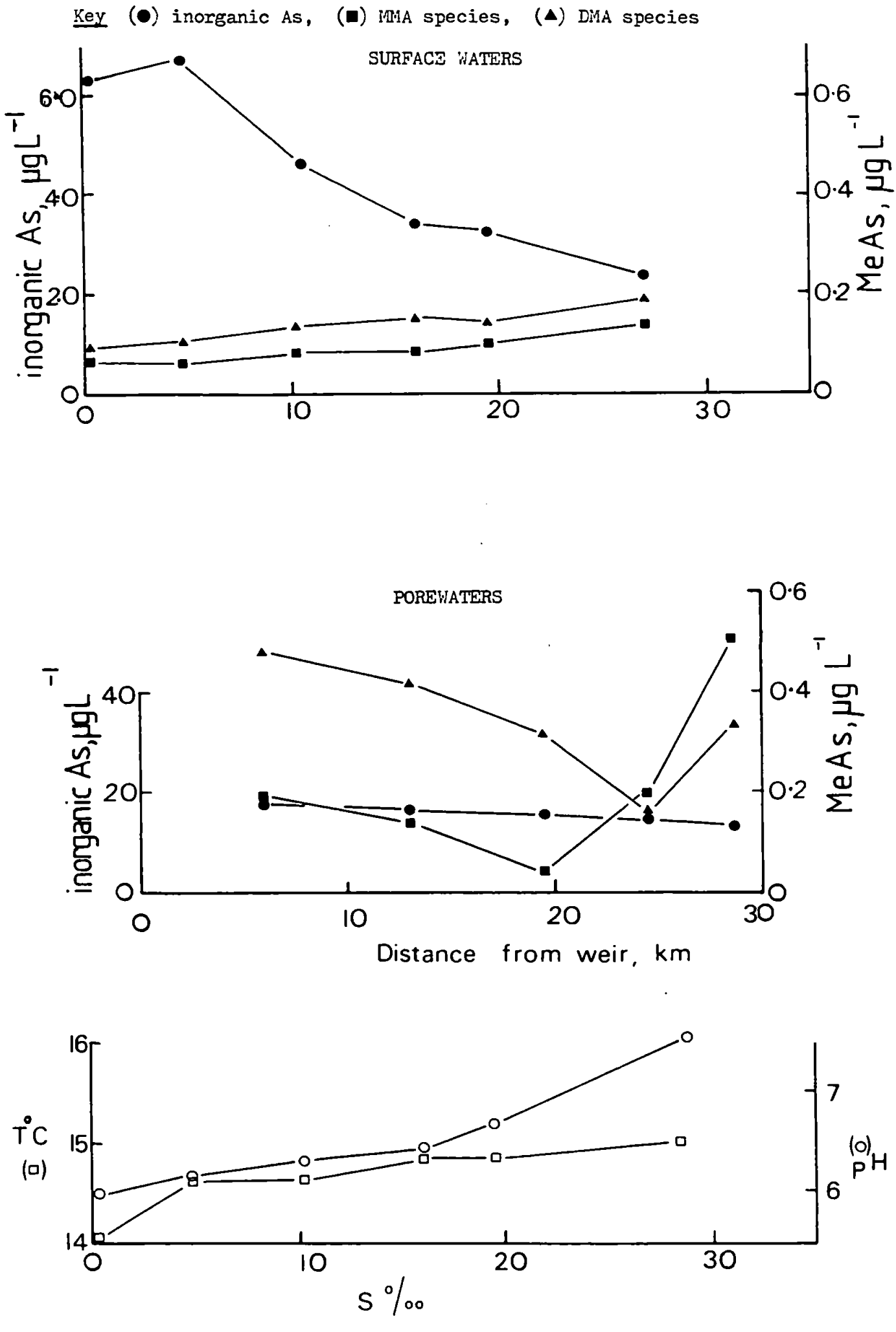


Figure 4.7 Tamar Estuary survey 27th May 1986

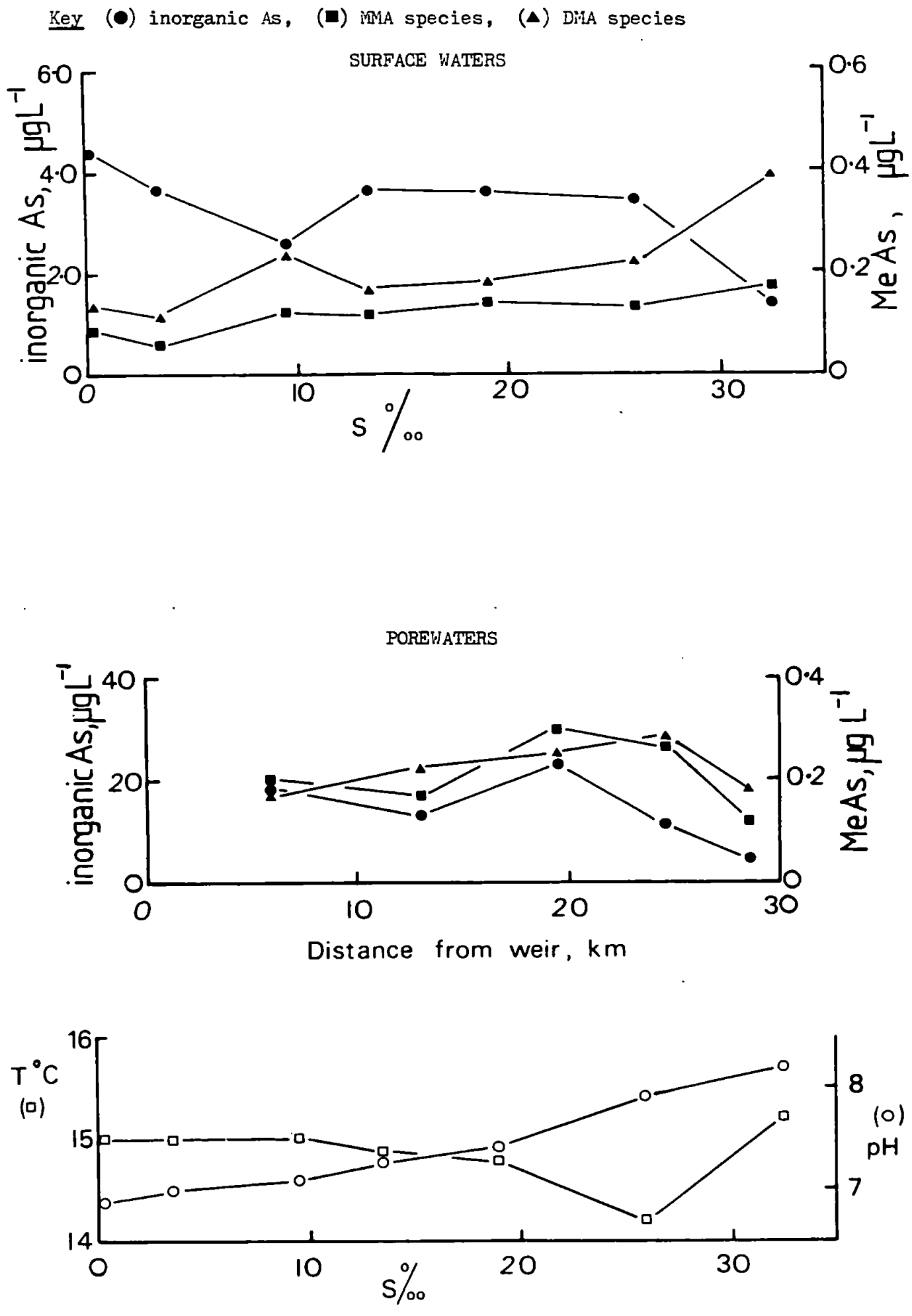


Figure 4.8 Tamar Estuary survey 11th June 1986

indicative of extended estuarine inputs including porewaters (Knox et al., 1981). However, substantial removal of As from solution may occur and on 3 occasions (21st July 1985, 28th April and 27th May 1986, Figures 4.3, 4.6 and 4.7 respectively) removal processes significantly altered the general broad mid-estuarine maxima profile normally observed. The classical non-conservative behaviour of As in the Tamar has been attributed to the loss of As from solution onto freshly precipitated colloidal Fe, the processes being most noticeable at the freshwater-brackish water interface (Langston, 1983; Millward and Marsh, 1986).

During the present study the concentration of dissolved methylated As species in the Tamar was found to account for a maximum of 10% of the 'total' inorganic As. The concentration of dissolved methylated As species (classified here as either monomethylarsenic species (MMA) or dimethylarsenic species (DMA)) ranged from <0.02 to 0.6 μg As l^{-1} over the seasonal cycle compared with 0-1.2 μg As l^{-1} for a summer month (20-27th August 1984) reported by Apte (1985). From the profiles of monthly survey work (Figures 4.1-4.8) several trends in the concentration of dissolved methylated As species are apparent. Freshwater sites (salinity <1‰) were observed to have lower concentrations of methylated As species (maximum 0.1 μg As l^{-1}) compared with marine sites (salinity >20‰, maximum concentration of 0.6 μg As l^{-1}) and this trend was observed throughout the seasonal cycle. A marine origin for methylated As species has been proposed by Howard et al., (1984). It is also evident from the monthly profiles that the preponderance of individual methylated As species varied throughout the seasonal cycle 1985-86. In winter months (February and March, Figures 4.4-4.5) MMA dominates over DMA. However, in April at the onset of increased biological activity, DMA and MMA concentrations became roughly equivalent (Figure 4.6) and from May to August the concentration of DMA tended to dominate that of MMA. The concentrations of MMA and DMA varied over the seasonal

cycle, in winter months a maximum concentration of MMA (0.6 μg As l^{-1}) and DMA (0.35 μg As l^{-1}) were observed. Whilst in summer months MMA (0.25 μg As l^{-1}) and DMA (0.4 μg As l^{-1}) maxima were obtained. The higher concentration of MMA compared with DMA in winter months could be accounted for if the DMA produced in summer months decomposes to MMA in winter months. Bacteria are known to be capable of demethylating methylated As species (Shariatpanahi, 1981). However, there is no apparent drastic decrease in the concentration of DMA in winter months to suggest this mechanism applies in the Tamar. Apte (1985) observed no marked decrease in DMA concentration over a period of 96 h when bacterially active natural waters were amended with DMA (spiking level 0.5-1.0 μg As l^{-1}). The work of Apte (1985) is in agreement with work in the present study (refer to Section 4.1.3). It may therefore be postulated that there is a further source of methylation, perhaps in the porewaters, this is discussed in Section 4.1.2.3.

4.1.1.2 River Carnon and Associated Restronguet Creek

During 2 surveys carried out on consecutive days in June 1985 detectable levels of dissolved inorganic and methylated As species were observed in the surface waters of the Carnon system (see Figures 4.9 and 4.10). Similar trends to those observed for As concentrations in the Tamar were apparent. In the Carnon system levels of inorganic As were approximately 3 fold greater than those observed in the Tamar reflecting the greater anthropogenic inputs into the Carnon system. During the summer inorganic As ranged from 0.5 to 12.0 μg l^{-1}. The behaviour of dissolved inorganic As in the Carnon was essentially non-conservative as evident on the 3rd June 1985 (Figure 4.9). The methylated As species tended to increase on moving from freshwater to marine sites and DMA (maximum concentration 0.7 μg As l^{-1}) dominated over MMA (maximum concentration 0.15 μg As l^{-1}). These concentrations are similar to

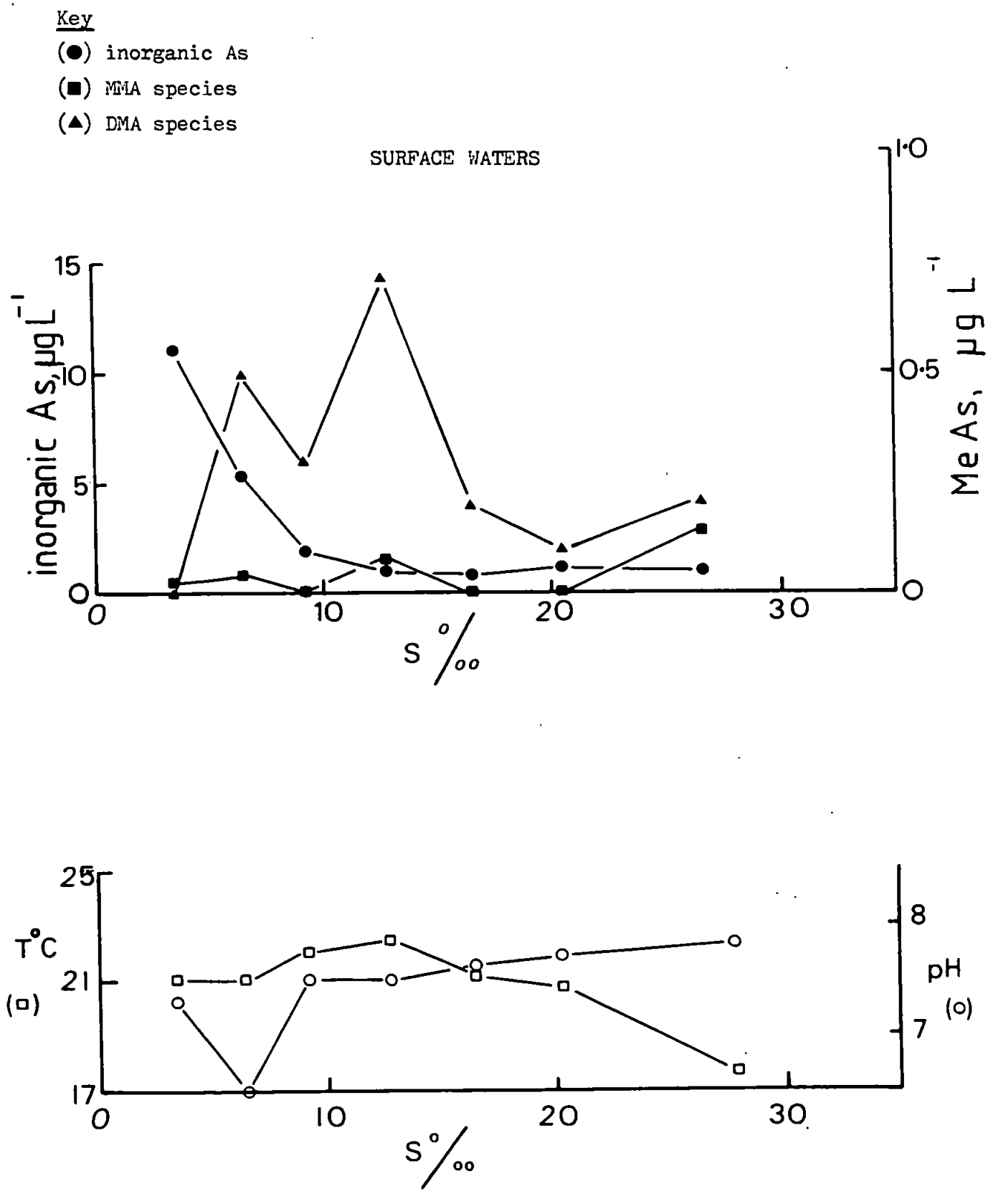


Figure 4.9 River Carnon and Restronguet Creek survey 3rd June 1985

Key

(●) inorganic As

(■) MMA species

(▲) DMA species

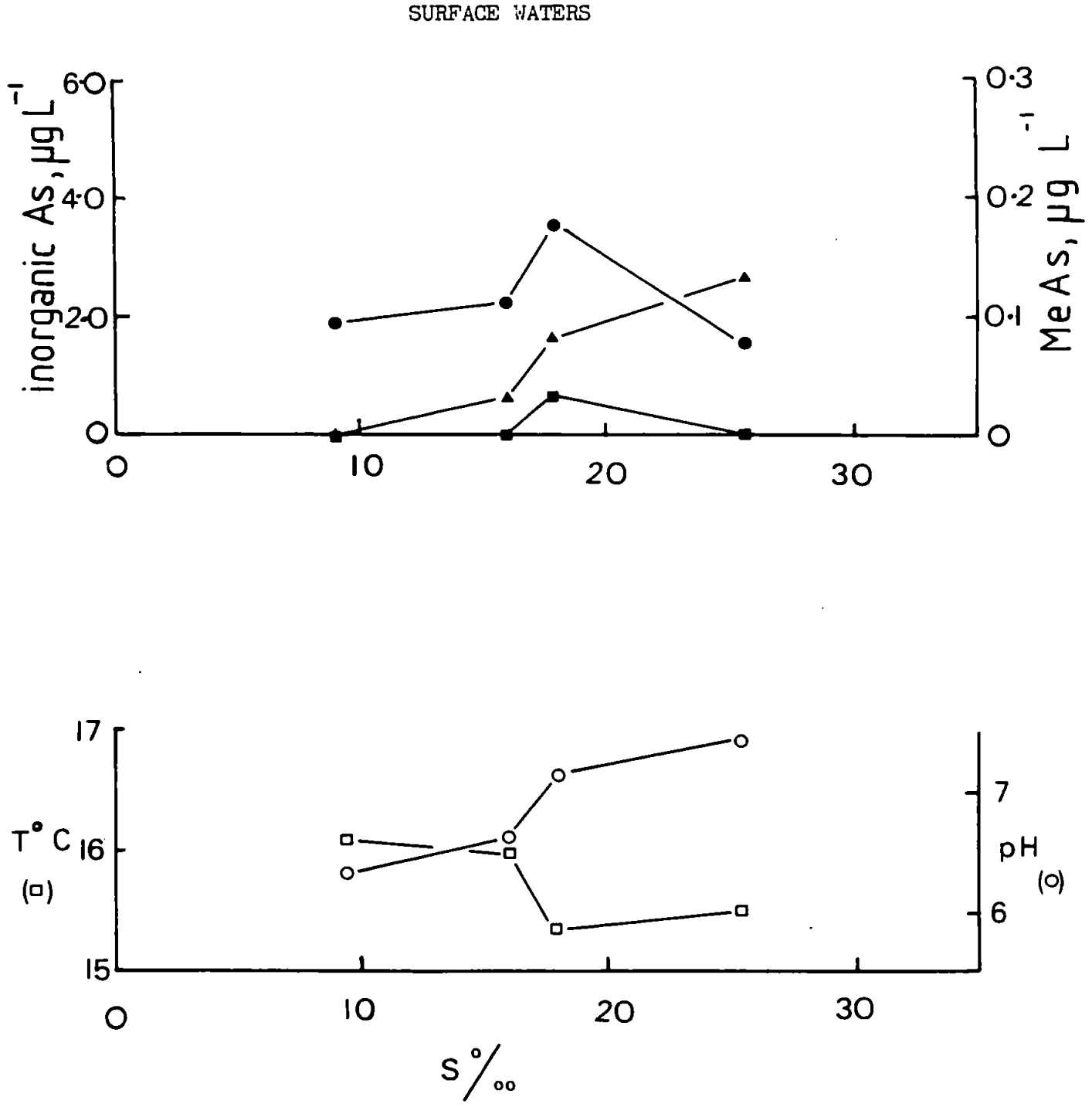


Figure 4.10 River Carnon and Restronguet Creek survey 4th June 1985

those observed in the Tamar. Further, methylated As species were observed at noticeably higher concentrations on the 3rd June 1985 compared with 4th June 1985 survey. Average water temperatures had fallen from 21°C on 3rd June 1985 to 16°C on 4th June 1985. The drop in temperature (5°C) would markedly lower the biological activity in the water column and in turn release of methylated As species to the water column would be lowered. This may account for the variation in the concentration of methylated As species observed on 3rd and 4th June 1985.

4.1.1.3 Beaulieu Estuary

In 2 further surveys detectable concentrations of inorganic and methylated As species were observed in the surface waters of the Beaulieu Estuary (refer to Figures 4.11 and 4.12). During a summer survey (17th-18th June 1985) dissolved inorganic As concentrations were found to vary from 0.05-0.4 $\mu g\ l^{-1}$. These concentrations compare with 0.05-0.6 μg As l^{-1} (as inorganic As) reported by Waslenchuk (1979) for relatively unpolluted estuaries in S.E. USA. Such concentrations are a factor of 10 lower than the inorganic As concentrations reported for the polluted Tamar and Carnon systems. The profiles of inorganic As indicate non-conservative behaviour with concentrations increasing seaward. High concentrationsof As species at high salinities are indicative of a marine planktonic source of As as suggested by Howard et al., (1984). The concentration of dissolved methylated As species were observed to be similar to inorganic As levels. The concentration of methylated As species was highest at marine sites and within this trend DMA (maximum concentration 0.6 μg As l^{-1}) tended to dominate over MMA (maximum concentration 0.5 μg As l^{-1}). These concentrations and general trends are similar to those observed in the Tamar and Carnon systems. Howard et al., (1984) studied the Beaulieu and reported inorganic As concentrations of 0.1 to 1.0 $\mu g\ l^{-1}$ and methylated As

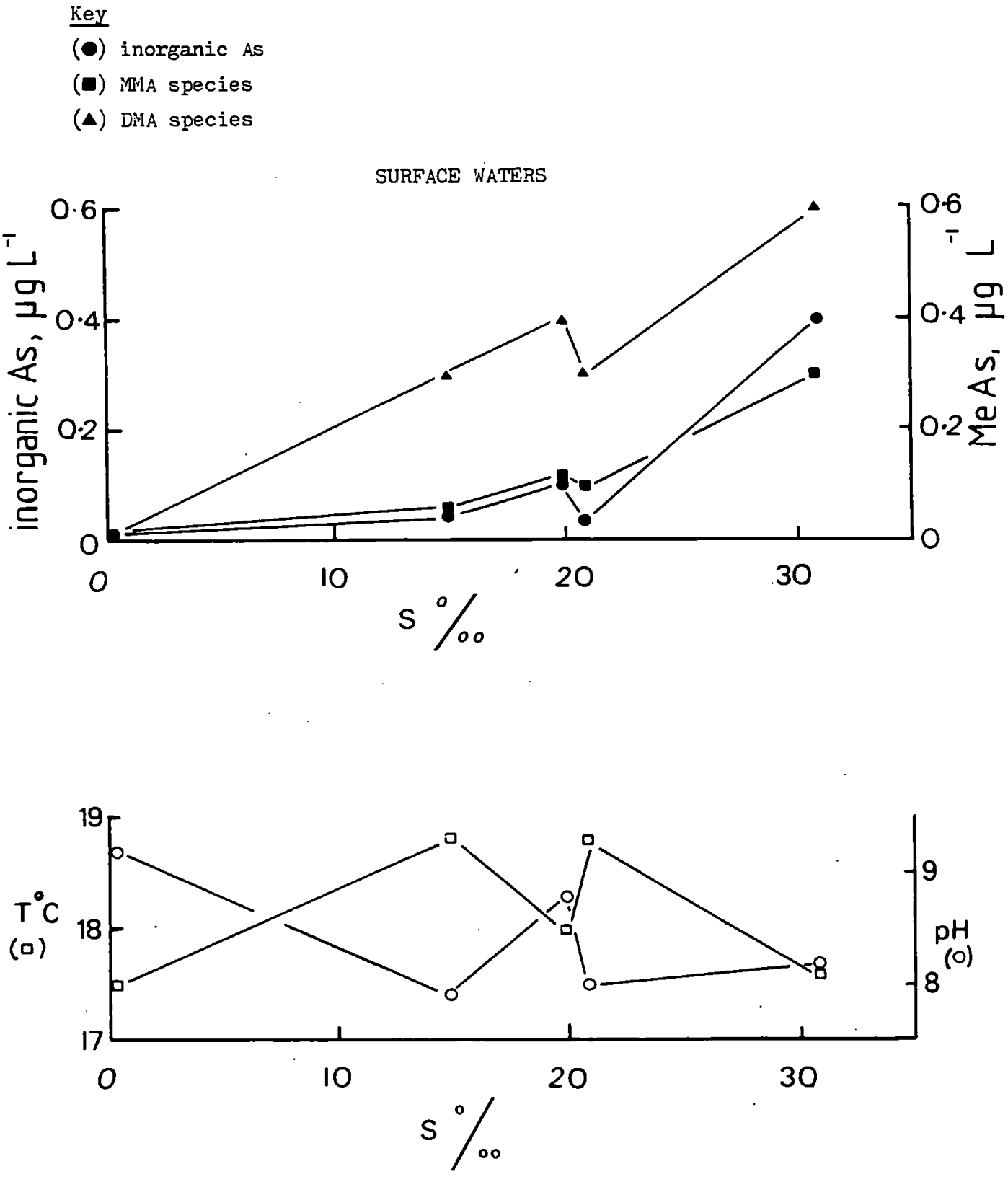


Figure 4.11 Beaulieu Estuary survey 17th June 1985

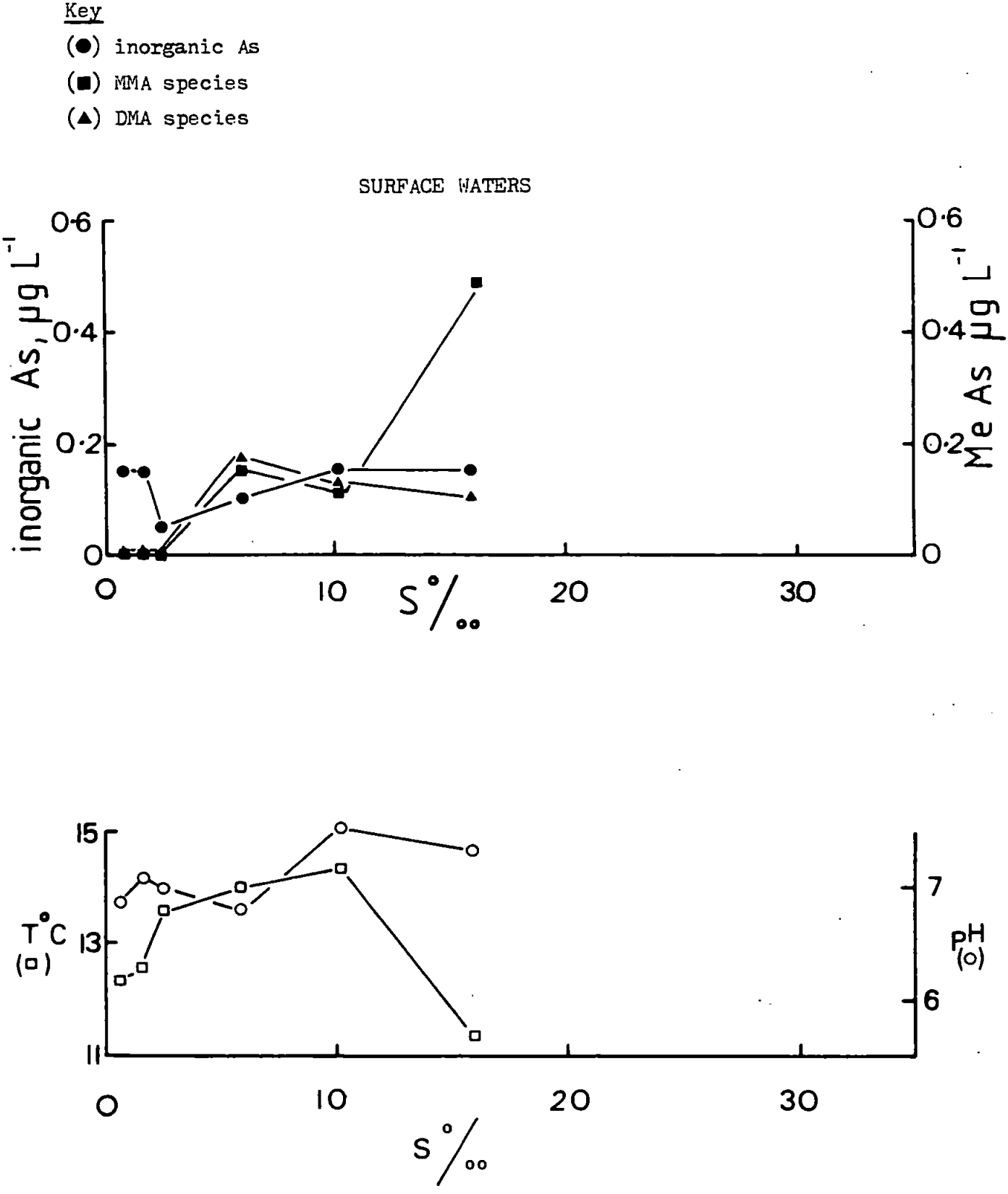


Figure 4.12 Beaulieu Estuary survey 18th June 1985

concentrations of 0.1 to 0.45 µg As l^{-1}, which are similar to the concentrations observed in the Beaulieu Estuary in this thesis.

4.1.2 Sources of Methylated Arsenic in the Tamar

Although several similar trends have been found to occur in the Tamar, Carnon and Beaulieu estuarine systems the knowledge of the sources and processes involved in As methylation are poorly understood. It is likely that several processes are responsible for the complex distribution of dissolved methylated As species in the 3 estuaries studied. In an attempt to understand more fully the cycling of As in the estuarine environment studies were undertaken to identify the sources of methylated As species. The Tamar system has been studied in detail and similar trends occur in the Carnon and Beaulieu systems. Thus the hypothesis developed from the studies in the Tamar should be applicable elsewhere.

4.1.2.1 Macro-algal Studies

Several studies on the As concentration and speciation of macro-algae indicate that these organisms are a significant source for methylated As species (Johnson and Braman, 1975; Andreae, 1978; Klumpp and Peterson 1979; and reviewed by Phillips and Depledge, 1985). In this study macro-algal samples were collected during an initial survey in December 1985 in the St. Johns' Lake area of the Tamar Estuary. Samples were digested as described in Section 2.3.3.2 and analysed as described in Section 2.2.2 and 2.2.4. The hydride generation methodology employed provides data on the reducible As species present whilst the GFAAS methodology yields data on total As. From Table 4.1 both Ascophyllum nodosum and F. vesiculosus contained detectable concentrations of inorganic and organoarsenic species. In hydride generation analysis following either hydrochloric or nitric acid digestion both inorganic As

Table 4.1 Total and reducible arsenic concentrations in Macro-algae

		HYDRIDE GENERATION ($\mu g\ g^{-1}$)								GFAAS ($\mu g\ g^{-1}$)
		Hydrochloric Acid digest				Nitric Acid digest				Nitric Acid digest
Species	Section	Inorganic As:	MMA:	DMA:	Total reducible As	Inorganic As:	MMA:	DMA:	Total reducible As	Total As
Ascophyllum nodosum	Stem	0.14 (0.11-0.16)	<0.05	3.41 (2.53- 4.04)	3.55	0.27 (0.21-0.31)	0.53 (0.42-0.66)	5.52 (4.81- 6.80)	6.32	7.03 (6.30- 7.73)
	Sporangia	0.09 (0.07-0.12)	<0.05	6.14 (5.38-6.83)	6.23	0.22 (0.17-0.26)	0.26 (0.18-0.35)	9.18 (7.45-11.63)	9.66	14.29 (13.07-16.41)
Fucus vesiculosus	Stem	0.15 (0.10-0.21)	<0.05	4.10 (3.20- 4.65)	4.25	0.63 (0.35-0.81)	0.43 (0.39-0.49)	4.50 (3.56- 5.00)	5.56	7.56 (6.01- 9.32)
	Sporangia	0.21 (0.17-0.27)	<0.05	8.10 (6.40-10.58)	8.31	0.69 (0.55-0.91)	0.51 (0.46-0.54)	9.90 (7.94-11.81)	11.1	13.78 (10.36-15.94)

Data are the mean of 3 analyses (Figures in parentheses refer to range in concentration). Samples collected from St. Johns' Lake, Tamar Estuary, Cornwall, S.W. England (December 1985). Inorganic As = As(III) + As(V), MMA = monomethylarsenic species, DMA = dimethylarsenic species.

and MMA were observed at sub μg g^{-1} (dry wt.) concentrations, whilst DMA was observed at significantly higher concentrations. Average macro-algal concentrations of species reducible to $(CH_3)_2AsH$ of 3.4 to 9.9 μg As g^{-1} were measured when an hydrochloric acid digest followed by hydride generation analysis was employed (Table 4.1) and this compares with the figure of 2.79 μg As g^{-1} for F. vesiculosus species reported by Howard et al., 1981. The methodology employed in the present study was identical to that reported by Howard et al., 1981 who collected algal samples from Dorset (S.W. England).

Klumpp and Peterson (1979) reported a maximum DMA concentration of 82 μg As g^{-1} in Ascophyllum nodosum. Klumpp and Peterson (1979) employed a strong acid digestion procedure (nitric/perchloric acid digest) followed by hydride generation analysis and samples were collected from Restronguet Creek, Falmouth (S.W. England). Data on methylated As concentrations are available for various algal species (Phillips and Depledge, 1985), however, because of the use of different digestion procedures much of the data is not compatible with work reported in this thesis. In the present study hydrochloric acid digests extracted lower concentrations of As than nitric acid digests. Since nitric acid is an oxidising acid and hydrochloric acid is not, greater decomposition of algal tissue would occur when nitric acid is employed with subsequently higher cellular concentrations of As being extracted. From spiking experiments (refer to Section 2.3.3.2) As forms reduced to monomethylarsenic species and dimethylarsenic species by nitric or hydrochloric acid digests did not undergo significant decomposition or rearrangement reactions. From Table 4.1 the summation of individual reducible As species (inorganic As + MMA species + DMA species) obtained by hydride generation does not fully account for the total As concentration obtained by GFAAS analysis. In the case of the macro-algae Ascophyllum nodosum (sporangia

section of plant) the average total As concentration of 14.3 $\mu g\ g^{-1}$ exceeded the summed hydrochloric acid digest-reducible concentration (average of 6.2 μg As l^{-1}) and the summed nitric acid digest-reducible concentration (average of 9.7 μg As l^{-1}). A significant percentage of As present in the reproductive organs (sporangia) of Ascophyllum nodosum therefore appears to be in a non-reducible organic form, possibly as arsenosugars (Phillips and Depledge, 1985). Klumpp and Peterson (1979) noted that oxidising acids (nitric or perchloric acids) may decompose the complex organoarsenic compounds present in macro-algae to the simple methylated forms, namely DMA and MMA. Hydride generation determination of As in algae after nitric acid digestion would therefore be likely to overestimate the simple methylated As content of algae, the extent being dependent on the degree of organic decomposition of the sample. Since data generated from nitric acid digests of macro-algal specimens is considered contentious all further studies employed hydrochloric acid digestion procedures.

In a further detailed survey macro-algal specimens were collected from previously selected sediment and porewater sites (Figure 2.3) located along the length of the Tamar Estuary. The As content and methylated forms observed of the major algal species found at each site is reported in Table 4.2. Samples were collected in March 1986 and in general the DMA content of St. Johns' Lake macro-algal samples is 4-5 fold greater than that of identical specimens collected in December 1985. The increased DMA content appears to reflect the increase in biological activity that normally occurs during the months of March and April. The exact seasonal cycling of macro-algae varies from estuary to estuary and species to species and little data is generally available on the life cycle of algae in the Tamar Estuary. Klumpp (1980) has studied

Table 4.2 Concentration and speciation of arsenic in macro-algae specimens collected from the Tamar Estuary (March 1986)

Site	Distance from Calstock (km)	Specimen	Plant section	Arsenic concentration (A) ($\mu g\ g^{-1}$) inorganic As:	MMA:	DMA
Halton Quay	7.0	*Fucus vesiculosus*	Stem	0.45 (0.37-0.53)	0.15 (0.10-0.20)	14.20 (12.0-15.9)
			Sporangia	0.85 (0.70-1.04)	0.44 (0.31-0.61)	33.80 (30.3-38.3)
Cargreen	13.5	*Ascophyllum nodosum*	Stem	0.97 (0.71-1.15)	<0.02 (<0.02)	14.60 (12.1-17.2)
			Sporangia	0.46 (0.35-0.53)	0.19 (0.15-0.22)	29.80 (27.7-31.4)
		Fucus serratus	Whole plant	0.94 (0.76-1.09)	0.07 (0.06-0.08)	26.90 (23.8-29.8)
Riverside	18.5	*Ascophyllum nodosum*	Stem	0.06 (0.04-0.07)	0.09 (0.07-0.11)	6.10 (4.2- 8.2)
			Sporangia	0.08 (0.06-0.09)	0.15 (0.12-0.19)	22.70 (19.6-26.3)
St. Johns' Lake	22.5	*Ascophyllum nodosum*	Stem	1.42 (1.28-1.59)	0.13 (0.10-0.19)	13.57 (11.91-16.41)
			Sporangia	0.71 (0.59-0.91)	0.32 (0.26-0.39)	28.40 (26.3-30.6)

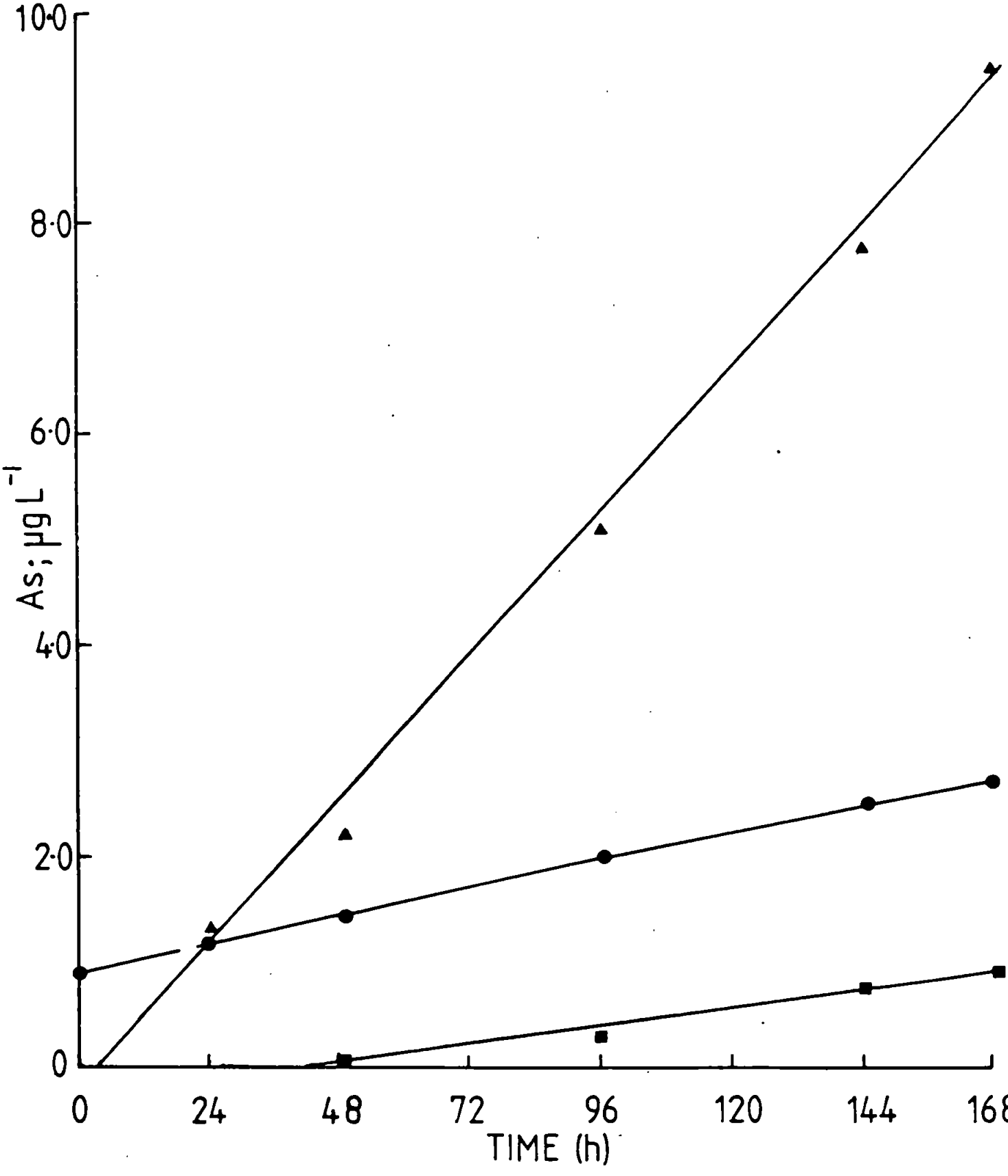
(A) Hydrochloric acid digestion, 70°C for 24 h ($\mu g\ g^{-1}$, dry wt.). Data quoted are the mean on 3 replicate analyses, figures in parentheses refer to the range in sample concentration. Inorganic As refers to total As(III) + As(V), MMA = monomethylarsenic species and DMA = dimethylarsenic species.

the uptake of inorganic As(V) by the species F. spiralis and reported that accumulation of As(V) increased by a factor of 2 when algae were incubated in waters at 30°C compared to 16°C. Given that the methylation of inorganic As is a known detoxification process for lower organisms it would be expected that the greater uptake of toxic inorganic As(V) with increased temperature would result in higher algal concentrations of the relatively non-toxic DMA species. The species Ascophyllum nodosum was observed at all sites, however, the species grew most prolifically at sites in the mid and lower reaches of the Tamar Estuary. The concentration and speciation of As in macro-algae collected at sites in the upper reaches of the Tamar were not significantly different from samples collected in the lower reaches. The variation in the concentration of 3 replicate analyses was greater than any discernable trends observed at individual sites. As with previous samples the reproductive organs (sporangia) of macro-algae specimens contained noticeably higher concentrations of DMA compared with the main stem of the plant. Although reported in the literature this phenomenon has not been explained. It is possible that toxic inorganic As is taken up to a greater extent in the sporangia, a young rapid growing part of the plant, thus detoxification via methylation of inorganic As would be more noticeable in this section of the plant.

In further studies the release of dissolved As species by the macro-algae Ascophyllum nodosum was examined under conditions which mimic those found in the estuarine environment. Macro-algal release rates were studied in 2 different regimes (i) summer simulation, aquarium water (seawater) temperature = 15°C, and (ii) winter simulation, aquarium water (seawater) temperature = 5°C (refer to Section 2.4.3.2.3 for fuller details). The results of the release experiments are shown in

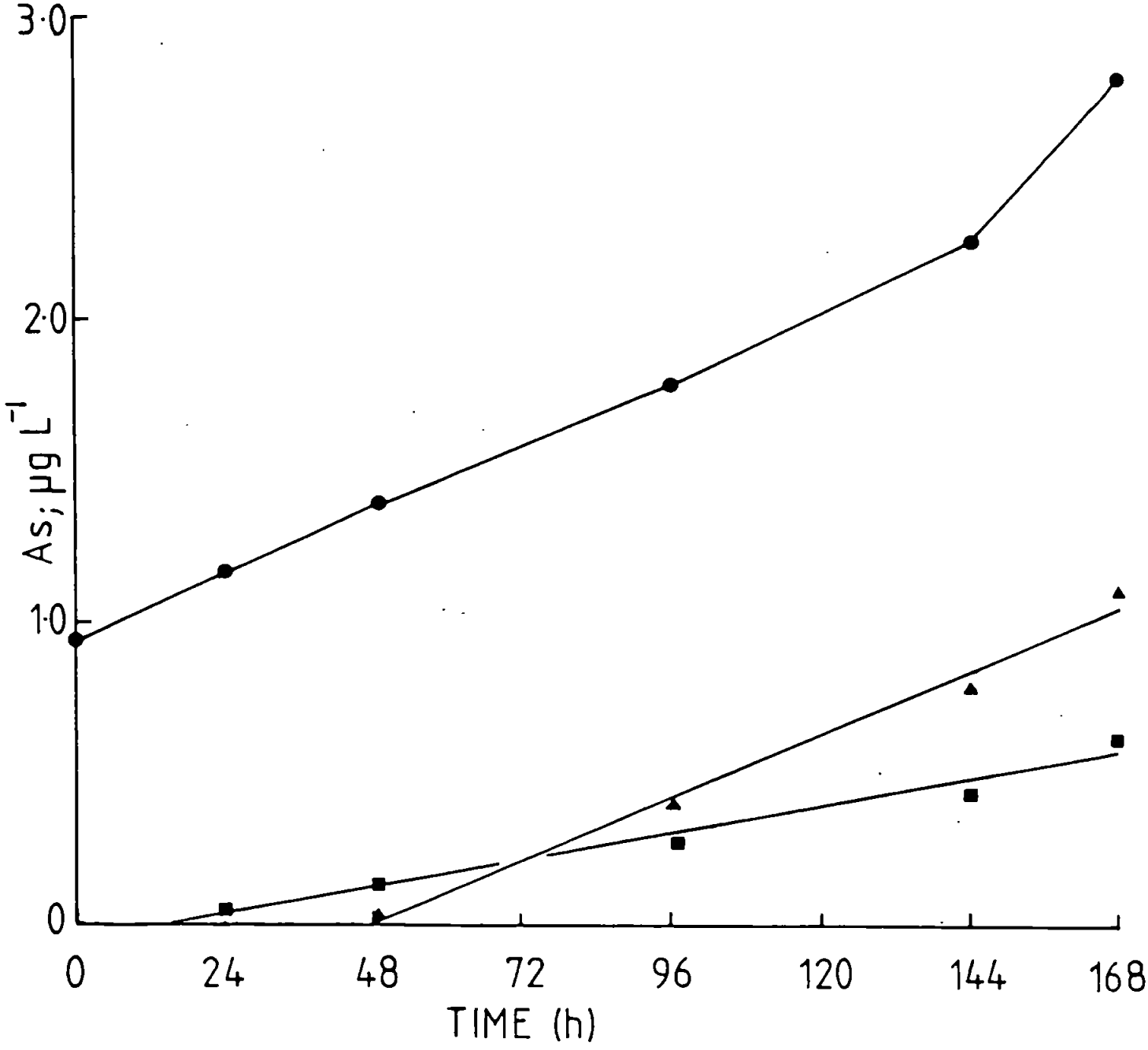
Figures 4.13 and 4.14 which show the release of As species by Ascophyllum nodosum at 15°C and 5°C respectively. The release of methylated As species into natural waters by algae may occur as a result of bacterial oxidation of arsenosugars present in outer cellular membranes (Bensen and Nissen, 1982). In this study the release of inorganic As did not increase with increasing temperature, however, the release of methylated species especially DMA was found to be a temperature dependent process. The higher concentration of DMA released into the water column during simulated summer compared to winter simulated conditions probably reflects the presence of greater microbial activity with subsequent greater bacterial oxidation of algae cells. The results therefore support the hypothesis that microorganisms can release DMA from macro-algal cells into surrounding waters.

Release rates for MMA and DMA were calculated from the slope of the profiles reported in Figures 4.13 and 4.14. It should be noted that during the 7 day period of study the concentration of As species did not reach steady state conditions. During simulated summer conditions release rates of 0.37 μg As kg^{-1} h^{-1} for MMA and 3.17 μg As kg^{-1} h^{-1} for DMA were calculated (based on kg wet wt. of algae). Under simulated winter conditions release rates of 0.20 μg As kg^{-1} h^{-1} for MMA and 0.48 μg As kg^{-1} h^{-1} for DMA were calculated. Data for release rates of methylated As species by estuarine fauna are not readily available for comparison. However, uptake rates of As(V) have been calculated for the macro-algae Ascophyllum nodosum. Klumpp (1980) reported steady state data which yielded uptake rates of 2.10 μg As kg^{-1} h^{-1}, under simulated summer conditions (water temperature 13°C, 12 h photoperiod). The inorganic As(V) uptake rate of 2.10 μg As kg^{-1} h^{-1} is similar to the release rate for DMA of 3.17 μg As kg^{-1} h^{-1} during summer months reported in this thesis work. The uptake and release data described above suggests that under steady state conditions once taken up by algae



Key (●) inorganic As
(■) MMA species
(▲) DMA species

Figure 4.13 Release of As species from Ascophyllum nodosum at 15°C



Key (●) inorganic As
(■) MMA species
(▲) DMA species

Figure 4.14 Release of As species from Ascophyllum nodosum at 5°C

inorganic As is rapidly methylated and released into the water column during summer months. The presence of large macro-algae colonies found in the Tamar Estuary may therefore significantly influence the cycling of As in the Tamar. This situation applies presumably equally to the Beaulieu and Carnon estuarine systems.

4.1.2.2 Laboratory Studies on the Diatom Skeletonema costatum

In further studies on the source of methylated As species in estuarine environments the diatom Skeletonema costatum was studied. Details of modelling experiments are given in Section 2.4.3.2.2 and the results are reported in Figures 4.15 and 4.16 which show the variation in dissolved As species and reactive phosphate, chlorophyll A and cell counts with time. The growth of Skeletonema costatum under simulated summer conditions in seawater media enriched with sodium arsenate (10 $\mu g\ l^{-1}$) resulted in a decrease of total cellular As concentrations (Day 0 cellular As concentration = 1.80 $\pm$ 0.1 $\mu g\ g^{-1}$, Day 10 cellular As concentration = 1.50 $\pm$ 0.1 $\mu g\ g^{-1}$, on duplicate analyses). In the culture medium a decrease in the total dissolved inorganic As (10.40-9.90 $\mu g\ l^{-1}$) and an increase in DMA concentration (<0.02-0.47 μg As l^{-1}) took place over the period of study. The maximum DMA concentration measured represented 4.5% of the total dissolved As concentration measured on the 7th day of the experiment. Within the culture flask the total dissolved As concentration (DMA + inorganic As) remained relatively constant between 10.30 to 10.56 $\mu g\ l^{-1}$. The abiotic control experiment (flask containing no Skeletonema costatum cells, autoclaved 10 l of distilled water spiked with sodium arsenate 10 μg As l^{-1}) showed negligible variations in As content (9.9-10.3 $\mu g\ l^{-1}$) when run concurrently with the biotic experiment and DMA was below the detection limit. The initial As concentration of the seawater media was 1.3 $\mu g\ l^{-1}$ inorganic As (DMA and MMA species were below the detection limit, 0.02 μg As l^{-1}).

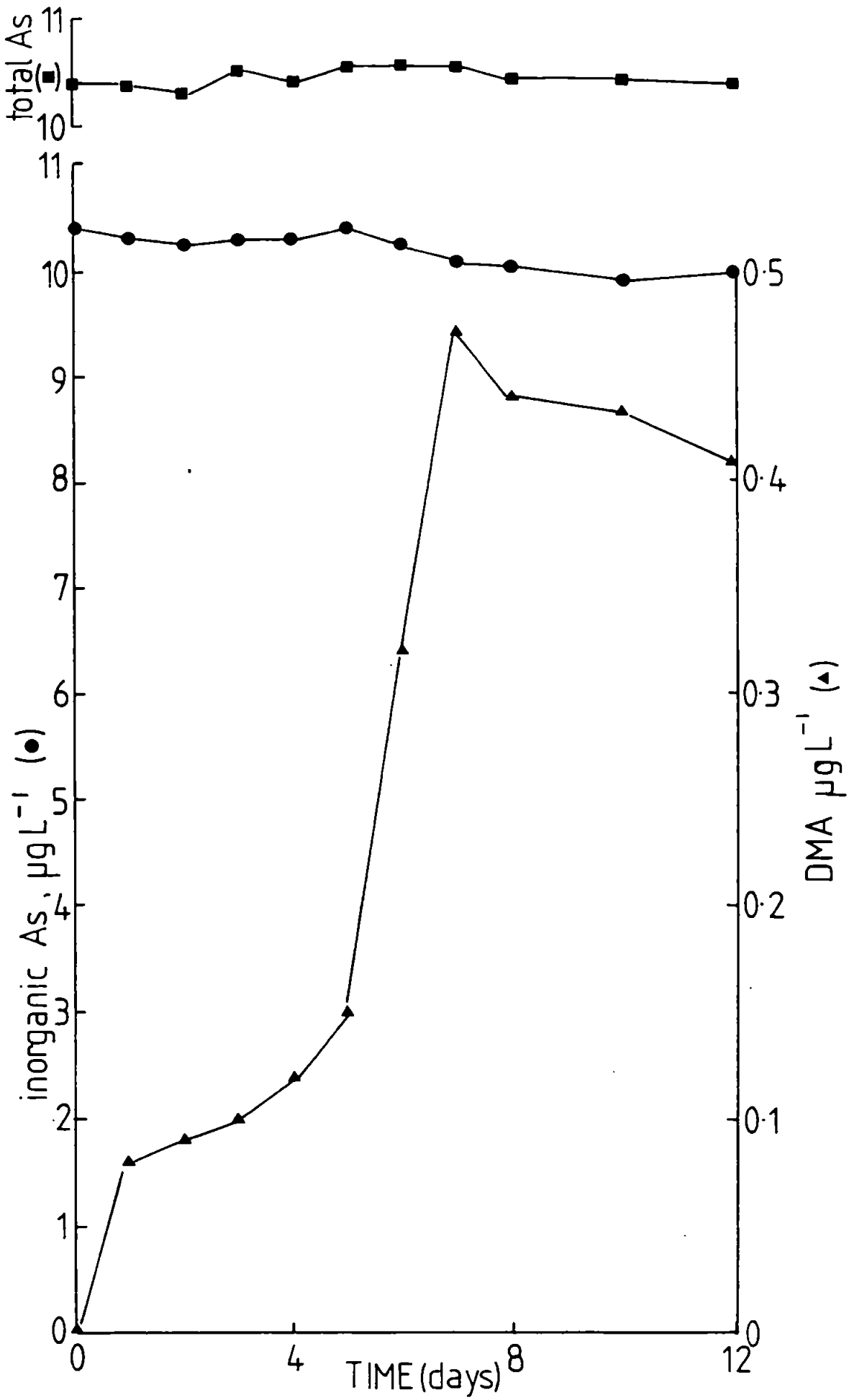


Figure 4.15 Variation in dissolved As speciation with time in a Skeletonema costatum culture

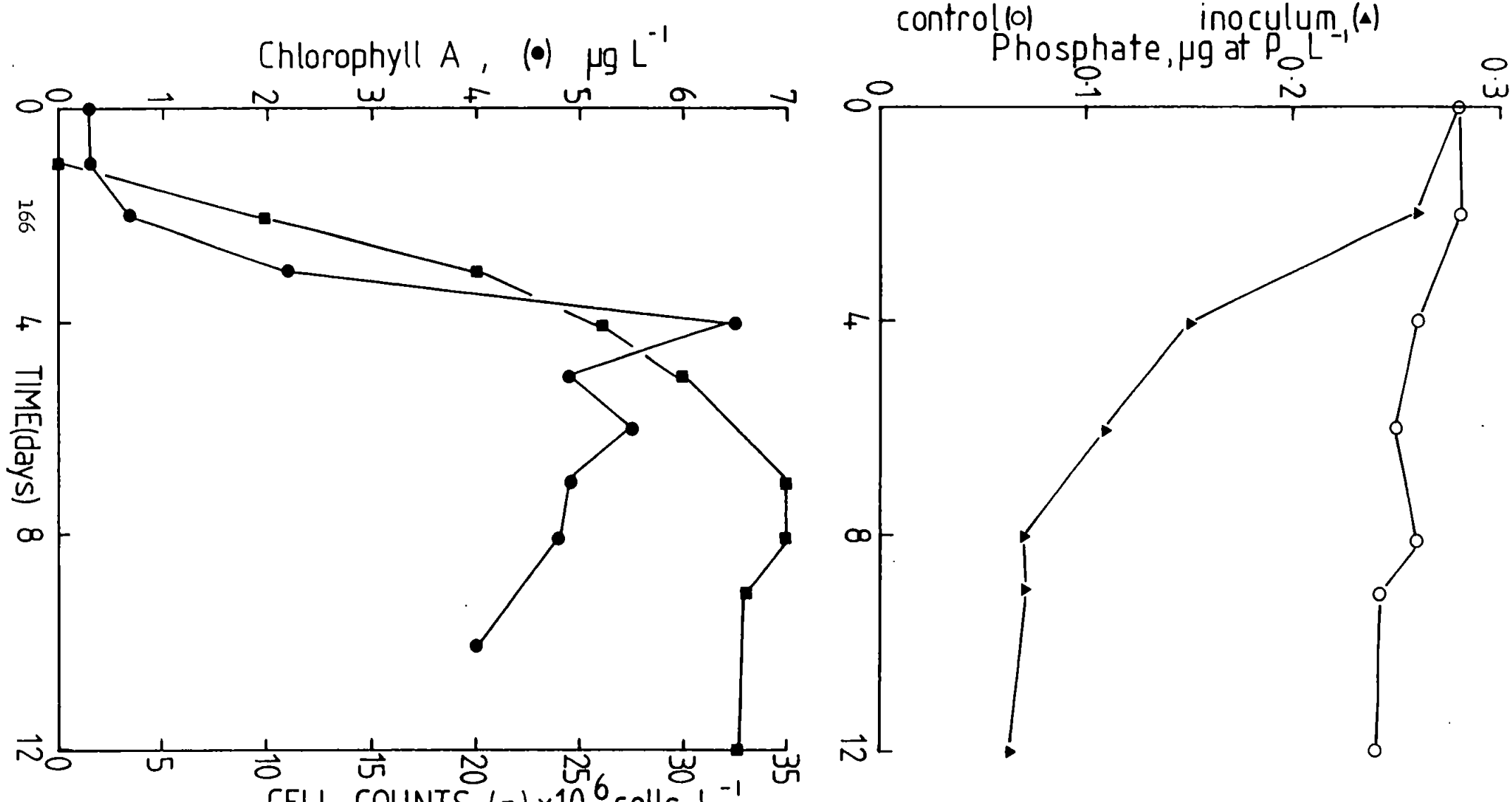


Figure 4.16 Variations in reactive phosphate, chlorophyll A and cell counts with time in a Skeletonema costatum culture

During the period of study MMA concentrations were always below the detection limit.

In a similar study on Skeletonema costatum Sanders and Windom (1980) observed an increase in total cellular As concentration of 22-29 $\mu g\ l^{-1}$ in response to As(V) additions of 6-25 $\mu g\ l^{-1}$ for a similar initial cell density of 1×10^6 cells l^{-1}. In the same studies Sanders and Windom (1980) noted that in a 5 μg As l^{-1} seawater solution 20% of the total As was methylated to DMA by Skeletonema costatum cells over a period of 11 days. In this present study the decrease in cellular As concentration was not reflected in an increase in dissolved As as evident from the relatively constant concentration of total dissolved As (Figure 4.15). This apparent anomalous behaviour of cellular As may be attributed to the difficulties in sampling such small masses of Skeletonema costatum cells (10^6 cells weigh approximately 1 mg, dry wt.). It is interesting to note that MMA concentrations were below the detection limit which compares with much lower MMA than DMA concentrations found in macro-algae. Apparently inorganic As is rapidly methylated to DMA by several phytoplanktonic species. Sanders and Windom (1980) did not detect any measureable concentrations of trimethylarsenic (TMA) in headspace gas of Skeletonema costatum culture flasks. In the present study the release of DMA into culture media coincided closely with cell growth and dissolved reactive phosphate (Figure 4.16). Release of DMA was observed throughout the logarithmic cell growth phase (1-7 days) and reached a steady state concentration during the stationary cell growth phase (7-12 days). The maximum chlorophyll A concentration was observed on day 4 with concentrations declining thereafter, suggesting release of chlorophyll A into the water column from dead cells. Disruption of Skeletonema costatum cells would result in a further rapid release of DMA to the seawater media. The profiles obtained from the experiments

undertaken in the present study may be used to crudely estimate the release of DMA into estuarine waters by Skeletonema costatum. From Figure 4.15 maximum DMA production of 0.47 μg As l^{-1} occurred after 7 days (168 h) when 35 x 10^6 cell l^{-1} were present. For a seawater media volume of 10 l this represents an average rate of production of 0.028 μg As h^{-1}. At the stationary phase the total mass of cells was calculated to be 130 mg hence a calculated release rate of 0.22 μg g^{-1} h^{-1} or 220 μg As kg^{-1} h^{-1} may be estimated. In interpreting the release rates for Skeletonema costatum it should be noted that the laboratory experiments employed a pure culture which was free from bacteria. In the environment the bacteria present would decompose cells allowing a faster release of methylated As species.

Recently Apte (1985) reported data for in situ studies on the release of As species in the waters of Loch Ewe (N.W. Scotland) during a diatom spring bloom in April 1983. The major diatom species present was Skeletonema costatum although Nitzschia delicatissima and Thalassiosira were also present. The release of DMA in a small experimental mesocosm (5 m diameter x 17 m depth) was restricted to waters below 3 m evidently poor circulation of water took place within the experimental tank. At depths approaching 17 m elevated levels of DMA were observed with a maximum DMA concentration of 0.9 μg As l^{-1} compared with a total As concentration of 1.4 μg l^{-1} occurring over an 18 day study period. The work of Apte (1985) suggests methylated As species may be released from phytoplankton as they die and sink through the water column. Little is understood of the phytoplankton sink-decay process in relation to the release of As species (Howard et al., 1982). However, the work described by Apte (1985) confirms that phytoplankton may release methylated As species to bottom sediments. Clearly this is an area for further investigation.

From work done in the present study a comparison of estimated summer release rates of DMA by macro-algae (maximum release rate 3.17 μg As kg^{-1} h^{-1}) and Skeletonema costatum (estimated release rate of 220 μg As kg^{-1} h^{-1}) suggests that on a mass to mass basis diatoms may play a greater role in the methylation of As than macro-algae. The actual mass of macro-algae and diatoms in estuarine environments changes over a seasonal cycle and little data is readily available for their total masses. As a result it is difficult to compare the absolute concentrations of methylated As species that macro-algae and diatoms contribute to the water column.

4.1.2.3 Porewater Source of Methylated Arsenic Species

In a study of the porewaters of the Tamar Estuary sediments and porewaters were collected from the same sites reported previously for the sampling of macro-algae (Figure 2.3). The results of the concentration and speciation of sediments and porewaters are reported in Table 4.3 and Figures 4.2-4.8, respectively. Due to logistical constraints on time and physical handling of sampling equipment porewater samples were not collected every month over the seasonal cycle.

From Table 4.3 sediment samples contained elevated concentrations of total As (average 35-78 μg As g^{-1}) compared with both water column and porewater samples. This proves the hypothesis that dissolved As is removed from solution on particulate phases. In general the total As concentration of sediment samples decreases with increasing distance from Calstock, reflecting the source input of As from mine adits and spoil heaps. The acetic acid leachable As concentration of sediments generally decreases, albeit in an irregular manner, upon moving from freshwater to marine sites. An acetic acid leachate represents that

Table 4.3 Total arsenic and acetic acid leachable arsenic in Tamar sediments

Site	Distance from Calstock (km)	Arsenic concentration Ⓐ (μg g^{-1}, dry wt.) Total As	Leachable
Calstock	0	77.9 (74.2-81.6)	8.8 (8.5-9.1)
Halton Quay	7	63.7 (61.4-65.9)	6.8 (6.6-6.9)
Cargreen	13.5	43.7 (42.9-44.5)	8.95 (8.4-9.5)
Riverside	18.5	41.9 (41.6-42.3)	4.6 (4.2-5.2)
St. Johns' Lake	22.5	35.2 (32.9-37.4)	4.7 (4.3-5.1)

Ⓐ Refer to Section 2.3.2.2.1

Data quoted are averages of duplicate samples. Figures in parentheses refer to the range in concentration observed.

fraction of bound metal which is conceivably 'available' to organisms in sediments, such data is important in assessing the maximum amount of particulate As that might be methylated in the porewaters of sediments.

During the period August 1985-June 1986 detectable concentrations of dissolved inorganic and methylated As species were present in the porewaters of the Tamar Estuary. From the profiles reported in Figures 4.2-4.8 dissolved inorganic As concentrations ranged from 0.4-60 $\mu g\ l^{-1}$ over the seasonal cycle monitored. The above data compares with 3-50 μg As(V) l^{-1} and 0.3-25 μg As(III) l^{-1} reported for porewaters in the Tamar by Knox et al., (1984). Knox et al., (1984) employed an identical method for porewater collection to that employed in this study, namely dialysis membrane collection. During the present study strong seasonal changes in porewater inorganic As concentrations were observed such that in winter concentrations range from 10-60 $\mu g\ l^{-1}$ compared with 0.4-20 $\mu g\ l^{-1}$ for summer. The increased winter concentration of inorganic As probably reflects the greater freshwater As input occurring in winter months. The subsequent adsorption of dissolved As onto particulate phases and accumulation in sediments followed by dissolution by microbial action would account for the higher winter porewater concentrations observed.

The concentration of dissolved methylated As species in porewaters varied to a lesser extent than did the inorganic As species. During summer DMA concentrations of <0.02-0.7 μg As l^{-1} and MMA concentrations of <0.02-0.5 μg As l^{-1} were observed which compared with winter concentrations of DMA of 0.1-0.4 μg As l^{-1} and MMA concentrations of 0.1-0.7 μg As l^{-1}. In general higher concentrations of methylated species were observed at marine sites (<0.02-0.7 μg As l^{-1}) compared with freshwater sites (<0.02-0.25 μg As l^{-1}). Within this trend DMA

concentrations generally exceeded MMA concentrations at freshwater sites. At marine sites MMA concentrations exceeded DMA concentrations during the months March to April after which the concentrations became more equable. An increase in porewater DMA concentrations in summer months may reflect increased *in situ* biomethylation to DMA or an increased influx of phytoplanktonic debris to the porewaters. Phytoplankton are known to contain high levels of DMA (see this work Section 4.1.2.1 and 4.1.2.2) and once incorporated in sediment degradation by bacteria may release cellular DMA into sediment porewaters. In this study the bacteria *Genus bacillus* and *Pseudomonas sp.* were identified in Tamar sediments. Porewaters therefore appear to be another significant source for methylated As species. These species may be released into the overlying water column either by diffusion or through resuspension of sediments during estuarine mixing which is usually most pronounced on spring tides (Morris *et al.*, 1986).

The experiments reported in this thesis identified porewater concentrations of methylated As species, however, previous workers have been unable to detect such species in anoxic or oxic sediment basins in USA coastal environments (Andreae, 1979; Peterson and Carpenter, 1986). Andreae (1979) employed a high pressure squeezing device to abstract porewaters from sediment samples, whilst Peterson and Carpenter (1986) employed high speed centrifugation. It has been reported that high pressure squeezing (Murthy and Ferrel, 1972) and centrifugation (Edmunds and Bath, 1976) may cause significant changes in chemical composition of porewaters. Furthermore noticeable changes in chemical composition may occur if samples are not filtered *in situ*. In early experiments in this study the changes in speciation of inorganic As in centrifuged porewater samples was examined using a coupled HPLC - hydride generation FAAS system (Ward, 1982). In unfiltered porewater samples approximately

50% of arsenite present originally was oxidised to arsenate within 2h. In the present study such changes are thought not to occur since in situ speciation studies are made using dialysis membranes and also any changes due to atmospheric oxidation are minimised by the transportation of samples encased in a coating of their native anoxic sediment.

4.1.3 Simulation of Porewater Arsenic Behaviour

Porewater modelling studies were undertaken to complement the environmental studies and provide a more comprehensive data base for porewater As behaviour. The approaches employed are summarised in Section 2.4.3.2.1. The environmental studies on As (refer to Section 4.1.1 and 4.1.2) indicated that inorganic As was a likely precursor in the formation of methylated As species and that an exotic organometallic precursor, as was the case for the Pb methylation study (i.e. $(CH_3)_3PbOAc$), may not apply in this case. For this reason sediment porewater systems were spiked with inorganic As species, sodium arsenate, (As(V)) and arsenic trichloride, (As(III)) and the formation of methylated As species in the illuminated overlying waters measured along with reactive phosphate. The results of modelling studies are reported in Figures 4.17-4.26. In duplicate flasks variation in As species was $\pm$ 10-15%.

The dissolved inorganic As concentration of overlying waters was variable and was not time dependent. However the formation of MMA and DMA in such sediment systems was time dependent.

From the profiles reported in 4.17-4.20 variations in the concentrations of DMA and MMA in the freshwater (Calstock) regime can be seen for concentrations of added As spike and temperature. During simulation of winter conditions (temperature 5°C) growth of MMA and DMA in a natural system not spiked with As occurred after an initial delay period of

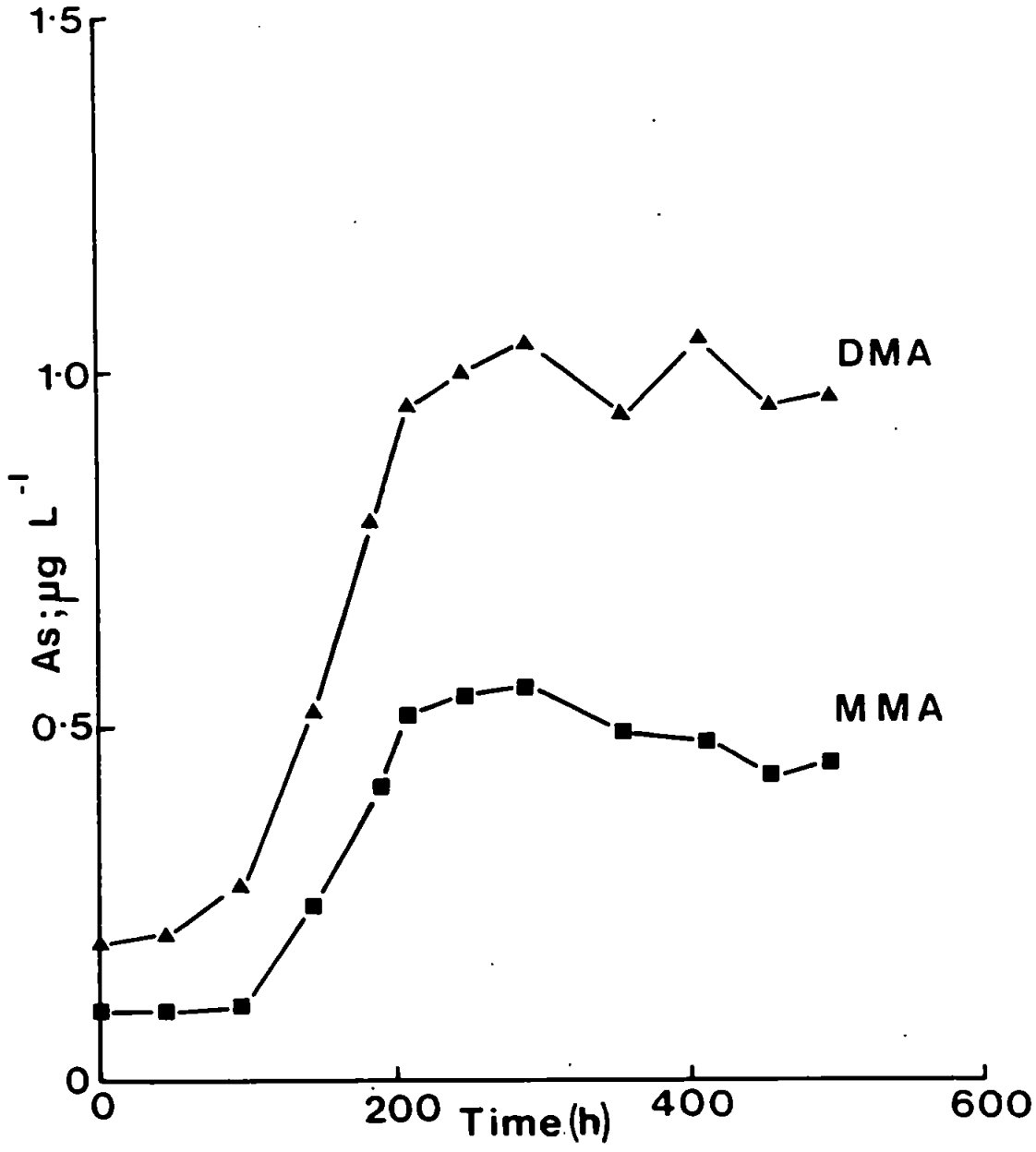


Figure 4.17 Variation in methylated arsenic species with time in a natural Calstock sediment system (5°C)

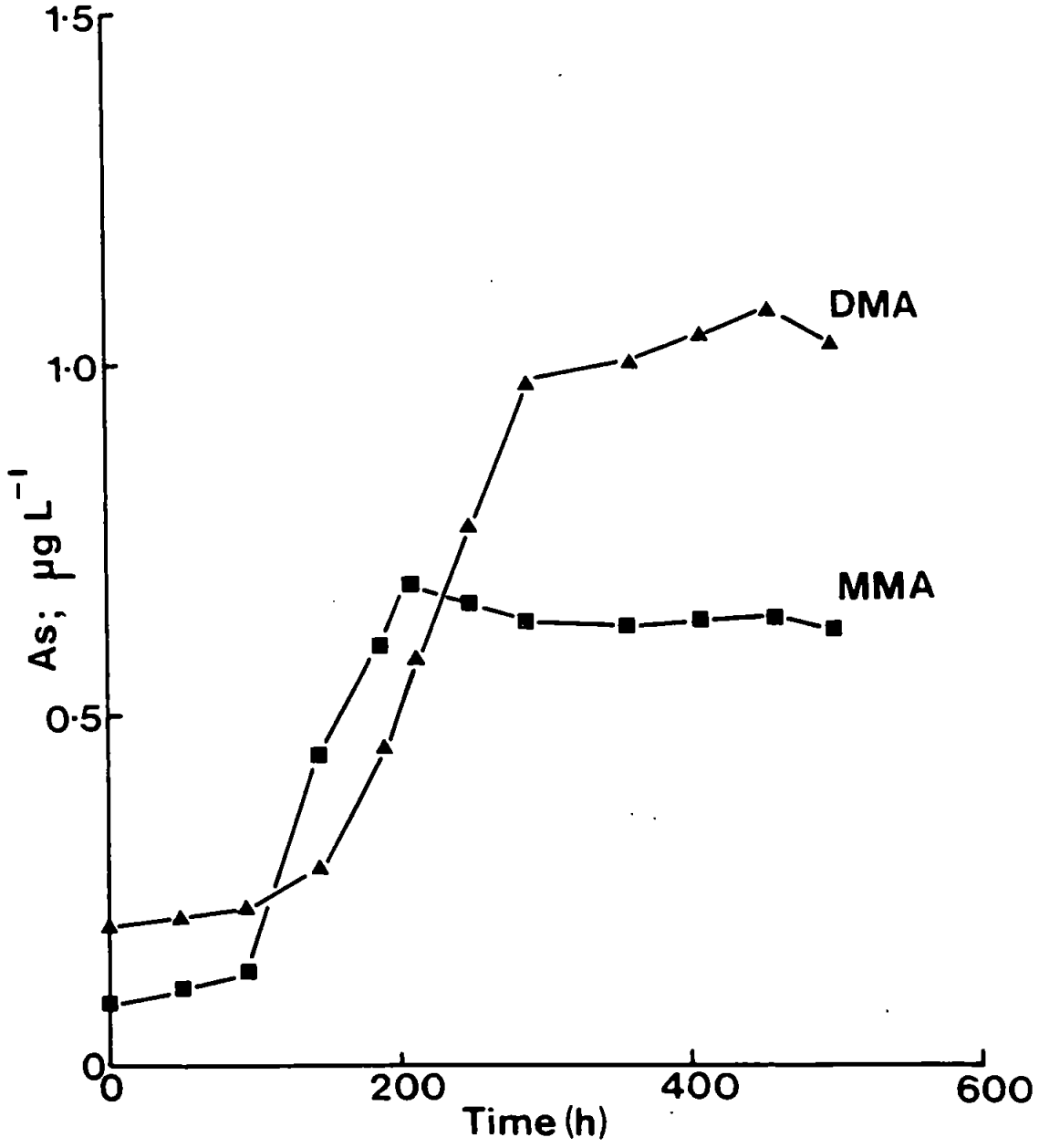


Figure 4.18 Variation in methylated arsenic species with time in a spiked (As(V) spike of 25 µg l^{-1}) Calstock sediment system (5°C)

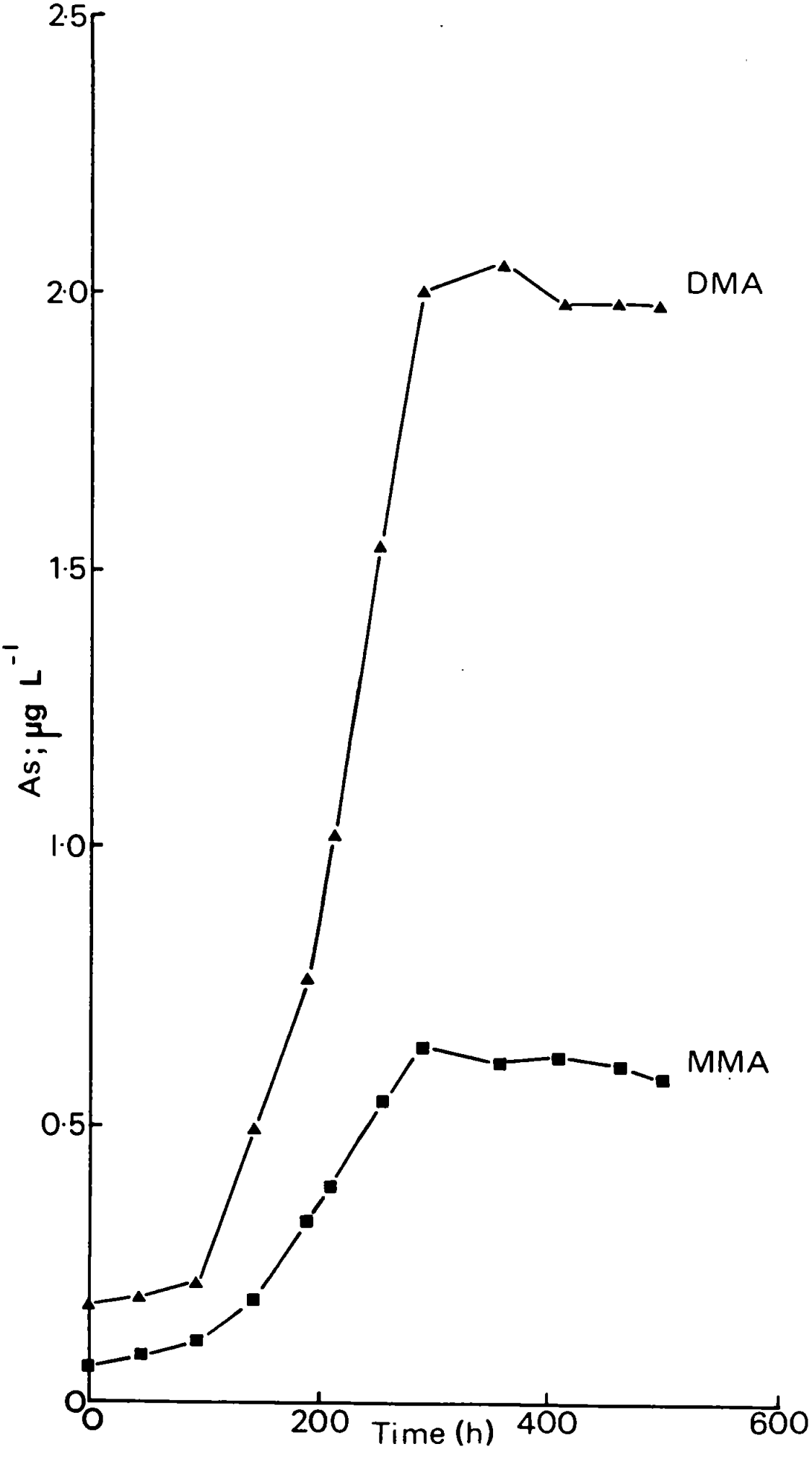


Figure 4.19 Variation in methylated arsenic species with time in a natural Calstock sediment regime (15°C)

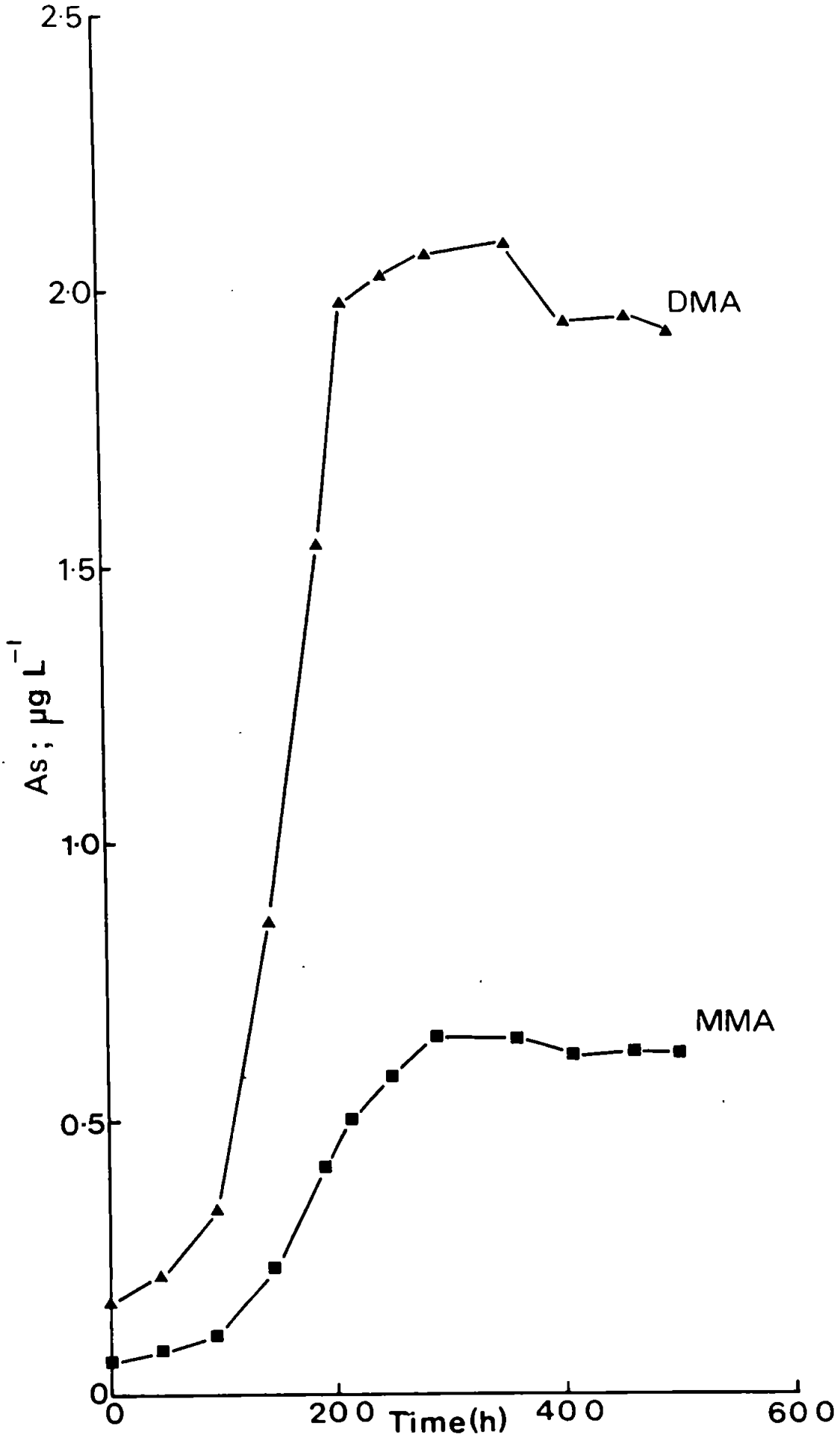


Figure 4.20 Variation in methylated arsenic species with time in a spiked (As(III) spike of 25 µg l^{-1}) Calstock sediment regime (15°C)

100 h (Figure 4.17), with maximum MMA and DMA concentrations of 0.5 and 1.0 μg As l^{-1} respectively. Both species reaching steady state concentrations after a period of 250 h. In a system spiked with 25 μg As(V) l^{-1} (Figure 4.18) a similar initial delay period (100 h) before DMA and MMA formation was observed with steady state maximum concentrations of MMA (0.7 μg As l^{-1}) and DMA (1.1 μg As l^{-1}) measured after 250-300 h respectively. In simulations of summer time conditions (temperature 15°C) an unspiked freshwater system (Figure 4.19) again showed growth of MMA and DMA only after an initial delay period of 100 h. Maximum steady state concentrations of MMA (0.6 μg As l^{-1}) and DMA (2.0 μg As l^{-1}) were reached after a period of 250 h growth. Similar maximum steady state concentrations of MMA and DMA were observed after only 200 h in a spiked (25 μg As(V) l^{-1}) sediment system (Figure 4.20).

In a marine system (St. Johns' Lake water) marked variations in DMA and MMA concentration occurred in summer compared with winter regimes. Under winter conditions in an unspiked sediment system no growth of DMA species above background concentrations was observed during a 1000 h study (Figure 4.21). However, after an initial delay period of 350 h MMA formation was observed and a maximum steady state concentration (1.0 μg As l^{-1}) was reached after 500 h. In a spiked system (25 μg As(V) l^{-1}) similar growth of DMA and MMA was observed with a MMA steady state concentration (1.0 μg As l^{-1}) measured after 500 h (Figure 4.22). Under summer conditions in an unspiked sediment system (Figure 4.23) growth of MMA occurred after an initial delay period of 100 h a maximum steady state concentration of MMA of 1.0 μg As l^{-1} being recorded, DMA formation occurring after 400 h with a maximum steady state concentration of 0.5 μg As l^{-1} after 550 h. Similar growth and steady state concentrations of MMA and DMA were observed in a spiked (25 μg As l^{-1}) marine system (Figure 4.24).

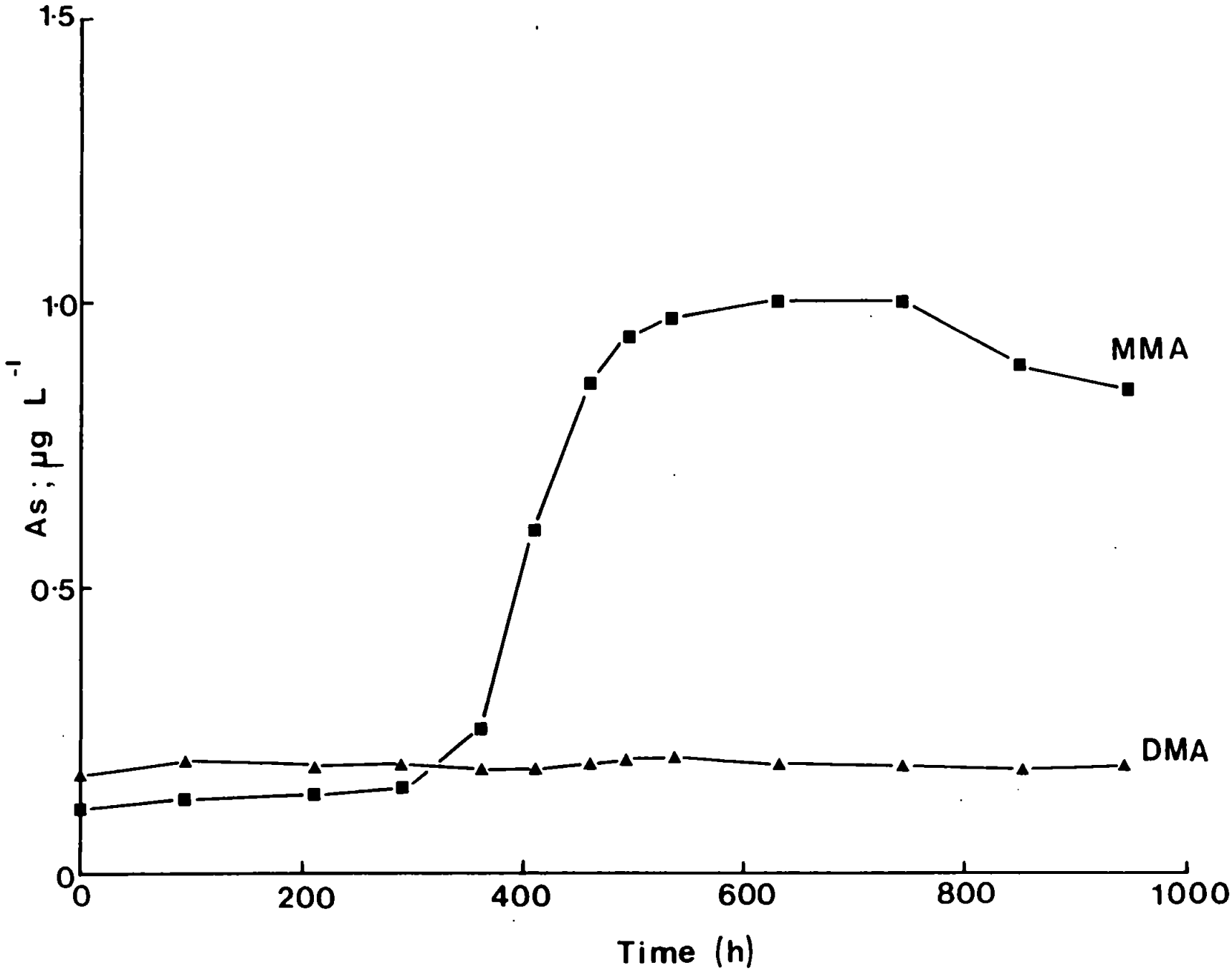


Figure 4.21 Variation in methylated arsenic species with time in a natural St. Johns' Lake sediment system (5°C)

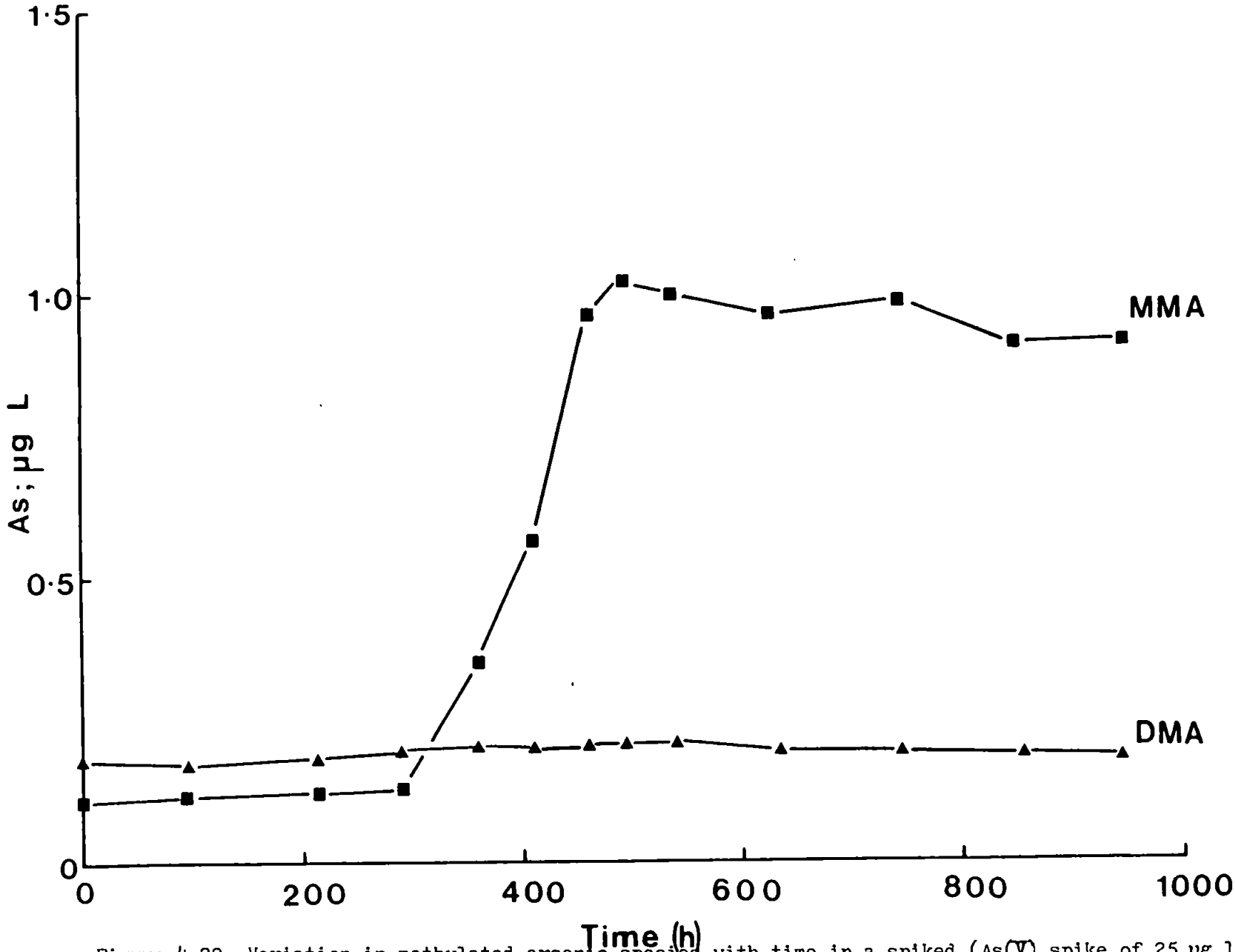


Figure 4.22 Variation in methylated arsenic species with time in a spiked (As(V) spike of 25 µg l^{-1}) St. Johns' Lake sediment system (5°C)

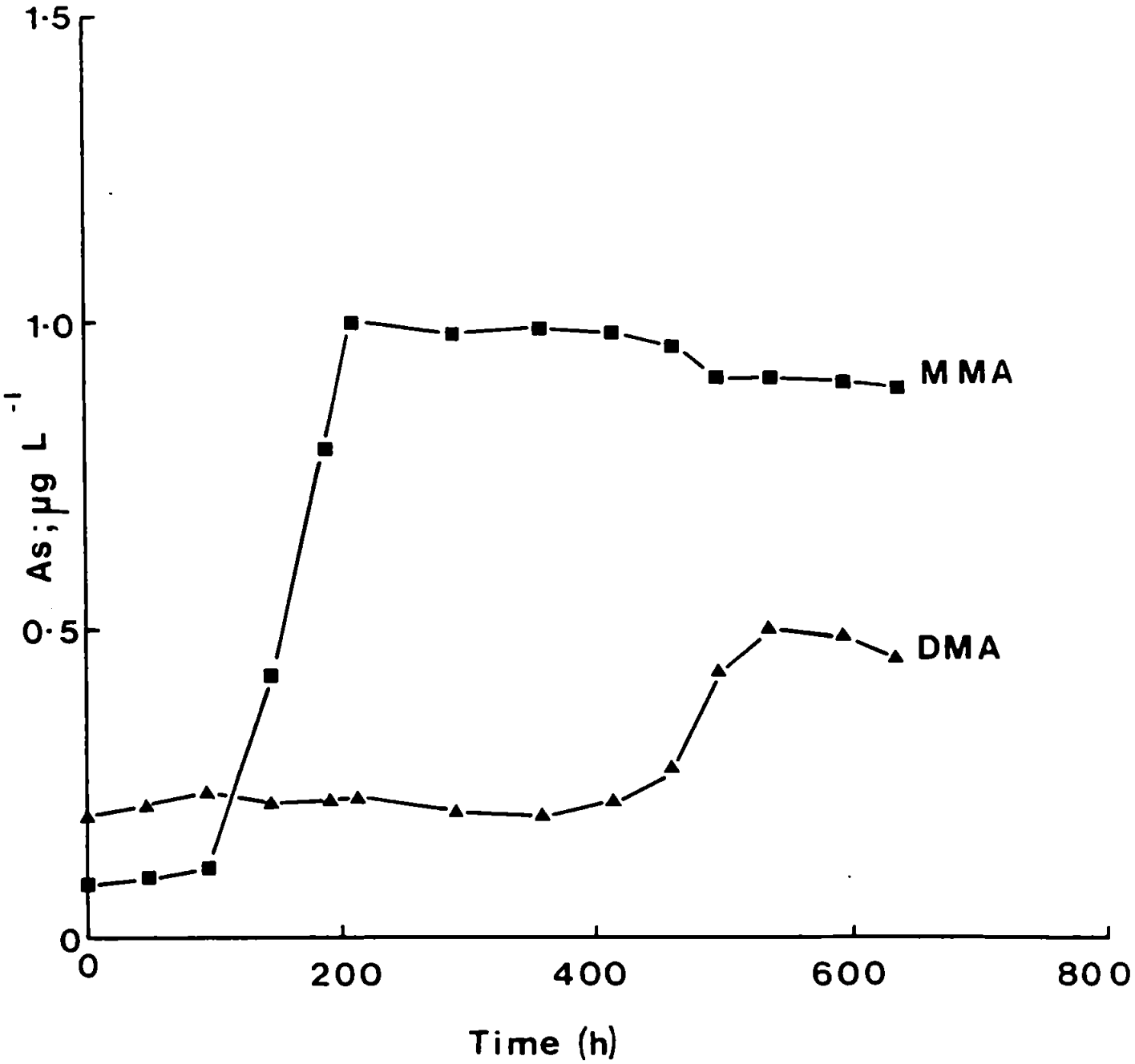


Figure 4.23 Variation in methylated arsenic species in a natural St. Johns' Lake sediment system (15°C)

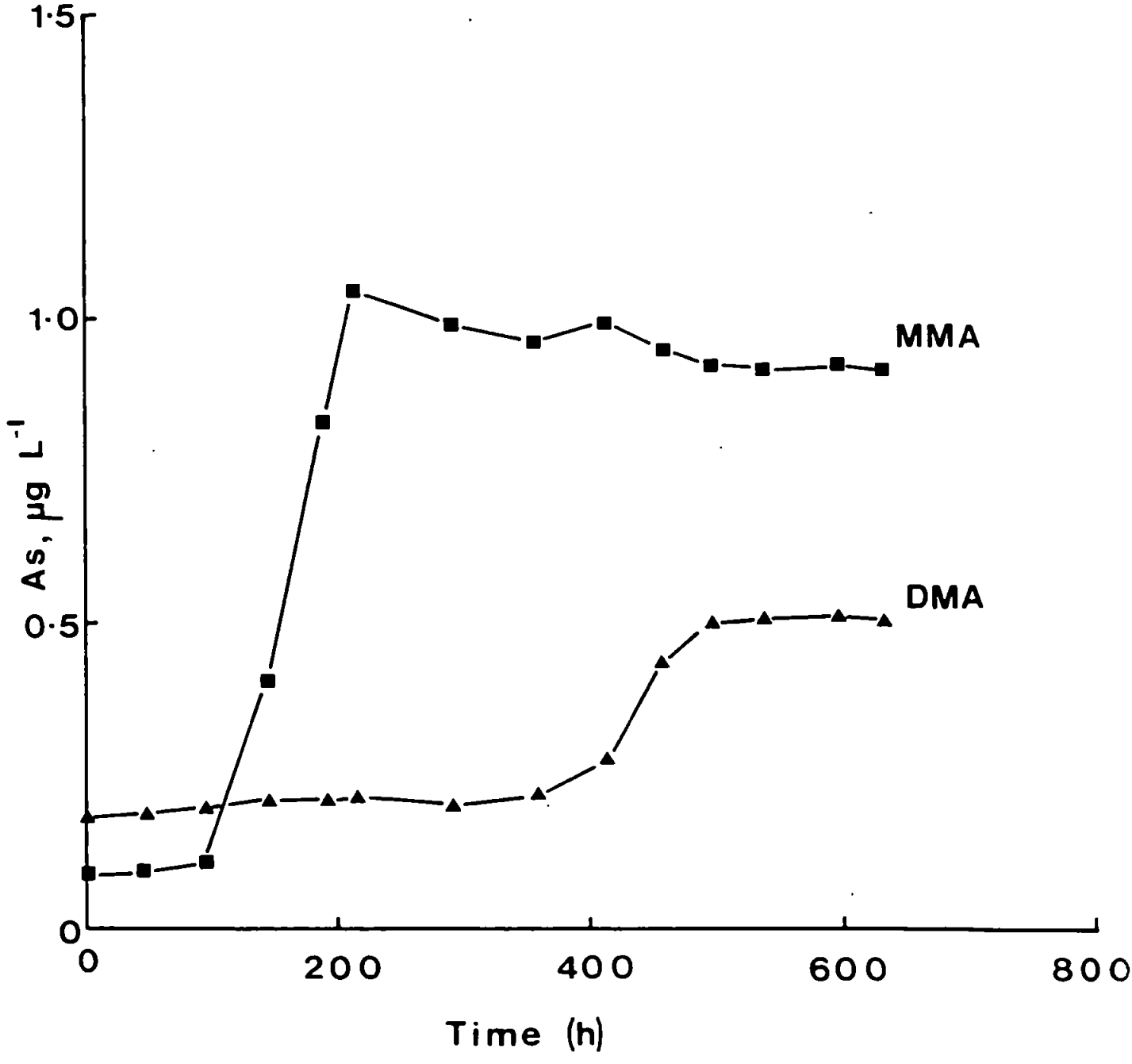


Figure 4.24 Variation in methylated arsenic species with time in a spiked (As(V) spike of 25 µg l^{-1}) St. Johns' Lake sediment system (15°C)

Several trends are apparent from the profiles reported in Figures 4.17-4.24. In most cases no significant increase in the concentration of methylated As species occurred when sediments were spiked with 25 μg As(V) l^{-1} compared to unspiked sediments. In the modelling of porewater - sediment biochemical processes high levels of 'available' As (refer to Section 2.3.2.2.1) were employed e.g. Calstock sediment 'available' As concentration 7.05 μg As g^{-1} and St. Johns' Lake sediment 4.45 μg As g^{-1}. Also high ambient porewater inorganic As concentrations (maximum 60 μg l^{-1}) have been reported for Tamar porewaters (see this work Section 4.1.2.3). The addition of 25 μg As(V) l^{-1} to such sediments would therefore not represent a significant input of available As as a result little perturbation in the natural sediment-porewater methylation process would occur, resulting in little or no increase in methylated products. It is pertinent here to mention that no significant growth of methylated species took place in an abiotic sediment system. An autoclaved sediment system (sediments amended with nutrients (0.1%) and inorganic As(V), 25 μg As l^{-1}) was observed to have an initial (Time = 0 h) MMA concentration of (0.06 μg As l^{-1}) and DMA concentration of (0.08 μg As l^{-1}) and a final (Time = 600 h) MMA concentration of (0.08 μg As l^{-1}) and DMA concentration of (0.11 μg As l^{-1}) Further, a noticeable increase in DMA concentrations in freshwater and marine sediment systems was observed in summer conditions compared with winter conditions. The normal increased biological activity associated with higher temperatures would give rise to increased biomethylation of As and thus probably explain the increased DMA concentrations observed. Another discernable trend observed in the profiles reported in Figures 4.17-4.24 was the relatively rapid production of methylated As species in freshwater systems compared with marine systems. In the unspiked freshwater sediment system (Figure 4.17) production of DMA and MMA reached a steady state in 250 h which compares with 500 h for MMA in the equivalent marine sediment system (Figure 4.21).

In the estuarine environment freshwater sediment production of methylated As species would have a greater production rate than marine sediment. The most marked difference between the 2 sediment regimes studied is that in freshwater regimes DMA production exceeds MMA production whilst the reverse is the case for the marine regime. This trend is apparent in winter and summer conditions. The prevalence of DMA or MMA in various sediment systems may be attributed to the presence of different microorganisms in each sediment. Wong et al., (1977) reported that the freshwater bacteria: Aeromonas sp. and Flavobacterium sp. produced DMA in preference to MMA. In the present study only Pseudomonas sp. and the marine bacterium Genus bacillus were positively identified. Data on the As methylation capability of these bacterial species is not presently available.

The concentration of reactive phosphate was studied in a 15°C marine sediment system amended with nutrients only (nutrient broth 0.1%, d-glucose 0.03%, yeast extract 0.03%) in order to assess if the quantity of nutrients added had markedly perturbed the natural sediment system. An initial concentration of 75 µg at P l^{-1} was measured in the sediment-porewater system studied (refer to Figure 4.25) which compares with 100-300 µg at P l^{-1} reported for Tamar porewaters (Watson et al., 1985), as such the sediment systems amended with nutrients appear to mimic the environment closely. The change in reactive phosphate with time is shown in Figure 4.25 and the system examined is comparable with the sediment system reported in Figure 4.23. From Figures 4.23 and 4.25 the initial decrease in phosphate was found to coincide with the initial formation of MMA (0-100 h). However, further rapid growth of MMA occurred during a plateau in the uptake of phosphate (100-200 h) and growth of DMA took place only after phosphate concentrations were reduced from 75 to 15 µg at P l^{-1}.

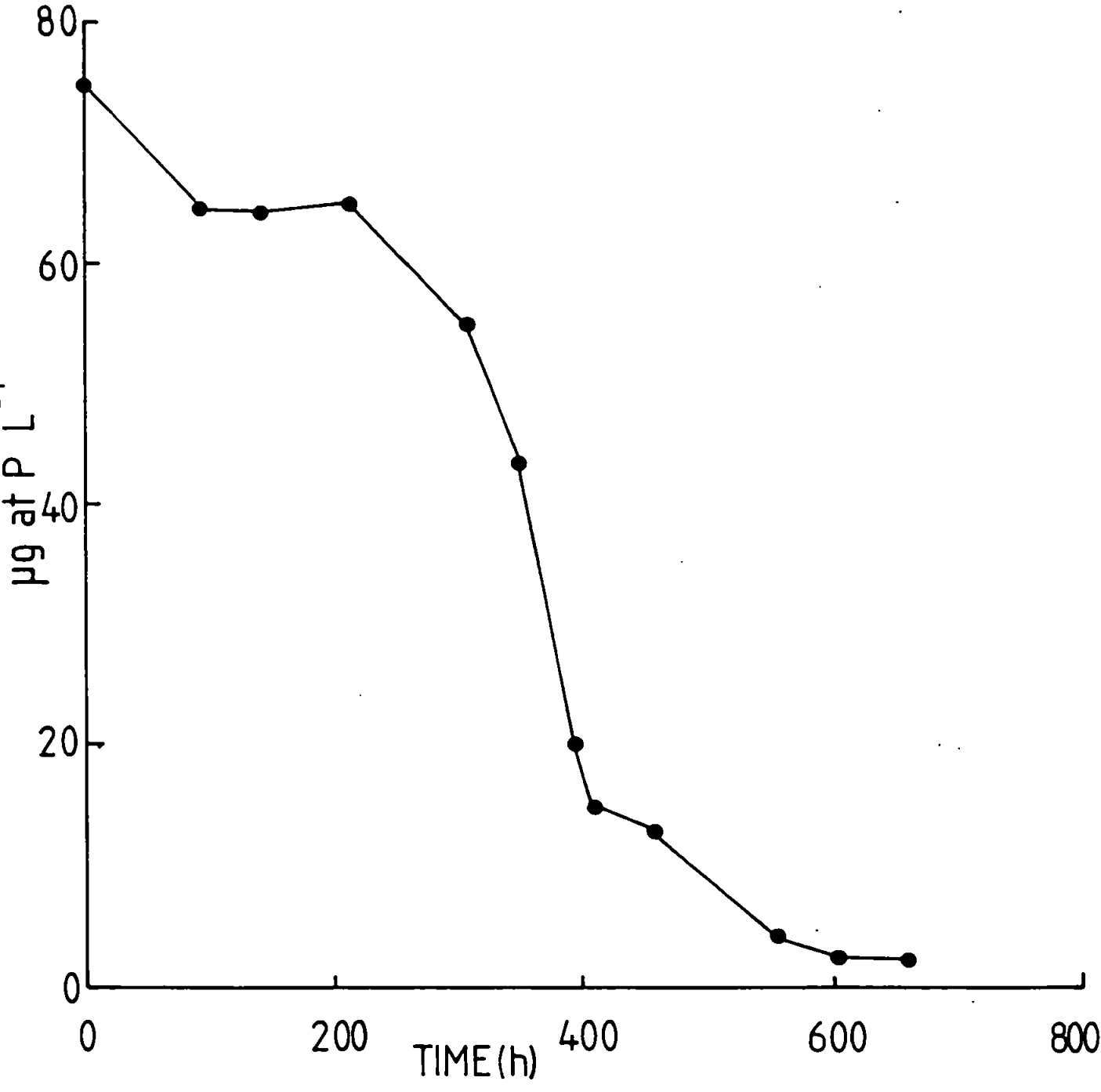


Figure 4.25 Variation in reactive phosphate with time in a St. Johns' Lake sediment system (15°C)

Microorganisms are thought to be unable to discriminate on uptake between arsenate and phosphate. At best discrimination between phosphate and arsenate is only by a factor of 10 fold for microorganisms (Apte, 1985). Since no loss of phosphate is observed in abiotic control studies (see Section 4.1.2.2, Figure 4.16) the coincidental decrease in dissolved phosphate concentration and increase in methylated As concentrations observed in this study is strong evidence for the hypothesis that microorganisms take up arsenate and phosphate simultaneously with poor discrimination. It is proposed that methylation of As by microorganisms occurs by the following steps, uptake of arsenate followed by reduction to arsenite and methylation to MMA and DMA, (Challenger 1945, refer to Figure 1.1). Substantial uptake of arsenate with subsequent growth in methylated As species would only occur when phosphate and arsenate concentrations approach equivalence. The period of delay ($\approx$100 h) in growth of methylated As species in sediment systems may represent the time required for arsenate and phosphate levels to approach equimolar concentrations. To examine the methylation process further a marine sediment system was spiked with As(III) (in the form of (arsenic trichloride)) and the formation of methylated As species observed over a period of 600 h (see Figure 4.26). If methylation of As occurs according to Challengers' pathway the addition of an As(III) species rather than an As(V) species might be expected to produce a different rate of production of methylated As species. The As(III) spiked sediment system (Figure 4.26) is comparable to the As(V) spiked system (Figure 4.24). In As(III) spiked sediment systems MMA formation occurred after an initial delay period of 100 h and a maximum steady state concentration of 0.9 μg As l^{-1} was reached after a period of 300 h compared with only 200 h for the comparable As(V) spiked system. Conversely DMA production in the As(III) spiked

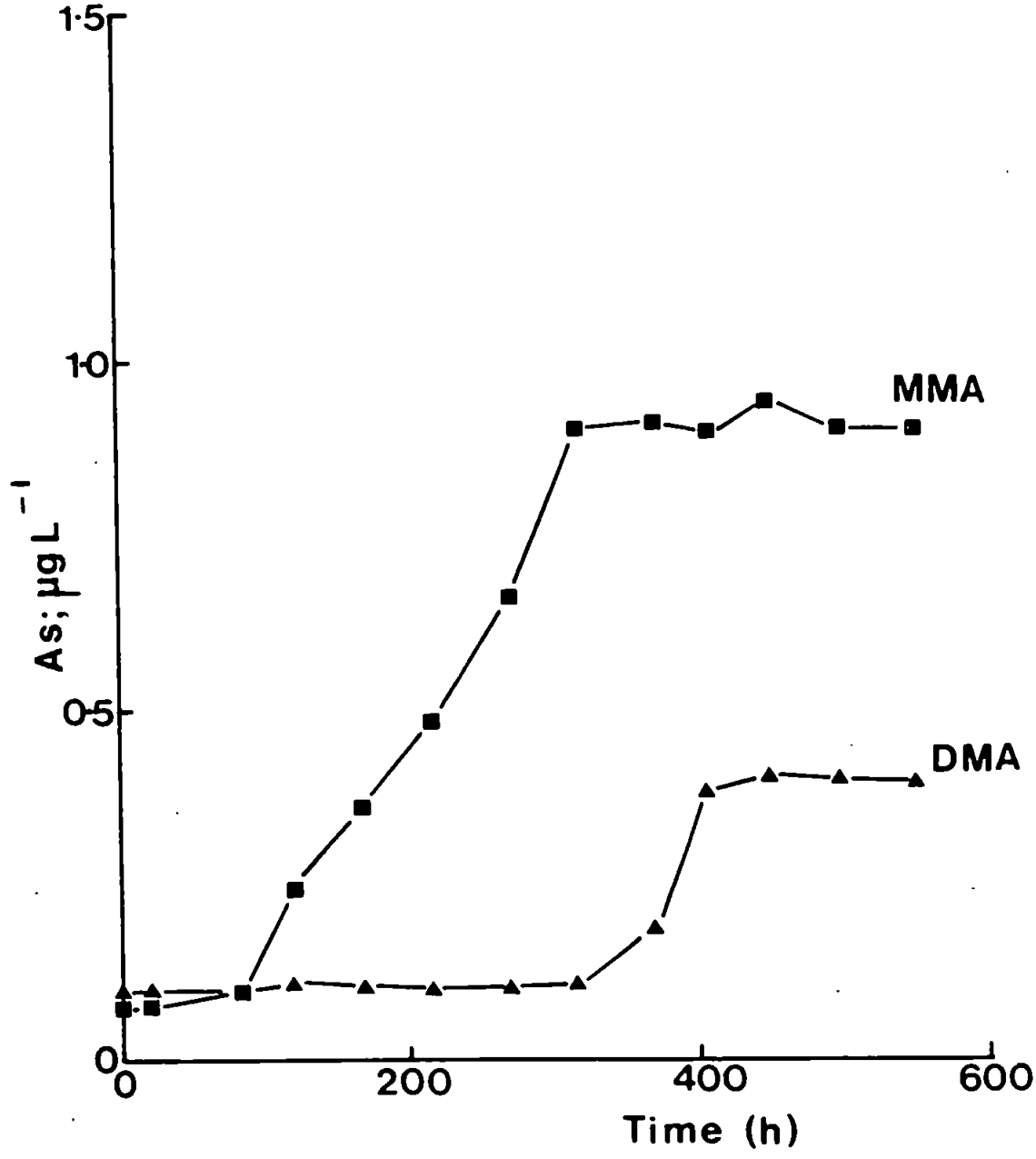


Figure 4.26 Variation in methylated arsenic species in a spiked (As(III) spike of 25 µg l^{-1}) St. Johns' Lake sediment system

sediment flask reached a maximum steady state concentration of 0.4 μg As l^{-1} after 450 h compared with 500 h for the equivalent As(V) spiked sediment system. If microorganisms take up As as arsenate prior to methylation then production of methylated As species would be expected to occur more rapidly in the presence of added As(V) than As(III) as was the case in Figure 4.24 compared with Figure 4.26. The slower production of MMA species in As(III) spiked sediment systems compared with As(V) spiked sediment systems may reflect the relatively slow chemical and microbial oxidation of arsenite to arsenate.

4.1.4 Proposed Estuarine Cycle for Arsenic

The behaviour of inorganic As has been observed to be largely independent of salinity, marked removal of As only occurring in freshwater areas. It has been suggested that the broad estuarine maxima observed in the Tamar Estuary is related to the injection of inorganic As from the mudflat porewaters most apparent in the lower reaches of the estuary. The dilution of seawater with freshwater accounts for the lower inorganic As concentrations observed at marine sites. The above phenomena are applicable to estuaries containing relatively high inorganic As loads. Where estuarine concentrations of inorganic As are lower than seawater concentrations (typically 1-1.5 μg As l^{-1}) e.g. Beaulieu Estuary, the flushing characteristic of the estuary dominates the cycling of As.

Many of the phenomena associated with the cycling of inorganic As are also observed for the biologically mediated cycling of methylated As species. From environmental studies 3 major sources of methylated As species were identified during the course of this present study, namely

1. Macro-algae
2. Marine diatoms
3. Porewaters

Consideration of the individual sources and their contribution during winter and summer months may be combined to form a tentative estuarine cycle for methylated As species.

During spring/summer months ($\geqslant 15^{o}C$) the phytoplankton activity in the photic zone of the water column increases due to the increase in light, temperature and available nutrients. Phosphate nutrients with chemically similar arsenate are taken up by phytoplankton in the water column. The As is rapidly methylated to DMA species. The phytoplankton are grazed upon by bacteria which through the use of oxidase enzymes release relatively large quantities of DMA to the water column. In the case of the marine diatom Skeletonema costatum an estimated release rate of 220 μg As kg^{-1} h^{-1} was observed in this present study. Similarly macro-algae Ascophyllum nodosum release preferentially DMA to the water column with release rate of 3.17 μg As kg^{-1} h^{-1} for DMA compared with 0.37 μg As kg^{-1} h^{-1} for MMA being observed. Although the rate of release of DMA from diatoms is much higher than for macro-algal specimens absolute concentrations of DMA released are related to the total mass of diatoms or macro-algae present in a given estuary. In the Tamar Estuary extensive colonies of macro-algae are observed and it is tempting to suggest that they are the dominant source of DMA species in summer. If phytoplankton are considered to be the dominant source of methylated As species in summer, as indicated by work in this thesis, the high levels of DMA compared with MMA observed during summer months can be accounted for. The generally higher concentrations of methylated As species in marine water compared with freshwaters is explained by the greater density of phytoplankton at marine rather than freshwater sites. It should be noted that the actual concentration of As species is also related to the advective transport in an estuary.

If the rate of phytoplankton release of MMA and DMA exceeds the current velocities of the estuary then high concentrations of such species will be maintained for the period of biological growth. Ultimately the methylated As species will be flushed from the estuary into the oceanic circulation, depending on the concentration of inorganic As that has been methylated to DMA or MMA species (in the case of the Beaulieu 50% of the total inorganic As concentration is in the form of methylated As species) this may result in a significant transportation of the element from the estuarine environment.

Towards the end of summer considerable phytoplankton debris may fall through the water column and be incorporated in sediments. This debris may undergo further bacterial oxidation in sediments resulting in the release of DMA and MMA species to porewaters. In winter months a transition in the water column takes place where the predominance of methylated As species occurs, and MMA concentrations exceed DMA concentrations. It is evident that the lower winter biological activity would not produce this observed phenomena. It is possible that bacterial demethylation of DMA species to MMA species may account for the observed behaviour, however, no drastic decrease in average summer DMA concentrations (0.4 μg As l^{-1}) compared with average winter DMA concentrations (0.35 μg As l^{-1}) occur to substantiate this hypothesis. A more tempting hypothesis would be to suggest that the porewater source of methylated As species dominates in winter months. From observations in this study porewaters contain large concentrations of methylated As species, which during the increased flow and mixing of estuarine waters in winter months may release periodic injections of As species into the overlying water column. The work presented here has shown that in marine porewaters during winter months MMA concentrations exceed those for DMA and that MMA is released preferentially to DMA

into the water column. The reverse was found for porewaters from freshwater sites. The large expanse of mudflats observed in the lower reaches of the Tamar Estuary (as is also the case for the Beaulieu and Carnon systems) indicates that the greatest contribution of methylated As species would be made by marine porewaters rather than freshwater porewaters. If this is the case the observed higher MMA than DMA water column concentrations in winter months may be accounted for. The following calculations were undertaken in order to provide some semi-quantitative estimate of the relative contribution of methylated As species from algal and porewater sources to the water column.

The porewater calculation was based on an estimated area of St. Johns' Lake of 2×10^6 m^2 (taken from an ordnance survey map) and that a sediment depth of 5 cm was involved in the porewater infusion mechanism (Ackroyd, 1983). This gave a total sediment volume of 10×10^4 m^3 and it is known from this study that 60% of this volume is occupied by porewater. From this a porewater volume of 6.0×10^7 l was calculated. The average depth in St. Johns' Lake was taken as 1 m resulting in a water volume of 2×10^9 l overlying the sediments during a tidal cycle. During summer surveys, typical MMA porewater concentrations of 0.4 μg As l^{-1} were observed, thus the porewaters could contain 30 g of As as MMA species. If all of this was injected into the water column during tidal mixing, the water column concentration of MMA species in St. Johns's Lake could be enhanced by 0.015 μg As l^{-1}. Similarly the concentration of DMA could be enhanced by 0.01 μg As l^{-1}. In winter surveys, typical MMA porewater concentrations of 0.7 μg As l^{-1} were observed which would result in an enhancement of 0.03 μg As l^{-1} to the water column in St. Johns' Lake. Similarly the concentration of DMA could be enhanced by 0.01 μg As l^{-1}.

In order to examine the contribution to the water column from algae

in St. Johns' Lake an algal density of 10 kg m^{-2} (10 kg algae wet wt. per m^2) was assumed. The aerial coverage of algal colonies was taken as 5×10^5 m^2 (i.e. one tenth of the total area of St. Johns' Lake) resulting in a total algal mass of 5.0×10^6 kg (wet wt.). Ascophyllum nodosum was assumed to be the major species of algae present. In summer conditions Ascophyllum nodosum release rates of DMA of 3 µg As kg^{-1} h^{-1} were observed in the present study. This would give rise to a DMA production of 15 g As h^{-1}. Assuming algae were covered by water for 10 h over a tidal cycle the algae would release 150 g of As as DMA. Assuming full mixing of the water column and that the water column has no background DMA concentration algae release could result in a DMA water column concentration of 0.10 µg As l^{-1} in summer. In winter release rates for DMA were shown to be approximately a factor of 10 lower than summer release rates. Hence in winter (assuming the same summer aerial coverage of algae) algal release could give rise to a DMA water column concentration of 0.01 µg As l^{-1}.

In summary, during the summer preferential release of DMA species by algae may account for a DMA water column concentration of 0.1 µg As l^{-1}, whilst the porewater infusion mechanism provides significantly less at a concentration of 0.01 µg As l^{-1}. This emphasizes the dominance of the macro-algal source over the porewater source in summer. In summer the water column concentration of DMA in St. Johns' Lake ranges from 0.2-0.4 µg As l^{-1}, algae release by Ascophyllum nodosum alone could therefore account for 25 to 50% of the water column DMA concentration. In winter months the release of DMA species by algae is substantially lower and can only account for a DMA water column concentration of 0.01 µg As l^{-1}. Furthermore, the contribution of MMA to the water column is only 0.005 µg As l^{-1}. However, porewater infusion now becomes more important since it contributes a water column concentration of MMA species

of 0.03 μg As l^{-1}. This emphasizes the dominance of the porewater source compared with the macro-algae source in winter. In addition, the porewaters in St. Johns' Lake are also expected to release more MMA than DMA species to the overlying water column. In winter the concentration of MMA (the dominant methylated As species) in the water column of St. Johns' Lake ranges from 0.2-0.5 μg As l^{-1}. The infusion of MMA species from porewaters could therefore account for 10-15% of the water column MMA concentration. Although these estimates are formed on the basis of fairly crude assumptions they do indicate the relative contributions of methylated As species in terms of both concentration and species type from algal and porewater sources do have a seasonal dependence. Similar calculations could be undertaken for other areas in the Tamar Estuary e.g., Cargreen where large mudflat areas are covered by extensive algal colonies.

If marine sites are regarded as the major source areas of methylated As species in the Tamar, this is not unlikely since extensive mudflats and algal colonies are observed in the lower rather than upper reaches of the estuary, the above statements can be summarised in terms of a first order conceptual model of dissolved As behaviour. The conceptual model is depicted in Figures 4.27 and 4.28. The arrows in each figure represent the relative source strength of phytoplankton and porewater sources of methylated As species. As discussed previously similarities in the observed distribution of As species in several estuaries in S. England indicate the general applicability of this conceptual model.

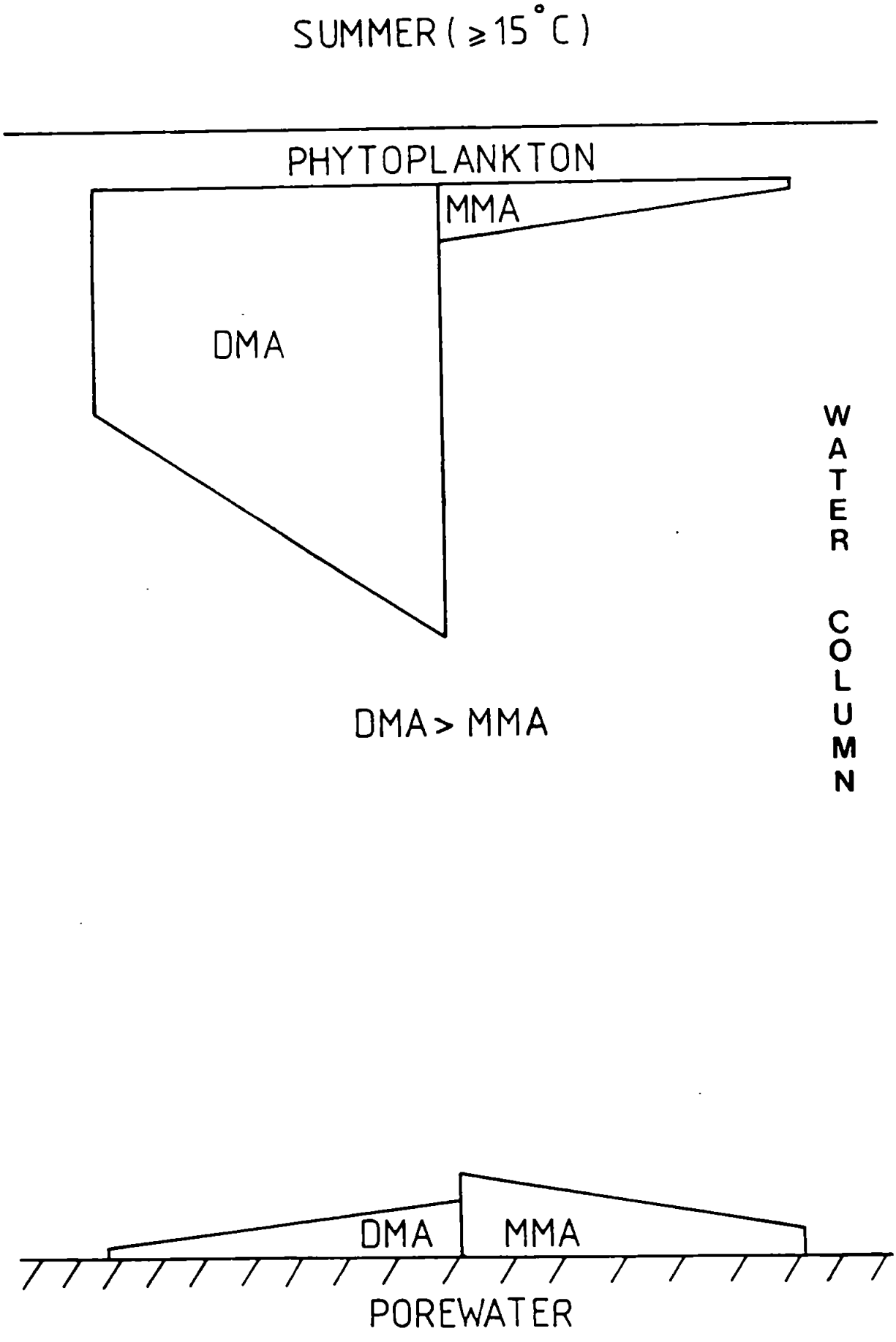
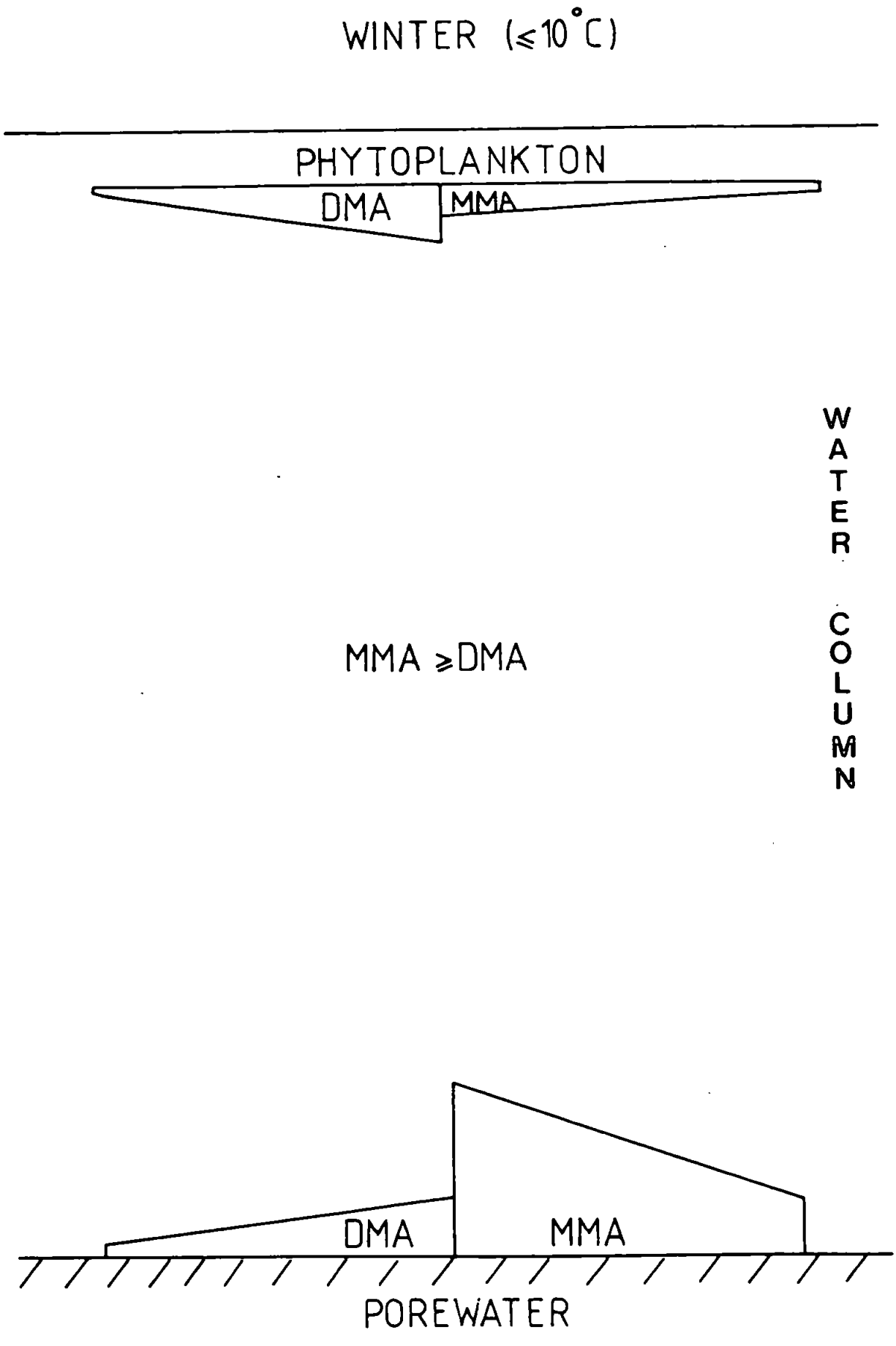


Figure 4.27 Relative contributions of methylated arsenic species to the water column from phytoplankton and porewaters in summer



4.28 Relative contributions of methylated arsenic species to the water column from phytoplankton and porewaters in winter

CHAPTER FIVE

CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

5.1.1 Lead

Both inorganic and methylated Pb species have been observed in rain, drain and estuarine water samples collected in the Tamar Estuary and its catchment area. In estuarine water samples only 1 methylated Pb species ($(CH_3)_3Pb^+$) was observed above the detection limit at a maximum concentration of 10 ng Pb l^{-1}. Methylated Pb species were only observed at sites where land derived petroleum inputs were evident and no detectable levels of such species were observed in porewater or sediment samples collected from the Tamar. Similarly methylated Pb species were not observed above the detection limit in water samples collected from the Carnon and Beaulieu Estuaries. The work on Pb methylation illustrates the general need for improvements in the detection limit capabilities of currently available techniques. From this work it is possible to conclude that data on the distribution of methylated Pb species in estuarine waters may be readily forthcoming if the GC-AAS Pb detection limit is improved by a factor of x 10 to x 100. The above statements are generally applicable to studies on the more volatile methylated metal species e.g., dimethylselenide.

In laboratory modelling experiments the conversion of trimethyllead acetate ($(CH_3)_3PbOAc$) to tetramethyllead (TML) occurred in both abiotic and biotic systems. In abiotic distilled water systems exponential growth of TML in headspace gases was observed and in both light and dark regimes the reaction was found to be first order with respect to TML concentration. The rate constant for the forward reaction (k_1) was 0.053 h^{-1} and 0.064 h^{-1} for the light and dark regimes respectively. The half-lives ($t_{\frac{1}{2}}$) for the 2 reactions were calculated to be 13 h in illuminated conditions and 10 h for darkened conditions. In abiotic systems a maximum conversion of $(CH_3)_3PbOAc$ to TML of 0.32% was

observed and this compares with 2.8% for biotic systems. The addition of sodium sulphide (1000 μg Na_2S l^{-1}) to biotic systems increased the yield of TML with a maximum conversion of 4.9% being observed. This confirms the importance of the sulphide mediated chemical disproportionation pathway in the conversion of $(CH_3)_3PbOAc$ to TML and suggests that a biologically mediated pathway need not be invoked for the reaction, as reported by Craig (1980). Work by Craig and Rapsomanikis (1985) indicates that the environmental methylation of inorganic Pb may occur *via* chemical as well as biologically mediated pathways. In studies in this thesis the methylation of the more environmentally significant Pb (II) species ($Pb(NO_3)_2$, $PbCl_2$, $Pb(OAc)_2$) was not reproducible although when detected a biologically mediated methylation process was invoked. Maximum conversions of $Pb(NO_3)_2$, (0.026%); $PbCl_2$, (0.028%); $Pb(OAc)_2$, (0.05%) were observed. The general lack of reproducibility of observations on Pb methylation has been reported by previous workers (Wong *et al.*, 1975; Schmidt and Huber, 1976). Since the environmental methylation of Pb was not proven to occur reproducibly a conceptual model of the biogeochemical cycling of Pb in estuarine waters was not proposed. The significance of metal methylation in the cycling of Pb in the environment is therefore difficult to assess. However, from the results reported in this thesis and consideration of an estimated atmospheric TML half-life of 10 h for summer (Hewitt and Harrison, 1985) it is possible to speculate that a significant concentration of Pb could be transported out of the estuarine environment in the vapour phase as TML. For example if Pb is methylated in estuarine sediments and subsequently released into the overlying air mass a gentle sea breeze of 20 km h^{-1} could transport the air mass some 200 km in 10 h during which time a 50% reduction in the initial concentration of TML would have taken place due to photolytic decomposition of TML. A significant concentration,

some 50% of the original atmospheric TML concentration, would therefore have travelled 200 km. The methylation of Pb in the estuarine environment would therefore be an important process in the transportation and general biogeochemical cycling of Pb in the estuarine environment.

5.1.2 Arsenic

Both dissolved inorganic and methylated As species have been identified in the water column of 3 estuaries in S. England which have strong hydrochemical and hydrodynamic contrasts in parameters such as pH and overall water column chemistry. Furthermore methylated As species were observed in the water column of the Tamar Estuary over a full seasonal cycle. From this work it is possible to conclude that there appears to be a wide range of conditions under which methylation of As can occur and that probably no unique process is responsible for the existence of methylated As species in the Tamar, Carnon and Beaulieu estuarine systems.

In this study 3 main sources of methylated As species have been identified these being diatoms (Skeletonema costatum), macro-algae and sediment porewaters. Strong seasonal changes in As speciation were observed in the Tamar and these have been related to the seasonal variations in the contribution of methylated As species to the water from the 3 sources described above. From the results of estuarine surveys and laboratory modelling experiments a first order model of dissolved As behaviour for the Tamar Estuary has been proposed. In summer the plankton source is considered to predominate over the porewater source. Both Skeletonema costatum and macro-algae were observed to preferentially release dimethylarsenic species (DMA) to the surrounding water column. The release rates for DMA from Skeletonema costatum were estimated to be 220 μg As kg^{-1} h^{-1} for summer conditions (15°C

water column temperature) and this compares with 3.2 μg As kg^{-1} h^{-1} of DMA released by the macro-algal species Ascophyllum nodosum under similar conditions. Macro-algae (Ascophyllum nodosum species) release of DMA may account for 25-50% of the DMA concentration observed in the water column of St. Johns' Lake, the largest mudflat area (extensively colonized by macro-algae) observed in the Tamar Estuary. The above observations may account for the general predominance of dimethylarsenic species over monomethylarsenic species (MMA) observed in the water column in summer. In winter, however, a transition takes place where the predominance of methylated As species occurs and the concentration of MMA exceeds that of DMA. This transition has been attributed to the increased importance of the porewater source of methylated As species in winter. The macro-algal release of DMA (Ascophyllum nodosum species) was observed to be a factor of x 10 fold lower in winter conditions compared with summer conditions, reflecting the decreased importance of the phytoplankton source in winter. On the basis of reasonable assumptions, porewater injections from St. Johns' Lake sediments in winter may account for 15% of the water column MMA concentration observed in St. Johns' Lake. Macro-algal release of methylated As species could only account for $<5\%$ of the observed water column methylated As concentrations. At present detailed information on porewater release rates for metal species are not readily available. In this study from the limited data available the porewater source has been estimated to account for a maximum of 15% of the observed water column methylated As concentration. However, if release rates for methylated As species from porewaters are found, from in situ observations, to be higher than estimated in this study the porewater source may account for $>15\%$ of the observed water column methylated As concentrations.

During summer surveys in the Carnon and Beaulieu estuarine systems

similar trends to those observed in the Tamar were discernable and it is therefore possible to conclude that the first order conceptual model developed for the Tamar is applicable to several other estuarine systems. In contrast to Pb methylation studies As methylation has proven to be highly significant in the cycling of As in the estuarine environment. In modelling studies reproducible methylation of inorganic As species (sodium arsenate and arsenic trichloride) occurred in model sediment-porewater systems. In a freshwater regime the maximum conversion of inorganic As to the preferred methylated As species (DMA) was 8.0%. Whilst in a marine regime the maximum conversion of inorganic As to the preferred methylated species (MMA) was 4.0%. In the Tamar and Carnon systems methylated As species accounted for approximately 10% of the total dissolved inorganic As concentration (arsenate and arsenite combined) and this compares with a figure of 50% for the Beaulieu system. It is clear that large concentrations of inorganic As can be methylated by biologically mediated processes in the estuarine environment with subsequent long distance transportation of As directly into the major oceanic circulations. The methylation of As may therefore be regarded as a highly significant process in the general biogeochemical cycling of As in estuarine waters.

5.1.3 Methylated Forms of Other Elements

Apart from Pb and As studies on the methylation of Hg, Sb, Ge and Se have been reported (refer to review in Chapter 1). Although Se methylation studies on a limited number of atmospheric samples have been reported (Jiang et al., 1983) little work on the distribution of methylated Se species in the estuarine environment is cited in the literature. In this thesis a preliminary study on the methylation of Se in the estuarine environment was undertaken.

The Se work is included in this chapter because on an occasion dimethylselenide, $Se(CH_3)_2$, was detected (at 0.5 μg Se l^{-1}, which is close to the detection limit of 0.15 μg Se l^{-1} for $Se(CH_3)_2$) in a surface water sample collected from the Carnon Estuary (25th June 1984, water conditions; temperature 17.4°C; pH 7.5; salinity 15.5‰, refer to Section 2.3.1.2.1 for experimental conditions employed in the analysis of organoselenium compounds). The chromatogram obtained in the determination of $Se(CH_3)_2$ in the column water sample obtained from the Carnon Estuary is shown in Figure 5.1(A). The presence of $Se(CH_3)_2$ was confirmed by comparison of the retention times of unspiked (Figure 5.1(A)) and spiked ($(CH_3)_2$ Se spike, Figure 5.1(B)) samples. The concentration of total dissolved inorganic Se in the River Carnon and associated Restronguet Creek ranged from 5.8-7.6 μg l^{-1} (freshwater sites sampled, salinity <2.1‰; analysis by GFAAS refer to Section 2.2.2). In further water column studies (samples collected during full estuarine surveys over the salinity range 1-25‰ in the Carnon, 5th June 1985; Beaulieu 18th June 1985; Tamar 30th July 1985 to 25th July 1986 monthly survey studies) the initial identification of $Se(CH_3)_2$ was not subsequently confirmed. Methylated Se species were also below the detection limit in porewater samples collected in the Tamar (over a seasonal cycle; August 1985 to June 1986, monthly survey studies) in which concentrations of dissolved inorganic Se of 21-50 μg l^{-1} were observed.

5.1.4 Comparison of Methylation Behaviour of Pb, As and Se

In discussing the significance of metal methylation in estuarine waters a consideration of the rate of formation and decomposition of methylated species as well as a knowledge of the yield and partition characteristics (gas, liquid or solid phases) of such species is required.

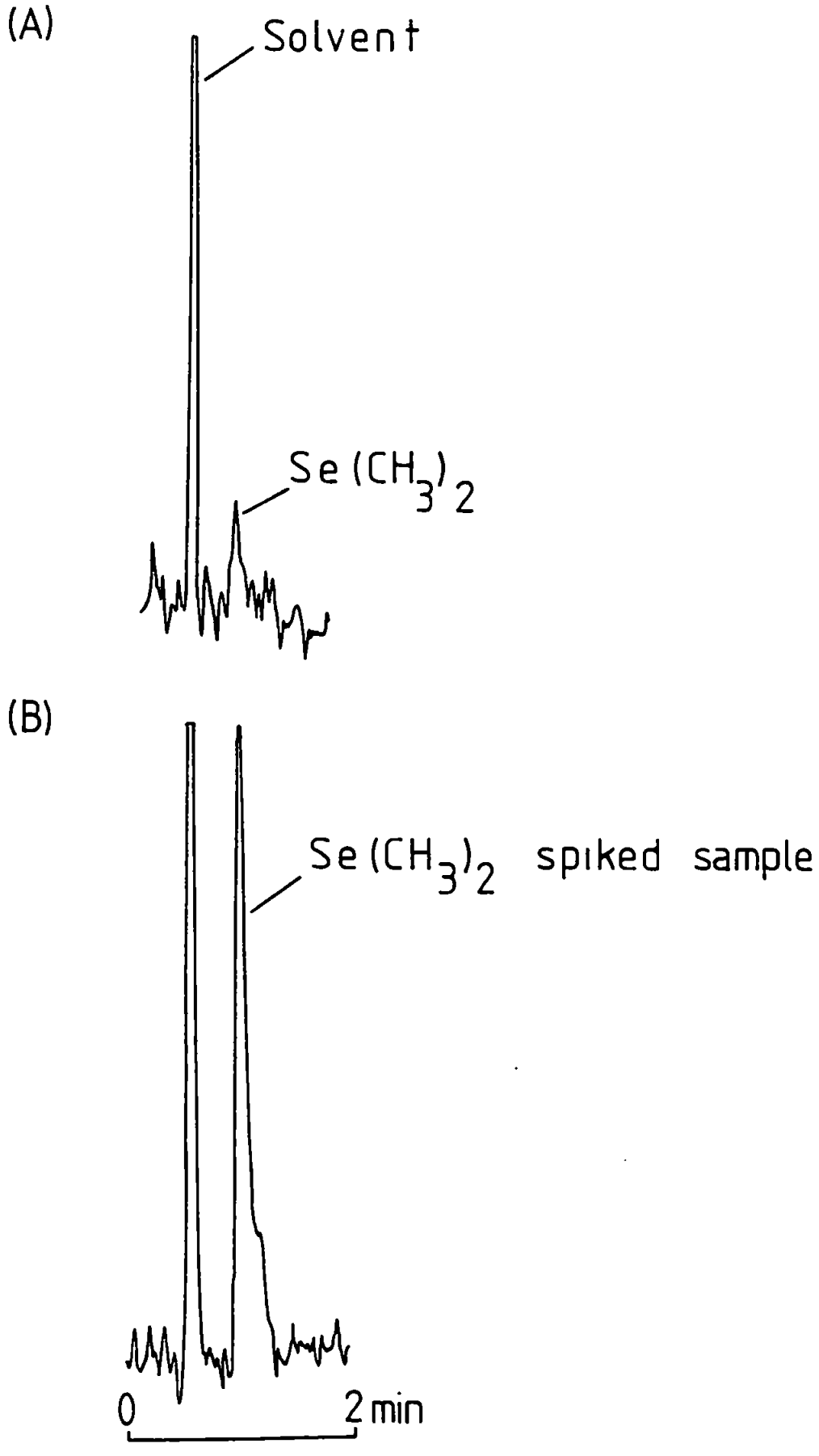


Figure 5.1 Chromatogram of dimethylselenide in a Carnon Estuary water sample (A) and (B) the same sample spiked with dimethylselenide.

The rate of methylation and demethylation is related to the stability of the methylated species in question. In the estuarine environment the stability of methylated species with respect to light, hydrolysis and biologically mediated dealkylation are to be considered important. The mean bond dissociation energies ($\bar{D}$) of methylated metal species may be used as a comparative guide to the stability of such species. In the case of tetramethyllead (TML) the mean bond dissociation energy is 155 KJ mol^{-1} and this compares with figures 230 and 247 KJ mol^{-1} for the methylated species trimethylarsine ($(CH_3)_3As$) and dimethylselenide ($(CH_3)_2Se$), respectively (Craig, 1986). The mean bond dissociation energy for alkyllead species is therefore significantly lower than those of methylated As or Se species and consequently the former species is likely to be more unstable with respect to light and hydrolysis.

In estuarine waters visible light (400-700 nm range) entering the water column is attenuated by absorption by the pure seawater itself and by dissolved organic material. As a result visible light of wavelengths below 500 nm rarely penetrates the water column to any great depth (Riley and Chester, 1971). Visible light of wavelength 500 nm has an energy corresponding to 230 KJ mol^{-1} which is sufficient to cleave the metal-carbon bond in TML. This may explain the apparent absence of such species in the surface waters of estuarine systems. It should be noted that the energy of light at a wavelength of 500 nm is also similar to the energy required to cleave the metal-carbon bond in both methylated As and Se compounds.

The hydrolytic stability of methylated metal species is of great importance to the persistence of such species in the aquatic estuarine environment. The rate of hydrolysis is related to the polarity of the metal-carbon bond such that highly polarised bonds are unstable

to water. Photolytic decomposition of TML results in the formation of the ionic tri- and dialkyllead species which are soluble in water and are themselves thermodynamically unstable to hydrolysis to metal hydroxide and hydrocarbon products. The methylated species dimethylarsinic acid and monomethylarsonic acid are water soluble and stable. The species dimethylselenide is water stable but only sparingly soluble in water. In biological systems decomposition of methylated species usually takes place by dealkylation. Biologically mediated dealkylation has been reported for methylated Pb species (Schmidt and Huber, 1976). Methylated As species are very stable to dealkylation process in plants and animals (Craig, 1986), however, certain soil bacteria are known to be able to demethylate monomethylarsonic acid (Shariatpanahi et al., 1981). Little is known of the biologically mediated dealkylation of methylated Se species. The relative stability of methylated Pb, As and Se species with respect to light, hydrolysis and biological dealkylation therefore appears to follow the order As > Se > Pb.

In discussing the significance of metal methylation in estuarine waters perhaps most important is a consideration of the yield and partition characteristics of the methylated species in question. The discharge of both domestic and industrial waste into estuarine waters frequently results in high levels of metal being incorporated into bottom sediments. From modelling experiments undertaken in this study it has been shown that significant quantities of both Pb and As pollutants can be remobilized from the metal-sediment reservoir and transported out of the estuarine system as a result of methylation processes. In the case of Pb a maximum conversion of inorganic Pb to TML of 0.028% was observed. Hence a maximum of 0.028% of inorganic Pb pollutant present in estuarine sediment-porewaters could be methylated to TML and transported out of the estuarine system completely in the vapour

phase. In the case of As a maximum conversion of inorganic As to the methylated species of 8% was observed. Hence a maximum of 8% of inorganic As pollutant present in estuarine sediment-porewaters may be methylated and transported out of the estuarine system into the oceanic circulation in the dissolved phase. The transportation of Se is not well understood, however, the detection of methylated Se species in estuarine waters suggests that as was the case for Pb inorganic Se pollutants may be methylated to volatile species ($Se(CH_3)_2$) and transported out of the estuarine system. In many models on the cycling of metals in the environment methylation is not considered a significant transport mechanism (Martin and Meybeck, 1979). The work undertaken in this study suggests that metal methylation should be incorporated in such models as a significant transport mechanism.

Furthermore the methylation process may be regarded as an important detoxification process in the case of As, however, for both Pb and Se the methylated species are more toxic than the inorganic precursor. The significant amounts of inorganic Pb and Se that could be methylated in the estuarine environment may therefore pose a serious toxicity threat, particularly if Pb and Se are biaccumulated or biomagnified in the estuarine environment.

In conclusion from environmental and laboratory work undertaken in this thesis and a consideration of the stability of methylated Pb, As and Se species it is possible to state that metal methylation is a significant process in the transportation and biogeochemical cycling of these elements in the estuarine environment. Further the significance of metal methylation in the cycling of the metals Pb, As and Se, in estuarine waters, appears to follow the order:

$$As > Se > Pb$$

5.2 Suggestions for Future Work

From the work undertaken in this study several suggestions for future work may be proposed these are considered below.

(i) Improvements in the detection limits of both methylated Se and Pb species may be achieved by the use of preconcentration techniques such as 'Cryogenic Trapping' (reported in this work for As speciation studies) of volatile methylated species and closed loop gas stripping analysis (Grob and Zürcher, 1976).

(ii) Further water column surveys should be undertaken over a full seasonal cycle in a larger number of estuaries. The data base generated on methylated As, Pb and Se species may be used in the development of empirical models for the behaviour of each element. Water column studies should include depth profile studies to understand further the release of methylated metal species by phytoplankton during the sink-decay process.

(iii) Since the biologically mediated methylation of Pb and Se is most likely to occur and be observed in sediment porewater further in situ porewater studies should be undertaken. The studies should employ the preconcentration techniques suggested above in (i). Such studies may yield valuable data on the methylation/demethylation process occurring in porewaters as well as yield information on porewater release rates for methylated Pb, As and Se species. This information may be used to estimate more accurately the contribution of methylated metal species to the water column from the porewater source.

(iv) The methylation of Pb, As and Se by various pure cultures of bacteria and phytoplankton have been studied in detail. An important

area of future work would involve an assessment of the mass and major phytoplankton species present in estuarine environments. This would allow an estimation of the significance of the contribution of methylated metal species to the water column from phytoplankton.

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Lectures and Associated Studies

i. IMER Seminar, 7th September 1983, IMER Plymouth,
Dr. M. Andreae, 'Organometalloid compounds in natural waters'.

ii. RSC meeting, 28th October 1983, Plymouth Polytechnic,
Prof. J.P. Riley, 'Analytical chemistry applied to marine pollution problems'.

iii. IMER Seminar, 24th November 1983, IMER Plymouth,
Dr. R. Loughlin, 'Physiochemical factors governing the toxicity of Organotins'.

iv. RSC lecture, 17th February 1984, Plymouth Polytechnic,
Prof. J.N. Miller, 'Hunting the Snark-recent developments in ultra sensitive Molecular Spectroscopy.

v. RSC lecture, 16th November 1984, Plymouth Polytechnic.
Dr. D. Woodcock, 'Fungicides and the environment'.

vi. Departmental Colloquium, 8th February 1985, Plymouth Polytechnic,
Dr. C. Corfield, 'Gas Chromatography-Mass Spectrometry, useful applications of a technique come of age'.

vii. RSC lecture, 15th March 1985, Plymouth Polytechnic, Dr. P.S. Liss,
'The role of the Oceans in the chemistry of the atmosphere'.

viii. MBA/IMER Joint Seminar, 22nd July 1985, MBA Plymouth, Dr. F. Millero,
'The kinetics of inorganic redox reactions in natural waters'.

ix. Departmental Colloquium, 10th September 1985, Plymouth Polytechnic,
Dr. J. Harnly, 'Recent developments in Analytical Atomic Spectroscopy'.

x. NERC Conference, 16th-19th July 1985, Plymouth Polytechnic,
'Estuarine and coastal pollution - detection, research and control'.

xi. NERC Conference, 4th February 1986, Royal Society of Chemistry,
London, 'Global ocean flux study'.

xii. Series of lectures on 'Advanced Analytical Atomic Spectroscopy',
Plymouth Polytechnic, June-August 1984.

xiii. Weekly meetings of the Chemistry of Natural and Polluted Environments Research Group, Plymouth Polytechnic, October 1983 - July 1986.

xiv. Electron Microscopy Course at Plymouth Polytechnic, August 1985, 'Transmission Electron Microscopy'.

Meetings of the Royal Society of Chemistry and Marine Chemistry Discussion Group

i. Analytical Division of RSC, 26th-27th June 1984, 'Research and Development Topics in Analytical Chemistry', UMIST, Manchester.

ii. Analytical Division of RSC, 10-13th July 1984, 'Second Biennial National Atomic Spectroscopy Symposium', University of Leeds.

iii. Analytical Symposium of RSC, 18th-19th April 1985, 'The Bishop Symposium', University of Exeter.

iv. Analytical Division of RSC, 20th-26th July 1986, SAC 86/3rd Biennial National Atomic Spectroscopy Symposium, University of Bristol.

v. Marine Chemistry Discussion Group Meeting, September 1984, Swansea University.

vi. Marine Chemistry Discussion Group Meeting, September 1985, Guildford University.

Presentations and Publications

As a result of the work reported in this thesis the following papers have been presented and published.

(A) PRESENTATIONS

(1) 'Estuarine trace metal speciation by coupled Chromatography-Atomic spectroscopy',
Paper presented at the Marine Chemistry Discussion Group Meeting, University of Guildford, September 1985.

(2) 'The causes and significance of arsenic methylation in estuarine waters',
Poster presentation presented at the RSC Research and Development Topics in Analytical Chemistry Meeting, University College London, April 1986.

(3) 'Metal methylation and its significance in estuarine waters',
Paper presented at the RSC SAC/3rd Biennial National Atomic Spectroscopy Symposium, University of Bristol, July 1986.

(B) PUBLICATIONS

WALTON, A.P., Ebdon, L. and Millward, G.E. (1986).
The causes and significance of arsenic methylation in estuarine waters.
Anal. Proc. (In press).