UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

'HIDDEN' ARSENIC IN ESTUARINE SYSTEMS

BY

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A THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

SEPTEMBER 2000

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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The nature, distribution and cycling of dissolved hydride-reducible and 'hidden' arsenic in the surface and interstitial waters of estuarine systems has been studied by cryogenic trap hydride-generation atomic absorption spectroscopy (HG-AAS) methods. With 'hidden' arsenic compounds no volatile hydride is formed upon treatment with sodium borohydride and pretreatment methods are necessary. Batch ultra-violet irradiation pretreatment processes for the detection of 'hidden' arsenic were further improved by the addition of persulphate resulting in the effective breakdown of non-hydride reducible arsenic compounds to 'inorganic' arsenic forms in 4-8 hours. Photolysis with persulphate using a microwave driven lamp reduced the time required to 2.5 minutes for freshwater samples, but concurrent heating effects made it unsuitable for estuarine water samples. A novel inline photooxidation HG-AAS technique was also developed which converted both hydride-reducible organoarsenic and non-hydride reducible arsenic species to 'inorganic' arsenic with *ca.* 92±4% efficiency.

A seasonal study of the surface waters of Southampton Water and the estuary of the River Itchen revealed that during the summer months dissolved hydride-reducible 'inorganic' arsenic concentrations decreased as the hydride-reducible methylated arsenic concentrations increased. Dissolved 'hidden' arsenic concentrations were consistent with the release of complex organoarsenicals from planktonic organisms by active excretion, during senescence and grazing. These complex organoarsenic compounds are then broken down to simpler forms by chemical and biological processes. Deposition of these organoarsenic compounds into the sediments is accompanied by further breakdown and release into the overlying water column. Only once the decomposition has reduced the chemical complexity of the organoarsenicals can they be detected as hydride-reducible arsenic species.

Acknowledgments

I would like to firstly thank my supervisor Dr. A.G. Howard for his experienced supervision and advice during the course of this project. Other staff members within the Chemistry Department who provided valuable information include Professor J. Mellor and Dr. G.S. Attard.

Past and present lab colleagues (Dr. Duncan Russell, Dr. Melanie Newton, Dr. Jalal Al-Saad, Dr. Tiffany Yeh, Mark Harding, Catherine Freeman, Tamer Shafik and Laetitia Labonne) must be thanked for keeping me company and providing assistance during my period of occupation in the Environmental Analytical Group laboratories.

Within the Oceanography Department, the efforts of Dr. P. Statham and Dr. D. Purdie in providing information on the Southampton Water/Itchen River estuarine system were greatly appreciated. The company, assistance and good humour provided by Graham and Mike, the boat crew of the Research Vessel *Bill Conway*, when collecting samples in Southampton Water/Itchen River will be remembered for some time to come.

The help and friendly words provided by the tea ladies, glassblowers, storekeepers, technical staff and secretaries of the Chemistry Department were greatly appreciated.

The financial support provided by the EPSRC is gratefully acknowledged.

Lastly but not least, my parents and family must be thanked for their valuable support and contribution during my long period of tenure in the education system.

Contents

ABBREVIATIONS

AAS	atomic absorption spectroscopy
As_{IN}	inorganic arsenic
AES	atomic emission spectroscopy
AFS	atomic fluorescence spectroscopy
ASV	anodic stripping voltammetry
bp	boiling point
chlor 'a'	chlorophyll a
CT	cryogenic trap
DMA	dimethylarsenic
DMAA	dimethylarsinic acid
ECD	electron capture detector
EDL	electrodeless discharge lamp
EDTA	ethylenediaminetetra-acetic acid
FID	flame ionisation detector
GC	gas chromatography
GFAAS	graphite furnace atomic absorption spectroscopy
HCL	hollow cathode lamp
HPLC	high performance liquid chromatography
ICP	inductively coupled plasma
MMA	monomethylarsenic
MMAA	monomethylarsonic acid
mg	milligram
MS	mass spectroscopy
NAA	neutron activation analysis
ng	nanograms
RSD	relative standard deviation
ppb	parts per billion (in this thesis µg/l)
ppm	parts per million (in this thesis mg/l)
PTFE	polytetrafluoroethylene
u.v.	ultra violet
v/v	volume per unit volume
w/v	weight per unit volume
µg	microgram
XRF	x-ray fluorescence

Chapter 1

The chemistry of arsenic

1.1 Arsenic and its compounds

1.1.1 Introduction

The elements of Group 15, of which arsenic is a member, have the $ns^2\ np^3$ general electronic configuration and the ^4S ground state which implies the presence of three singly occupied electron orbitals. Arsenic, antimony and bismuth, however, differ from nitrogen and phosphorous due to their lower electronegativity values (Table 1.1) which decrease with increasing atomic number.

Element/property	Arsenic	Antimony	Bismuth
Atomic number	33	51	83
Electronic configuration	$[Ar]3d^{10}4s^2 4p^3$	$[Kr]4d^{10}5s^2 5p^3$	$[Xe]4f^{14}5d^{10}6s^2 6p^3$
Covalent radius (pm)	121	141	146
Ionic radius M^{3+} (pm)	69	90	120
Metallic radius (pm)	139	159	170
Electronegativity (Allred-Rochow)	2.20	1.82	1.67

Table 1.1 Atomic properties of arsenic, bismuth and antimony

Arsenic, antimony and bismuth tend to form three co-ordinated compounds, leaving a ns^2 lone pair of electrons within their structures. Hybridisation is known

to occur and can result in the formation of near tetrahedral structures [1]. It is also possible for all five valence electrons within arsenic, antimony and bismuth to be involved in bonding with other elements resulting in the formation of bipyramidal, square pyramidal and octahedral structures.

Arsenic tends to exhibit metalloid properties and often exists with the hexagonal-rhombic structure. In contrast, bismuth and some antimony compounds tend to show metallic behaviour due to the increase in cationic/metallic behaviour down the group. It is also well established that arsenic, nitrogen and phosphorous are able to form a number of compounds which are chemically analogous [2].

The redox potential for the As(III)/As(V) interchange reaction is dependent on the pH of the solution:

$$H_3AsO_4 + 2H^+ + 2e^- \longrightarrow HAsO_2 + 2H_2O \quad E^\circ = +0.56V \quad (1.1)$$

$$[AsO_4]^{3-} + 2H_2O + 2e^- \longrightarrow [AsO_2]^- + 4OH^- \quad E^\circ = -0.67V \quad (1.2)$$

This reaction scheme suggests that in strong acidic media, arsenic acid oxidises iodide to iodine (E° I_2/I^- = 0.54V) but in neutral and alkaline environments, iodine oxidises arsenite to arsenate.

In addition to the most commonly found + (III) and + (V) oxidation states, arsenic is known to form compounds in the 0 and - (III) oxidation states. Compounds in the + (I), + (IV) and + (VI) oxidation states also exist but are rare.

Elemental arsenic is insoluble in water but dissolves in nitric acid to form arsenic acid and burns in air to form arsenic trioxide.

1.1.2 As(III) compounds

Arsenic reacts with the halogens, oxygen, carbon, hydrogen, oxygen and sulphur to give As(III) species. The resulting structures are often trigonal pyramidal or tetrahedral in structure but due to the ns^2 lone pair on the arsenic component, structural distortions are known to occur. For example, the arsenic dithiocarbamate complex $[As(S_2CNEt_2)_3]$ [3] which contains six co-ordinated arsenic (III) species has three long (2.85Å) and three short (2.35Å) As-S bonds.

Natural forms of As(III) exist in the mineral arsenolite, principally in the form of arsenic trioxide (As_2O_3). Two different structures of arsenic trioxide are known – claudetite I and II – differing only in the arrangement of the arsenic atoms. Arsenic trioxide is known to exist in a number of allotropic forms: one is the gaseous dimer As_4O_6 where the arsenic atoms in a tetrahedral environment have three direct As-O pyramidal bonds (Figure 1.1).

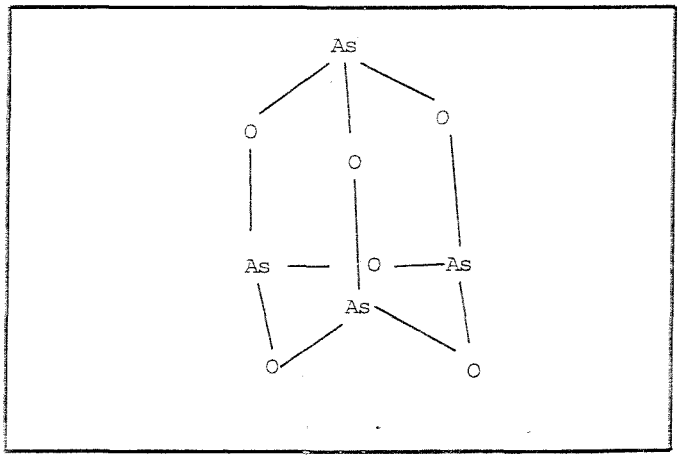

Figure 1.1: Structure of As_4O_6 in the vapour phase (adapted from reference [4])

Arsenic trioxide is thought to exist as $As(OH)_3$ in neutral or acidic media. The meta-arsenite salt which has the $As(ONa)_2(OH)$ or Na_2HAsO_3 formulation

polymerises in the solid state with a continuous sequence of pyramidal AsO_3 groups linked together by As-O-As bonds (Figure 1.2). The arsenic atom is at the centre of the pyramidal structure with the oxygen atoms attached to each corner of the pyramid face. For each unit cell, four AsO_3 chains are packed together by sodium ions. This therefore suggests that a more accurate structural formula would be $(NaAsO_2)_n$.

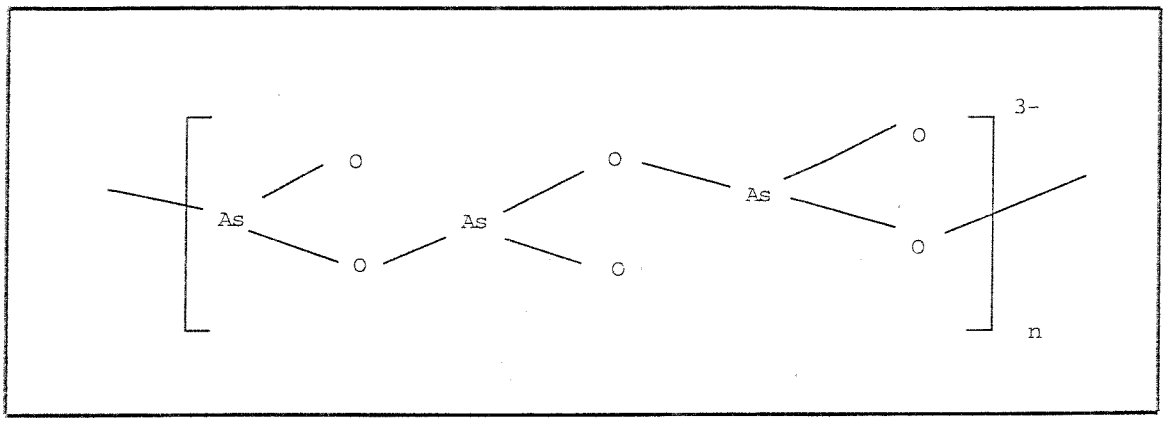

Figure 1.2: **X-ray solid state structure of $[NaAsO_2]_n$. Adapted from reference [4]**

A biologically important group of three co-ordinated arsenic compounds are the thioarsenites. These compounds have the $R_nAs(III)$ bonded to one or more SR groups. As the As(III) species are thought to inhibit enzymatic activity, studies have focussed on the interactions between the thioarsenites with thiol-containing enzymes and proteins. Selected examples of thioarsenite compounds are shown in Figure 1.3.

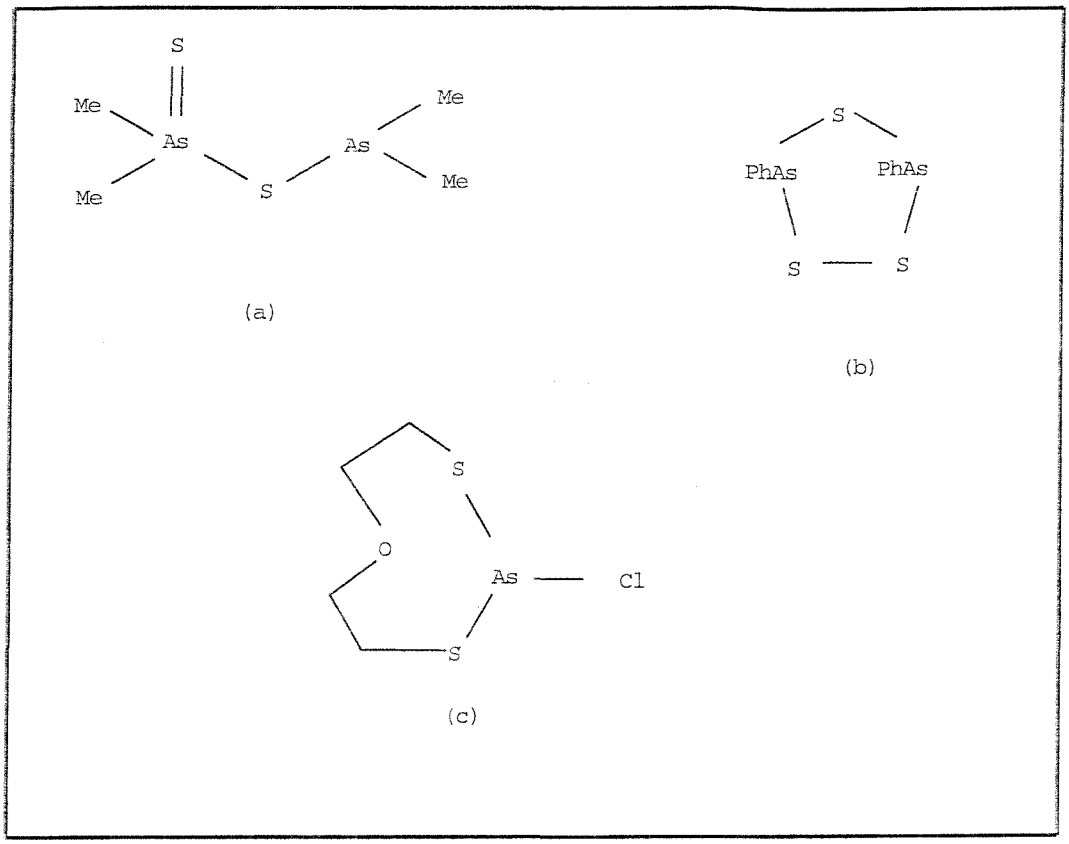

Figure 1.3: Structures of some As-S containing thioarsenites as determined by X-ray crystallography. Adapted from reference [4] ((a) $(Me_2As)_2S_2$ (b) $Ph_2As_2S_2$ (c) 5-Cl-1-O4,6S-5-arsaocane)

One significant property of As(III) compounds is the ability of the ns^2 lone pair to act as a Lewis base, leading to the formation of tetrahedral four co-ordinated species. These R_4As^+ tetraalkylarsonium ion forms include the biochemically important arsenic species of arsenobetaine and arsenocholine as well as the arsenosugar and tetramethylarsonium species.

Arsenic hydrides and other similar species where hydrogen is replaced by an alkyl group are commonly known as arsines or alkylarsines. These compounds may have one of the following general formulations: AsH_3, $AsRH_2$, AsR_2H or AsR_3. However, the polarity of the As-H bond is unclear which is in contrast to the slightly electropositive nature of the As species in the As-S, As-C and As-Cl bonds. One significant property of the arsines is their ability to decompose easily

to arsenic, which is deposited on hot surfaces as a mirror. This property forms the basis of the Marsh test where arsenic compounds are reduced by zinc in HCl solution.

1.1.3 As(V) compounds

In aqueous media, four co-ordinated As(V) compounds are commonly found. Arsenic acid, H_3AsO_4 is formed by dissolving arsenic pentoxide in water. Alkylated forms of arsenic acid are known with R groups replacing OH. Examples of such compounds include the simple monomethyl (H_2MeAsO_3) and dimethyl (HMe_2AsO_2) arsenic species.

The arsenic (V) oxide, arsenic pentoxide (As_2O_5), consists of both tetrahedral AsO_4 and octahedral AsO_6 groups linked together in a 3-dimensional framework with bond lengths of 1.68 and 1.82 Å respectively. In contrast with the synthesis of P_2O_5, As_2O_5 cannot be prepared directly from the oxidation of the element. It can, however, be prepared via the dehydration of the hydrates of arsenic acid [4]:

$$H_3AsO_4.2H_2O \xrightarrow{-30^{\circ}C} H_3AsO_4.\tfrac{1}{2}H_2O \xrightarrow{36^{\circ}C} H_5As_3O_{10} \xrightarrow{170^{\circ}C} As_2O_5 \qquad (1.3)$$

Reactions with the halogens, alkyl groups, sulphur and hydrogen with arsenic(V) species lead to the formation of five co-ordinated arsenic(V) compounds such as AsF_5, As_2S_5, $AsCl_4F$ and Me_2AsCl_3. These compounds exist with the trigonal bipyramidal structure.

1.1.4 As(I) compounds

Arsenic compounds with an oxidation state of 1 have been determined such as $PhAs[Mn(CO)_2Cp]_2$ and $[Cr(CO)_5](Cl)As[Mn(CO)_2Cp]$. These compounds have approximately planar geometry around the arsenic species as shown in Figure 1.4.

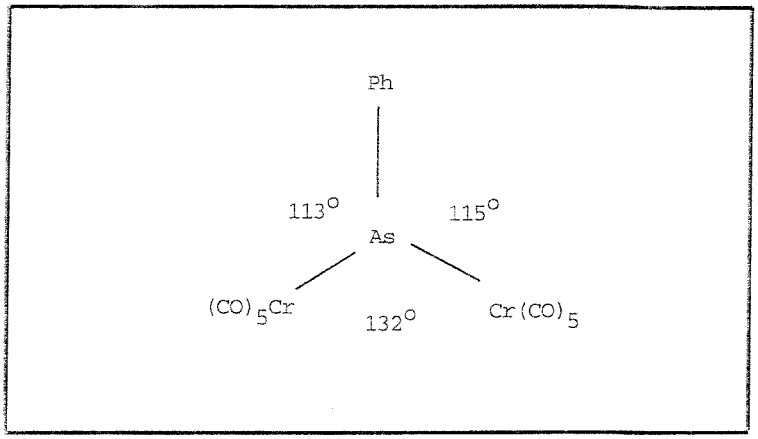

Figure 1.4: Structure of a As(I) co-ordinated compound. Adapted from reference [4].

The solid state structures of some $(RAs)_n$ compounds have been evaluated such as $(PhAs)_6$, for example which is a six membered ring with a 'chair' configuration. It was noted that the As-As bond distances in the $(PhAs)_6$ structure were unequal with one of the As-As bonds having a distance of 2.464Å in contrast to the other two As-As distances of 2.456Å and 2.457Å [4].

Compounds containing As=As double bonds were structurally identified in 1983 [5]. One example is $[(2,4,6\text{-t-}Bu_3C_6H_2)As{=}AsCH(SiMe_3)_2]$ which has an As-As bond distance of 2.22Å which is smaller than the average As-As bond distance of 2.45Å. [6]. It was observed that bulky substituent species are necessary for the stabilisation of the As=As double bond as is the case for the formation of free diphosphenes [6].

1.1.5 Four and six co-ordinated arsenic compounds

An individual four co-ordinated arsenic compound can be formed by the reaction between arsenic (III) species and OH radicals [5]. Likewise, a reaction, which takes place between arsenic (V) species and the $(SO_4)^-$ ion leads to the formation of a six co-ordinated arsenic complex [5].

An unusual bicyclic compound containing both four and six co-ordinated arsenic species has been characterised (Figure 1.5) [7].

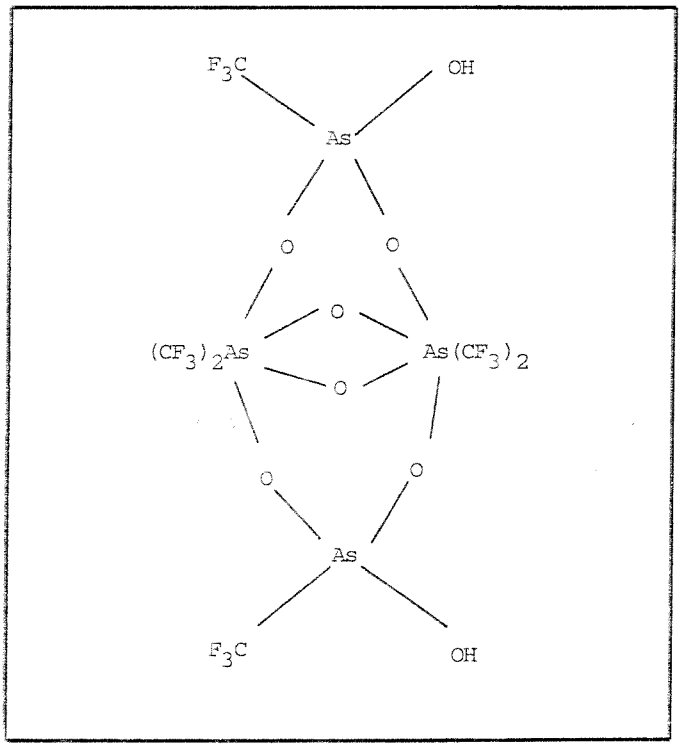

Figure 1.5: **Structure of a bicyclic compound containing both 4 and 6 co-ordinated arsenic species. Adapted from reference [7].**

1.1.6 Nomenclature of commonly encountered arsenic species

Four commonly encountered compounds have caused confusion in reports of work carried out in environmental systems. Some of the most commonly encountered analytical procedures cannot for example distinguish dimethylarsinate (DMAA) from other dimethylarsenic (DMA) species. As an example, in the initial work of Braman and Forebeck [8], it was suggested that dimethylarsinate was found in significant quantities in the marine environment. This was not however the case as they had not used a species-specific procedure that was capable of distinguishing dimethylarsinate from other dimethylarsenic species. Subsequent reports in the literature have erroneously referred to dimethylarsinate as having been identified as the major form of dimethylarsenic in the marine environment [9]. Therefore, in this thesis, mono- and di- methylated arsenicals are assigned as MMA or DMA, unless they are measured using a species-specific procedure.

1.2 Commercial and medicinal uses of arsenic and its compounds

1.2.1 Production and industrial uses of arsenic and its compounds

The majority of arsenic compounds are synthesised by reaction with arsenic trioxide, which is formed as a secondary product in the smelting of copper, zinc, silver and gold ores. Arsenic related compounds have found widespread use in the agricultural, glass manufacturing and the wood preservation industries.

Arsenate is widely used in the agricultural industry, particularly in the production of cotton. This is due to arsenate's ability to act as a drying agent, which prevents the build up of crop moisture, which would otherwise damage the cotton-harvester machinery. It has been reported that the arsenate drying properties can

also assist in the destruction of cotton stalk material, which is frequently attacked by insects [10]. This has the benefit of reducing cotton disease losses. Unfortunately, the use of arsenate as a cotton plant-drying agent has necessitated land use controls with livestock forbidden to graze in areas treated with arsenate. Land that has become contaminated with arsenate can be treated with lime or gypsum. This enables the arsenic species to be converted to the insoluble calcium arsenate forms which prevents runoff to water sources [11].

Metal arsenates such as lead arsenate and calcium arsenate were once frequently used as insectides in orchards and on vegetables. However, the development of organic insect control agents has rendered the use of metal arsenates obsolete.

Approximately 17% of the United States arsenic applications budget is attributed to the glass manufacturing industry. The incorporation of arsenic selenide (As_2Se) into glass formulations helps to reduce the intensity of a green colour (due to the presence of iron) present in container glasses [10].

In the wood preservation industry, certain arsenic compounds play a vital rôle as wood preservation agents. For example, chromated copper arsenate, a mixture of chromic and cupric arsenate, has been found to be an effective method in preventing wood infestation by microbes and fungae [10].

1.2.2 Medicinal uses of arsenic and its compounds

Arsenic related chemicals have been used throughout the centuries in medicine. One notable chemical, Fowler's solution, consisting of 1%, $KAsO_2$, was once widely used for the treatment of skin conditions, epilepsy and asthma. However, its use appears to be widely abandoned as unfortunate side effects such as vomiting and diarrhoea were reported when patients accidentally ingested the chemical solution in significant quantities.

One of the first widely recognised chemotherapeutic agents was Salvarsan (3,3'-diamino-4,4'-dihydroxyarsenobenzene). Salvarsan and its derivatives were used widely for the treatment of syphilis, venereal and tropical parasitic diseases. It was reported that a daily dose of 0.5g, which was injected intravenously over an extended period of time, had no apparent side-effects [10]. The use of penicillin and antibiotics have largely replaced Salvarsan in the treatment of venereal diseases in recent times.

A small number of arsenic containing drugs are known to be in use today. One example is a derivative of melarsoprol (Figure 1.6) which is used in the treatment of advanced cases of sleeping sickness. It has been reported that glycobiarsol, which is a bismuth salt of N-glycoloylarsanilate, is still effective in the treatment of tropical parasitic diseases such as amebiasis and moniliasis [4].

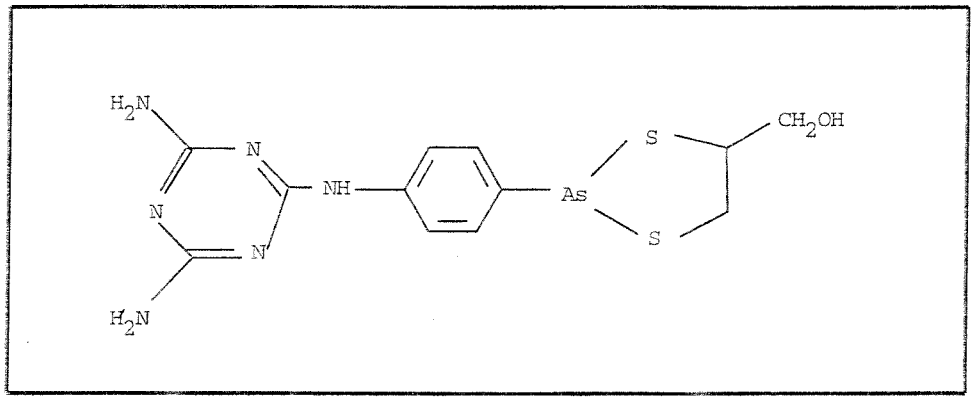

Figure 1.6: Chemical structure of melarsoprol. Adapted from reference [4].

1.2.3 Arsenic as a poison

Between the thirteenth and sixteenth centuries, 'white arsenic' (arsenic trioxide) proved to be an effective poison as it was odourless, tasteless and difficult to detect until the introduction of the Marsh Test in the early nineteenth century. One

case involving arsenic poisoning occurred at the University of St. Andrews in 1943 where sausage meat was contaminated with significant quantities of arsenic trioxide (between 125mg and 2.5mg/kg) [12].

The oral lethal dose of arsenic trioxide for humans is *ca.* 100mg. After several hours ingestion of a significant quantity of arsenic trioxide, symptoms include abdominal pain, vomiting, diarrhoea, cold clammy skin, a feeble pulse and weak respiration. Unless treated effectively, death can occur at any time between 24 hours and 4 days [13].

1.2.4 The toxicity of arsenic and its compounds

The toxicity of arsenic and its compounds are dependent upon the chemical form of the element. From Table 1.2 it can be observed that the arsine gases are amongst the most toxic forms of arsenic compounds whereas arsenobetaine is relatively non-toxic.

The acute toxicity of arsines arise from their potential haemolytic properties which can result in renal failure [14]. However, arsine species are very unstable and are not normally found in the natural environment due to their conversion to relatively non-toxic forms of arsenic compounds *via* alkylation and arylation [15].

Arsenite reacts covalently with sulphur atoms present in thiol groups on the active sites of a number of enzymes and tissue proteins such as keratin in human skin, nails and hair. Such a reaction deactivates the enzyme/tissue protein's active sites [16]. In humans, symptoms of acute exposure to arsenite include nervous disorders, kidney damage and chronic intoxication associated with decreased motor coordination.

Lewisite, a three co-ordinated arsenic species, was developed as a poison gas during the Second World War. The reaction mechanism appears to involve the

Compound	Toxicity (mg As/kg)	Comment
Arsenic trioxide	20.0 34.5	LD_{50} rat LD_{50} mice
Arsenite	14.0	LD_{50} rat
Arsenate	20.0	LD_{50} rat
Monomethylarsonate	700 - 1800 1800	LD_{50} rat LD_{50} mice
Dimethylarsinate	700 - 1200 1200	LD_{50} rat LD_{50} mice
Phenylarsonic acid	50.0	LD_{50} rat
Trimethylarsine oxide	10600	LD_{50} mice
Arsanilic acid	75 - 216	LD_{50} rat
Arsine	3.0	LD_{50} rat
Trimethylarsine	8000	LD_{50} mice
$Me_4As^+I^-$	82.0	LD_{50} mice
Arsenobetaine	> 10,000	LD_{50} mice

Table 1.2: The toxicities of selected arsenic compounds (adapted from reference [17])

reaction of the arsenic(III) species with the sulphydryl groups present in enzymes in the human body [18]. Once the reaction had taken place, selected active sites of the affected enzymes would be deactivated resulting in a reduction in the human body's metabolism. This process could be reversed by using British Anti-Lewisite (2,3 dimercaptopropanol) but the reverse mechanisms involved are not well understood.

For arsenate, the toxicity mechanism is not clear. A possible mechanism may involve the inhibition of ATP synthesis by uncoupling oxidative phosphorylation, which results in a breakdown of energy metabolism [19]. Arsenate is also known to displace phosphate in substituted monosaccharide compounds (for example glucose-6-phosphate is replaced by glucose-6-arsenate) [20].

Generally speaking, the organoarsenic species are less toxic than their inorganic counterparts due to metabolic conversion resistance to the toxic forms of arsenic [21]. It has been reported that arsenobetaine and arsenocholine do not have the ability to bind to the thiol groups in the enzyme active sites and are therefore resistant to conversion to the toxic forms of arsenic [13].

Arsenic compounds were originally thought to be carcinogens but repeated experiments involving the induction of tumours by oral exposure to arsenic compounds in mice and rats at maximum tolerated dosages for extended periods of time have yielded negative responses [21]. Despite this, arsenic is still listed as a carcinogenic agent by several official bodies such as the International Agency for Research for Cancer and the Occupational Safety and Health Administration.

Strict official controls of arsenic levels in food and water are in place to ensure personal safety and well being. Today, legally enforceable limits appear to be in the region of 1mg/kg for foodstuffs [13] and 50µg As/l for water supplies [22].

Until relatively recently arsenic was thought to be a non essential element in organisms but, however, evidence for its essentiality has been presented arising from deprivation studies on mammals [23]. Arsenic is thought to be involved in manganese and zinc metabolism and stimulates haemoglobin production.

1.3 The Environmental Chemistry of Arsenic

1.3.1 Occurrence and extraction of arsenic in the Earth's crust

Arsenic ranks as the 20th most abundant metal present in the Earth's crust [24]. Over 245 arsenic-containing minerals are known to exist in the Earth's crust with the principal ores being arsenopyrites (FeAsS), realgar (AsS_2) and orpiment (As_2S_3). The concentrations of arsenic in most rocks range from between 1mg/kg in sandstones up to 13mg/kg in shales but values between 300-5000mg/kg have been reported in African soils associated with gold deposits. Table 1.3 gives an overview of the arsenic levels in various rock materials in the Earth's crust.

The mobilisation of arsenic in land from ores is a result of weathering processes involving the dissolution and oxidation of mineral bound arsenic. Depending upon the underlying geology, arsenic concentrations in soil range from between 0.1 to 42mg/kg [25]. The retention of arsenic species is dependent upon the adsorption affinities of soil constituents. The presence of iron hydroxides, clay and alumina minerals can result in the binding and complete immobilisation of arsenic species. A study in arsenic contaminated soils in Wolfsberg (Carinthia, Austria) revealed that arsenate was the dominant arsenic species in the porewaters of the soil material studied [26]. However, evidence was presented for the microbiological conversion of the arsenate species in the porewaters of the soil material to the arsenite, monomethylarsenate (MMAA) and dimethylarsinate (DMAA) forms.

Crust type	Total arsenic concentration (mg As/kg)
Soils	1 – 40 0.1 – 40 300 – 5000
Rocks	
Sulphides (e.g. realgar and orpiment)	3 – 15.0
Igneous (e.g. basalt and granite)	0 – 113
Metamorphic (e.g. quartzite, slate)	0 – 17.6
Sedimentary (including coal)	0.1 – 32.0
Marine (e.g. shale)	0.1 – 490

Table 1.3: Table of arsenic concentrations in the Earth's crust (adapted from reference [17])

1.3.2 Arsenic in the atmosphere

Generally speaking, estimates of arsenic transport to and from the atmosphere average at around 30 x 10^9g As/year [27]. Natural sources by which arsenic is known to enter the atmosphere include sea spray, volcanic activity and forest fires while the remaining levels of arsenic come from anthropogenic activity such as ore processing activities in the chemical industry and waste incineration. Removal processes such as dry deposition and rainfall counter balance these inputs of arsenic into the atmosphere.

Arsenic in the atmosphere is thought to be in particulate form, with speciation controlled by redox chemistry, depending upon the concentration levels of H_2O_2, O_2 and SO_2 present [28]. Walsh has suggested that 90% of atmospheric arsenic is thought to be present above urban areas with the remainder present in the oceanic environment [27]. A recent study made on the city of Isfahan in Iran which suffers from traffic congestion problems revealed that over 50% of the total arsenic present in the city atmosphere was in the form of As(III) species [29].

1.3.3 Arsenic in the aquatic environment

The majority of the arsenic release into the water column comes from both anthropogenic activities, biological activity and natural sources. The arsenic species can mobilise themselves in the water column by means of dissolution, suspended particles or as part of the bulk sediment load. Once mobilised, the arsenic species normally end up entering the oceanic environment.

Concentration levels of dissolved arsenic species in the oceans are in the range 1.0-1.8µg As/l [30] with an average lifetime of 6 x 10^4 years [31]. Table 1.4 gives a listing of arsenic concentrations and speciations in selected hydrosphere environments. It is also noted from Table 1.4 that the majority of arsenic in the

Water environment type	Arsenic concentrations (µg As/l)					
	Total arsenic	As(V)	As(III)	As_{IN}	MMA	DMA
Rivers/Lakes						
Tagus (Portugal)		2.21-6.39	0.11-0.94		trace	trace
Keg Lake (Canada)		58.0	5.9			
Humber 1989 (UK)				1.05	0.04	0.11
Thames 1990 (UK)				2.12	0.04	0.14
Dover Strait (UK)				1.30	0.01	0.04
Seawaters						
South California Blight		0.16-1.45	0.009-0.87		trace-0.03	0.011-0.26
Indian Ocean	1.3-2.2					
Groundwaters	0.001-800					
Rain	0.019-0.46					

Table 1.4: Arsenic concentrations (in µg As/l) for various water environments (with acknowledgment to [17],[108] and [109]).

natural water environment occurs in the form of As(V) but other arsenic species such as As(III), MMA and DMA also exist.

Arsenic removal from the oceans occurs by deposition onto organic sediment material, arsenic volatilisation into the atmosphere or biological take-up by marine organisms. Francesconi and Edmonds gave a review on arsenic uptake by marine organisms in [32].

The freshwater algae *Chorella vulgaris* has been studied extensively to determine its effects when exposed to arsenic and its compounds. *Chorella vulgaris* has a high resistance to arsenic toxicity with levels of tolerance up to 10,000 mg As/kg being reported. However when *Chorella vulgaris* is exposed to arsenite, growth is inhibited at 10 mg As/kg [33]. Other studies on *Chorella vulgaris* reveal that it has the ability to take up and methylate arsenate *in vivo* to form mono-,di- and trimethylated arsenic species but their structutal forms have not been identified [34]. A commercial study made by the company Nostoc determined the optimum conditions for arsenic bioaccumulation with a view to using the algae as an arsenic removal agent in polluted water environments [35].

1.3.4 Arsenic in the terrestrial environment

Terrestrial plants and vegetables that have not been contaminated with arsenic have arsenic concentration levels of approximately 0.2mg As/kg and 0.4mg As/kg respectively. These levels are generally lower than those found in marine plants [36]. One terrestrial plant taken from an arsenic mine, the *Andropogon scoparis* bluestem, was found to have evolved a resistance to high levels of arsenic [37].

Animals, including humans have been reported to be able to methylate inorganic forms of arsenic. A study carried out by Buchet revealed that after human ingestion of a fixed quantity of sodium arsenite, the inorganic arsenic species were methylated to form mono- and dimethylarsenic species. There was no

evidence in the study of any other arsenic species being present [38]. However, other studies of arsenic speciation in human urine revealed that out of a total arsenic concentration of 17.2µg As/l, only 2µg As/l was attributed to inorganic (As(III) + As(V)), MMA and DMA arsenic species, implying the presence of unidentified forms of arsenic [39]. Arsenobetaine has only recently been confirmed as one of the unidentified forms of arsenic in human urine [40].

1.4 Arsenic in marine biota

1.4.1 The uptake of arsenic by marine organisms

Marine organisms such as seaweed [41], molluscs [42] and crustacea [43] have the ability to take up and store within their cellular structures the dissolved arsenate present in the water column. The other dissolved arsenic species (apart from arsenite) present in the water column may be taken up by marine organisms but it is normally assumed that these compounds are the products of biochemical mechanisms rather than the starting products of arsenic metabolism [44]. The actual mechanisms involved in the entry of arsenic into marine organisms are not clear but it is well established that marine organisms must have the ability to assimilate phosphate in areas of very low nutrient concentrations. As the chemical properties of arsenate and phosphate are similar, it is unavoidable that some arsenate is taken in by some marine organisms.

Radiolabelled ^{74}As arsenate was introduced to the brown algae (*Fucus spirallis*). After 5 minutes exposure, 'inorganic' arsenic was found to be the dominant arsenic form alongside with two water soluble organoarsenic compounds and a small amount of lipid soluble arsenic species. Over a period of time the arsenate concentration decreased while the proportion of the water soluble organoarsenic compounds and lipid soluble arsenic species increased. After 16 hours the lipid-soluble arsenic species comprised 60% of the arsenic budget in the algae. Klummp and Peterson were then able to conclude that the incorporation of

arsenate by *Fucus spirallis* was by a mechanism in which the arsenate is first converted to the water soluble arsenic compound and then to a lipid soluble arsenic form [45].

In culture, arsenate uptake is observed to decrease when phosphate uptake increases in selected marine organisms (such as the phytoplankton *Skeletonema costanum* [44] and *Champia parvula* [46]). Such behaviour suggests that discrimination between arsenate and phosphate occurs when phosphate concentrations are in plentiful supply. However, in other marine organisms [46] the presence of a high concentration of phosphate leads to a higher rate of incorporation of arsenate into the cellular structure of the organism.

As some forms of algae are consumed by other marine organisms, it is often assumed that trace element levels accumulate along the food chain. However no evidence has been presented that this is the case for arsenic [32].

1.4.2 Arsenic incorporation into marine organisms

Marine organisms have been shown to have the ability to accumulate significant quantities of arsenic within their cellular structures. The arsenic concentrations of selected crustacea from Scottish coastal waters are, on average, approximately 6000 times higher than the arsenic concentration in the seawater [32]. The high levels of arsenic found in most marine organisms therefore suggest that they retain the arsenic, though not necessarily in the form of arsenate.

A significant quantity of arsenic found in marine organisms occurs in the form of complex methylated arsenic species such as arsenobetaine, arsenocholine, and arsenosugars. The exact mechanisms involved in the formation of those complex methylated arsenic species are as yet, unclear.

Two pathways have been postulated for the metabolism of arsenate species in marine organisms:

a. The methylation of inorganic arsenic species

The work of Challenger on microbial methylation [47] still provide a basis for the understanding of how arsenate is converted to methylated arsenic species. In the presence of the *Scopulariopsis brevicaulis* mold, arsenate is transformed to trimethylarsine by the sequential reduction and oxidative methylation of the arsenic species as shown in Figure 1.7. As Challenger was not able to identify the monomethylarsenic (MMA), dimethylarsenic (DMA) and trimethylarsine oxide (TMAO) intermediates during the reaction pathway, he noted that when MMA, DMA and TMAO were added to *Scopulariopsis brevicaulis*, trimethylarsine was formed. The source of the methyl groups was suggested to have been S-adenosylmethionine which had already proved its abilities as a methyl group donating agent in enzyme systems. However, the origin of the electrons involved in the reduction of arsenic is unclear.

A recent re-evaluation of Challenger's work has been carried out by Cullen and Reimer [48] where small quantities of arsenite, arsenate, MMAA and DMAA were added to the cultures of *Scopulariopsis brevicaulis*. It was observed that the intermediates suggested by Challenger were present but the major end product was TMAO rather than trimethylarsine. What was not clear was whether tetramethylarsonium species were present in the *Scopulariopsis brevicaulis* cultures studied as its presence was not reported by Cullen and Reimer.

b. Microbiological transformations involving arsenic species

Marine microorganisms have the ability to transform arsenic to other forms but the mechanistic aspects are poorly understood. It has been suggested that the energy released by seawater bacteria when they convert arsenate to arsenite

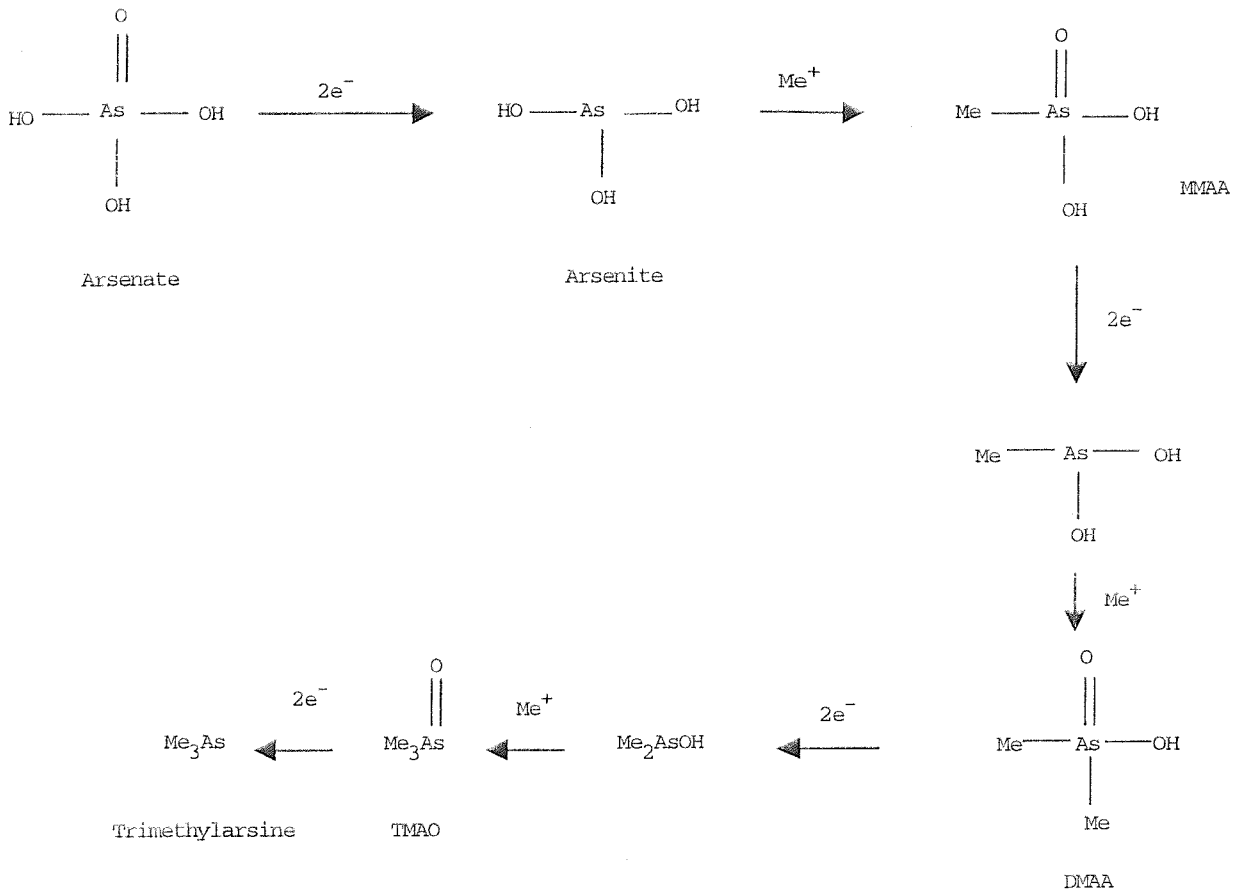

Figure 1.7: **Challenger's pathway for the methylation of arsenic by microorganisms [47].**

allows for microbial growth [49]. For example, a microorganism isolated from freshwater sediments was reported to derive energy by converting arsenate to arsenite [50]. Johnson [51] and Sanders [52] have reported that microorganisms in seawater also have the ability to demethylate and oxidise methylated arsenic compounds.

The introduction of arsenobetaine (Figure 1.8), a naturally occurring biochemical arsenic species, to microorganisms derived from aerobic sediments, algae and the intestine of molluscs revealed that in all cases arsenobetaine easily decomposed to trimethylarsine oxide (TMAO) and DMAA. With the sediment, however, further degradation to arsenate was observed [53].

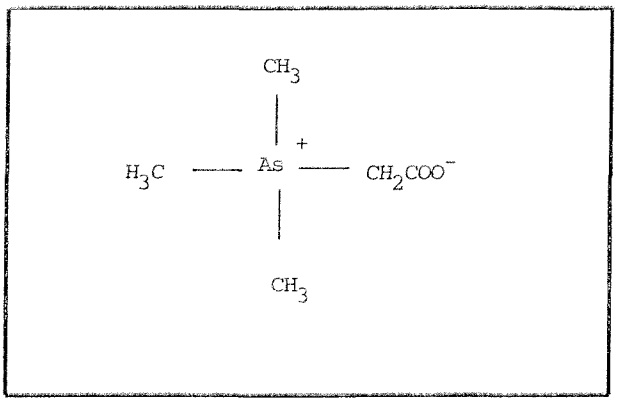

Figure 1.9: The structure of arsenobetaine

Cullen and Nelson [54] carried out a number of experiments involving the mussel *Mytilus edulis* cultured in media containing either ^{3}H[MMA] or ^{3}H[DMA]. ^{3}H-labelled arsenobetaine was formed from both ^{3}H[MMA] and ^{3}H[DMA]. Interestingly, ^{3}H-labelled arsenobetaine was also found in the seawater. Higher levels of arsenobetaine were present in the seawater when the mussels were absent. This behaviour was attributed to the microbial biosynthesis of arsenobetaine and its accumulation by the mussels.

1.4.3 Arsenic compounds in marine algae

It is well established that inorganic arsenic, water soluble organoarsenic and lipid soluble arsenic species all contribute towards the arsenic budget of marine algae. The most commonly reported organoarsenic species found in marine algae are the arsenosugars which were first isolated and identified by Francesconi and Edmonds [55]. In addition, the work of Morita [56-57] has contributed significantly to the elucidation of the structures of lipid soluble arsenic species found in certain marine algae.

1.4.3.1 Arsenosugars

Since the identification of two novel arsenosugars in the marine alga *Ecklonia radiata* [53], a total of 15 different arsenosugar forms have been identified in marine algae (Figure 1.9). The majority of the arsenosugar forms are dimethylarsenoylribosides differentiated by slight differences in the non-sugar component.

Francesconi and Edmonds [32] have postulated that arsenate is the primary source of arsenic for the formation of arsenosugars but the transformations involved are not yet clear. However, they have proposed a reaction scheme for the formation of arsenosugars (Figure 1.10) making use of Challenger's arsenate methylation reaction scheme with a slight modification. In Challenger's mechanism S-adenosylmethionine was used solely as a methyl donor whereas Francesconi and Edmonds have suggested that adenosylmethionine (Ado-Met) acts both as a methyl and ribosyl donor. After the donation of two methyl groups, Ado-Met transfers its adenosyl component forming the arsenic-containing nucleoside intermediate (4) shown in Figure 1.10. Upon glycosidation this results in the formation of the wide variety of arsenosugars present in marine algae species.

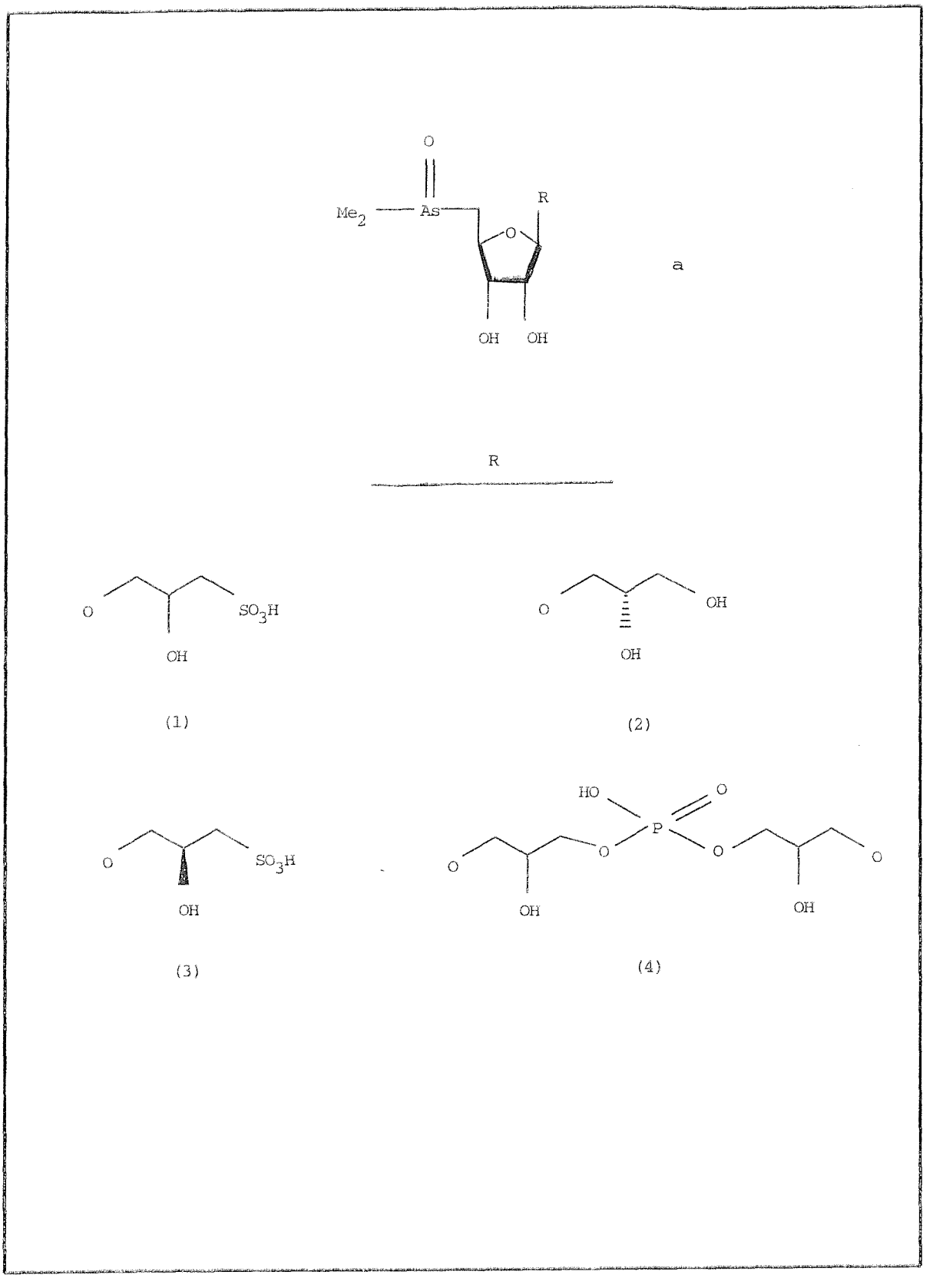

Figure 1.9: Structures of some dimethylarsenosugars [32].

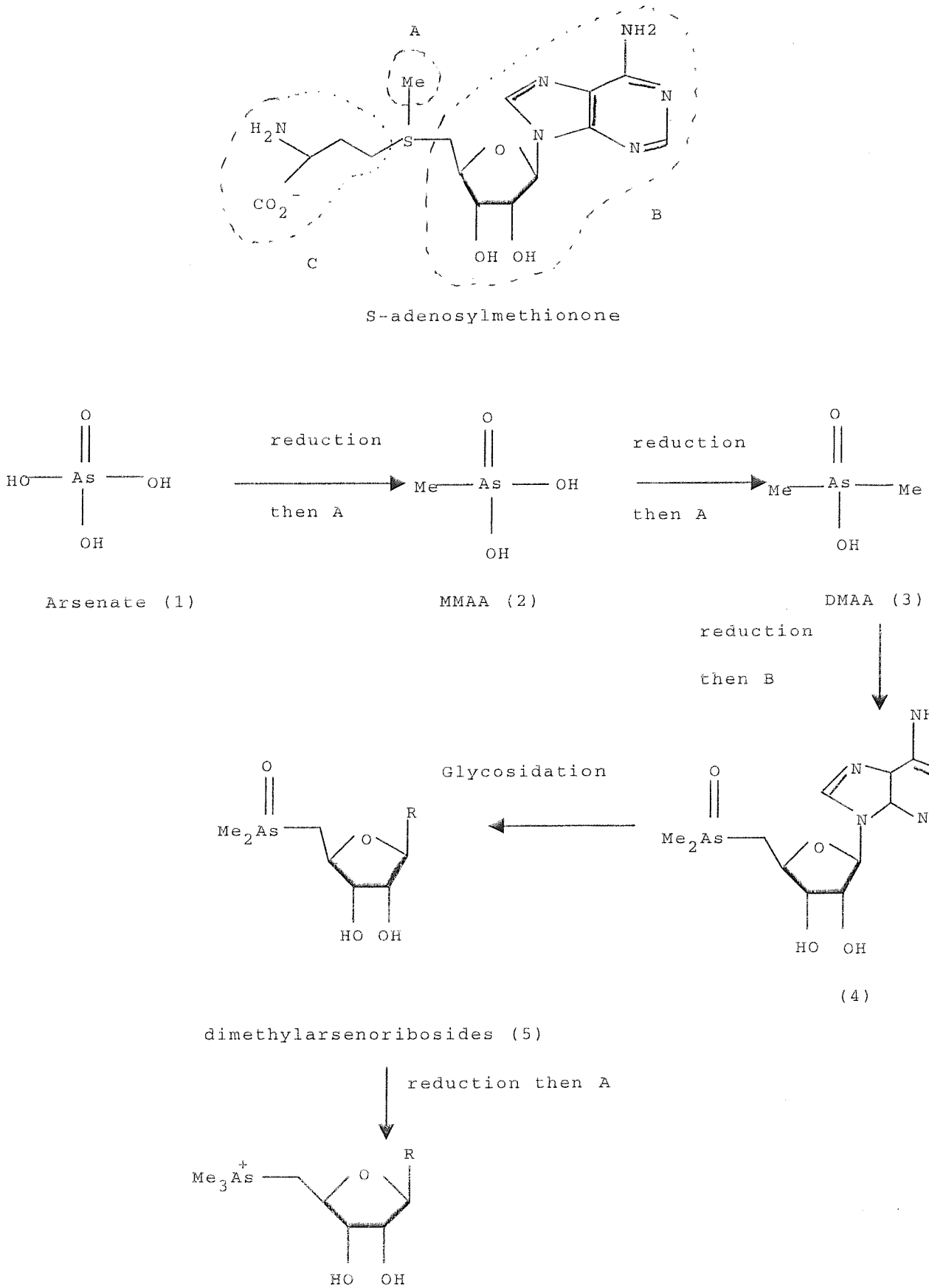

Figure 1.11: Proposed mechanistic pathway for arsenosugar biosynthesis [32]

Evidence supporting Francesconi and Edmond's reaction mechanism comes from two different sources. One study involving the kidney of the giant clam (*Tridacna maxima*) revealed that the arsenic-containing nucleoside intermediate (4) shown in Figure 1.10 was present [58]. The second source of support involved the work of Cantoni [59] who mentioned that the transfer of the individual alkyl groups present in the Ado-Met structure to a suitable receptor such as the dimethylarsinate species shown in structure (3) of Figure 1.10 is very feasible. As an example, it has been suggested that the transfer of group C in the Ado-Met structure is involved in the biotransformation of a nucleophilic nitrogen compound (Figure 1.11).

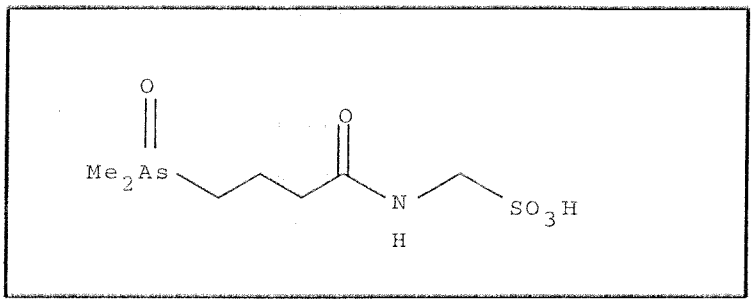

Figure 1.11: Structure Y

The order of methylation and adenosylation proposed in Figure 1.10 is believed to be correct, but Francesconi and Edmonds [58] have noted that adenosylation may precede methylation. However, in marine algae very little evidence has been presented to suggest that this may be the case.

In addition to the dimethylarsinoylribosides, trimethyl arsenosugars have also been isolated from marine algae, but in very small quantities (< 1%) [32]. This suggests that the methylation of arsenate to arsenosugars by marine algae ceases at the trialkyl stage. However, as the rates of formation of

tetramethylarsonioribosides are very high, the chances of detecting these arsenosugar species in marine algae are likely to be very low.

Dimethylarsenosylribosides are thought to be involved in algal cellular redox reactions [32]. Under reducing conditions, dimethylarsenosylribosides are decomposed to arsines. These arsine species can then be regenerated, under mildly oxidising conditions to their original dimethylarsenosylriboside forms.

1.4.3.2 Arsenolipids

There are a number of reports in the literature on the total arsenolipid concentrations present in marine algae but very little information is forthcoming on the actual structures of the individual arsenolipid species. Initial studies by Francesconi and Edmonds [60] on the glycerophospho compound illustrated in Figure 1.12 present in marine algae samples led to the suggestion that the arsenolipids were related to the phospholipids. In light of this information, Morita [57] was subsequently able to isolate and identify the structure of one of the arsenolipid compounds present (Figure 1.13) in the brown alga *Undaria pinnatifida*.

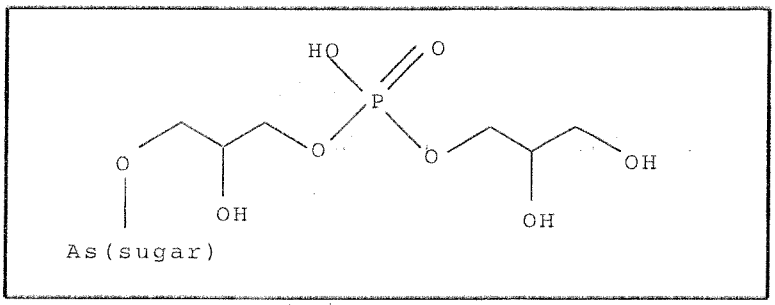

Figure 1.12: Glycerophospho compound
(for As-sugar structure see Figure 1.9 (a))

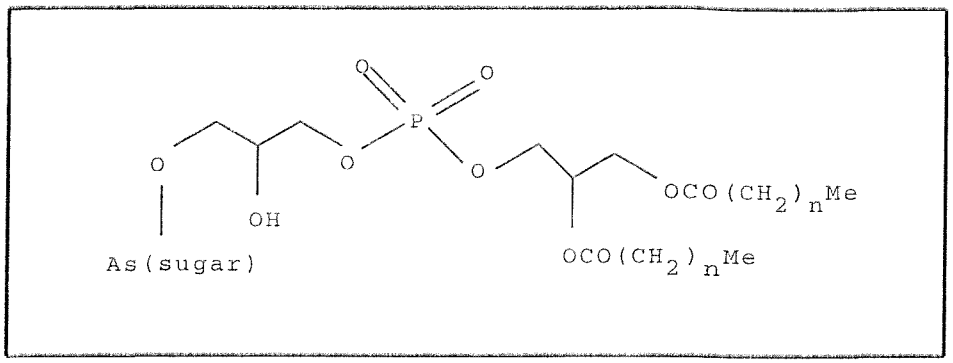

Figure 1.13: Arsenosugar structure isolated by Morita (for As-sugar structure see Figure 1.9(a))

It is possible that other arsenolipid compounds present in marine algae are acylated derivatives of the arsenosugar structure shown in Figure 1.14 [61].

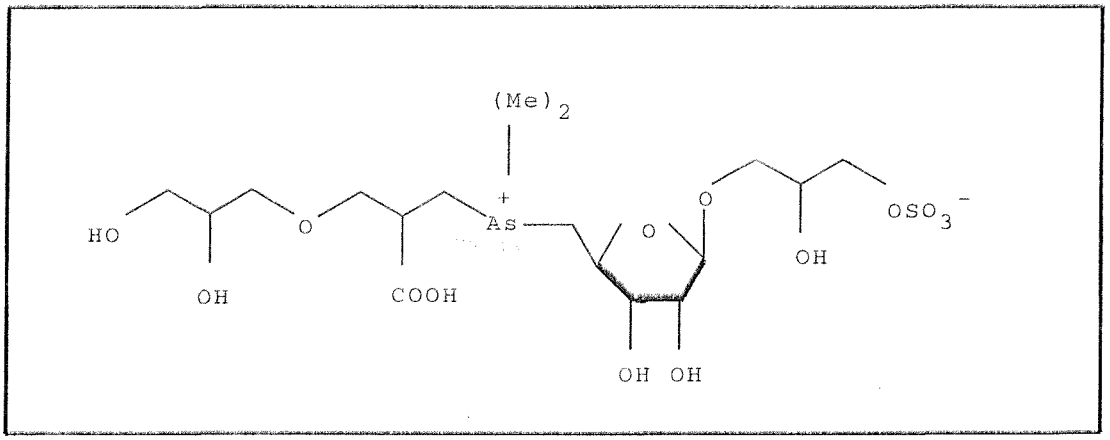

Figure 1.14: Acylated derivatives of this arsenosugar are possible arsenolipids.

1.4.4 Arsenic speciation in marine animals

One of the first arsenic species identified in a marine animal was arsenobetaine when it was isolated from the rock lobster (*Panulirus cygnus*) [62]. Subsequent research has revealed that arsenobetaine is the predominant form of arsenic present in most marine animals. Table 1.5 gives a listing of arsenic concentrations and arsenobetaine content of selected marine animals.

Animal	Arsenic content (mg/kg, wet weight)	Arsenobetaine content (% of total arsenic)
FISH		
Elasmobranchs (7)	3.1-44.3	≥ 94
Teleosts (17)	0.1-166.0	48 to > 95
CRUSTACEANS		
Lobsters (4)	4.7-26.0	77 to > 95
Prawns (5)	5.5-20.8	55 to > 95
MOLLUSCS		
Bivalves (4)	0.7-2.8	44-88
Gastropods (6)	3.1-116.5	58 to > 95

Table 1.5: Arsenic concentrations and arsenobetaine content of selected marine animal species. Adapted from [32].

Other arsenic species present in marine animals include the tetramethylarsonium ion (TeMA) [63] which, in some bivalve molluscs can be present in higher concentrations than arsenobetaine. In some marine animals arsenosugars, which may originate from marine algae, are present in sizeable concentrations, especially in the giant clam (*Tridacna maxima*) [56]. This is believed to be due to the

presence of a symbiotic unicellular alga within its cellular structure, which can accumulate large quantities of arsenosugars.

Minor components of arsenic in marine animals include inorganic arsenic which accounts for approximately 2% of the total arsenic burden. Improvements in the sensitivity and detection limits of analytical methods have led to the identification of trimethylarsine (TMAO) in fish [64], arsenocholine in marine turtles [65] and trimethylarsenic (TMA) in prawns and lobster [66].

1.4.4.1 Arsenobetaine

Arsenobetaine has been detected in a wide variety of marine animals from the plaice (*Carcharinus obscurus*) [67], the American lobster (*Homarus americanus*) [68] to the octopus (*Paroctopus deofleini*) [69]. Arsenobetaine can account for over 90% of the total arsenic burden in certain marine animals. In the cephalopods, for example, arsenobetaine can comprise over 95% of the total arsenic present [32].

One pathway for the formation of arsenobetaine in marine animals is thought to involve phospholipid biosynthesis with the arsenic species substituting for nitrogen due to the chemical similarities between arsenobetaine and glycine betaine [82]. The arsenic species are thought to originate from either arsenocholine or arsenoethanolamine. However, Francesconi and Edmonds point out in their review that no experimental evidence has as yet been presented to support the presence of arsenobetaine or its related derivatives in the phospholipid metabolism pathways of marine animals [32].

Subsequent research has led to the development of the theory that arsenobetaine originates from the dimethylarsinosylribosides of marine algae [32]. Dimethylarsinoylribosides have been shown to degrade readily to dimethylarsinoylethanol (DMAE) which provides the two carbon chain necessary

for arsenobetaine. Further treatment of DMAE by oxidation and methylation, can result in the formation of arsenobetaine. Unfortunately, no evidence has been forthcoming to support the presence of DMAE in marine animals and efforts to methylate DMAE under environmental conditions have proved to be unsuccessful. A recent study [71], however, has revealed the presence of both dimethylarsinoylacetic acid (Figure 1.15) and arsenosugars in a mussel extract. If the presence of dimethylarsinoylacetic acid is confirmed in other marine organisms, then a strong case can be made for the interconversion of dimethylarsinoylacetic acid (Figure 1.16) to DMAE and its subsequent transformation to arsenobetaine.

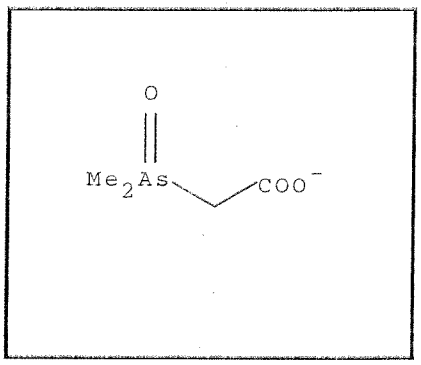

Figure 1.15: Structure of dimethylarsinoylacetic acid

As mentioned in Section 1.4.3.1, trimethylarsonioribosides have been isolated from marine algae. Their conversion to arsenobetaine would involve a much simpler process than the dimethylarsinonylriboside forms as the mechanism would only involve a two stage process: the cleavage of the C3-C4 carbon-carbon bond in the riboside component of the arsenosugar, leading to the formation of arsenocholine and its subsequent oxidation to arsenobetaine [72]. Simulation studies have revealed that when a fixed quantity of trimethylarsonioriboside is added to the marine animal under marine environmental conditions, there is a high rate of formation of arsenobetaine. The high rate of arsenobetaine conversion can be attributed to the fact that no additional methylation mechanisms are involved in

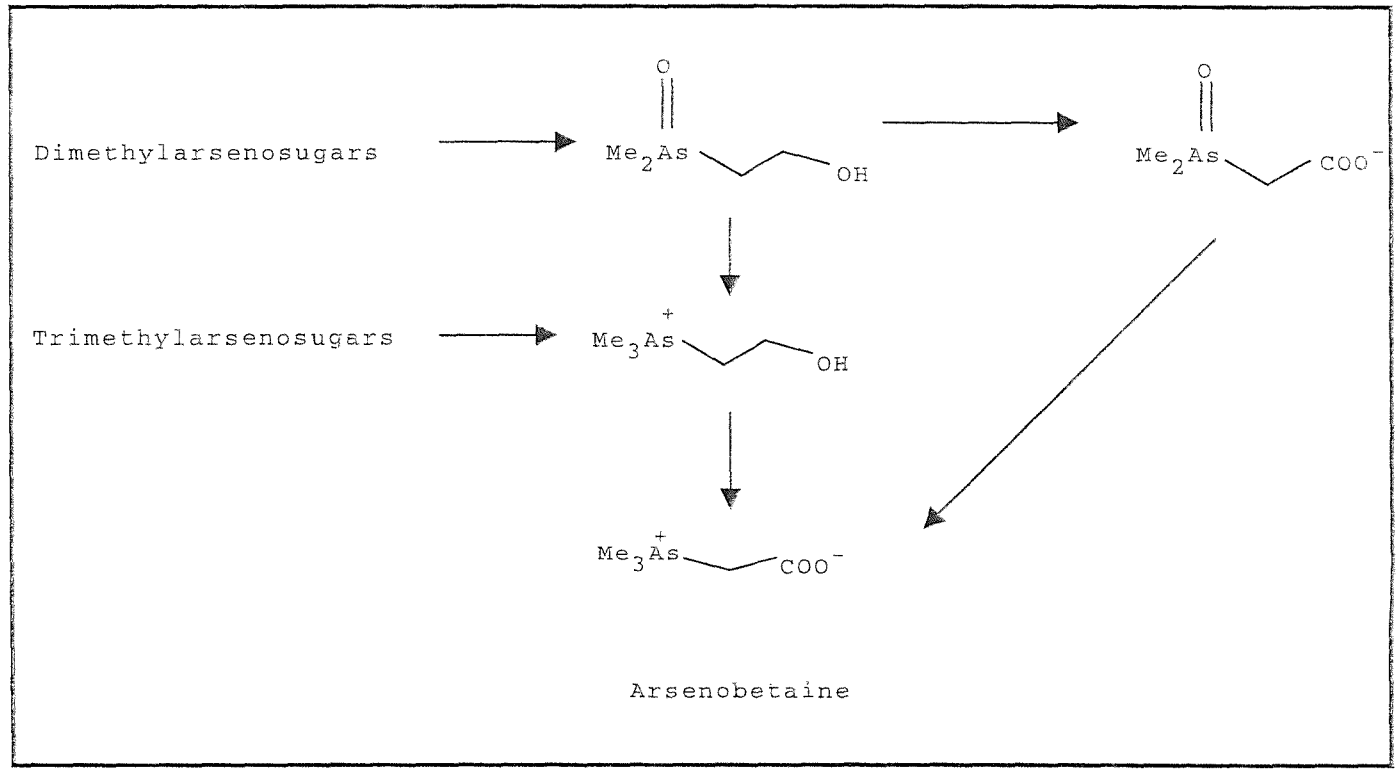

Figure 1.15: Possible pathway for the formation of arsenobetaine [34].

the transformation of trimethylarsonioriboside species to arsenobetaine. However as the trimethylarsonioriboside species only comprise 1% of the arsenic budget in marine algae, it is not clear how much of a contribution the trimethylarsonioriboside species make towards the formation of arsenobetaine in marine animals [32].

1.4.4.2 Arsenosugars

The presence of arsenosugars in marine animals is currently believed to be due to the digestion of microalgae or the presence of symbiotic organisms. Arsenosugars have been detected in shrimp [73] (*Crangon crangon*) and gastropods [74] (*Thais bitubercularis, Thais distinguenda* and *Morula musiva*).

A study of the mussels (*Bathymodiolus puteoserpentis*) from a hydrothermal vent in the Mid Atlantic Ridge, revealed that a significant quantity of dimethylarsinoylribosides were present [75]. This was attributed to the activities of the symbiotic bacteria present in the mussel species. The authors of the study, however, express doubts as to why the arsenosugar species are formed. Two suggestions were put forward: either that the arsenosugars were formed as a direct result of detoxification mechanisms present within the cellular structure of the mussel or that arsenosugar production is part of the mussel's normal physiological functions.

1.4.4.3 Arsenocholine

Arsenocholine has been found in trace quantities in fish (< 0.1%), molluscs and crustacea [64]. A derivative of arsenocholine, phosphatidylarsenocholine has been isolated and identified in low quantities in the digestive gland of a lobster [56]. In studies of the starspotted shark (*Mustelus manazo*), arsenocholine was found in the hydrolysates of the muscle tissue implying that arsenolecithins were present [76].

1.4.4.4 Arsenolipids

Dimethylated arsenolipids have been suggested to be present in the starspotted shark (*Mustelus manazo*) [76].

1.4.4.5 Tetramethylarsonium ions

Tetramethylarsonium forms of arsenic are found in trace quantities in the gill of the clam (*Meretrix lusoria*) [77].

1.4.4.6 Trimethylarsine oxide

TMAO is thought to be biosynthesised in two ways: as one of the products formed by the degradation of arsenobetaine by microorganisms or by the methylation of arsenate by microbes present in ingested flora in the gut of marine animals [78]. TMAO has been detected in certain species of molluscs and crustacea [67].

1.4.4.7 Trimethylarsine

TMA has been isolated and identified in six species of prawns and two species of lobster. TMA is thought to originate from the methylation of arsenate by microbes in the digestive glands of the marine animal species concerned [66].

1.5 Aquatic arsenic

1.5.1 Arsenic inputs into the Oceans

1.5.1.1 Input from rivers

Arsenic levels in river waters, where there is little or no evidence of anthropogenic activity, are in the range 1.0-2.0μg As/l [79,80]. As the concentration levels of arsenic are so small, the transport of dissolved arsenic from such river waters to the Oceans is low. In contrast, in rivers and estuarine waters where there is evidence of substantial anthropogenic activity, arsenic transport to the Oceans is much higher depending upon the concentrations of both the particulate and dissolved arsenic species that are present. For example in Europe, where there is a higher level of industrial activity than North America, the average water column concentrations are 3.5 and 0.5μg As/l respectively [81].

The presence of arsenic in particulate matter can either increase or decrease the dissolved arsenic content in the water column by desorption or adsorption mechanisms [82]. However, most particulate arsenic species are inert and rarely result in a change in the overall arsenic budget [83]. One significant method of arsenic removal in areas of increasing ionic strength and pH is the adsorption of arsenic by hydrated iron(III) oxides. The hydrated iron (III) oxides are thought to act as a reservoir for dissolved arsenic compounds such as As(V) species in the South California (US) [50] The removal of those dissolved arsenic compounds can significantly alter the arsenic flux to the Oceans.

1.5.1.2 Inputs from the atmosphere

In the presence of anthropogenic activity, atmospheric arsenic transport to the Oceans is likely to be significant. For example, urban dust particles containing arsenic compounds can easily dissolve up to 50% of their arsenic content into the

water column [84]. This may lead to higher levels of dissolved arsenic than expected in the water system studied. In areas of little or no anthropogenic activity, the atmospheric arsenic species can derive from the colloidal dispersion of solid or liquid particles from the land mass. These solid/liquid particles rarely contain significant quantities of arsenic. This is the case observed in the Georgia Bight (U.S.A) system [85].

Methylarsenic species, which are thought to originate from biomethylation transformations [86], have been found to comprise 10% of the total atmospheric arsenic budget [87].

1.5.2 Occurrence and speciation of arsenic in natural waters

The concentration of dissolved arsenic in the deep waters of the Pacific and Atlantic Oceans have been reported to be within a narrow concentration band of between 1.0-1.8μg As/l with inorganic forms of arsenic being the dominant arsenic species [88,89]. In contrast, estuarine water environments provide a more variable arsenic concentration range with a higher probability of locating a greater variety of arsenic species [90].

Evidence has been presented to support the presence of As(V) (likely to be in the form of arsenate), As(III) (suggested to be in the form of arsenite), monomethylarsenic (MMA), dimethylarsenic (DMA), monomethylarsenate (MMAA), dimethylarsinate (DMAA) [91] and a group of arsenic species known collectively as 'hidden' arsenic in natural waters [90]. 'Hidden' and 'refractory' arsenic forms are species of arsenic which are not capable of being detected by conventional hydride-based analytical methods unless pretreated by photochemical or digestion methods.

1.5.3 Factors influencing arsenic speciation

1.5.3.1 Biological effects on arsenic speciation and concentration

It is well established that marine organisms play a crucial role in controlling the concentrations and speciation of arsenic in the water column. For example, in phytoplankton cultures arsenate is taken up and metabolised to form As(III), MMA and DMA species before leaving the phytoplankton cells. A quantity of arsenate, however, is retained within the phytoplankton cell structure and is converted to complex organoarsenic compounds. The actual route to these complex organoarsenic compounds is species dependent.

The majority of the dissolved methylated arsenic which is found in the water column during the summer season is a direct result of biological activity. The work of Howard reveals that there is a time lag after the commencement of the spring phytoplankton bloom before MMA and DMA are identified in the water column. The MMA and DMA species were thought to be released from bacterial and abiotic decay of plankton and by grazing zooplankton [92].

A number of major biochemical steps are currently believed to result in the storage of methylated arsenic as arsenosugars in flora and arsenobetaine in marine animals. Once the arsenosugars and arsenobetaine are released into the water column they have a limited lifetime as they breakdown to dimethylarsenic compounds. Arsenobetaine, in particular, has been found to be an unstable compound and decomposes easily to trimethylarsine oxide (TMAO), DMAA and inorganic arsenic. DMAA has been found to be a very difficult compound to demethylate and consequently is thought to have a relatively long lifetime in the water column [93].

'Hidden' and 'refractory' arsenic compounds present in the water column are thought to originate from the degradation of the products released from the

substantial reservoir of dimethylarsenic species held in marine organisms during the organism's life and after death. Evidence has been presented to suggest that the marine organism's excretion and decomposition routes contribute jointly to the presence of dimethylarsenic in the water column but the relative contributions of the two different routes have yet to be established [91].

Marine bacteria and yeasts are also involved in arsenic speciation. For example, Reimer and Thompson presented the first evidence of arsenic biomethylation by bacteria in the interstitial waters of sediments in Rupert Inlet and Alice Arm (British Columbia, Canada) [94]. Bacteria and yeast have also been shown to be involved in arsenic demethylation pathways [95], As(V) reduction [49] and in the formation of volatile arsines [95] in the sediment.

1.5.3.2 The effects of the sediments on arsenic concentration and speciation

Sediments have long been acknowledged to be a major reservoir and a possible source of arsenic for the water column. A study of sedimentary material in the Yellowknife region of British Columbia (Canada) revealed that the arsenic concentration levels of the sediments were much higher than those recorded for the water column. For example, in Peg Lake, the total dissolved arsenic present in the water column was calculated to be 422µg As/l whereas the total dissolved arsenic concentration in the 0-16cm sediment layer was found to be 11900µg As/l, a 28 fold increase in concentration [96]. It was also noted that the water column transport of dissolved arsenic species from high arsenic concentration areas to low arsenic concentration areas did not necessary result in a significant increase in the low arsenic concentration area. This implies that some of the dissolved arsenic was incorporated into the sediment material.

Adsorption, diffusion and precipitation mechanisms are involved in the incorporation of arsenic into the sediments. These mechanisms tend to prevent the transport of high levels of dissolved arsenic species which may be present at the

mouth of a river, as was the case in the Moira Lake (Ontario, Canada), to the open sea [97]. Once arsenic is bound to the sediment, arsenic transport to the open sea will be much slower than if it were in the form of mobile dissolved arsenic.

Arsenic has a particular affinity for hydrous iron (III) oxides. The adsorption of the inorganic forms of arsenic onto hydrous iron (III) oxides has been extensively studied with both the As(III) and As(V) species observing Langmuir type isotherm behaviour where absorption is limited by surface saturation. The presence of alumina and clay minerals such as kaolinite and montmorillinite can assist in the complete removal (at pH 4) of combined iron(III) oxide-arsenic forms from the water column. It has also been reported that arsenic has an affinity for hydrated manganese (IV) oxides, but they are not as effective as hydrated iron(III) oxide [98].

The redox potentials of the sediments are controlled by the bacterial degradation of organic material and the oxidising agents (such as O_2, SO_4^{2-}, NO_3^-, Fe^{3+} etc.) are taken up in a stepwise fashion according to their availability. In sediments containing high concentrations of organic matter where there is a high rate of sedimentation, the organic matter will be consumed rapidly which makes it difficult to distinguish the depths where the reduction of the individual oxidation agents will begin to occur. In sediments of low organic content, it is easier to observe the depths where individual oxidising agents make their presence felt. Reimer has observed that during the reduction of insoluble iron(III) to the more soluble iron(II) species, arsenic dispersal into the sediments began to occur [99]. This behaviour was observed at a depth well above the region where sulphate reduction occurs.

Andreae's study of inorganic arsenic speciation of high organic content, intense sulphate reduction and low redox potential sediment material in the Santa Barbara Basin (U.S.A) revealed that As(V) and As(III) species behaved differently in terms of transport to and from the sediment material [80]. For example, the

concentration of As(V) in the upper fringes of the sediment material (1.1μg As/l) was lower than that recorded in the water layer above it (1.7μg As/l). This implied that there was a mass transport of arsenate from the water column to the sediment. With the As(III) species, the reverse behaviour was observed: As(III) concentrations in the upper sediment material was at 0.3μg As/l whereas in the water layer the As(III) concentration was 0.2μg As/l which suggested an As(III) flux to the water column from the sediment material.

Studies on the inorganic content of sediments in the Pontevedra Estuary [100] (Vigo, Spain) revealed that due to high levels of anthropogenic activity which brought about a low level of dissolved oxygen, the concentration of As(III) species in the sediment was much higher than the As(V) concentration. However, there was no relationship between the As(III) concentration and depth.

The presence of methylated arsenic species in the interstitial waters of sediment material has been taken as evidence supporting the argument that biological methylation/demethylation pathways play a critical role in the cycling of arsenic in the marine environment. Methylated arsenic species have been located in sediment material in estuarine systems such as the Tamar (Devon, UK) and in lakewater environments such as the Yellowknife region in British Columbia (Canada). In the Tamar estuarine system, methylated arsenic species can contribute up to 1-4% of dissolved arsenic in localised interstitial waters [101] whereas in selected sites of the Yellowknife region, methylated arsenic species comprised between 0.2-0.8% of the interstitial waters dissolved arsenic budget [96].

Takamatsu has hypothesised that the presence of methylated arsenic species in the sediment material of lakewaters is due to their release from organic matter which originated from phytoplankton cultures at or near the sediment surface [102]. Studies of phytoplankton cultures in freshwater or marine aquatic systems have revealed that the extent of arsenic methylation is governed by how efficiently

arsenic assimilation occurs within the culture and the culture's ability to discriminate effectively between arsenate and phosphate [103]. However this is disputed by Anderson and Bruland [104]. Further studies are needed to confirm the origin and forms of the methylarsenic species present in the sediments.

In the Tamar estuary, methylarsenic species present in the interstitial waters of the sediments were thought to originate from either the microbial methylation mechanisms present in the sediments or by the breakdown of organic debris originating from marine organisms. Ebdon [101] has concluded that the microbial methylation mechanism was the most favourable explanation for the presence of methylated arsenic species in the sediments as there were significant quantities of methylated arsenic species when biological activity was at its lowest during the study period.

A study of the effects of sediment burrowing organisms (*Macoma balthica* and *Nereis succinea*) on arsenic release to the water column revealed that an increase in dissolved arsenic content in the form of both inorganic arsenic and methylated arsenic species is observed at the time when the organism disturbed the sediment surface. However, if the organism is not present at the affected sediment area, the dissolved methylated arsenic species is absent. The burrows formed by the organisms in the sediment resulted in an increased surface area, which eventually led to a 5-fold improvement in arsenic species diffusion [105].

1.5.4 Aquatic arsenic speciation – an overview

1.5.4.1 Inorganic arsenic

Based on the pE-pH diagram for arsenic species (Figure 1.17), it can be suggested that the majority of arsenic in highly oxygenated seawaters (at a pH of 8.2 and where pE > 10) should be in the form of arsenate. However, early work on the form of arsenic in seawater revealed that As(III) concentrations were notable and

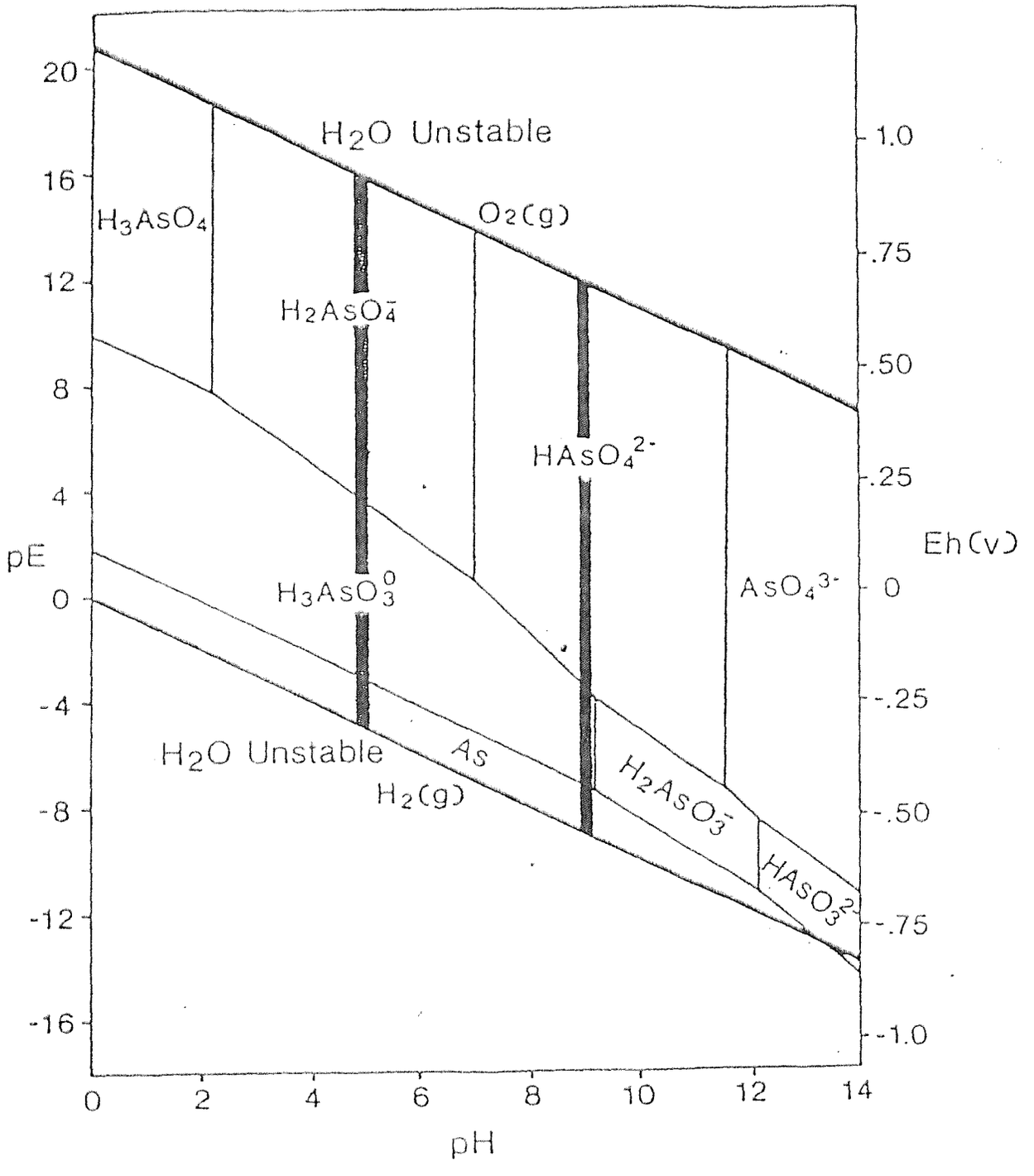

Figure 1.17 The pE-pH diagram for arsenic in water at 25°C. The area within the vertical lines represents the common pE-pH domains for natural waters. Extracted from reference [17]. (pE = -log [E])

sometimes higher than those of As(V) [106]. Johnson [49,107] has attributed this behaviour to phytoplankton and marine bacteria as these organisms are capable of reducing As(V) to As(III). Further work on phytoplankton cultures has revealed that the biologically induced reduction of As(V) results in the formation of As(III), MMA and DMA [92].

In temperate climates, during winter environmental conditions As(V) is the dominant arsenic species in natural waters. Such behaviour has been observed in the Thames Plume (UK) [108] and Humber Estuary (UK) [109] under winter environmental conditions.

Studies carried out by Bright and Reimer [96] on As(V) levels in selected lakewaters within the Yellowknife region (British Columbia, Canada) revealed that the arsenate concentration levels varied widely from 0.7μg As/l (Yellowknife Bay site 2) to 520μg As/l (Kam Lake). An explanation for this wide variation in arsenate concentration levels can be attributed to the discharge of gold mine effluent containing arsenic bound material in Kam Lake whereas Yellowknife Bay Site 2 is within a freshwater environment with a history of very little anthropogenic acitivity.

Arsenite is thermodynamically unstable in oxygenated waters (Figure 1.18). However, arsenite concentrations can be significant in oceanic surface waters where high levels of microbic activity are present [41].

As(III) species can also enter the water column by anthropogenic activities. For example in the Tejo River Estuary in Portugal, dissolved As(III) levels were highest in the surface waters taken from near a pyrite roasting plant which emitted approximately 1000-2000 tonnes of arsenic each year. Such high levels of dissolved As(III) were thought to be due to the incomplete oxidation of the As(III) species in the surface waters [110].

As(III) species can be produced in response to redox changes in the water column. One example of this behaviour occurs when the water layers become stratified resulting in the deep water layer becoming anoxic in character. Studies of the Baltic Sea, where the deep water layers become anoxic during unusual meteorological conditions, revealed that between 56-76% of the total inorganic arsenic content was present as As(III) [111].

1.5.4.2 Dissolved MMA and DMA

There are regional variations in the dissolved concentrations of MMA and DMA. For example in freshwater environments [96], methylated arsenic can make up 10-20% of the total dissolved arsenic budget. In estuarine coastal environments, during periods of intense biological activity, dissolved methylated arsenic concentrations can reach up to 60% of the total dissolved arsenic [92].

A study on methylarsenic species in Southampton Water and Itchen River (Hampshire, UK) revealed that dissolved MMA and DMA were only observed between the months of May-October where the water temperatures were at or above 12°C [112]. Further evidence to support the temperature dependence of dissolved methylarsenic formation has been given by a study on the River Beaulieu (Hampshire, UK) where below temperatures of 9-12°C, there was no evidence of MMA or DMA [17].

Braman and Foreback emphasise that methylarsenic species are unlikely to undergo chemical transformations in seawater [8]. However, microbial activity in seawater has been shown to demethylate the methylarsenic species [113].

1.5.4.3 Dissolved MMAA and DMAA

Recent developments in analytical techniques have permitted the detection of the pentavalent methylated arsenic species in the water column. Howard has

established the presence of dissolved dimethylarsinate in the River Beaulieu (Hampshire, UK) and at Lepe Beach (Hampshire, UK) with average concentrations of 0.07μg As/l and 0.34μg As/l respectively [91]. The mechanisms behind the formation of DMAA species in the water column are not yet established but the favoured route is by the breakdown of dimethylarsenosugars from marine flora.

Hasegawa was able to confirm the presence of pentavalent methylarsenic species in Lake Biwa (Japan) with DMAA being the dominant species in both the photic zone and the deep water layer [114]. In the photic zone DMAA concentrations increase linearly with temperature. However, in the deep-water layer, the DMAA concentration was not proportional to temperature as DMAA was formed in significant quantities during the winter period. Such behaviour has been attributed to the formation of pentavalent methylarsenic species from the decomposition of complex methylarsenic compounds and/or the products of bacterial methylation.

By utilising a novel method of pH discrimination before analytical measurement, Bright and Reimer [96] were able to reveal the presence of pentavalent methylarsenic species in the water column of sub-Arctic lakes in British Columbia with the highest levels of MMAA and DMAA being recorded at Kam Lake with 0.55 and 0.72μg As/l respectively. Interestingly, trimethylarsinate (TMAA) and an unidentified pentavalent arsenic species attributed to gold mine stack emission output were also identified at this site. It was, again emphasised that the decomposition of complex organoarsenic compounds and bacterial arsenic methylation could play a vital rôle in the formation of pentavalent methylarsenic compounds.

1.5.4.4 'Hidden' and 'refractory' forms of arsenic

Two decades ago, it was thought that only As(III), As(V), MMA and DMA species were present in the water column [103]. However, the photochemical and

alkaline digestion treatment of estuarine water has revealed the presence of previously 'hidden' or 'refractory' organoarsenic compounds forming methylarsines upon hydride-generation analysis [90,115]. These compounds are now thought to originate from the decomposition of complex organoarsenicals such as arsenosugars or from inorganic arsenic compounds tightly bound to dissolved humic or fulvic material [90]. Possible 'hidden'/'refractory' arsenic candidates include arsenobetaine, arsenosugars and arsenocholine.

By using a base digestion method for the decomposition of organoarsenicals to HG-AAS detectable forms, de Bettencourt was able to confirm the presence of 'refractory' forms of arsenic. Approximately 20% of the total arsenic present in the lower waters of the Tagus estuary (Lisbon, Portugal) was attributed to 'refractory' arsenic forms [115]. However, de Bettencourt has admitted that on this occasion the base digestion technique was not effective in the complete decomposition of possible 'refractory' arsenic compounds: arsenosugars and arsenobetaine were effectively decomposed whereas arsenocholine and TeMA did not.

De Bettencourt carried out further studies on Tagus estuarine waters [116] using extensive preconcentration and purification procedures with the aim of identifying the 'refractory' arsenic species. It was suggested that arsenobetaine, arsenocholine and trimethylarsine oxide were examples of the 'refractory' arsenic species found in Tagus estuarine waters. If further studies confirm the presence of these arsenic species in other estuarine systems, then gaps in our understanding of the estuarine arsenic cycle will be filled.

Howard and Comber in their study of Southampton Water and the Solent estuarine water environment (Hampshire, UK) revealed that 'hidden' arsenic formation reached a maximum during the early spring and summer seasons. This was attributed to the high levels of phytoplankton activity recorded during those times. The majority of the 'hidden' arsenic after photochemical treatment was in

the form of dissolved DMA (79% increase) but lesser increases in dissolved MMA (47%) and dissolved inorganic arsenic species (25%) were noted [90].

A recent study made of 'hidden' arsenic forms in Uranouchi Inlet (Japan) and Lake Biwa (Japan) revealed that after appropriate photochemical treatment, 'hidden' arsenic forms can be classified according to their resistance to photochemical degradation: the u.v. 'labile fraction' is the amount of 'hidden' arsenic that decomposes easily after a short period (2.5 hours) of photochemical treatment whereas the u.v. 'resistant fraction' is the difference between the u.v. 'labile fraction' and the total arsenic measured by microwave digestion. Hasegawa has estimated the u.v. 'labile fraction' of 'hidden' arsenic in the Uranouchi Inlet and Lake Biwa to be within the ranges of 15-45% and 4-26% of the total arsenic respectively. The highest value of u.v. 'resistant fraction' 'hidden' arsenic occurred in Uranouchi Inlet when there was an increased presence of DMA species in the water column. However, the reasons for this unusual behaviour are not yet clear [117].

1.6 Summary

Figure 1.18 gives an overview of the arsenic species that are present in the four different individual components which make up the aquatic ecosystem.

1.7 Conclusion

This chapter has aimed to give an overview of arsenic's contribution to man, its industrial uses, applications and rôle in the environment with emphasis on the aquatic ecosystem. It can be seen that certain aspects of the arsenic cycle are as yet unclear, leaving gaps in our understanding how arsenic species convert from one form to another.

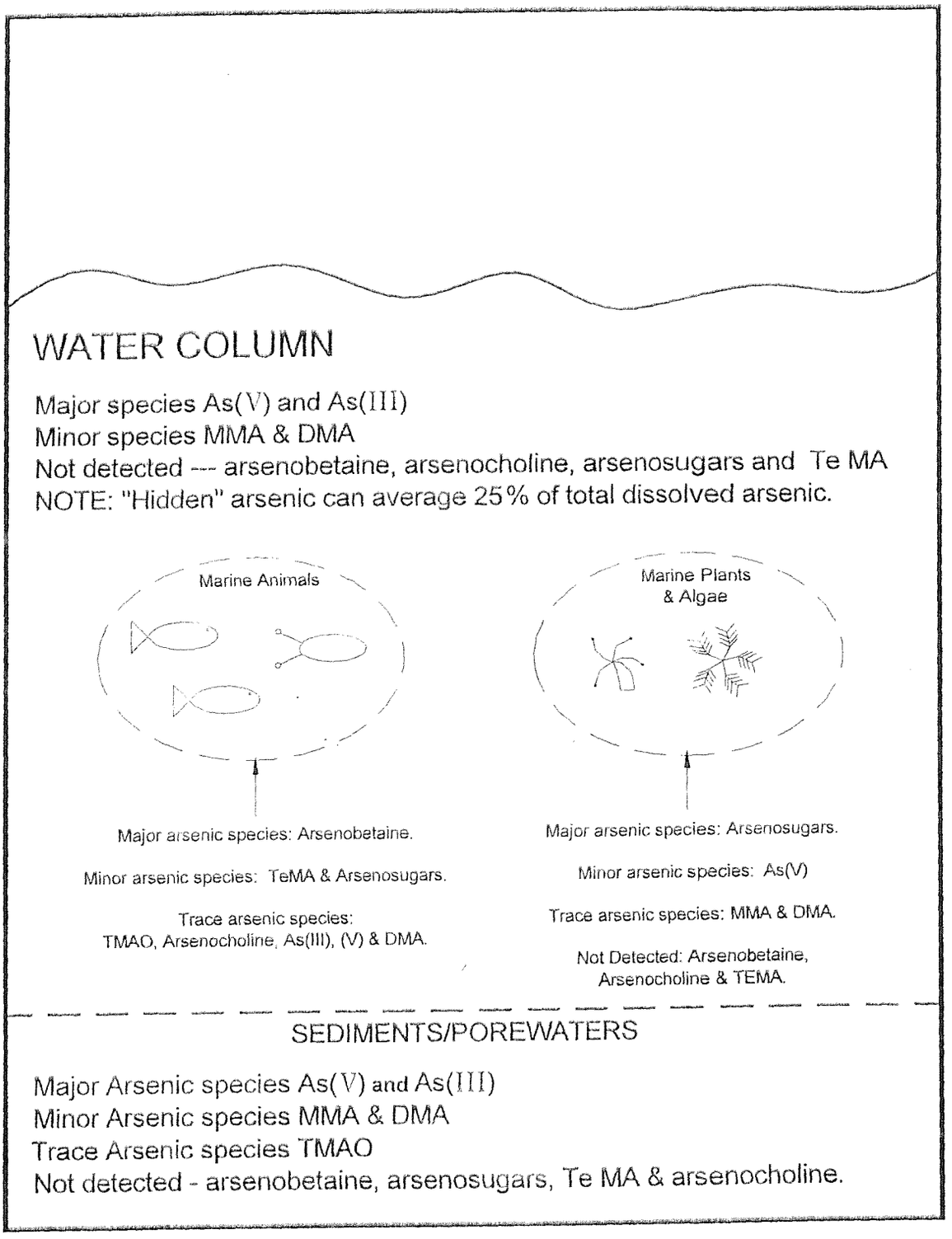

Figure 1.18: Arsenic species present in the aquatic system

In the estuarine arsenic ecosystem, fragments of information on arsenic speciation and content have been pieced together to show how arsenic species are converted to other forms. However, certain aspects of the estuarine arsenic cycle are as yet unclear. For example, the original structures of 'hidden' arsenic species present in the estuarine water column system have not yet been elucidated. By developing appropriate techniques for the detection of arsenic species in the estuarine system and scrutinising carefully information given in the literature, improvements in our understanding of the estuarine arsenic cycle are likely to follow.

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Chapter 2

The determination of arsenic species

2.1 Introduction

This chapter aims to review the analytical techniques that are available for the determination of arsenic. Particular emphasis will be placed on techniques that are capable of giving speciation information.

2.2 Sample pretreatment and storage

2.2.1 Sample preservation

To ensure that an accurate measurement can be made of arsenic concentrations in environmental samples, care must be taken to ensure sample integrity between sample collection and analysis, especially when there is a delay between these stages. Provided that the proper protocols are adopted for the preservation of the sample, the risks of sample deterioration can be minimised. Any sample deterioration in environmental samples may result in an inaccurate determination of arsenic speciation and content.

Techniques that assist in the preservation of arsenic in environmental samples have been extensively studied but controversy has developed over which preservation technique is the most suitable. Despite this, some techniques have been commonly adopted. These include the collection of environmental water samples in polyethylene containers, which have been acid-washed and rinsed thoroughly in deionised water prior to use. Immediately after water sample collection, filtration with 0.4-0.45μm membrane filters is advised [1,2].

There appears to be a degree of confusion in the literature over which technique is the most suitable preservation method for environmental samples containing arsenite. As arsenite is known to oxidise very slowly in seawater, other factors such as temperature, light, pH and salinity can affect arsenite levels dramatically [3]. For example, Andreae [4] reported that when the water temperature rises by 10°C, the initial arsenite oxidation rate doubles. A summary of how arsenite concentrations are affected by various preservation techniques is given in Table 2.1. For estuarine water samples, Comber reported that the most effective preservation technique for arsenite-containing samples was to freeze and store the minimum possible volume in liquid nitrogen [7].

Preservation method	**Results**	**Reference**
None	As(III) to (V) oxidation kinetically slow with very little evidence of sample decomposition after 3 weeks.	[5]
	Samples containing 1-10µg As/l As(III) oxidised to As(V) after 4 days.	[6]
Stored in dark	As(III) oxidation rate slowed considerably in sample treated in the dark.	[7]
Frozen	As(III) species decompose at an unpredictable rate.	[7]
Freezing and storage in liquid nitrogen	Very little change in As(III) concentration after a 1 week treatment period.	[7]
Refrigeration	As(III) species behave erratically after only 1 days refrigeration.	[7]

Table 2.1: Examples of As(III) preservation techniques and their effects.

For arsenate preservation [8], the quick freezing of the environmental sample in either liquid nitrogen or in ice-methanol slurry is recommended prior to sample transfer to a freezer with a maximum temperature of –30°C. This technique also

appears to be suitable for the preservation of arsenite and the methylated arsenicals MMAA and DMAA [8].

Andreae has reported that if MMAA and DMAA samples are untreated after sample collection unexplained changes in arsenic speciation occurs [8]. To prevent an occurrence of this, it is suggested that 0.05mol/l of hydrochloric acid is added to each environmental sample. This allows the environmental sample to be preserved for several months but is also likely to cause speciation changes.

2.2.2 Sample digestion methods

Wet and dry ashing pretreatment methods, employing a wide variety of reagents, have been extensively used for the decomposition of arsenic compounds present in environmental samples to determine total arsenic concentration levels. These techniques have been used for the determination of total arsenic concentrations in biological tissues [9,10], fish [11], sediments [12] and plants [11].

Wet ashing procedures involve the digestion of the environmental sample in acidic media using a combination of acids such as nitric acid, sulphuric acid and perchloric acid. One such wet ashing technique involves the dissolution of the environmental sample in nitric acid overnight with the assistance of heat. Sulphuric and perchloric acid are added to the sample residue and heated for a further 3-4 hours before being diluted appropriately for analysis [13].

A procedure involving dry ashing [11] involves the mixing of the environmental sample with a magnesium oxide-magnesium nitrate heptahydrate slurry before transfer to a furnace where it was ashed overnight at a temperature of 500°C. The sample residue is then dissolved in hydrochloric acid, diluted appropriately and analysed.

In a study of both wet and dry ashing pretreatment methods employed in the determination of the total arsenic concentrations in fish, Tam and Conacher [11] revealed that there was no significant difference in the resulting arsenic levels. However, the authors emphasised that the dry-ashing procedure is the preferred pretreatment technique as it results in lower blank values and avoids the use of perchloric acid, a strong oxidising agent, which if used incorrectly can have fatal consequences.

However, ashing procedures can be problematic. For example, if hydrochloric acid is used in the digestion process, volatile $AsCl_3$ halides (boiling point 130°C) may be formed which results in lower than expected total arsenic concentrations[14]. Addition of an excess quantity of nitric acid to environmental samples containing high halide levels can assist in overcoming this volatilisation problem. The development of microwave digestion [15,16] procedures has reduced the times necessary for the effective matrix decomposition with geological and biological samples.

2.2.3 Photo-oxidation methods

Batch photolysis techniques have been successfully employed to increase the range of arsenic compounds that can be detected by conventional analytical techniques as well as permitting total arsenic determination. By employing a batch irradiation technique involving the use of a 200W mercury arc lamp, Comber [17] was able to report an average 25% increase in the concentration levels of dissolved arsenic species found in estuarine water samples. Cullen and Dodd [18] revealed that by using a batch irradiation technique involving a 1200W-mercury lamp, organoarsenic compounds were converted to arsenate.

Inline photo-oxidation techniques adopting the use of an oxidant and a low powered mercury lamp have also proved beneficial in increasing the range of arsenic compounds that can be detected by conventional analytical techniques.

For example, Hunt [19] was able to extend the range of arsenic compounds that could be identified by high performance liquid chromatography-hydride generation methods (HPLC-HG-AAS) using a 4W Sylvania mercury lamp and the inline addition of potassium persulphate. One advantage of this technique over batch irradiation is the shorter times necessary for the effective decomposition of arsenic compounds.

2.3 Analytical techniques for the determination of arsenic and its speciation (excluding atomic spectroscopy and cryogenic trap techniques)

2.3.1 Traditional arsenic detection techniques

The Marsh and Gutzheit tests were once commonly used to detect arsenic. Both tests involve the use of a zinc/HCl solution, which reduces arsenic species to arsine. In the Marsh Test, any arsines formed were decomposed on hot surfaces as a mirror. Quantitative measurements were possible by comparing the mirror surface with other mirrors produced by standard solutions. With the Gutzheit Test, any arsines generated change the colour of saturated mercuric bromide paper. Arsenic concentration can be determined by comparing the colour of the mercuric bromide paper with a colour-coded chart. A detection limit of 100µg As/l has been quoted for some commercially available Gutzheit Test kits [20].

Arsenic concentrations can be determined gravimetrically by precipitating any arsenic present as arsenic trisulphide (As_2S_3), magnesium pyroarsenate ($Mg_2As_2O_7$) or ammonium uranyl arsenate (which is subsequently weighed as U_3O_8) [21]. Caution should be exercised when using some gravimetric techniques as the precipitation of arsenic as arsenic trisulphide is only capable of detecting ternary forms of arsenic.

Titration techniques involving the reactions between arsenite and potassium bromate are possible [21]. However, for the detection of arsenate, it is necessary to first convert it to arsenite.

2.3.2 Colorimetric methods

2.3.2.1 Molybdenum blue

When arsenate reacts with ammonium molybdate, it results in the formation of a blue complex $H_3As(Mo_2O_5)_5Mo_2O_5$ which can be measured colorimetrically at 865nm. A detection limit of 0.8µg As/l in water has been quoted [22]. However, potential interferences arising from the presence of phosphate (which also forms a blue complex) and silicate must first be overcome.

2.3.2.2 Silver diethyldithiocarbamate

When silver diethyldithiocarbamate is dissolved in an organic base (such as pyridine) it will react with arsine (formed by reducing arsenic in a zinc/HCl solution) to form an intense red complex which can be measured colorimetrically at 540nm [23]. This technique, which has a detection limit of 0.1µg As/l, has been used to determine arsenic levels in marine algae [24] and fish [25]. Interference problems have been reported when high levels of antimony and mercury [26] are present but in estuarine waters, the concentration levels of these compounds are low.

2.3.2.3 Rhodamine B

The reaction between potassium iodate and arsenic in mildly acidic media results in the release of iodine which bleaches the rhodamine pink colour. This colour change can be detected spectrophotometrically at 553nm. Pillai [27] reports that for arsenite, Beer's Law is obeyed in the concentration range 0.04-0.4mg/l and

has successfully applied this colorimetric technique for arsenite detection in biological and environmental samples.

2.3.3 Neutron activation analysis (NAA)

Intense bombardment of arsenic with neutrons at 559keV in a nuclear reactor produces ^{76}As, which decays to ^{76}Se along with the formation of a β particle and gamma radiation, offering a sensitive method for arsenic determination [28]. With an absolute detection limit of 1ng arsenic, this technique has great potential in arsenic trace analysis but high background peaks arising from the sodium, antimony and bromine isotopes can affect the arsenic signal. However, Landsberger [29] has recently managed to reduce the high background effects of sodium and bromine, which has resulted in an improvement of the arsenic detection limit.

Neutron activation analysis ensures sample integrity but the instrumentation used is not widely available as very few laboratories have a nuclear reactor. However, extensive studies have been made in recent years of arsenic determination in American coals [30], marine algae [31], sediments [32], natural waters [33] and animal organs [34]. Neutron activation analysis has also been used to identify the compounds formed by the irradiation of solid arsenobetaine [28].

2.3.4 Electrochemical methods

Electrochemical techniques offer a relatively straightforward and sensitive means of determining arsenite and total arsenic concentrations. In electrochemistry, it is possible to distinguish between As(III) and As(V) species, as As(III) forms can be reduced electrochemically to elemental arsenic while As(V) species do not have this electrochemical property [35]. Consequently it is necessary to reduce any As(V) species present in a sample matrix to arsenite by pretreating it with a solution of iodide or mannitol before a measurement of total arsenic can be made.

This pretreatment step is often carried out under strong acid conditions at elevated temperatures, which requires the adoption of special precautions. Once all the arsenic forms have been converted to As(III), total arsenic determination is possible using differential pulsed polarography [36] or by either anodic [37] or cathodic [38] stripping voltametric techniques.

A recent study of anodic stripping techniques has led to the development of a portable instrument capable of determining arsenic concentrations and speciation in environmental water samples [20]. The technique firstly determines the As(III) concentration when the deposition potential is fixed at –0.2V before the addition of an oxidising agent (potassium permanganate) which allows for the conversion of all arsenic forms to arsenate. Once all of the arsenic forms are converted, the deposition potential is adjusted to –1.6V to permit a measurement of total arsenic. This technique has a detection limit of 0.5µg As/l but interferences from copper can affect the arsenic signal.

2.3.5 X-ray fluorescence (XRF)

Studies on the determination of arsenic using conventional X-ray fluorescence methods have been hampered by poor detection limits (0.02-0.1mg As/l) thereby restricting their use in trace arsenic analysis. However, X-ray fluorescence ensures sample integrity and is suitable for arsenic determinations of small quantities of environmental material [39]. X-ray fluorescence has been successfully employed in arsenic determination of environmental samples in riverwaters [40], coal [41] and biological materials [42].

By utilising a metal-loaded activated charcoal pretreatment method, it was possible for Latva *et al* [43] to separate As(III), As(V), DMA and phenylarsonic acid from a sample matrix prior to energy dispersive X-ray fluorescence spectrometry. This technique would not be suitable for the analysis of estuarine

water samples where arsenic concentrations are frequently in the low µg/l (< 5µg As/l) range as the detection limit of the technique was 20µg As/l.

2.3.6 Gas chromatography (GC)

As the majority of arsenic compounds are not volatile enough for direct GC determination, prederivatisation is frequently necessary. For example, thioglycol methylate (TGM) [44] is used to form complexes for the GC separation of MMAA and DMAA. GC interfaced with mass spectroscopy (GC-MS) has been used for the determination of the arsenic content of environmental samples such as seaweeds [45,46] and fish [47]. Detection limits of one GC-MS study were found to be in the range of between 0.8-0.95µg As/kg [47].

2.3.7 Supercritical fluid chromatography

Reaction of a mixture of arsenite and arsenate (pretreated using potassium iodide and sodium thiosulphate) species with lithium bis(trifluoroethyl)dithiocarbamate prior to supercritical fluid chromatography (SFC) with a flame ionisation detection (FID) resulted in the separation and quantification of the As(III) and (V) species [48]. This technique allows the As(III) and (V) forms to be preconcentrated as their chelate forms prior to SFC separation. However, with a detection limit of 88µg As/l and with a significant quantity of chemicals to be added to a sample matrix, it would be difficult to apply this technique to estuarine water samples.

2.3.8 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) has become a well-established technique for the separation of arsenic compounds. Arsenic separation can be achieved by using a strong anion exchanger (SAX) with an isocratic or gradient-step elution, a cation exchanger with isocratic elution or by ion-pair methods. It is

also possible to separate arsenic anions (e.g. arsenate and arsenite) and cations (e.g. arsenobetaine and arsenocholine) in a single step by using a column switching technique which involves an anion exchanger and a reversed-phase separation column. Once all of the arsenic species have been separated by HPLC, a detection system is used to identify the arsenic species present. Examples of detection systems include inductively coupled plasma-mass spectroscopy (ICP-MS) [49,50] and hydride generation-atomic absorption spectroscopy (HG-AAS) [19,51-52]. Unfortunately for environmental samples containing trace levels of arsenic, the detection limits of some interfaced HPLC systems such as HG-HPLC-AAS (between 1 to 4.7µg As/l) can be too high.

Recent studies using HPLC-UV-HG-atomic fluorescence spectrometry (AFS) techniques resulted in the separation of arsenite, arsenate, MMAA and DMAA and arsenobetaine. Detection limits were in the range of between 0.06 to 0.14µg As/l [53].

Environmental applications of HPLC methods include the detection of the tertramethylarsonium ion in shrimp (*Crangon crangon*) [54] and the investigation of arsenic speciation in scallops taken from off the Newfoundland coast of Canada [55].

2.3.9 Mass spectrometry (MS)

A mass spectrometer is often used as the detector in HPLC-inductively coupled plasma (ICP) systems. The advantages of HPLC-ICP-MS systems include arsenic species discrimination, the removal of any interferences that may affect the arsenic signal as well as the ability to measure concentration levels of elements other than arsenic that may be present in the sample matrix. One major stumbling block in using HPLC-ICP-MS systems for the analysis of arsenic speciation in estuarine waters is the presence of chlorine interferences as ^{40}Ar-^{35}Cl and As have similar m/z values [56].

A HPLC-ICP-MS system utilising an anion-exchange column with a phosphate buffer in 2% v/v acetonitrile was developed by Demesmay *et al* [50]. To ensure the separation of arsenite, arsenate, MMAA, DMAA, arsenobetaine and arsenocholine, an ionic strength step gradient was required. Detection limits were in the range of 0.3-1μg As/l.

HPLC-ICP-MS systems have been used to investigate the arsenic content and speciation in the sediments of a lake in the St Lawrence Valley (Canada) [57] as well as assisting in the identification of water-soluble arsenic species in the marine brown algae (*Fucus distichus*) [58]. Ebdon investigated arsenic speciation in blood plasma using HPLC-ICP-MS techniques and revealed that arsenobetaine was the only arsenic species detected [59]. Detection limits of HPLC-ICP-MS systems vary but are within a consistent band of between 0.25-6μg As/l.

Nanoelectrospray quadrupole time-of-flight mass spectrometry techniques [60] have been employed in the detection of arsenosugars in the picogram range. Applications of this technique include the identification of a dimethylated arsenosugar in an algal extract.

2.3.10 Capillary electrophoresis

A capillary electrophoresis (CE) technique interfaced with ICP-MS was used for the determination of arsenite, arsenate, MMAA and DMAA in a sample matrix. This technique involves the introduction of the sample using a method based upon a direct injection nebuliser (DIN). Such a DIN adapted system permitted the introduction of a sample matrix under different capillary electrophoresis conditions as well as allowing the DIN to be optimised independently of the capillary electrophoresis set-up. Lin *et al* [61] has suggested that this novel system (CE-ICP-MS) couples the high separation efficiency of the CE technique

with the high elemental sensitivity and selectivity of the ICP-MS system. Detection limits were in the range of between 0.02-0.1μg As/l.

2.4 Hydride Generation techniques

Hydride generation (HG) techniques are used widely in arsenic speciation analysis due to their simplicity, high efficiency and their ability to be interfaced with a wide variety of analytical tools and detectors. The HG procedure involves the use of a reducing agent such as zinc/hydrochloric acid mixture or sodium borohydride solution, and subsequent stripping of any arsines generated from a liquid-based sample matrix. By converting arsenic species to arsines, the analyte is separated from the sample matrix, reducing some potential interference problems.

In recent years, sodium borohydride solution has found widespread use as the preferred reagent for HG procedures in arsenic trace analysis by acting both as a hydride generator and a reducing agent [62]. For example, in the reaction between sodium borohydride and arsenic oxy-anions, where the arsenic species are present in the +5 oxidation state, the first step is thought to involve the reduction of the arsenic species to the +3 state:

$$R_nAs(O)(OH)_{3-n} + H^+ + BH_4^- \longrightarrow R_nAs(OH)_{3-n} + H_2O + BH_3 \quad (2.1)$$

where R is a methyl group and n ranges from 0 to 3.

The resulting arsenic species then react with the borohydride to allow the formation of arsines:

$$R_nAs(OH)_{3-n} + (3-n)BH_4^- + (3-n)H^+ \rightarrow R_nAsH_{3-n} + (3-n)BH_3 + (3-n)H_2O \quad (2.2)$$

A possible pathway for this reaction is as follows [66]

$$(3\text{-}n)BH_4^- \longrightarrow (3\text{-}n)BH_3 + (3\text{-}n)H^- \quad (2.3)$$

$$R_nAs(OH)_{3\text{-}n} + (3\text{-}n)H^- + (3\text{-}n)H^+ \longrightarrow R_nAsH_{3\text{-}n} + (3\text{-}n)H_2O \quad (2.4)$$

$$BH_3 + 3H_2O \longrightarrow H_3BO_3 + 3H_2 \quad (2.5)$$

The first step in this pathway involves the formal generation of the BH_3 and H^- species (2.3). These products then react with the $R_nAs(OH)_{3\text{-}n}$ species to form arsines ($R_nAsH_{3\text{-}n}$) as well as water and hydrogen gas (2.4). The remaining $(3\text{-}n)BH_3$ will be hydrolysed to form boric acid (2.5).

The reaction between sodium borohydride and arsenic species is pH dependent. For reaction 2.1 to proceed as efficiently as possible, the arsenic oxy-anions must not be present as a negatively charged species. This means that arsenic oxy-anions must be highly protonated to enable their conversion to arsine.

As arsenic acid has a low acid dissociation constant of 2.3 (Table 2.2), the reaction with sodium borohydride must be carried out at a low pH, hence the addition of 1-2 mol/l hydrochloric acid [62]. For arsenite, however, which is reduced over a larger pH range from between 1 to 5, only mildly acidic conditions are required. Speciation information can be gained simply by varying the pH of the solution being studied. For example by carrying out a reaction at pH 5, the level of arsenite present can be ascertained while by changing the pH to 1, it is possible to calculate the levels of total 'inorganic' arsenic. However, in real samples, where methylated arsenic species are present interferences can occur between 'inorganic' arsenic species and methylated arsenic species unless an effort is made to separate the methylated arsines from the 'inorganic' arsines (AsH_3) [62].

Species	Abbreviation	pK
$As(OH)_3$	As(III)	$pK_1 = 9.2$
$As(O)(OH)_3$	As(V)	$pK_1 = 2.3$
		$pK_2 = 6.8$
$CH_3As(O)(OH)_2$	MMAA	$pK_1 = 4.0$
		$pK_2 = 8.6$
$(CH_3)_2As(O)(OH)$	DMAA	$pK = 6.3$

Table 2.2: Acid dissociation constants of some arsenic acids (adapted from reference [62])

In online HG procedures, any arsine gases formed by reaction between sodium borohydride and the sample matrix are transported by means of a carrier gas such as nitrogen to the detector. Online HG procedures can be easily adapted to give arsenic speciation information by the addition of a separation procedure such as HPLC [19] prior to the HG step or the insertion of a cryogenic trap after the HG set-up [63]. However, some arsenic species do not form a volatile arsine upon reaction with sodium borohydride without prior pretreatment [17]. Caution should therefore be exercised in determining 'total' arsenic concentration levels using HG procedures, as without the appropriate pretreatment procedures in place, misleading 'total' arsenic concentrations are likely to be found.

The HG procedure suffers from a number of interferences, which arise mainly from elements that interact with the reducing agent, transition metals and oxoanions [64]. A number of studies have been made of the effects of interferands on various arsenic forms, but caution must be exercised when interpreting interference data as experimental and detection methods may vary from one study to another. It is also important to consider the environmental context of the

sample as the concentrations of interfering species are likely to be low in estuarine waters while in sediments there may be high concentrations of certain elements, which suppress the formation of arsines. Interferences can be reduced by the use of masking agents such as L-cysteine [65] and ethylenediaminetetracetic acid (EDTA) [63], ion–exchange procedures or by extraction methods [63].

Online HG procedures can be used for trace analysis of arsenic speciation in the nanogram-picogram range. For example, the HG procedure can be interfaced with a cryogenic trap (CT) and an atomic absorption spectrometric detector (AAS) for the analysis of estuarine water samples. Detection limits are in the range of 0.01-0.015μg As/l [7]. It has also been reported that a HG procedure which utilises a GC and a photo-ionisation detector (PID) which separates As(III) and (V) forms of arsenic has an almost identical detection limit range to that of the HG-CT-AAS procedure (0.008 and 0.018 μg As/l) [66,67].

2.5 Cryogenic trap techniques

In trace arsenic analysis, one of the most commonly used techniques for the concentration and speciation of the arsine gases formed by the HG procedure prior to their release to a detector is a cryogenic trap. The cryogenic trap procedure involves the condensation of any arsines formed by the HG method in liquid nitrogen and their eventual release to the detector according to their volatility. A heater can be employed to enhance the volatility of the trapped arsines [68]. This heating process has the added benefit of ensuring a controlled temperature gradient. A major problem in the use of cryogenic traps is that water condenses onto the cryogenic trap during sample passage. This creates excessive back pressure. It has been suggested that the incorporation of a drying agent such as sodium hydroxide, that is changed regularly during system operation [69], or the insertion of a 'dead space' within the cryogenic trap reduces the water condensation problem significantly [70].

Cryogenic traps are U-shaped in design and are usually made of borosilicate glass. Within the trap, packing material such as hydrofluoric acid etched glass beads [71], silanised glass wool [72] or Chromosorb W-HP coated with 15% w/w silicone OV-3 is used [67]. Caution should, however, be exercised when silanising or etching packing material as there may be high levels of arsenic impurities present in the reagents used thereby affecting sample analysis. It is frequently necessary to condition the trap packing material by running a number of standard solutions of known concentration to ensure peak separation and reproducibility [7].

With the assistance of a cryogenic trap, arsine gases are released to the detector according to their volatility. This therefore makes it possible to detect 'inorganic' arsenic, monomethylarsenic, dimethylarsenic and trimethylarsenic separately. Coupled with HG methods, this speciation information can be achieved at very low levels of detection, in the nanogram-picogram range [7,63], thus making the technique suitable for arsenic species determination in estuarine waters.

There are some limitations of cryogenic trap systems. For example, without pH discrimination, it is not possible to measure As(III) and (V) concentrations separately [62]. It should also be noted that it is not possible for dimethylarsinate and other dimethylarsenic forms to be distinguished separately using HG-cryogenic trap systems unless separated previously [73].

For the analysis of estuarine water samples, where arsenic concentrations are frequently in the nanogram-picogram range, cryogenic trap systems have proved to be valuable due to their relative ease of operation and simplicity. Hunt has reported that the insertion of an HPLC pump and column prior to a HG-cryogenic trap-detector manifold system (HPLC-HG-CT-detector) gives more information on arsenic speciation, but the detection limits are much higher than with cryogenic trap systems (HG-CT-detector) alone [19].

Cryogenic trap systems interfaced with a number of analytical tools such as HG-atomic absorption spectroscopy (AAS) [63], HG-atomic emission spectroscopy (HG-AES) [74] and GC-MS [45] have been used extensively for the investigation of arsenic speciation in estuarine waters [17,75,76] and lakewaters [77,78].

2.6 Atomic spectroscopy

2.6.1 Atomic absorption spectroscopy (AAS)

The detector most commonly interfaced with HG-CT systems for the trace analysis of arsenic is an atomic absorption spectrometer. AAS detectors are relatively inexpensive, able to detect arsenic in the nanogram-picogram range and easy to adapt with any additional equipment that may be necessary for arsenic analysis. AAS detectors can, however, suffer from absorption problems from atmospheric or flame sources, which in turn, have an effect on the arsenic signal and sensitivity [79].

For arsenic, the major resonance lines are at 189.0, 193.7 and 197.3nm, which lie close to or within the vacuum UV region of the electromagnetic spectrum. It has been reported that the lower wavelengths provide a more sensitive means of arsenic detection but they suffer from increased signal-to-noise characteristics. Hollow cathode lamps (HCL) are frequently used as the light source due to their low cost, high sensitivity and low arsenic detection limits; but they have a short lifespan (usually 6-7 months of continuous use) [80]. Other light radiation sources such as electrodeless discharge lamps (EDL) have been used. EDL lamps offer a longer lifespan and better sensitivity but are much more expensive than the HCL [81].

2.6.1.1 Flame atomic absorption spectroscopy (FAAS)

In flame atomic absorption spectroscopy, the sample matrix is aspirated into a mixed air-acetylene flame by means of a nebuliser. However, due to the high absorption of arsenic emission wavelengths by the flame, the sensitivity of the method is poor. Replacement of the air/acetylene flame with an argon/hydrogen mixture leads to a 15% decrease in the absorption of the arsenic emission wavelengths but due to the lower flame temperatures, chemical interferences arising from the presence of molecular arsenic species (such as As_2 and As_4) affected the sensitivity [82].

FAAS is frequently used to check arsenic concentrations of standard solutions from samples to be analysed by other cryogenic trap HG-AAS methods. However, the concentrations must be above the detection limits, which are in the range of between 0.1-3.0mg As/l [67]. As some environmental samples contain arsenic in the nanogram-picogram range, it would not be possible to utilise FAAS techniques due to their high detection limits.

2.6.1.2 Quartz tube atomiser AAS

Quartz tubes atomisers require the sample matrix to be introduced in a gaseous form. Consequently, for samples containing arsenic, HG procedures are commonly used. Atomisation occurs either by heating of the quartz-T-tube externally or by burning a flame inside the atomizer tube [83]. The atomisation mechanisms for both systems are thought to be similar [84].

In HG-AAS systems utilising an externally heated quartz-T-tube and with sodium borohydride reduction, arsenic atomisation is achieved with the assistance of hydrogen gas, formed by the decomposition of sodium borohydride and an added flow of oxygen. Other gases introduced by the HG-AAS manifold such as

nitrogen, water vapour, carbon dioxide, hydrochloric acid and sodium borohydride fumes do not affect the arsenic atomisation [85].

For an arsenic AAS signal to be observed, the overall reaction

$$AsH_3 \longrightarrow As(g) + \text{products} \quad (2.6)$$

must occur.

A number of possible mechanisms have been proposed:

Temperature

Early reports in the literature [86] suggested that the hydrides were atomised by thermal decomposition alone:

$$AsH_3 \xrightarrow{\text{heat}} As(g) + \tfrac{3}{2} H_2 \quad (2.7)$$

The temperature that is required for this reaction is approximately 2000°C [87] but significant atomisation can occur at temperatures between 650 and 1000°C [88]. On the basis of this evidence, factors other than thermal decomposition must be involved to allow arsine atomisation to occur at lower temperatures.

Welz and Melcher [89] found that when oxygen is present in the HG-AAS manifold system, the minimum temperature required for arsenic atomisation decreases from 1000°C to approximately 650°C. It was also noted that with no hydrogen gas present, arsine atomisation efficiency decreases by a factor of 4, implying that both hydrogen gas and oxygen gas play critical rôles in arsine atomisation.

Presence of oxygen gas

It has been reported [87,90] that small amounts of oxygen gas introduced into the HG-AAS manifold system in the form of air are critical for arsine atomisation to occur. Hunt [67] has suggested as only low levels of oxygen are required for increased sensitivity, the oxygen present in the laboratory environment and dissolved oxygen present in the liquids introduced into the HG-AAS manifold may influence the sensitivity of atomiser systems.

Quartz-T-pieces, which have larger diameters or have become contaminated, both require higher levels of oxygen gas for efficient atomisation [91]. This behaviour might be due to the catalytic properties of oxygen at the quartz surface allowing the arsenic atoms to decompose.

Atomisation mechanism

Both hydrogen and oxygen are necessary for the atomisation of the arsines. The mechanism for arsenic atomisation is thought to involve the formation of OH, H and O radicals which either immediately allow or catalyse AsH_3 species decomposition and atomisation. One example of such a mechanism is as follows [89,92]:

$$H_2 \longrightarrow H + H \qquad (2.8)$$

$$H + O_2 \longrightarrow OH + O \qquad (2.9)$$

$$O + H_2 \longrightarrow OH + H \qquad (2.10)$$

$$OH + H_2 \longrightarrow H_2O + H \qquad (2.11)$$

Welz and Dedina [91] have reported that the formation of the hydrogen radical (reaction (2.8)) is the most likely candidate for initiating arsenic atomisation.

With AsH_3 present, arsenic atomisation may then occur by collision with hydrogen radicals according to the following equations [91]:

$$AsH_3 + H \longrightarrow AsH_2 + H_2 \quad (2.12)$$

$$AsH_2 + H \longrightarrow AsH + H_2 \quad (2.13)$$

$$AsH + H \longrightarrow As + H_2 \quad (2.14)$$

Radical recombination is thought to take place according to:

$$H + O_2 + X \longrightarrow HO_2 + X \quad (2.15)$$

where X is another molecule such as the quartz surface on the T-tube or water.

A reason why low quantities of oxygen are critical may be that radical recombination is slower than formation and the presence of a small quantity of oxygen allows the radical density to be maintained [91].

The above reaction mechanism proposed has its uncertainties on thermodynamic grounds, but Agterdenbos and Bax [92] have suggested that the radicals catalyse arsine atomisation.

Etching of quartz-T-tubes in 40% v/v hydrofluoric acid for approximately 30 minutes is necessary to improve peak sensitivity, probably by reducing recombination rates. If radical recombination is increased, the arsine species may decompose before detection [91].

Extensive studies have been made of quartz-tube atomiser AAS techniques in the analysis of arsenic in environmental samples due to their low detection limits, ease of operation and rapid sample throughput. The interfacing of quartz-tube atomiser systems with HG-CT techniques has found widespread application in the study of arsenic in environmental samples. Examples include the investigation of

arsenic speciation in estuarine waters [17,77-78,93], lakewaters [79], porewaters [94] and soils [95]. A novel technique for the investigation of biological materials using an online HPLC-microwave digestion-HG-AAS procedure utilised a quartz tube and an air-acetylene flame in the AAS set-up. Detection limits were between 2.5-5.9μg As/l [96].

2.6.1.3 Graphite furnace atomic absorption spectroscopy

Graphite furnace atomic absorption spectroscopy (GF-AAS) has found widespread application in the determination of arsenic in environmental and biological samples. Unfortunately, loss of arsenic species which have a high volatility in the sample matrix may occur during the drying and ashing stages of the GF-AAS procedure resulting in an inaccurate picture of arsenic concentration levels. To overcome this problem, treatment with a chemical modifier such as palladium nitrate [97] or nickel(II) nitrate [98] is recommended. As the chemical modifiers have high ashing temperatures (for nickel(II) nitrate 1400°C), the incorporation of the arsenic containing sample matrix with the chemical modifier will prevent the accidental volatilisation of arsenic forms at lower temperatures. When arsenic forms interact with the nickel(II) nitrate modifier, the reaction mechanism is thought to involve the formation of nickel arsenide which allows the various arsenic species present in the sample matrix to have identical sensitivities.

However GF-AAS techniques can suffer from interference problems which can affect the arsenic signal. In order to overcome interference problems, standard additions are often carried out. Some examples of interferands include H^+, SO_4^{2-}, NO_3^- and Fe(III) [99]. Preconcentration methods are frequently used to remove arsenic species from a sample matrix. For example, ammonium pyrrolidinedithiocarbamate (APDC) incorporated in an anion-exchange resin is used as a preconcentration method to selectively extract arsenite from a sample matrix [100].

Detection limits of GF-AAS techniques are frequently in the picogram range. This makes the technique suitable for the determination of arsenic in urine [101] and natural waters [97].

2.6.2 Atomic fluorescence spectroscopy (AFS)

In recent years with the development of a reliable high intensity excitation source in the form of a boosted discharge hollow cathode lamp, atomic fluorescence spectroscopy (AFS) techniques are now more widely used in trace arsenic analysis. AFS interfaced with other analytical techniques have been extensively studied to determine arsenic speciation in environmental systems. For example, a comparison study between HPLC-ultraviolet-HG-AAS and HPLC-ultraviolet-HG-AFS couplings revealed that the detection limits of the AFS (0.06 to 0.14μg As/l) system were approximately twenty times lower than the AAS (1.1 to 3.9μg As/l) method [102]. HG-AFS based techniques have been utilised to determine arsenic speciation in seawaters [102], reference and biological materials [103].

2.6.3 Atomic emission spectroscopy

2.6.3.1 Flame emission spectroscopy

Flame emission spectroscopy is not suitable for arsenic trace analysis due to its lack of sensitivity.

2.6.3.2 Plasma emission spectroscopy

Three types of plasma based atomic emission spectroscopic methods have been used in the determination of arsenic:

Direct current plasma emission (DCP-AES)

A review of the literature has revealed that this technique is not widely used in the determination of arsenic. A HG procedure utilising DCP-AES resulted in the analysis of volatile arsenic forms [104]. These HG based DCP-AES systems were developed further for the determination of arsenic in the water column. Detection limits were approximately 0.15µg As/l [105].

Microwave induced plasma emission (MIP-AES)

Conventional MIP-AES systems have found limited use in the determination of arsenic. This is likely to be due to the inability of the plasma to tolerate volatile hydrides, the continuous introduction of aerosols and gases. However, a high powered nitrogen MIP system has been recently developed to overcome these problems. A detection limit of 2.99µg As/l was quoted. MIP-AES systems have been used to determine arsenic levels in carbon and stainless steels [106].

Inductively coupled plasma emission (ICP-AES)

ICP-AES systems have been used for the investigation of arsenic and its speciation in environmental samples. The advantages of ICP-AES systems include multi-element analysis where the same sample matrix can be used for the analysis of arsenic, bismuth and antimony content without sample matrix changeover for each individual element and short sample passage to detection times. However, ICP-AES systems are expensive to operate when compared to QFAAS methods.

ICP-AES systems have been used to determine arsenic content and speciation in UK and Korean cigarettes [107], fish [107] and lakes [108].

It has been reported that the development of a HG-ICP-AES system utilising sodium borohydride as a reducing agent permitted the detection of As(III) in groundwater samples without encountering any severe interference effects from any arsenate present in the sample matrix. Detection limits of 1µg As/l were quoted for both 'inorganic' arsenic forms [109]. Other HG-ICP-AES systems utilised L-cysteine as a pre-reducing agent to determine arsenic(III) concentrations in sediments. For this system, arsenic(III) had a detection limit of 0.6µg As/l [110].

A study of interferences affecting a continuous flow HG-ICP-AES system revealed that certain transition metals such as Cu, Pd, Ni and Co affected system performance. It was reported by Jamoussi *et al* [112] that the addition of a controlled quantity of cyanide improved system performance by eliminating the majority of the interferent effects as well as improving the system's sensitivity and selectivity. A detection limit of 0.82µg As/l was reported for arsenite. However, special precautions must be taken when handling cyanide.

2.7 Conclusion

An overview of the various analytical techniques that are available for the determination and speciation of arsenic in environmental samples has been given in this chapter. Careful consideration must be given to selecting the appropriate analytical technique for the determination of arsenic in environmental samples. In particular, questions such as:

i. is it necessary to determine the total arsenic concentration or the concentration of the individual arsenic species present in the sample matrix or both?
ii. in what concentration range is arsenic found?
iii. are certain interferands known to exist in the sample matrix likely to pose a problem in determining arsenic content?

iv. how long does it take for each arsenic analysis to occur?

v. what steps can be taken to preserve the sample matrix prior to arsenic analysis?

vi. is the equipment available and cost-effective to operate?

must be considered before carrying out arsenic analysis.

In natural waters, arsenic determinations are often carried out using hydride-generation atomic spectroscopic methods (HG-AAS). HG-AAS systems are the preferred mode of arsenic analysis due to their ease of operation, low levels of detection, the lack of severe interference effects affecting arsenic outputs and their short sample passage to detection times. However, other techniques such as ICP-AES and ICP-MS have been utilised to determine arsenic content and speciation in natural waters but factors such as cost limit their widespread use in arsenic analysis.

One limitation of HG-AAS techniques is that they are not capable of detecting 'hidden' forms of arsenic without prior pre-treatment or by using a chromatographic separation tool such as HPLC. By designing the most suitable tool for arsenic determination in natural waters, improvements in our understanding of arsenic's role in the marine biogeochemical cycle are likely to follow.

2.8 Experimental objectives

Recent advances in our understanding of the compounds present in the marine water column have necessitated a reappraisal of the techniques used to measure arsenic; up to 50% of previously reported levels of methylated arsenic having been missed by conventional analytical methods. This type of arsenic is known as 'hidden' arsenic [17]. Very few studies have been reported in the literature of the role, nature and seasonal distribution of 'hidden' arsenic in estuarine waters and

the sediments. Thus, the main objective of the experimental work is to develop appropriate analytical techniques that will allow a re-evaluation of the nature and distribution of 'hidden' arsenic in estuarine waters and the sediments. Any new information gained should allow a better understanding of the marine biogeochemical cycle in estuarine systems.

The proposed approach is to firstly improve the sensitivity of the cryogenic trap HG-AAS methods developed by previous laboratory workers to determine very low levels of arsenic in seawater and in more complex matrices such as the sediments which suffer from metal ion interferences. Prederivatization of the arsine complexes with L-cysteine, thioglycolic acid and sodium thioglycolate will be investigated and any improvements should, in turn, improve the attainable detection limits.

Secondly methods used to determine the nature of the 'hidden' forms of arsenic will be developed. Research by previous workers on an online photo-oxidation system interfaced with HPLC-uv-HG-AAS showed that it was possible to determine the nature of 'hidden' arsenic forms but problems with detection were encountered at low concentrations. Photo-oxidation methods have been widely used as pretreatment agents in environmental samples to promote the breakdown of complex species to simpler forms thus permitting their detection by analytical techniques. Unfortunately, pretreatment methods can result in a loss of arsenic speciation information and it can be difficult to ascertain whether all of the 'hidden' arsenic species have been converted to the arsenic hydride reducible forms. By investigating batch and inline photo-oxidation systems for the detection of 'hidden' arsenic forms, it will be possible to devise experiments to ascertain the effects that each pretreatment method has on the sample matrix arsenic content and speciation.

When the pretreatment methods for the determination of 'hidden' arsenic are established, it will be possible to devise fieldwork experiments, which will focus

on evaluating the nature and distribution of both 'hidden' and non-'hidden' forms of arsenic in estuarine waters and the sediments. For the investigation of 'hidden' arsenic speciation in estuarine waters, a sampling survey will be carried out on Southampton Water and the River Itchen (Hampshire, UK) at regular intervals during a one-year period. With this sampling survey, the analytical techniques developed for the determination of 'hidden' arsenic forms will be assessed in terms of suitability for arsenic determination in estuarine waters as well as evaluating the factors that govern the seasonal release of 'hidden' arsenic. Any results gained should, therefore, allow a better understanding of arsenic's role in estuarine waters.

Interstitial water extracts will be collected from the Rivers Test (Hampshire, UK) and Tamar (Devon/Cornwall) where arsenic speciation will be investigated and its relationship to arsenic content and speciation in the water column will be studied in detail. It has been suggested that sediments could be a possible source of 'hidden' arsenic in the water column. Experiments will, therefore, be devised using the pretreatment methods discussed previously to ascertain 'hidden' arsenic content and speciation to determine its role in the marine biogeochemical cycle.

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Chapter 3

Hydride generation-atomic absorption spectroscopy techniques for the determination of arsenic

3.1 Introduction

This chapter gives an overview of the hydride generation-AAS methods that were employed to determine trace levels of arsenic. The analytical methodologies are largely based upon the work of Arbab-Zavar [1] and Hunt [2] but have been further developed by the incorporation of pre-derivatisation steps [3,4].

3.2 Glassware and Reagents

All glassware was soaked overnight in 3% v/v hydrochloric acid (Fluka, 32% (w/v), 'Arsenic free') and rinsed at least three times in deionized water prior to use.

Four stock arsenic solutions were prepared using arsenic trioxide (BDH, Poole, UK), sodium arsenate (BDH, Poole, UK), disodium methylarsonate (Pfaltz and Bauer, Waterbury, CT) and dimethylarsinic acid (Pfaltz and Bauer, Waterbury, CT). Each stock solution contained 1000mg As/l and was prepared by the dissolution of the appropriate compound in deionized water. The arsenite standard was prepared by dissolving arsenic trioxide in 1 mol/l sodium hydroxide (BDH, AnalaR) before neutralising with 1 mol/l hydrochloric acid (Fluka, 32% (w/v), 'Arsenic free'). Stock solutions were diluted on the day of use to give working standards. The concentrations were checked by flame atomic absorption spectroscopy (FAAS) prior to HG-AAS analysis.

Sodium borohydride (Avocado, 98%+ (w/w)) was prepared daily as a 2% (w/v) aqueous solution.

Potassium persulfate (BDH, AnalaR) was prepared as a 2%(w/v) solution in 1% (w/v) sodium hydroxide (BDH, AnalaR).

3.3 An automated method for the determination of total dissolved arsenic

This technique is based upon the system described by Hunt [2] and is appropriate for measuring total dissolved hydride-reducible arsenic levels between 10 and 40µg As/l [2].

3.3.1 Overview of the system

The apparatus (Figure 3.1) is fully automated and consists of three distinct sections: a hydride generator, a free flowing gas/liquid separator and an atomisation/detection system.

A peristaltic pump (Ismatec mp-13) controls the flow of the sample, acid and borohydride in the system. The sample was acidified prior to the addition of borohydride. The resulting solution passed through a 14-turn reaction coil (length 8cm, i.d. 0.2cm) to allow for arsine generation to occur and for the arsine to be stripped from the liquid. Nitrogen carrier gas then swept the mixture to a custom-built, free flowing, gas-liquid separator. The volatile hydrides were dried with sodium hydroxide pellets (BDH, GPR) and swept with air into an electrically heated, hydrofluoric acid etched, quartz-T-tube (Figure 3.2) heated at *ca.* 950°C. Detection was achieved using either a Varian AA-5 or AA-175 atomic absorption spectrometer. After approximately 1 hour of continuous operation the sodium hydroxide pellets were removed and replaced with fresh pellets [5].

A trace pattern for 5, 10 and 20µg As/ml of arsenite is illustrated in Figure 3.3.

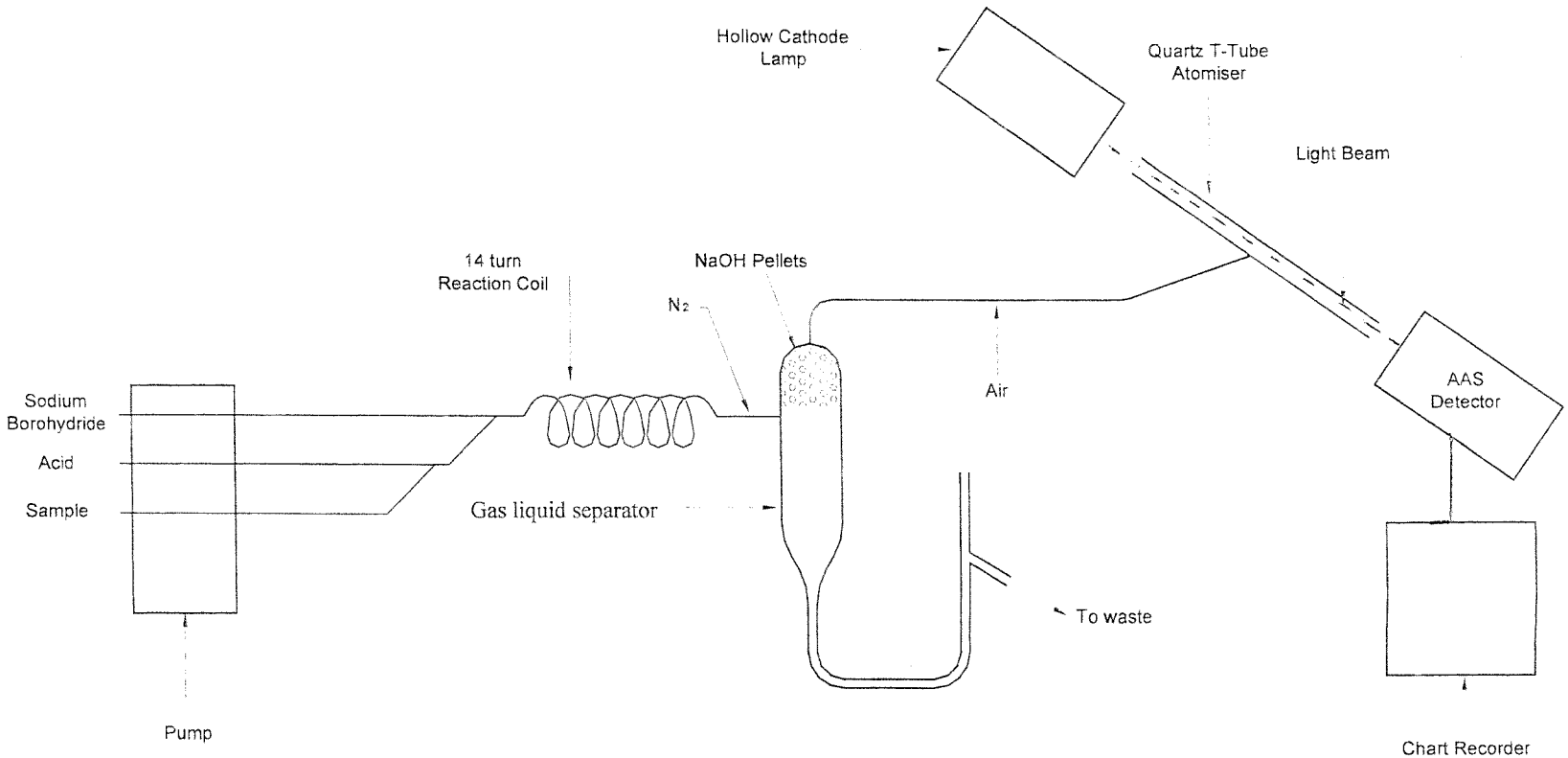

Figure 3.1 Schematic diagram of continuous flow gas-liquid separator

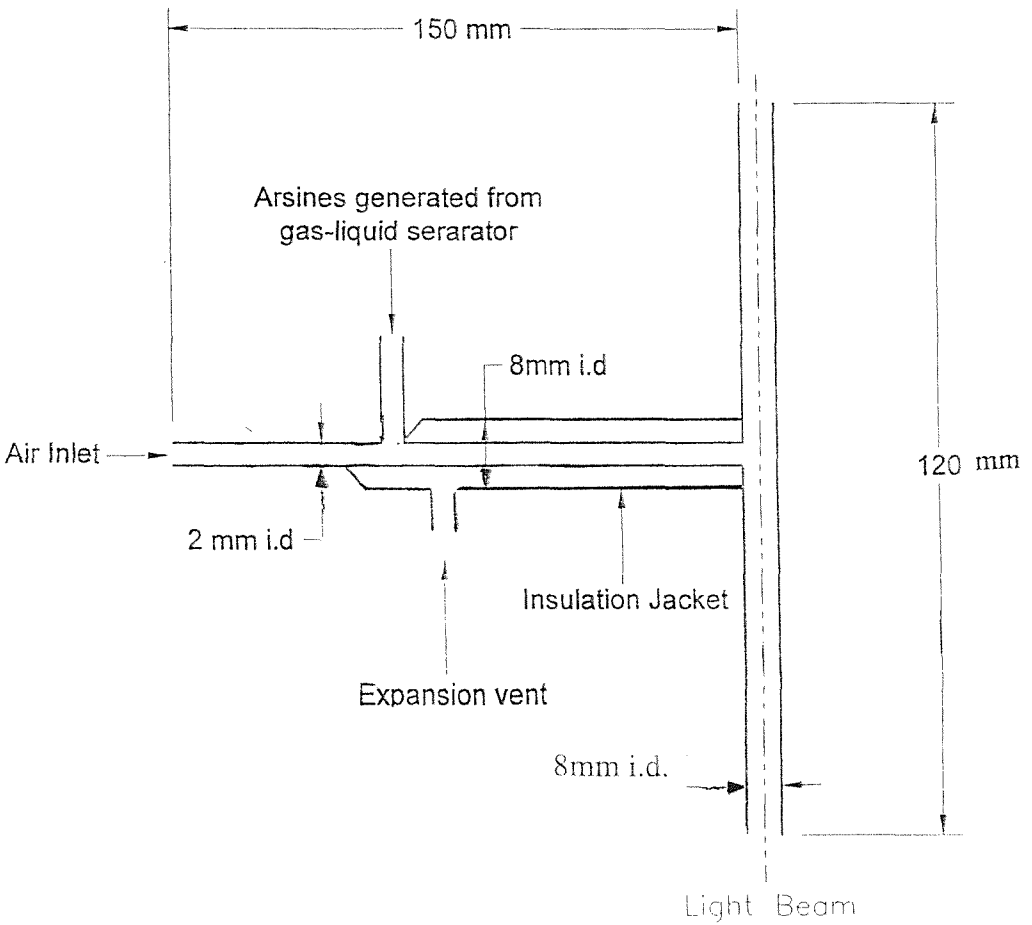

Figure 3.2: Dimensions of the quartz-T-tube assembly.

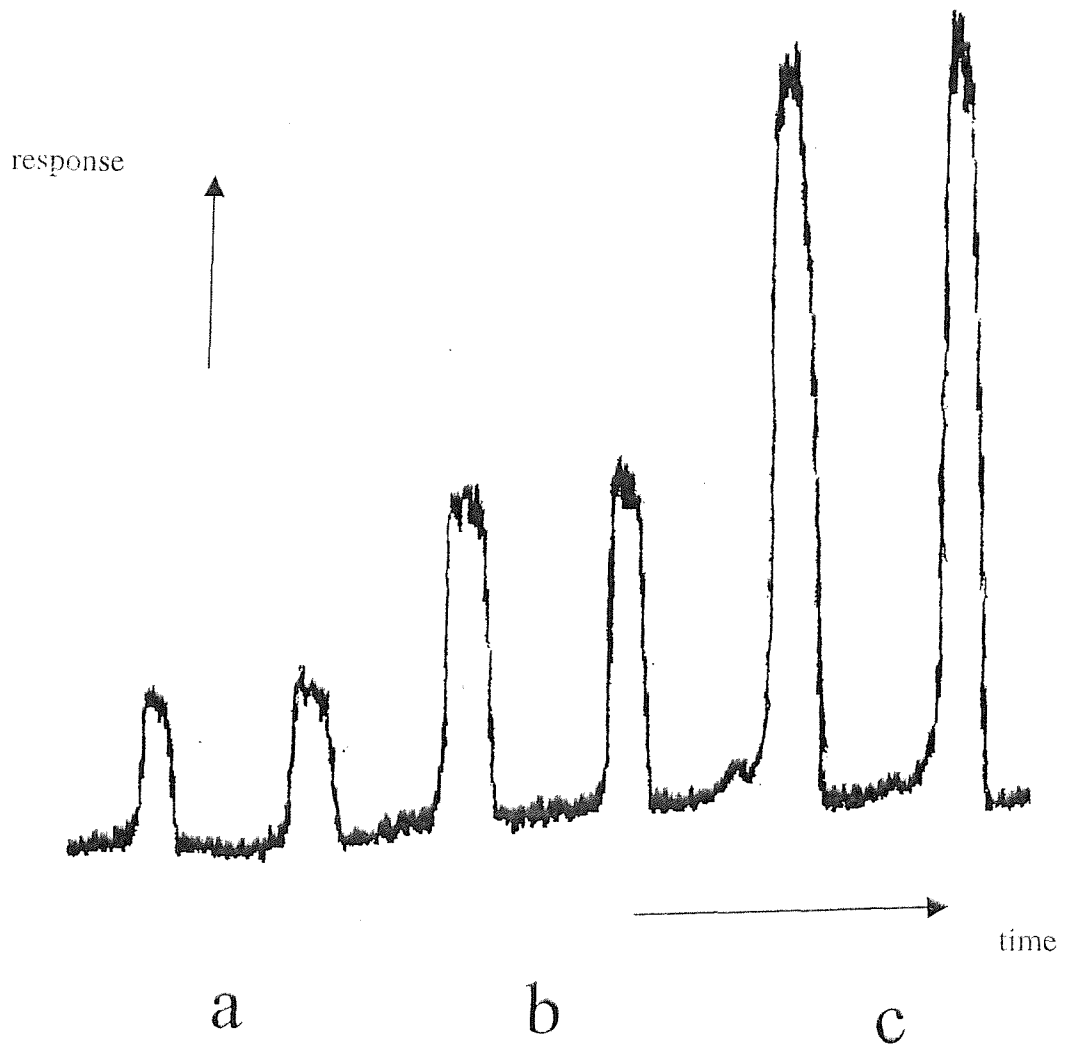

Figure 3.3: **Typical output from the continuous flow HG-AAS system for (a) 5 (b) 10 and (c) 20µg As/l arsenate.**

3.3.2 Operating conditions

The following operating conditions [2,5] were used throughout this work unless otherwise indicated:

Hydrochloric acid concentration:	1mol/l
Sodium borohydride concentration:	2% (w/v)
Nitrogen carrier gas flow rate:	150ml/min
Air flow rate:	10ml/min
Pump rate (all lines):	2.5ml/min
Lamp:	Arsenic hollow cathode lamp (Cathodeon)
Wavelength:	193.7nm
Lamp current:	7mA
Background correction:	OFF
Output:	Oxford 3000 Series or Tekmann 200 Series Chart recorder
Chart speed:	10mm/min
Atomiser furnace temperature:	approximately 950°C
Quantification:	By peak height measurement compared to external standards

3.3.3 Reproducibility, linear range and detection limits

Using the operating conditions described in Section 3.3.2, it was possible to calculate the reproducibility, linear range and detection limit of the automated method:

Reproducibility (RSD%, based upon 10 replicates of 10µg As/l arsenite):	3.2%
Approximate linear range:	5-40µg As/l

Detection limit (three times the standard deviation of the blank signal (3σ), based upon arsenite): 0.52μg As/l

3.4 A cryogenic trap method for the determination of arsenic species

3.4.1 Operating conditions

Unless otherwise stated, the conditions described in Section 3.3.2 were used.

3.4.2 Overview of the system

The apparatus consisted of a hydride generator, a cryogenic trap and an atomic absorption detection system. The hydride generation and detection systems were identical to those used in the automated system described previously. The only differences were the replacement of the free flowing gas-liquid separator by a custom built controlled flow gas-liquid separator (Figure 3.4) and the introduction of a cryogenic trap containing 15% w/w silicone OV-3 on Chromosorb W-HP (60-80 mesh) (Figure 3.5). The cryogenic trap was heated by a heating coil (Nickel 20% chromium wire, 24swg, length *ca.* 2m, diameter 0.5mm, resistance 4.49Ω/m) wound around the trap and attached to a variable transformer delivering *ca.* 21V.

The cryogenic trap was incorporated into the HG-AAS manifold between the gas/liquid separator and the quartz T-piece atomizer, and fixed into position using PVC tubing (Figure 3.6).

The sample inlet tube was placed in a deionized water reservoir, and the peristaltic pump was switched on causing deionized water, acid and borohydride to pump through the system. The cryogenic trap was placed in liquid nitrogen and the stopwatch was started. After 1 minute the sample tube was moved to the sample vial and 1ml of sample was introduced into the system. This was followed by a

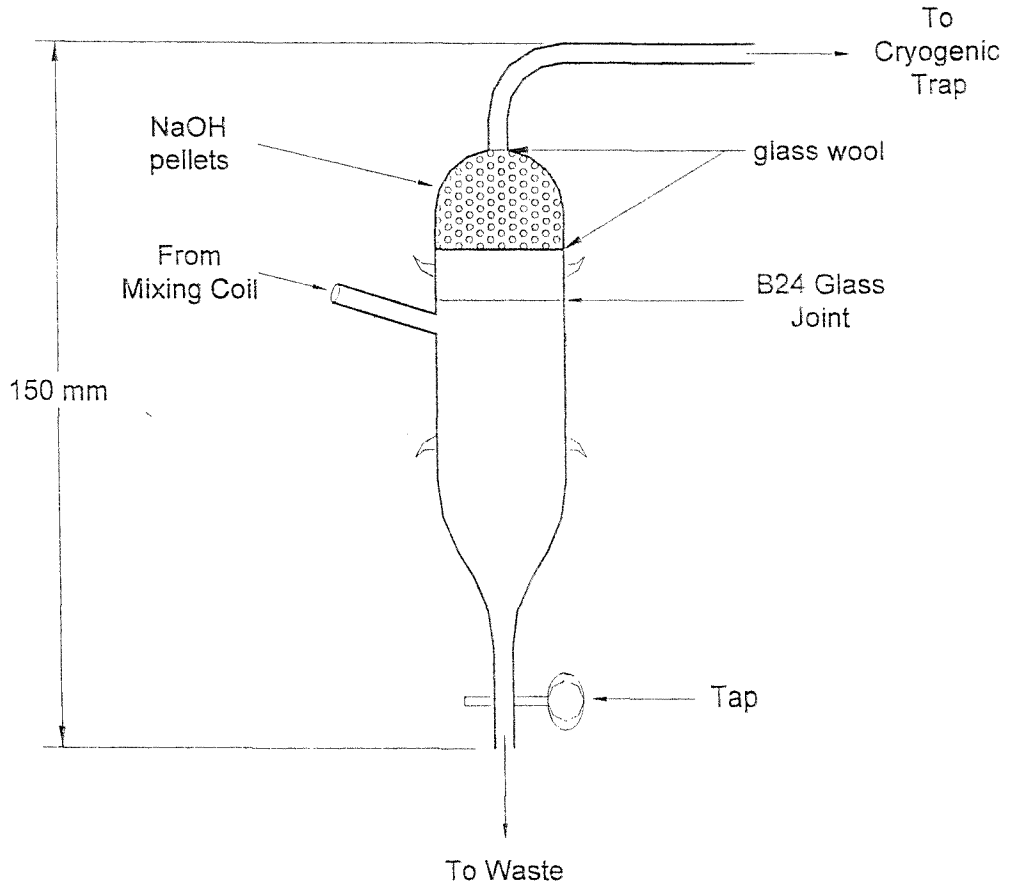

Figure 3.4: Dimensions for the controlled flow gas-liquid separator.

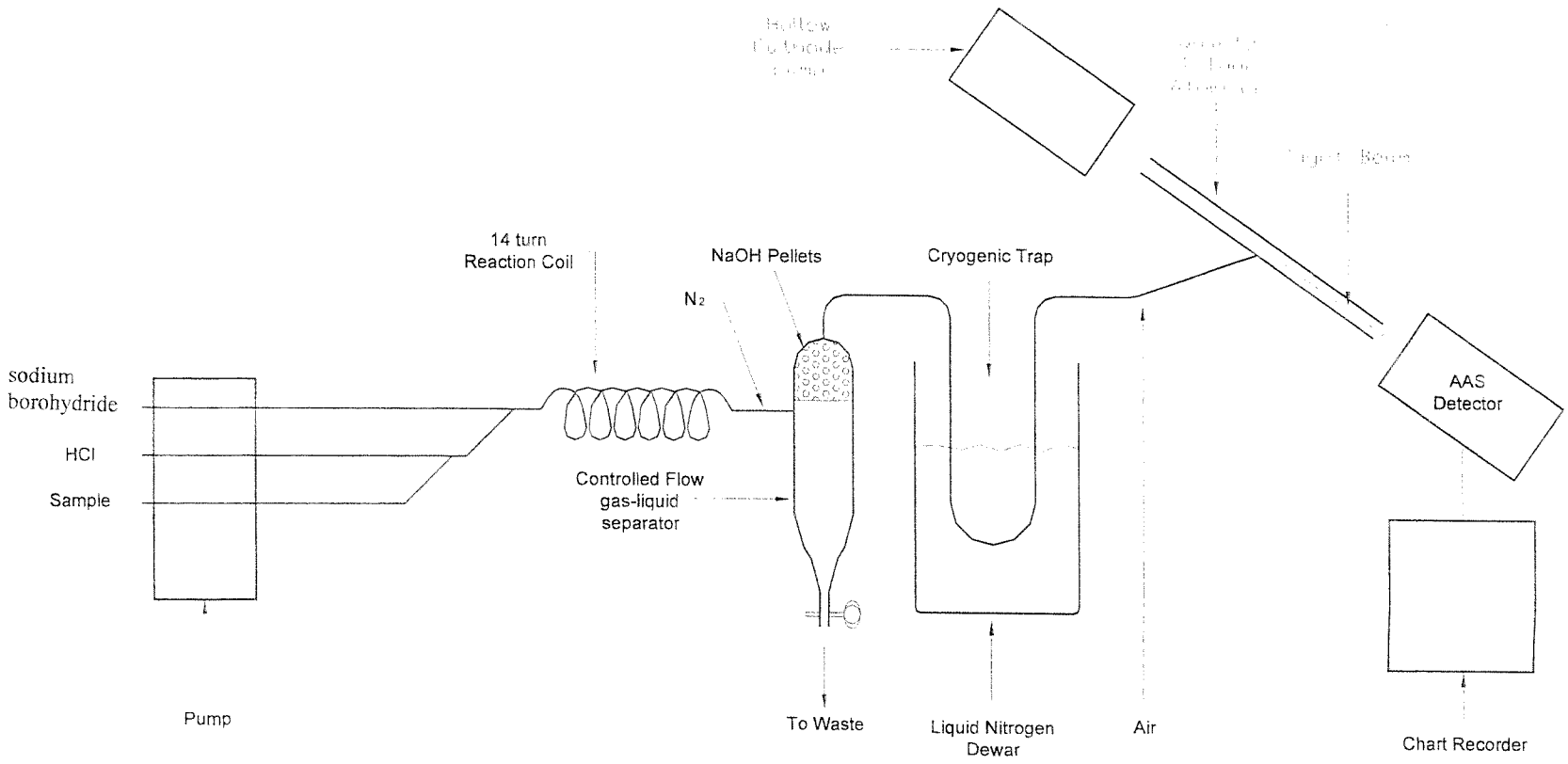

Figure 3.5: Schematic representation of the cryogenic trap HG-AAS system.

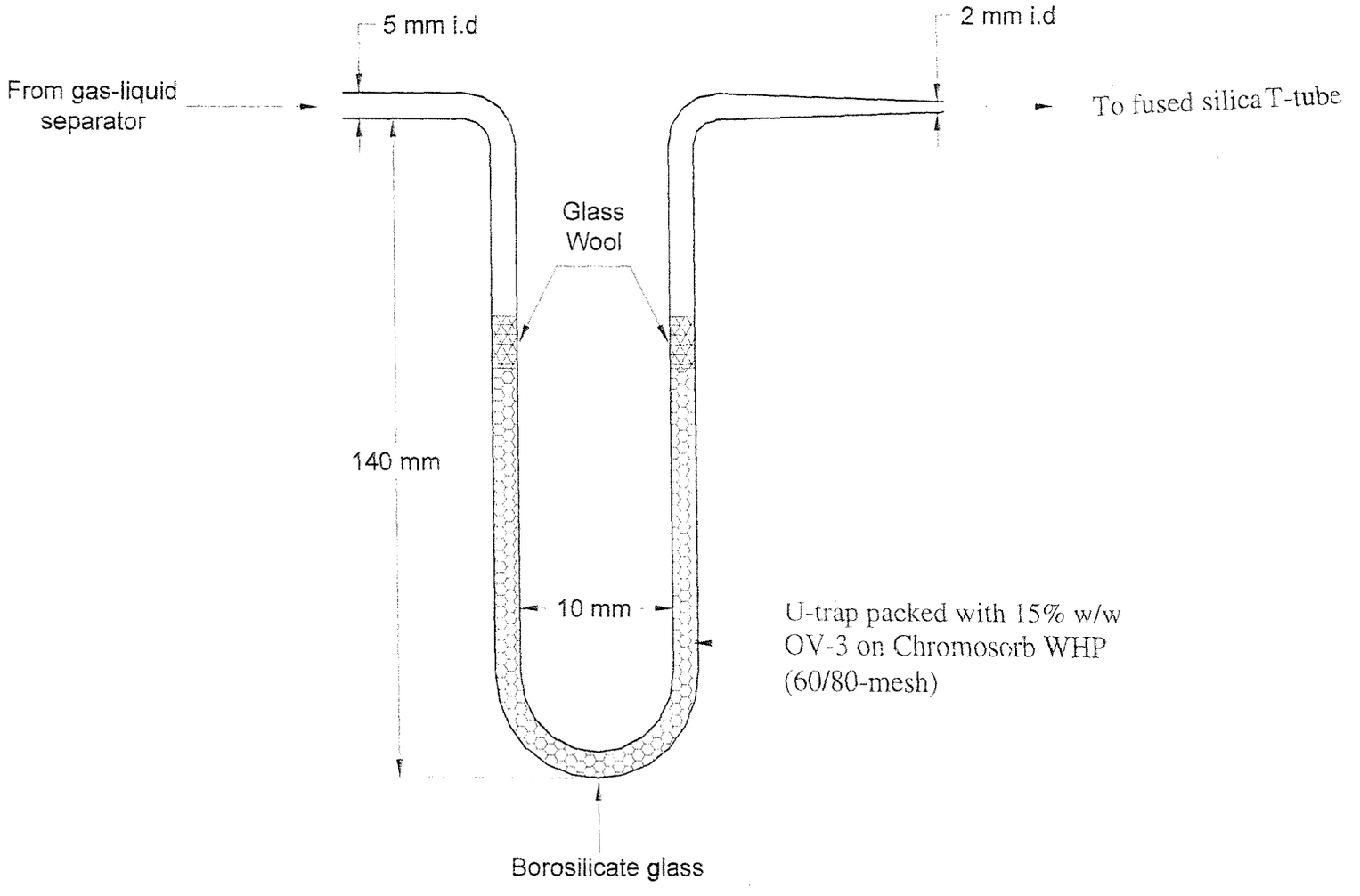

Figure 3.4 Cryogenic Trap Illustration

deionized water wash. When the timer read 180 seconds, the chart recorder was switched on, the liquid nitrogen was removed from the cryogenic trap and the electrical heater was switched on to allow the arsines to volatilise and be transported by the carrier gas into the detector. The arsine gases were given off according to their volatility (Table 3.1). The chart recorder was then switched off, the liquid in the gas/liquid separator was removed and the electrical heater switched off. After approximately 1 hour of continuous operation, the sodium hydroxide pellets were replaced.

Species	Boiling point/°C
AsH_3	-55.0
CH_3AsH_2	2.0
$(CH_3)_2AsH$	55.0

Table 3.1: Boiling points of arsines

To condition the cryogenic trap, two solutions containing 2ng As/ml and 10ng As/ml of arsenite, MMAA and DMAA respectively were analysed by the above procedure. Peak reproducibility and concentration versus peak output were checked prior to starting the actual measurements. A typical separation, showing the peak output from the hydride generation of total 'inorganic' arsenic, mono- and dimethylarsenic species, is shown in Figure 3.7.

3.4.3 Precision and detection limits

The precision and the detection limit of the system were found to be:

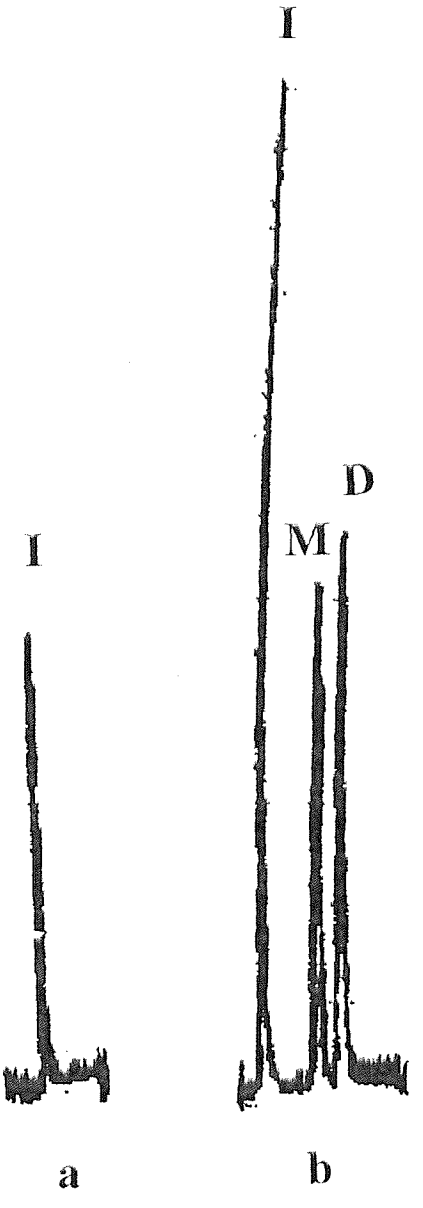

Figure 3.7: Typical output from the cryogenic trap HG-AAS system with (a) acid derived blank (I) and (b) 1μg As/l arsenite (I) MMA (M) and DMA (D).

Precision (RSD %, based upon 10 replicates)

'Inorganic arsenic':	2.2%
MMA:	6.9%
DMA	3.2%

Detection limit (three times the standard deviation of the blank signal (3σ), based upon 10 determinations)

'Inorganic arsenic':	8pg
MMA:	9pg
DMA:	9pg

3.5 Enhancement of the HG-AAS procedure

3.5.1 By derivatisation with L-cysteine

3.5.1.1 Introduction

As explained in Section 2.5, there are two major areas which could be improved with the cryogenic trap HG system – the sensitivity of the 4 different hydride reducible arsine species could be enhanced as well as improving the system's resistance towards interferences from metal ions. Howard and Salou [4] have reported that the addition of an appropriate quantity of L-cysteine leads to more consistent sensitivities to arsenate, arsenite, monomethylarsonate and dimethylarsinate as well as improved resistance towards interference from metal ions.

3.5.1.2 Experimental

For the investigation carried out in this study, three standard solutions were prepared, one containing arsenite, MMAA and DMAA, the second containing arsenate and the third, a blank, deionized water. The arsenic solutions were prepared from a 1000mg As/ml stock solution to contain, for each species, 1ng As/ml. When appropriate, excess L-cysteine (10mg/ml, Aldrich) was added to each solution and shaken vigorously before leaving to stand for approximately 15 minutes prior to analysis.

3.5.1.3 Results and discussion

Attention was initially focussed upon obtaining optimum conditions for the formation of the arseno-cysteine complexes, using the cryogenic trap procedure. An experiment was devised in which the HCl concentration was kept constant at 0.1 mol/l while the borohydride concentration was varied between 1-5% w/v. From the graph (Figure 3.8), it would appear that for arsenite, the peak height increases to a maximum at around 2% w/v but after 2% w/v the peak height decreases slightly. For both arsenate and DMAA, the peak heights are not as large as is the case with arsenite, but they follow a similar trend with respect to variation with borohydride concentration. However, for MMAA, the peak height reached a maximum at 1% w/v before remaining relatively constant. A borohydride concentration of 2% w/v was therefore selected for future work.

In order to investigate the effect of acidity on the analysis, the borohydride concentration was fixed at 2% w/v, while the HCl concentration was varied between 0.01mol/l and 2mol/l. With arsenate and arsenite solutions, maximum peak height was obtained at 0.01mol/l whereas with higher HCl concentrations, the peak height decreased dramatically (Figure 3.9). For DMAA, after reaching a maximum at 0.01mol/l, the peak height steadily decreases. With MMAA, the peak height steadily increases to a maximum at 1mol/l before decreasing

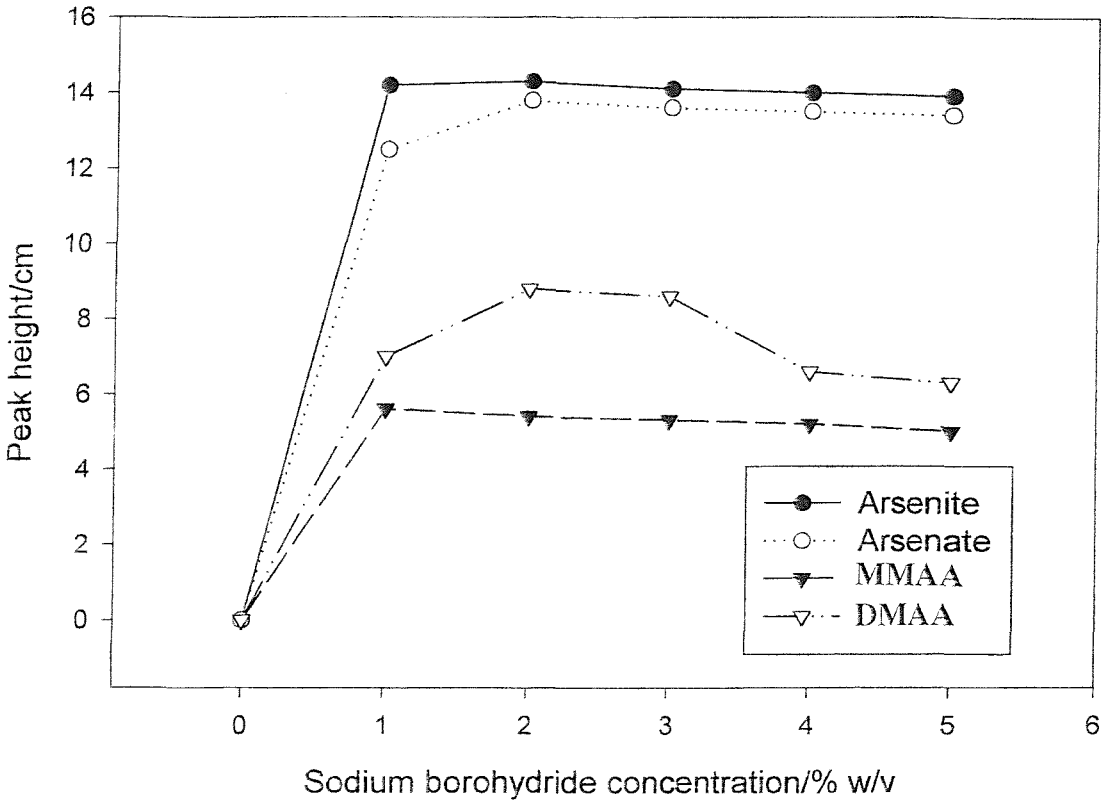

Figure 3.8: **Effect of sodium borohydride concentration on arsine yields (HCl acid concentration fixed at 0.1mol/l)**

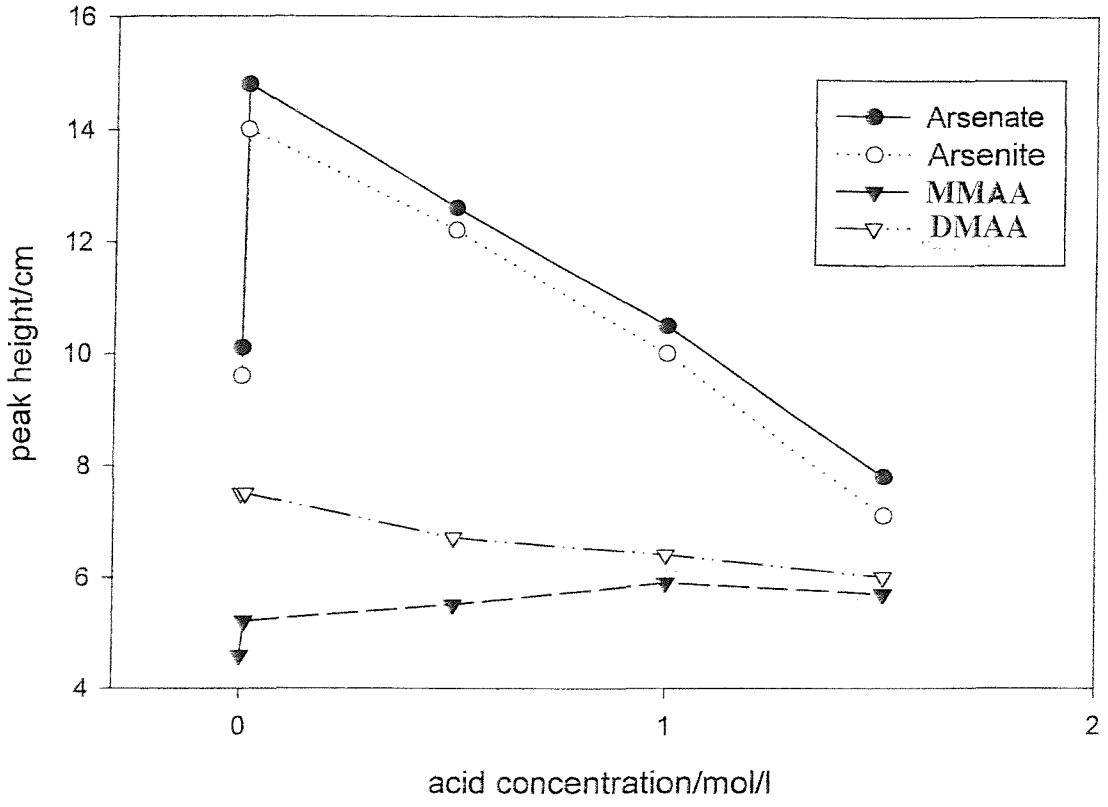

Figure 3.9: **Effect of HCl (acid) concentration on arsine yields (borohydride concentration fixed at 2% w/v)**

slightly. Therefore, the HCl concentration was fixed at 0.01mol/l for further analysis.

3.5.1.4 Kinetics of the formation of arseno-cysteine complexes

In order to monitor the kinetics of the formation of the arseno-cysteine complexes, an experiment was devised which made use of the automated method. To a volumetric flask containing a solution of 20ng As/ml of arsenite, 10mg/ml of L-cysteine was added. The volumetric flask was then shaken and the solution was analysed continuously by the automated HG-AAS method. A stopwatch was started after the L-cysteine was added to the solution and stopped after approximately 8 minutes when the chart recorder output was switched off. This experiment was repeated with standard solutions of arsenate, MMAA and DMAA. The time taken for the sample peak output to reach half its maximum peak output ($t_{\frac{1}{2}}$) was calculated as a measure of formation kinetics.

Table 3.3 shows that the formation kinetics for the 4 different arsenic-cysteine complexes are not all the same. For example, arsenite and DMA appear to react with cysteine much faster than arsenate and MMA. This therefore suggests that the maximum response from arsenate and MMA is obtained after several minutes at room temperature

Arsine species	Time taken to reach half maximum output /seconds
Arsenite	66 ± 2
Arsenate	105 ± 1
MMAA	118 ± 4
DMAA	88 ± 3

Table 3.3: Kinetics of formation of arsine species with L-cysteine

It is now necessary to find an alternative pre-derivatising agent which enhances peak sensitivity but with faster reaction kinetics than L-cysteine.

3.5.2 Pre-derivatisation with alternative sulphur reagents

3.5.2.1 Introduction

Pre-derivatisation of the four hydride-reducible arsenic species by L-cysteine has been shown to enhance the performance of the automated method for the determination of 'total arsenic' and in the cryogenic trap hydride AAS of arsenic species [6]. Unfortunately, as shown in the previous section, the reaction kinetics for the formation of the arseno-cysteine complexes for the four principal hydride-reducible arsenic species are not identical. This led to a study of other sulphur derivatisation agents such as thioglycolic acid and sodium thioglycolate in order to determine whether any advantages could be gained over L-cysteine.

3.5.2.2 Experimental

Standard solutions were prepared daily by diluting a stock 10µl As/ml solution to 1ng As/ml using deionised water. Four samples containing arsenate, arsenite, MMAA and DMAA, and a blank containing only deionised water, were prepared. 50µl of the sulphur liquid complexant (2-mercaptoethanol (Aldrich), 3-mercaptoethanol (Aldrich), 3-mercaptopropionic acid (Sigma) or thioglycolic acid (BDH)) were added to 1ml of each analyte solution containing 1ng As/ml for each arsenic species being studied. For the solid sulphur complexants (thiourea (BDH), sodium thioglycolate (Aldrich) and L-cysteine (Sigma)), the analyte solutions (1ng As/ml of each species) were prepared in an aqueous solution containing 2% w/v of the sulphur-containing species. After addition of the reagents, the resulting solution was left to stand at room temperature for approximately 30 minutes prior to its analysis step. The arsenic determinations were carried out using the automated flow hydride generation method with the

hydrochloric acid and borohydride concentrations fixed at 0.05mol/l and 2% w/v respectively.

3.5.2.3 Results

Each of the reagents was employed in the determination of arsenite, arsenate, MMAA and DMAA. As L-cysteine is known to enhance arsine yields, L-cysteine was used as a reference pre-derivatisation reagent against which the performance of the other sulphur containing reagents could be determined.

From Table 3.4 it can be seen that two sulphur containing compounds (thioglycolic acid and sodium thioglycolate) gave results that were considered to be similar to L-cysteine. The other sulphur compounds did not give arsenic signal outputs that were considered to be identical or higher than those obtained by L-cysteine under the experimental conditions in this experiment.

Compound	Relative peak height			
	Arsenite	Arsenate	MMA	DMA
L-cysteine	100±0	100±0	100±0	100±0
2-mercaptoethanol	41±7	60±3	67±5	72±7
3-mercaptoethanol	107±6	98±3	53±9	80±4
3-mercaptopropionic acid	74±4	85±3	92±4	89±7
thioglycolic acid	108±3	104±5	100±4	107±2
thiourea	38±7	38±5	13±4	40±5
sodium thioglycolate	105±9	98±4	94±6	97±5

Table 3.4: Responses of arsenic species in the presence of sulphur pre-derivatisation agents compared to those of L-cysteine (taken as 100±0)

3.5.2.4 Rates of reaction with thioglycolic acid and sodium thioglycolate

As a result of the information obtained previously, further investigations were carried out to compare the performances of thioglycolic acid and sodium thioglycolate with L-cysteine.

In Section 3.5.1.3, it was noted that when L-cysteine is added to the sample, maximum response is obtained after several minutes at room temperature. In order to investigate the reaction kinetics of the sulphur complexants, analyte solutions were prepared containing 2% w/v of sodium thioglycolate or 5% v/v of thioglycolic acid. These were employed using the procedure described in Section 3.5.1.2. Table 3.5 shows the variation of the peak height responses from the four main arsenic species against time.

Arsine species	**L-Cysteine**				**TGA**				**NaTGA**			
Arsenite	5.6	5.8	6.3	6.5	5.8	6.2	6.1	6.3	4.4	4.7	4.5	4.9
Arsenate	3.3	5.5	6.1	6.3	5.7	6.2	6.0	6.3	0.2	0.8	5.8	6.1
MMA	2.0	4.2	5.8	6.4	6.1	6.3	6.5	6.4	1.5	1.9	5.6	6.2
DMA	6.2	6.1	6.4	6.4	5.8	6.1	5.9	6.2	5.2	4.9	5.0	4.7
Time (minutes)	2	10	20	60	2	10	20	60	2	10	20	60

Table 3.5: Variation of peak height against time for the four hydride reducible arsine species (note TGA and NaTGA stand for thioglycolic acid and sodium thioglycolate respectively)

For sodium thioglycolate, the prederivatisation reaction with arsenate and MMA is not complete after two minutes. This, therefore, suggests that the higher pH of sodium thioglycolate solution could have a bearing on the reaction kinetics. With thioglycolic acid, however, the prederivatisation reaction is essentially complete

for all four arsenic species within two minutes. This suggests that thioglycolic acid is the preferred reagent for the enhancement of arsenic peak height signals. This is in accordance with the studies carried out by Howard and Salou [6].

3.5.3 Conclusions and discussion

The addition of L-cysteine to a solution containing hydride reducible arsenic species has been found to be beneficial in terms of improving signal peak output and by reducing the acid levels required for the analysis. However, the formation kinetics for the four hydride reducible arsenic species are not ideal: arsenite and DMA react with L-cysteine much faster than MMA and arsenate.

Braman and Foreback [7] have reported that the complete protonation of arsenic species such as arsenate and dimethylarsinate is required for their reaction with sodium borohydride but low pH conditions are necessary to achieve this protonation. Le [3] suggests that low pH conditions are not necessary, however, when L-cysteine is present. When L-cysteine is added to arsenate, a pre-reduction step is believed to occur before it reacts with sodium borohydride:

$$2CysSH + H_3AsO_4 \longrightarrow Cys\text{-}S\text{-}S\text{-}Cys + As(OH)_3 + H_2O \qquad (3.1)$$

where Cys-SH is the abbreviated form of L-cysteine.

The $As(OH)_3$ species then reacts with the L-cysteine to form arseno-cysteine complexes:

$$3CysSH + As(OH)_3 \longrightarrow As(\text{-}SCys)_3 + 3H_2O \qquad (3.2)$$

As the arseno-cysteine complexes are neutral species, it is not necessary to carry out their reaction with sodium borohydride under low pH conditions. This, in turn,

results in lower levels of acid being required for the analysis and improves the blank.

For arsenite, the rate of formation of the arsenic-cysteine complex is fast compared to the other three species studied. This is due to the arsenic species already being present in the +3 oxidation state, eliminating the need for reaction (3.1). With arsenate and MMAA, the kinetics are much slower due to the need for the pre-reduction step in (3.1) to occur.

With the sodium thioglycolate and thioglycolic acid containing samples the reaction mechanisms are believed to be similar to those described above and only low levels of acid (0.05 mol/l) were therefore required. This results in lower acid derived blank signals, improving detection limits and peak reproducibility.

One major disadvantage of using thioglycolic acid as an alternative reagent to L-cysteine was that special precautions had to be taken in the laboratory, as it was a toxic compound with an unpleasant smell.

3.6 Summary

The desire to study the behaviour of arsenic in the marine environment has resulted in the development of hydride generation-cryogenic trap (HG-CT) systems capable of measuring trace quantities of arsenic and its speciation in the nanogram-picogram range [8]. These cryogenic trap systems offer a relatively simple and inexpensive technique that can be used for the determination of arsenic speciation in estuarine water samples within a relatively short timeframe.

The use of pre-derivatising agents has been shown to improve further the usefulness of HG-CT methods. With the addition of L-cysteine to a sample matrix, peak sensitivity is improved and acid levels required for analysis are reduced. However the use of L-cysteine has its disadvantages as the formation

kinetics for the four arsenic species studied makes it impractical to incorporate the inline introduction of L-cysteine into the cryogenic trap HG-AAS system. Thioglycolic acid gives improved rates of arsine formation but its unpleasant smell in an open laboratory environment is unsatisfactory.

3.7 References

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Chapter 4

Pretreatment methods for the decomposition of organoarsenic compounds

4.1 Introduction

Organoarsenical species such as arsenobetaine and arsenosugars cannot be detected by conventional HG-AAS methods alone as arsenobetaine and arsenosugar species do not form volatile hydrides upon reaction with sodium borohydride. This has led to workers such as Howard [7] and de Bettencourt [8] to suggest that additional organoarsenical species other than the simple methylated oxy-anions are present in natural waters. These non-hydride reducible arsenic species are commonly referred to as 'hidden' or 'refractory' arsenic.

One analytical tool that can be used to permit the detection of 'hidden' or 'refractory' organoarsenicals is to partially predigest them prior to analysis by HG-AAS methods. For example, de Bettencourt [8] developed a base digestion procedure which enabled 'refractory' forms of organoarsenicals to be converted to hydride-reducible forms, thereby permitting their detection by HG-AAS methods. To enable the detection of 'hidden' arsenic, Howard [7] developed a photolysis pre-treatment step using a medium pressure mercury arc lamp which enabled previously unidentified forms of arsenic to be broken down to hydride reducible forms prior to analysis by HG-AAS techniques. All such pretreatment methods, however, can result in a loss of arsenic speciation information and it can be difficult to ascertain whether all of the 'hidden' or 'refractory' arsenic species have been converted to the hydride reducible forms.

Hasegawa [9] and Hanaoka [10] have both suggested that these previously undetected forms of arsenic play a vital role in linking arsenic speciation in

natural waters with the biological activity of marine organisms. Figure 4.1 shows a possible scheme for the cycling of arsenic in the marine environment.

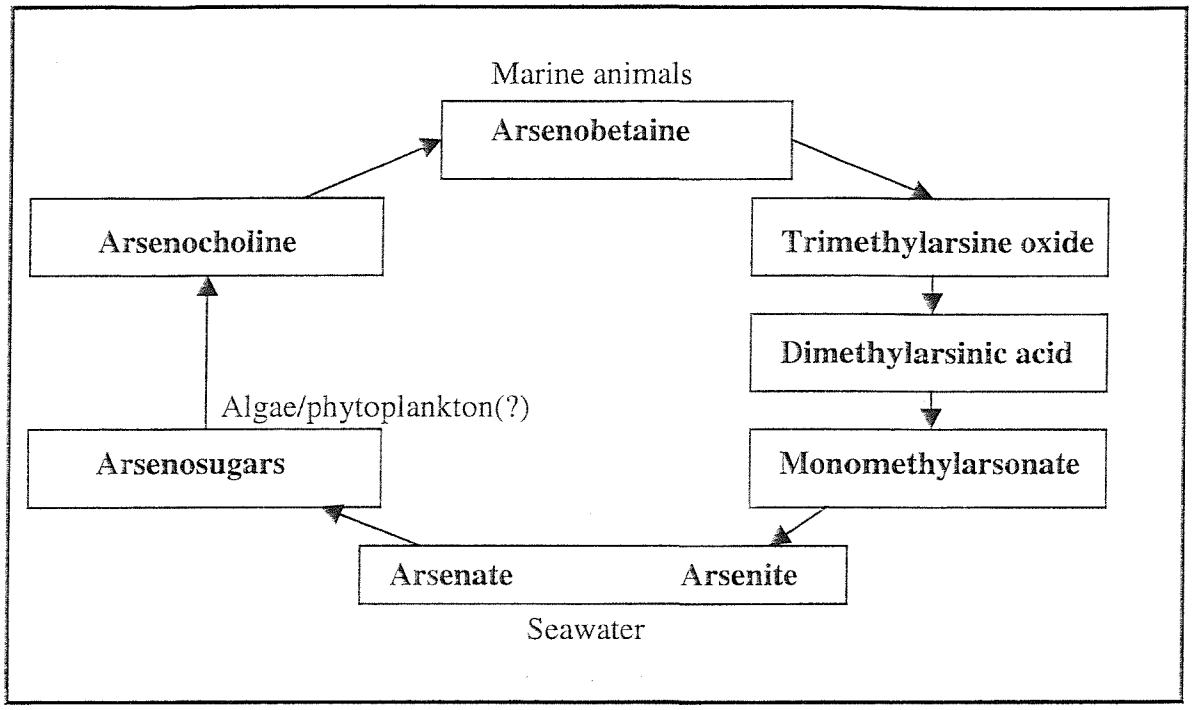

Figure 4.1: Arsenic cycling in the marine environment as suggested by Hanaoka [10].

This chapter describes the investigation of a number of analytical techniques for the study of 'hidden' arsenic.

4.2 Batch ultra-violet photolysis methods

4.2.1 Introduction

Batch photochemical methods for the decomposition of arsenic compounds derive from the work of Tam [11] and have been used for the detection of 'hidden' arsenic in natural water samples. Howard and Comber [7] reported that in their study of estuarine waters, an average 25% increase in the measured concentration

of dissolved arsenic species was obtained following sample irradiation using a 200W Wotan short arc mercury lamp.

4.2.2 Experimental

For batch u.v. irradiation experiments carried out in this section, a 200W Wotan medium pressure mercury lamp was used. The lamp was enclosed in a chamber fitted with an extraction fan. Samples were dispensed into quartz tubes (3mls) which were sealed using quartz stoppers and placed approximately 3cm away from the mercury lamp. After 18 hours irradiation [12], the samples were allowed to cool and analysed using cryogenic trap HG-AAS methods.

4.2.3 Results

4.2.3.1 The ultra violet emission wavelengths

The emission spectrum of the 200W Wotan mercury medium arc lamp was measured using a Varian AA-5 atomic absorption spectrophotometer in emission mode. Table 4.1 details the major emission wavelengths present. It should be noted that due to the variation of the photomultiplier response at different wavelengths, it is not possible to calculate accurately the emission intensities. Comparison of the major emission wavelengths obtained using the 200W Wotan mercury medium arc lamp with the literature values for the mercury emission spectrum reveal that the major emission wavelength at 254nm is absent. This is believed to be due to a glass envelope.

Light source	Emission wavelength/nm
Wotan mercury arc lamp	365.3, 404.3, 435.6, 546.5
Literature data [22] for the mercury emission spectrum	253.6, 365.0, 365.3, 365.5, 404.7, 435.8, 546.1

Table 4.1: Emission wavelengths of the Wotan 200W mercury lamp

4.2.3.2 The ultra-violet irradiation of hydride-reducible arsenic species

The effects of irradiation on standard solutions of arsenite, arsenate, MMAA and DMAA were investigated using the procedure described in Section 4.2.2.

Table 4.2 shows that there is no evidence of demethylation of the methylated arsenic species (MMAA and DMAA). Likewise, within experimental error, for arsenate and arsenite, there is no significant change in the 'inorganic' arsenic recovery.

Sample	Arsenic species detected prior to sample irradiation		
	'Inorganic arsenic'	MMA	DMA
1µg As/l arsenate	0.98 ± 0.03	not detected	not detected
1µg As/l arsenite	0.95 ± 0.01	not detected	not detected
1µg As/l MMAA	not detected	1.04 ± 0.05	not detected
1µg As/l DMAA	not detected	not detected	0.93 ± 0.02

All concentrations are measured in µg As/l

Sample	Arsenic species detected after sample irradiation		
	'Inorganic arsenic'	MMA	DMA
1µg As/l arsenate	1.02 ± 0.04	not detected	not detected
1µg As/l arsenite	0.97 ± 0.04	not detected	not detected
1µg As/l MMAA	not detected	0.94 ± 0.03	not detected
1µg As/l DMAA	not detected	not detected	1.06 ± 0.05

All concentrations are measured in µg As/l

Table 4.2: Table showing the results obtained before and after 18 hours photolysis of hydride reducible arsenic species using the 200W Wotan mercury lamp

4.2.3.3 Ultra-violet irradiation of non-hydride reducible arsenic species

Standard solutions of non-hydride reducible arsenic compounds (p-arsanilic acid ($C_6H_8AsNO_3$, Sigma), o-arsanilic acid ($C_6H_8AsNO_3$, Sigma), arsenazo (I) ($C_{16}H_{10}AsN_2O_{11}S_2Na_3$, Aldrich) and arsenazo (III) ($C_{22}H_{18}As_2N_4O_{14}S_2$, Aldrich)) were prepared to give standard solutions of 1000mg As/ml and diluted appropriately to 1ng As/ml. The samples were then prepared and irradiated using the method described in Section 4.2.2.

Table 4.3 shows that the analysis of the non-hydride reducible arsenic species prior to irradiation results in no arsenic signal. After sample irradiation, however, there was no evidence of MMA or DMA but the level of 'inorganic' arsenic increased. This evidence supports the view that the batch irradiation procedure could prove to be useful in the determination of the concentration levels of non-hydride reducible arsenic species in the estuarine environment.

Sample	Arsenic species detected prior to sample irradiation		
	'Inorganic arsenic'	MMA	DMA
1µg As/l o-arsanilic acid	not detected	not detected	not detected
1µg As/l p-arsanilic acid	not detected	not detected	not detected
1µg As/l arsenazo (I)	not detected	not detected	not detected
1µg As/l arsenazo (III)	not detected	not detected	not detected

Sample	Arsenic species detected after sample irradiation		
	'Inorganic arsenic'	MMA	DMA
1µg As/l o-arsanilic acid	0.92 ± 0.03	not detected	not detected
1µg As/l p-arsanilic acid	0.94 ± 0.04	not detected	not detected
1µg As/l arsenazo (I)	0.91 ± 0.02	not detected	not detected
1µg As/l arsenazo (III)	0.95 ± 0.03	not detected	not detected

All concentrations are measured in µg As/l

Table 4.3: Table showing the results obtained prior to and after 18 hours photolysis of non-hydride reducible arsenic species

4.2.3.4 Discussion

Treatment of samples by ultra-violet irradiation can assist in the photochemical decomposition of organoarsenic compounds when high powered lamps are employed. Such methods usually result in the complete decomposition of the organoarsenic compound to arsenate. For example, Cullen and Dodd [14] reported that by using a batch u.v. irradiation method with the samples placed in fused-silica tubes and left to photolyse using a 1200W-mercury lamp for 1 hour, it was possible to convert organoarsenic compounds to arsenate. In this study, the cleavage of the As-CH_3 bond in MMAA and DMAA was not evident. This may be

due to the comparatively low power of the lamp employed or due to the absence of the 254nm line from this particular lamp design.

With the non-hydride reducible arsenic species studied As-CH_3 groupings are absent and it should not be possible to generate methylated arsenic from them. within their chemical structures. The results suggest that sample decomposition to 'inorganic' arsenic species is close to completion for the four non-hydride reducible arsenic samples studied.

4.2.3.5 Conclusion

It has been shown that batch irradiation of organoarsenic compounds using a 200W Wotan mercury arc lamp can be a useful tool for the detection of 'hidden' arsenic compounds in estuarine waters/sediment samples. Of particular importance is the apparent conservation of the As-CH_3 bond therefore retaining some element of speciation information during the breakdown of 'hidden' arsenic species.

The long time (18 hours) required for effective u.v. irradiation can be time-consuming and only a limited number of samples can be treated at any one time. Further work on this technique, using estuarine water and sediment samples, is described in **Chapters 5 and 6.**

4.3 Batch ultra-violet irradiation of organoarsenic species with the addition of oxidants

4.3.1 Introduction

Oxidising agents such as potassium persulphate and hydrogen peroxide are commonly used to break down organic compounds into simpler forms that permit detection by quantitative methods. A study of the use of persulphate oxidising

agents in the decomposition of organoarsenic compounds to arsenate, was made by Atallah and Kalman [15].

It was thought that the addition of a controlled quantity of an external oxidant such as potassium persulphate or hydrogen peroxide could assist in reducing the times required for the effective u.v. irradiation of organoarsenic compounds. A study was therefore carried out to ascertain whether any advantages could be gained over the conventional batch u.v. photolysis methods described in the previous section.

4.3.2 Experimental

Experiments were devised in which a fixed quantity of either potassium persulphate solution (prepared as detailed in Section 3.2) or hydrogen peroxide solution (30% (v/v), GPR, BDH) was added to quartz test tubes each containing 3mls of 1µg As/l of the arsenic species studied. The quartz test tubes were then shaken well and the stoppers removed prior to their transfer to the u.v. lamp compartment. The samples were then irradiated, allowed to cool, shaken well and analysed by cryogenic trap HG-AAS.

4.3.3 Potassium persulphate oxidant

4.3.3.1 Photolysis time optimisation

Attention was initially focussed on optimizing the time required for the effective decomposition of both hydride and non-hydride reducible organoarsenic compounds to 'inorganic' arsenic. 100µl of 2% w/v potassium persulphate solution in 1% w/v sodium hydroxide (prepared as described in Section 3.2) was added to each of the quartz test tubes containing 3mls of the arsenic solution to be studied. The samples were then treated in a manner similar to that described in

Section 4.3.2 with the photolysis times ranging from between 0 (control) to 18 hours.

4.3.3.2 Results

From Table 4.4 it can be clearly seen that the MMAA and DMAA samples undergo a stepwise decomposition to 'inorganic' arsenic. With DMAA it would appear that the potassium persulphate oxidant starts to act immediately on its addition. After a one hour photolysis the proportion of the arsenic present as DMA has decreased and both MMA and 'inorganic' arsenic signals have appeared. This implies that the ultra-violet irradiation process breaks down the DMAA species to MMAA, which in turn decomposes to the 'inorganic' arsenic form. With 4 hours irradiation, DMAA sample breakdown is near completion but it is not until after 8 hours sample irradiation that the entire original DMAA sample is converted to 'inorganic' arsenic forms.

Similar behaviour was observed with the MMAA sample with sample decomposition to 'inorganic' arsenic being essentially complete after 8 hours u.v. irradiation.

For the non-hydride reducible arsenic species studied, there was no evidence of methylarsine generation without sample irradiation. With the control samples (0 hours photolysis, 100µl potassium persulphate solution), the potassium persulphate, was again found to generate a little hydride reducible arsenic. The non-hydride reducible arsenic species break down to 'inorganic' arsenic more easily than MMAA and DMAA with sample breakdown to 'inorganic' arsenic appearing to be complete after 4 hours irradiation.

Within experimental error, there was no significant change in the 'inorganic' arsenic signal output values for the arsenite and arsenate samples studied.

Sample	Arsenic species detected (μg As/l)	Photolysis time (hours)					
		0	1	2	4	8	18
Arsenate	'Inorganic' arsenic	1.04±0.02	0.98±0.04	1.02±0.05	1.05±0.03	0.98±0.04	1.01±0.04
	MMA	not detected	not detected	not detected	not detected	not detected	not detected
	DMA	not detected	not detected	not detected	not detected	not detected	not detected
Arsenite	'Inorganic' arsenic	0.96±0.02	0.94±0.02	1.02±0.03	0.95±0.03	0.98±0.03	0.93±0.02
	MMA	not detected	not detected	not detected	not detected	not detected	not detected
	DMA	not detected	not detected	not detected	not detected	not detected	not detected
MMAA	'Inorganic' arsenic	0.03±0.01	0.21±0.04	0.62±0.02	0.86±0.04	0.95±0.02	0.92±0.03
	MMA	0.92±0.03	0.68±0.02	0.18±0.14	0.02±0.01	not detected	not detected
	DMA	not detected	not detected	not detected	not detected	not detected	not detected
DMAA	'Inorganic' arsenic	not detected	0.02±0.01	0.28±0.04	0.82±0.05	0.92±0.02	0.86±0.05
	MMA	0.05±0.02	0.18±0.02	0.42±0.02	0.06±0.01	not detected	not detected
	DMA	0.87±0.03	0.70±0.04	0.13±0.04	not detected	not detected	not detected

Table 4.4(a): Batch photolysis + 100μl persulphate solution time optimisation for the hydride reducible arsenic species (n=4)

Sample	Arsenic species detected (µg As/l)	Photolysis time (hours)					
		0	1	2	4	8	18
o-arsanilic acid	'Inorganic' arsenic MMA DMA	0.08±0.02 not detected not detected	0.61±0.03 not detected not detected	0.87±0.02 not detected not detected	1.04±0.05 not detected not detected	0.96±0.03 not detected not detected	0.93±0.03 not detected not detected
p-arsanilic acid	'Inorganic' arsenic MMA DMA	0.05±0.02 not detected not detected	0.68±0.02 not detected not detected	0.90±0.03 not detected not detected	0.91±0.02 not detected not detected	0.92±0.04 not detected not detected	0.95±0.02 not detected not detected
arsenazo (III)	'Inorganic' arsenic MMA DMA	0.07±0.02 not detected not detected	0.57±0.03 not detected not detected	0.80±0.05 not detected not detected	0.94±0.01 not detected not detected	0.91±0.02 not detected not detected	0.86±0.04 not detected not detected
arsenazo (I)	'Inorganic' arsenic MMA DMA	0.04±0.01 not detected not detected	0.64±0.03 not detected not detected	0.77±0.04 not detected not detected	0.89±0.02 not detected not detected	1.03±0.04 not detected not detected	0.89±0.02 not detected not detected

Table 4.4(b): Batch photolysis + 100µl persulphate solution time optimisation for the non-hydride reducible arsenic species (n=4)

With the addition of persulphate solution, it would, therefore, appear that an optimum time for the effective photolysis of all of the organoarsenic species studied is between 4 and 8 hours.

4.3.3.4 Volume of persulphate solution

It was decided to ascertain whether it would be possible to reduce further the time required for the effective decomposition of the organoarsenic species by increasing the volume of potassium persulphate added. It should be noted that if a large quantity of potassium persulphate solution is added to the organoarsenic species studied, problems may arise during the analysis stage as an increase in potassium persulphate oxidant could lead to a decrease in the reducing ability of the sodium borohydride reagent in the cryogenic trap HG-AAS analysis, resulting in a lower than expected arsenic signal.

To each quartz test tube containing 3mls of a solution of the organoarsenic species studied was added a fixed quantity (from between 0 to 1ml) of 2% w/v potassium persulphate reagent (prepared as described in Chapter 3.2). The quartz test tubes were then shaken well and the stoppers removed prior to transfer to the u.v. lamp compartment where they were left to photolyse for 4 hours. The samples were then treated and analysed as described in Section 4.3.2.

4.3.3.5 Results

It was noted that for the arsenic samples containing 500µl and 1000µl potassium persulphate solution the uncorrected 'inorganic' arsenic signal (i.e. uncorrected for blank values) was much higher than those obtained for the arsenic samples containing 100-200µl potassium persulphate solution. This can be attributed to the arsenic impurity present in the potassium persulphate. Such high levels of

Sample	Arsenic species present (μg As/l)	Volume of persulphate solution added/μl				
		0	100	200	500	1000
arsenate	'Inorganic' arsenic	0.96±0.02	1.05±0.03	0.98±0.04	0.86±0.04	0.80±0.04
arsenite	'Inorganic' arsenic	0.98±0.04	0.95±0.03	0.93±0.02	0.93±0.02	0.76±0.04
o-arsanilic acid	'Inorganic' arsenic	not detected	1.04±0.05	0.96±0.02	0.90±0.04	0.83±0.05
p-arsanilic acid	'Inorganic' arsenic	not detected	0.91±0.02	0.94±0.03	0.86±0.02	0.76±0.02
arsenazo(III)	'Inorganic' arsenic	not detected	0.94±0.02	1.02±0.03	0.92±0.04	0.84±0.05
arsenazo (I)	'Inorganic' arsenic	not detected	0.89±0.02	0.92±0.03	0.85±0.02	0.79±0.03
MMAA	'Inorganic' arsenic MMA	not detected 0.94±0.02	0.86±0.04 0.02±0.01	0.90±0.02 0.04±0.02	0.78±0.02 not detected	0.72±0.05 not detected
DMAA	'Inorganic' arsenic MMA DMA	not detected not detected 0.93±0.02	0.82±0.05 0.06±0.01 not detected	0.92±0.04 0.02±0.01 not detected	0.82±0.04 not detected not detected	0.73±0.03 not detected not detected

Table 4.5: Persulphate optimization experiment for hydride and non-hydride reducible arsenic species (n=4)

uncorrected 'inorganic' arsenic signal outputs can pose difficulties in determining accurately the true corrected 'inorganic' arsenic output.

It was observed from Table 4.5 that for the samples containing both 500µl and 1000µl potassium persulphate solution, the corrected arsenic signal outputs are lower than expected for all of the arsenic species studied. This is probably due to the increased levels of persulphate which reduce the ability of the sodium borohydride to react with the arsenic species and hence decreases the expected arsenic signal peak output.

Increasing the persulphate addition from 100 to 200µl resulted in little improvement and the optimum volume for the potassium persulphate solution would therefore be within this range.

4.3.4 The use of hydrogen peroxide as an oxidising agent

4.3.4.1 Experimental

Encouraged by the success of the experiments described above using potassium persulphate reagent, it was decided to see whether any advantages or comparable results could be gained using hydrogen peroxide. The procedure described in Section 4.3.2 was followed with 100µl of hydrogen peroxide (30% (v/v), GPR, BDH) solution being added to each quartz test tube. The samples were left to irradiate for 4 hours. After cooling, the quartz test tubes were placed into a water bath heated at 90°C for approximately 1½ hours to remove any traces of hydrogen peroxide. Once all of the hydrogen peroxide had been decomposed or driven off, the samples were again allowed to cool. Analysis was then carried out using cryogenic trap HG-AAS methods.

4.3.4.2 Results

Analysis of the data in Table 4.6 suggests that for the 1µg As/l DMAA sample studied, there was evidence of all three hydride reducible arsenic species being present: DMA, MMA and 'inorganic' arsenic, with the 'inorganic' arsenic signal output being the largest. However, the arsenic mass balance for the DMAA sample is not close to unity.

For the 1µg As/l MMAA solution, there was also evidence of both MMA and 'inorganic' arsenic being present after sample irradiation which again suggests that MMAA sample decomposition is not complete. It was also noted, that again the total arsenic recovery after sample treatment was not complete.

With the non-hydride reducible arsenic species studied, it was noted that the conversion rate to 'inorganic' arsenic was not as high as could be obtained by using potassium persulphate.

It is possible that some of the arsenic may have vaporised after heat treatment, leading to a smaller than expected arsenic recovery. Alternatively, there may have been traces of hydrogen peroxide reagent present in the sample when analysed by HG-AAS, which would in turn reduce the effectiveness of the sodium borohydride reducing agent.

4.3.5 Discussion

The addition of a fixed quantity of either potassium persulphate or hydrogen peroxide to organoarsenic samples prior to u.v. irradiation has proved to be beneficial in reducing the time required for the decomposition of organoarsenic species. It would appear that potassium persulphate is the preferred reagent for assisting the decomposition of organoarsenic species. Treatment of the arsenic samples with hydrogen peroxide reagent led to the formation of oxygen which was

Sample	Arsenic species present	% recovery of each species present after H_2O_2 photolysis
arsenate	'Inorganic' arsenic	85±2%
arsenite	'Inorganic' arsenic	73±5%
MMAA	'Inorganic' arsenic MMA	53±4% 22±4%
DMAA	'Inorganic' arsenic MMA DMA	45±3% 21±3% 5±1%
o-arsanilic acid	'Inorganic' arsenic	76±3%
p-arsanilic acid	'Inorganic' arsenic	73±3%
arsenazo (III)	'Inorganic' arsenic	71±3%
arsenazo (I)	'Inorganic' arsenic	67±3%

Table 4.6: Batch photolysis + H_2O_2 reagent results for hydride and non-hydride reducible arsenic species

difficult to remove using gentle heating (30-40°C). By adopting a harsher heating regime it is possible that some of the arsenic present will have vaporised.

The results obtained with both reagents confirmed the theory suggested by Hunt [13] that the hydride reducible organoarsenic species undergo a stepwise demethylation process to arsenate.

The reaction mechanism for the photodecomposition of organoarsenicals [15,16] to hydride reducible forms using potassium persulphate is thought to involve a three-stage process. The first stage involves the formation of the $[SO_4]^{-\bullet}$ radical, which is formed by the excitation (a maximum absorption at 235nm [15]) of the persulphate ion:

$$[S_2O_8]^{2-} \xrightarrow{h\nu} 2[SO_4]^{-\bullet} \qquad (4.1)$$

The sulphate radicals then react with water to form hydroxyl radicals:

$$[SO_4]^{-\bullet} + H_2O \rightleftharpoons HSO_4^- + OH^{\bullet} \qquad (4.2)$$

At alkaline pH values, $SO_4^{-\bullet}$ radical concentrations decrease close to zero, resulting in a high proportion of $OH^{\bullet}$ radicals being present. It is thought that the $OH^{\bullet}$ radicals have the ability to decompose organic species to CO_2, thereby suggesting that the $OH^{\bullet}$ radical is involved in the photodecomposition of organoarsenic species to arsenate [15]:

$$\text{organoarsenic species} + OH^{\bullet} \longrightarrow \text{arsenate} + H_2O + CO_2 \qquad (4.3)$$

The mechanism of the reaction of hydrogen peroxide and the organoarsenic species when subjected to u.v. irradiation is thought to occur in a similar manner to that shown for potassium persulphate solution. This involves a two-stage

process instead of three [16]. The first step involves the formation of the hydroxyl radical formed by the photodecomposition of the hydrogen peroxide species:

$$H_2O_2 \xrightarrow{h\nu} 2OH^{-\bullet} \qquad (4.4)$$

The oxidation by the hydroxyl radicals is, again, dependent upon the pH of the solution. Studies determining the optimum pH range for the formation of the hydroxyl radical have revealed that the most effective pH range is between 6-8, which lies within an acid-neutral-base boundary. The reaction between the hydroxyl radical and the organoarsenic species results in the formation of arsenate, water and carbon dioxide as shown in reaction (4.3).

Atallah and Kalman [15], in their report of the photodecomposition of organoarsenic species, reported that HNO_3 has been used as an alternative oxidising agent to hydrogen peroxide and persulphate reagents. However, they emphasise that the best results are obtained using persulphate reagents rather than hydrogen peroxide or nitric acid.

4.3.6 Conclusion

The addition of a controlled quantity of an external oxidising agent such as potassium persulphate solution prior to sample irradiation has proved advantageous in breaking down selected hydride and non-hydride reducible organoarsenic species. By applying this method in the determination of 'hidden' arsenic species in estuarine waters and sediment samples, it is likely that the times required for the photolysis of non-hydride reducible arsenic species will be reduced. Care must however be taken to ensure that the potassium persulphate does not interfere with other ions that may be present in the estuarine water/sediment sample that could lead to a reduction in the expected arsenic signal.

Further work on this batch irradiation technique using estuarine water and sediment samples is described in detail in **Chapters 5 and 6.**

4.4 Online cryogenic HG-AAS method for the determination of 'hidden' arsenic using a low power u.v. lamp and potasium persulphate oxidant

4.4.1 Introduction

Some understanding of the nature of 'hidden' arsenic species in estuarine waters can be obtained by HPLC systems interfaced with, for example, HG-AAS (HPLC-HG-AAS) [17] with online photo-oxidation. A major stumbling block, however, is that the 'hidden' arsenic concentrations in estuarine waters/sediment samples are too low to permit detection by such methods.

It was therefore suggested that the incorporation of a low power u.v. lamp into the cryogenic HG-AAS system, together with the use of an added oxidant such as persulphate, might prove beneficial to determining the low levels of 'hidden' arsenic that are present in estuarine water/sediment samples.

4.4.2 Preparation

In addition to the standard solutions of MMAA and DMAA prepared previously, standard solutions of non-hydride reducible arsenic compounds (10μgAs/ml) were prepared from each of p-arsanilic acid ($C_6H_8AsNO_3$, Sigma), phenylarsonic acid ($C_6H_{10}AsN_2O_{11}S_2Na_3$, Aldrich), o-arsanilic acid ($C_6H_8AsNO_3$, Sigma), arsenazo(I) ($C_{16}H_{10}AsN_2O_{11}S_2Na_3$, Aldrich) and diluted to give 1μg As/l working standards when necessary. The concentrations of the stock solutions were checked by FAAS.

4.4.3 Experimental

The apparatus employed in this work is based upon the cryogenic trap procedure described in Chapter 3. The only major difference to the instrument construction was the addition of a 4W-mercury lamp wound with PTFE tubing (370cm in length, o.d. 1.6mm, i.d. 0.8mm) (Figure 4.2). The lamp was covered with aluminium foil and a glass sleeve painted to prevent the exposure of the operator to u.v..

With the deionised water, acid, persulphate and borohydride pumping through the system, the cryogenic trap was placed into liquid nitrogen and the timer was started. After 1 minute, the sample tube was moved to the sample vial and 1ml of sample was introduced into the system. The sample and the persulphate solution were mixed together and irradiated using the Sylvania 4W G4TS mercury lamp prior to acidification with hydrochloric acid and its subsequent reduction with sodium borohydride. After all the sample had been taken up from the sample vial, the system was allowed to run for approximately 3 minutes to allow the arsine gases to be condensed within the cryogenic trap prior to the removal of the liquid nitrogen dewar. Once the liquid nitrogen dewar had been removed, the electrical heater was switched on to *ca.* 21V to allow the arsine gases to volatilise and be transported by the carrier gas into the detector and the chart recorder was switched on. With the arsenic signal recorded, the chart recorder was switched off, the liquid solution in the controlled flow gas/liquid separator was removed and the electrical heater switched off. After approximately 1 hour of continuous operation, the sodium hydroxide pellets were replaced by fresh pellets.

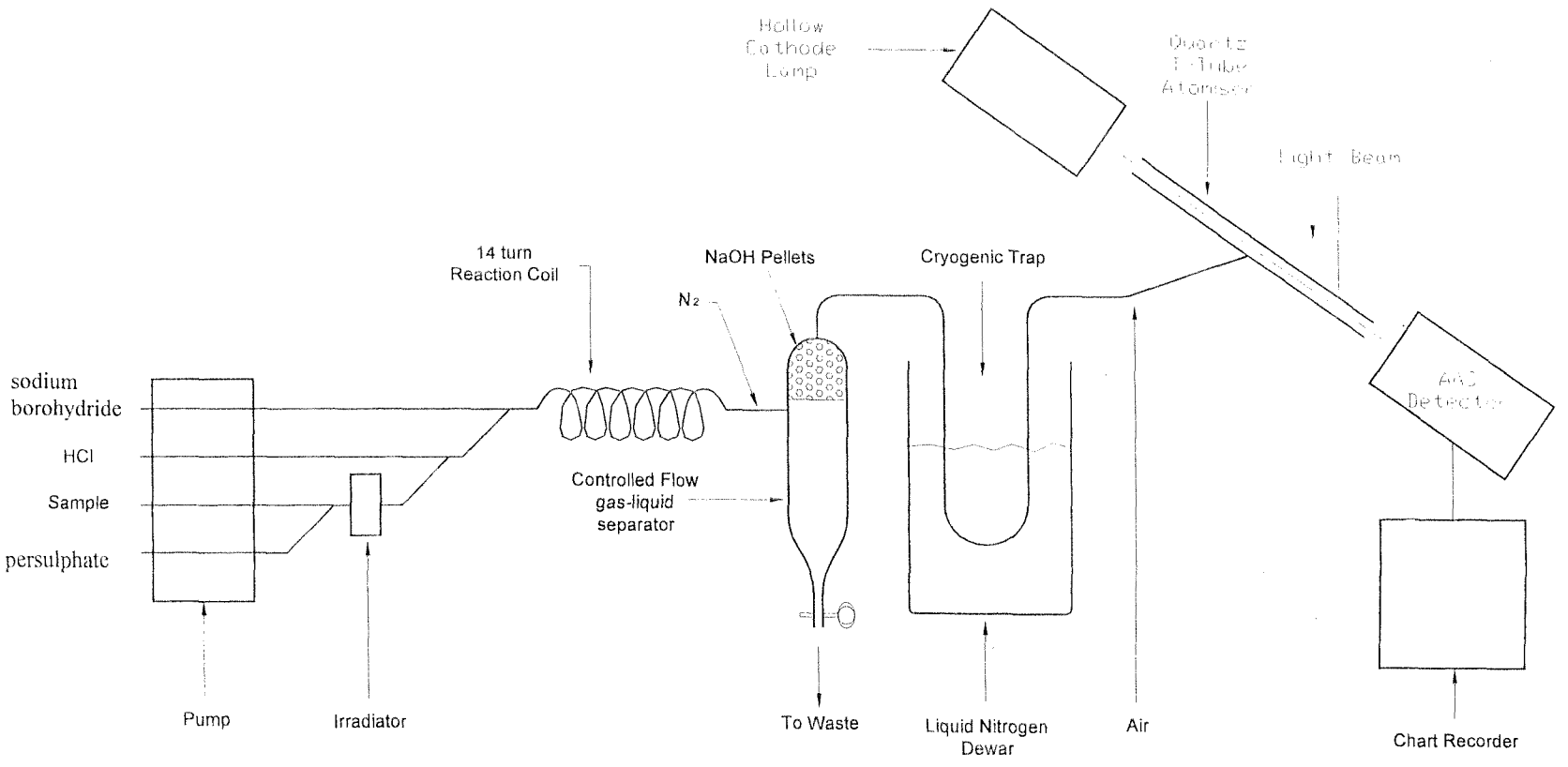

Figure 4.2: Schematic representation of the persulphate inline photooxidation cryogenic trap HG-AAS apparatus.

4.4.4 Results

4.4.4.1 Introduction

Initially, there were two main problems. The first was the build up of pressure within the system causing tubing to burst. In order to alleviate this problem, the diameters of the pump tubes were reduced. The second problem was poor reproducibility. This was resolved by allowing a slightly longer period of time for the material to purge from the trap before the next sample run.

A summary of the system parameters used in the final configuration is given in Table 4.7.

4.4.4.2 The ultraviolet emission spectrum of the Sylvania 4W mercury lamp

The emission spectrum of the low powered Sylvania mercury lamp was measured by a similar procedure to that described in Section 4.2.3.1. Table 4.8 shows that the high energy mercury emission line at 254nm which was absent from the emission spectrum of the 200W Wotan mercury arc lamp is present. The line at 506nm is not attributed to the mercury emission spectrum but due to the inert gas filling agent.

Air flow rate	10ml/min
Nitrogen carrier gas flow rate	150ml/min
HCl flow rate	1.25ml/min
HCl concentration	1 mol/l
Sodium borohydride flow rate	1.25ml/min
Sodium borohydride concentration	2% w/v
Persulphate solution flow rate	0.75ml/min
Persulphate concentration	2% w/v
Sodium hydroxide concentration	1% w/v
Sample flow rate	1.25ml/min
Light source	Arsenic hollow cathode lamp
Wavelength	193.7nm
Current	7mA
Sample size	1ml
Trap heating before next sample	1 minute
Equilibration in liquid nitrogen	1 minute
Lamp used	Sylvania 4W mercury lamp

Table 4.7: System parameters for the online photo-oxidation cryogenic trap system

Light source	Emission wavelength/nm
Sylvania 4W mercury lamp	253.4, 313.6, 365.2, 404.8, 435.6, **506.4**
Literature data [22] for the mercury emission spectrum	253.6, 365.0, 365.3, 365.5, 404.7, 435.8, 546.1

Table 4.8: Emission lines for the 4W Sylvania 4W mercury lamp

4.4.4.3 Optimisation of the sample timing

An experiment was devised to optimise the time required for the sample to pass through the system. For this experiment, the potassium persulphate concentration was fixed at 2% w/v in a 1% w/v sodium hydroxide solution. 1μg As/l of arsenazo(III) was then passed into the system and the time allowed for the sample to be processed prior to the removal of the liquid nitrogen dewar was varied from between 1 minute to 6 minutes. After each arsenazo(III) measurement, the system apparatus was flushed out by carrying out five blank measurements to ensure that any traces of arsenazo(III) that may have remained within the system apparatus were removed.

Figure 4.3 shows that a very small 'inorganic' arsenic peak output occurred if the time taken for the sample to pass through the system apparatus was fixed at 1 minute. This would imply that transit time had not yet been reached. The 'inorganic' arsenic peak height then increases to a maximum at approximately 4.5 minutes. The peak height obtained at 4.5 minutes was compared to standard solutions of arsenate and it was found that 1μg As/l of arsenazo(III) gave an

'inorganic' arsenic peak output of approximately 0.94 ± 0.03ng of arsenic, suggesting a high conversion rate.

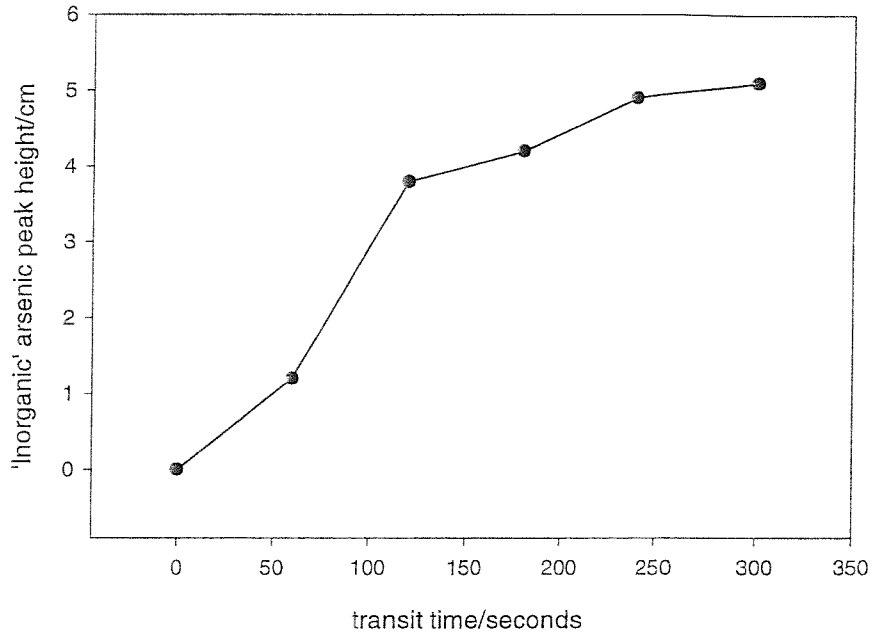

Figure 4.3: Variation of peak height against transit time for 1μg As/l arsenazo(III)

The optimum time of 4.5 minutes was altered to 5 minutes in order to ensure that the entire sample had passed through the system before the material was released from the trap. Subsequent experiments used a sample throughput time of 5 minutes.

4.4.4.4 Optimisation of the potassium persulphate solution for the hydride reducible arsenic species

A 2% w/v potassium persulphate solution was used in the early stages of this chapter as it was reported by Atallah and Kalman [15] to be the most effective solution concentration available for the photodecomposition of organoarsenicals. It was subsequently decided to investigate further whether a variation in the percentage composition of either potassium persulphate or sodium hydroxide present in the solution would prove beneficial to the operation of the online cryogenic trap HG-AAS method.

For the sodium hydroxide variation experiment, the potassium persulphate composition was fixed at 2% w/v, while the sodium hydroxide composition was varied between 0 and 4% w/v. From Figure 4.4, it can be seen that with 1µg As/ml arsenite, the 'inorganic' arsenic conversion efficiency reached a maximum with 1% w/v NaOH. Between 1% and 2% w/v NaOH, the 'inorganic' arsenic yield decreased. For 1µg arsenate, the peak height remained unchanged until 1.5% w/v where it decreases. With MMAA or DMAA conversion to 'inorganic' arsenic is incomplete with NaOH concentrations below 0.5%. Under these low base conditions there was evidence of methylated arsenic present. All evidence of residual methylated arsenic disappears when 1% w/v NaOH is employed. It was noted that for MMAA, after reaching an 'inorganic' arsenic peak height maximum at 2% w/v NaOH, the 'inorganic' arsenic peak height decreased between 2% and 4% w/v NaOH. With DMAA, the 'inorganic' arsenic conversion efficiency maximum was reached at 1% w/v before decreasing slightly between 1% and 4% NaOH. A NaOH concentration of 1% was therefore selected for future work.

A second experiment was carried out in which the persulphate concentration was varied between 0-5% w/v whilst the NaOH concentration was kept constant at 1% w/v. For the arsenite and arsenate samples, the maximum 'inorganic' arsenic

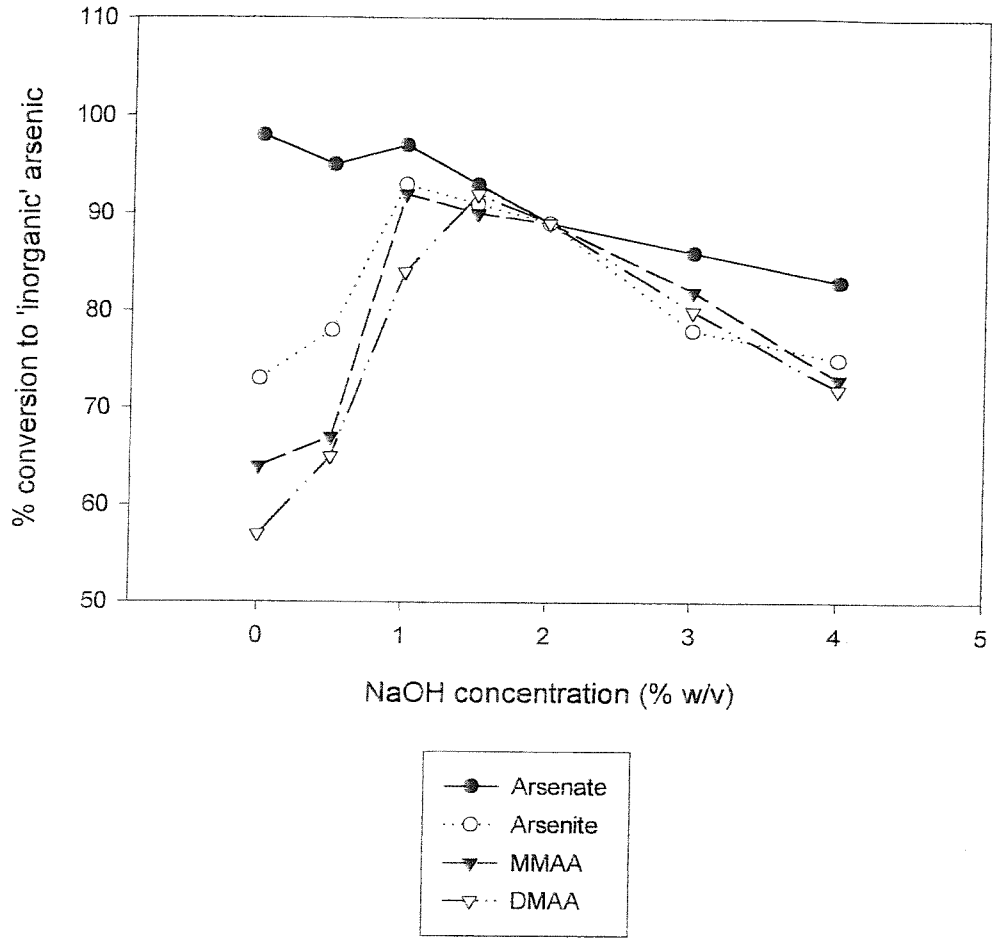

Figure 4.4: Graph showing the effect of NaOH on 'inorganic' arsenic conversion efficiency.

yield was obtained with a persulphate composition of 0.5% w/v (Figure 4.5). With persulphate compositions between 0 and 1.5% w/v MMAA decomposition is not complete. With 2% w/v persulphate concentration, however, there is no evidence of residual MMA and a maximum inorganic arsenic peak is observed. With higher persulphate concentrations, the 'inorganic' arsenic peak output decreases steadily. Similar behaviour is observed with DMAA. A persulphate concentration of 2% w/v was therefore selected for future work.

4.4.4.5 Results

With both the sample throughput timing and oxidising agent solution concentrations optimised, attention then focussed on the analysis of both the non-hydride and hydride-reducible organoarsenic species. Table 4.9 shows the results that were obtained

Arsenic species	Conversion efficiency of arsenic species to 'inorganic arsenic
arsenate (control)	100%
arsenite	94±2%
MMAA	90±3%
DMAA	96±5%
arsenazo(III)	94±4%
arsenazo(I)	91±4%
o-arsanilic acid	89±5%
p-arsanilic acid	91±5%
phenylarsonic acid	95±3%

Table 4.9: Conversion efficiencies of arsenic species after treatment by the inline photo-oxidation method (n=4).

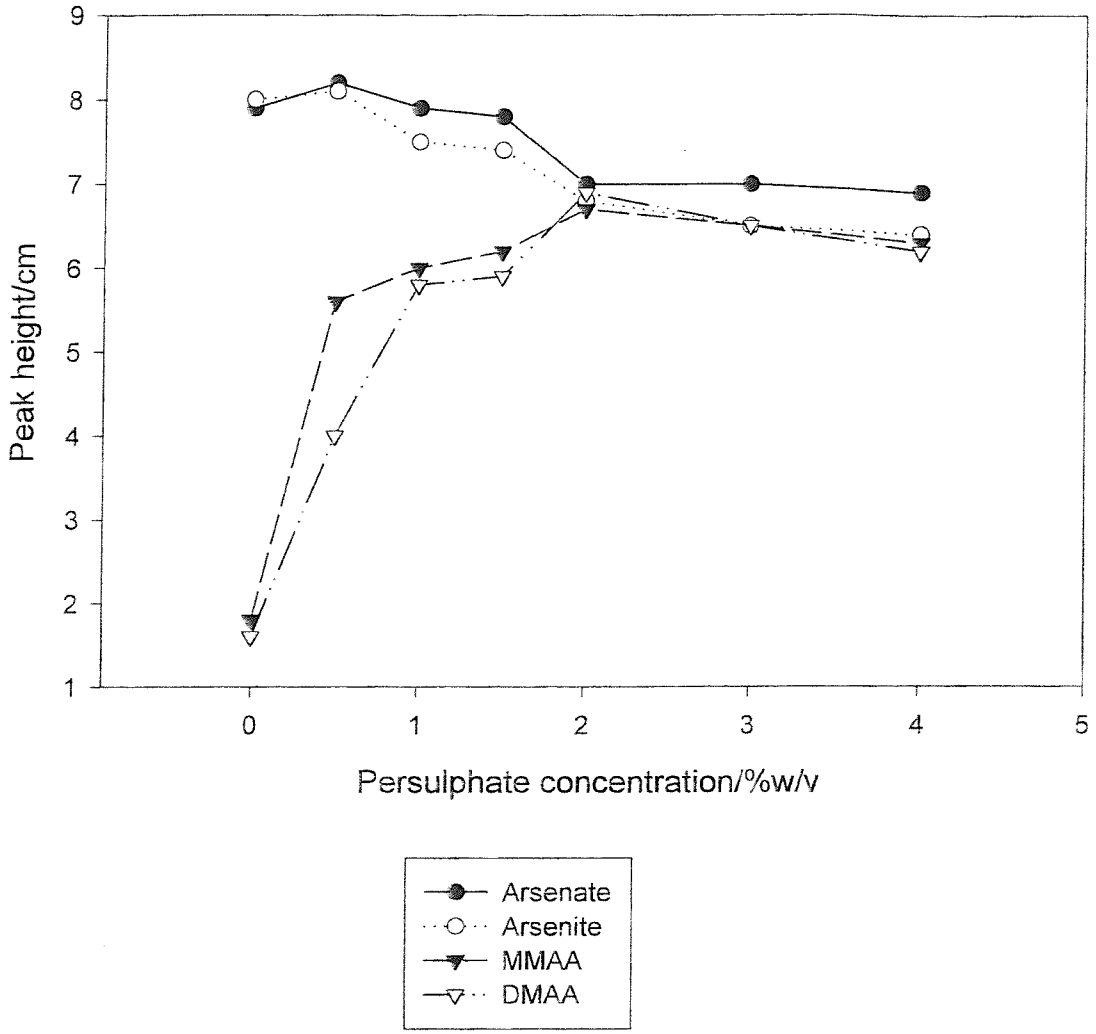

Figure 4.5: Graph showing the effect of persulphate concentration on peak height.

For the hydride reducible arsenic species, it can be seen that a high proportion of DMAA and MMAA is converted to 'inorganic' arsenic with no evidence of residual methylated arsenic.

The second batch of samples consisted of non-hydride reducible arsenic species. Once again, a high conversion to 'inorganic' arsenic was achieved.

4.4.5 Discussion

A simple online photo-oxidation system incorporating a low powered mercury lamp and chemical oxidant has been developed. This technique offers a sensitive and rapid method for the determination of total arsenic in a sample.

With the optimisation experiments, it was noted that, in general, with high NaOH concentrations (> 1-2% w/v) the peak height of the arsenic species decreased significantly. This is likely to be due to the excess OH^- species neutralising the acid present in the HG procedure. This would, in turn, decrease the borohydride reducing properties. With high concentrations of persulphate (> 2% w/v), arsenic signal output is reduced. This is due to the excess persulphate reacting with the sodium borohydride. Attalah and Kalman reported that high concentrations of borohydride were necessary in their study when high levels of persulphate were employed in order to reduce both the persulphate and arsenic species [15].

4.4.6 Conclusion

A novel online photo-oxidation system has been developed which allows the arsenic content of a sample to be measured even when the arsenic is bound in a non-hydride reducible organoarsenic form. Further work using this technique is described in **Chapters 5 and 6**.

4.5 Microwave photolysis

4.5.1 Introduction

The University of Southampton in co-operation with Jenact Ltd. have developed a new generation of u.v. lamps which emit u.v. radiation when exposed to microwaves in an adapted conventional microwave oven. The u.v. lamp consists of a beaker shaped quartz vacuum chamber containing argon and a trace of mercury. The lamp emits a mixed mercury-argon emission spectrum (Figure 4.6). Labonne [18] has successfully employed this emission source in the photolytic conversion of organoselenium compounds to inorganic selenium species.

The main objective of this section was to ascertain whether this novel method of photolysis would be effective in the decomposition of organoarsenic species.

4.5.2 Experimental

The u.v. lamp, which was bell shaped in design, contained a quartz carousel holding six quartz test tubes in which samples could be placed. The u.v. lamp was powered by a Sanyo microwave oven which had been modified by Newton [19] to give a continuous power output. For the experiments carried out in this study, the power of the microwave was fixed at approximately 620W.

Samples containing 3mls of 1μg As/l arsenite, arsenate, MMAA, DMAA, o- and p-arsanilic acid and arsenazo(I) and (III) were dispensed into individual quartz test tubes in the microwave lamp. The lamp assembly was then transferred to the microwave and the samples were irradiated for varying periods of time. The samples were allowed to cool prior to analysis by cryogenic trap HG-AAS methods.

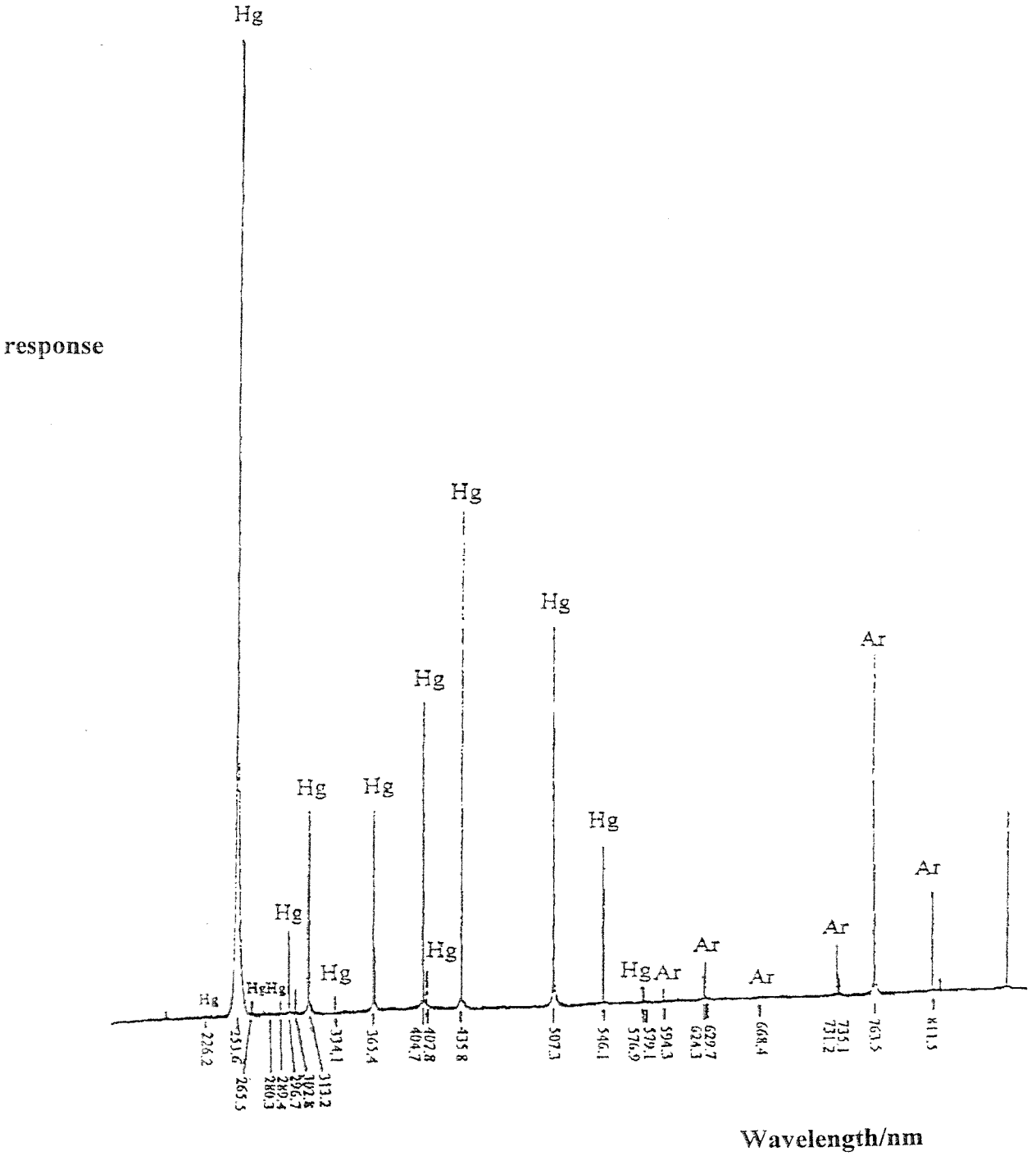

Figure 4.6: Emission spectrum of the microwave excited u.v. lamp [18].

4.5.3 Results

4.5.3.1 Optimisation of irradiation time for the hydride reducible arsenic species

An initial experiment was carried out to assess the rate of demethylation of DMAA, MMAA and arsenic loss during irradiation. Irradiation was carried out from 0-240 seconds and the samples were then analysed by the method described in Section 4.5.2. Figure 4.7 shows the results that were obtained.

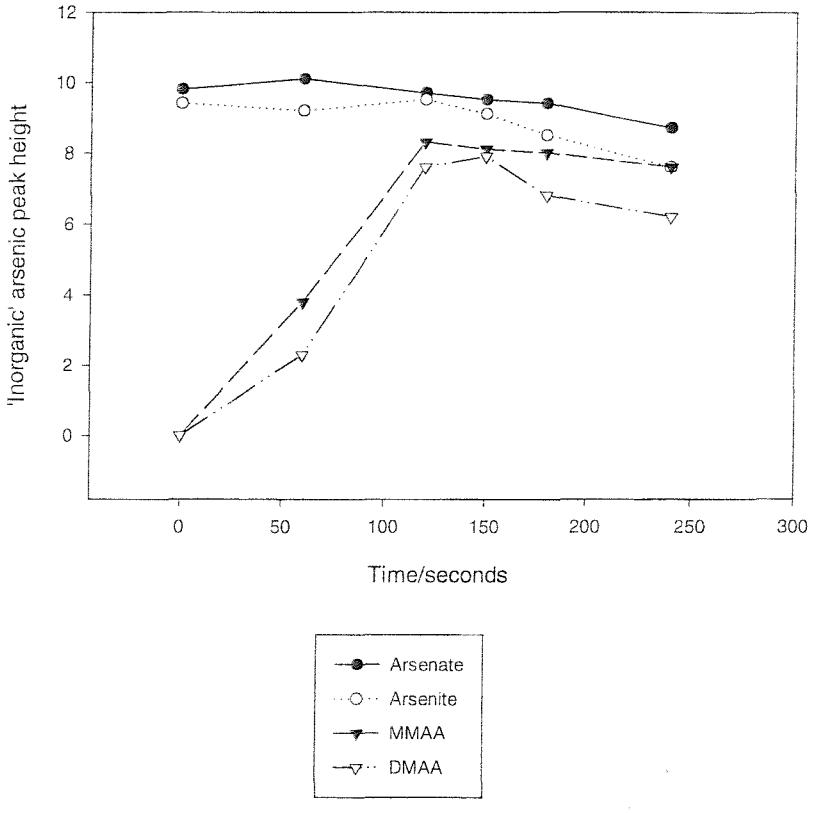

Figure 4.7: Microwave photolysis time optimization (n=4)

The peak output for the 'inorganic' arsenic species arsenate remained relatively constant between 0 and 180 seconds. After 180 seconds the 'inorganic arsenic peak output began to decrease, implying that the heat generated by the microwaves leads to some volatilisation of the arsenic species. With arsenite, a similar trend was observed with a relatively constant peak output up to 150 seconds. After 150 seconds, the 'inorganic' arsenic peak output decreased significantly.

After 120 seconds irradiation of MMAA, there was evidence of only 'inorganic' arsenic being present. After 150 seconds, the proportion of 'inorganic' arsenic decreased dramatically. For DMAA (Figure 4.8), a peak height maximum of 'inorganic' arsenic was obtained at 150 seconds but subsequently, the 'inorganic' arsenic peak height output decreased significantly. By considering the results of the four hydride reducible arsenic species, it was decided to fix the time necessary for the effective microwave photolysis of hydride reducible arsenic species at 150 seconds.

4.5.3.2 'Inorganic' arsenic conversion efficiencies

Table 4.10 shows the efficiencies with which both the hydride reducible and non-hydride reducible arsenic species are converted to 'inorganic' arsenic by the microwave photolysis method. Arsenate and arsenite were relatively unchanged by microwave photolysis. For the organoarsenic species, there was no evidence of methylated arsenic remaining but the conversion to 'inorganic' arsenic was lower than expected. This suggests that sample decomposition to 'inorganic' arsenic is incomplete or that arsenic is being lost during the photolysis.

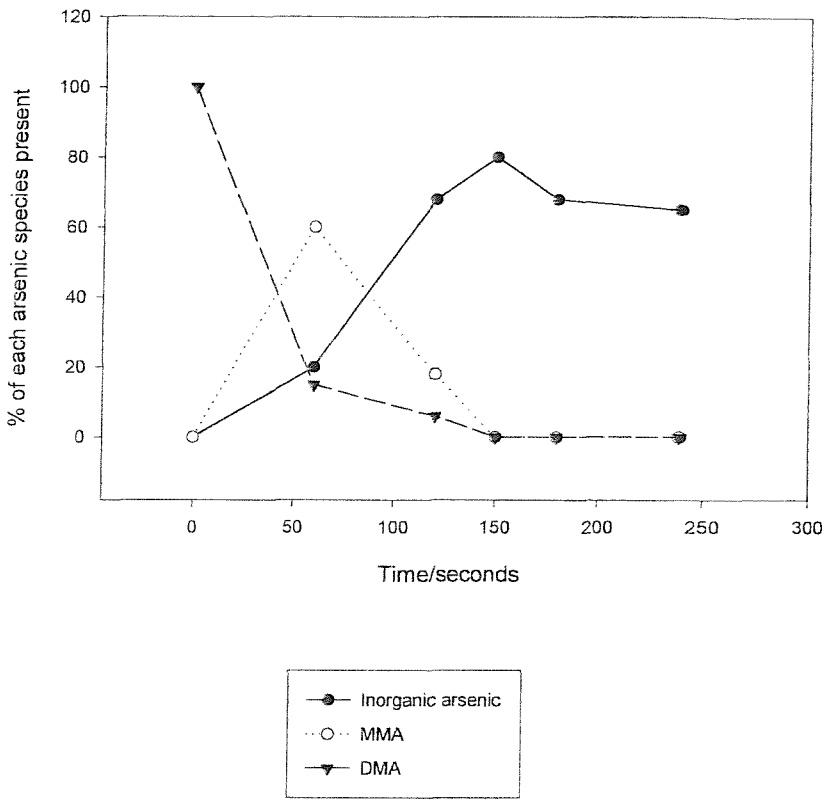

Figure 4.8: Microwave photolysis of DMAA over time (n=4)

Arsenic species	% conversion efficiency to 'inorganic' arsenic
arsenate	98±2%
arsenite	93±4%
MMAA	83±3%
DMAA	81±5%
p-arsanilic acid	62±4%
o-arsanilic acid	68±4%
arsenazo(III)	67±4%
arsenazo (I)	64±5%

Table 4.10: Microwave photolysis results (n=4)

4.5.3.3 Addition of potassium persulphate reagent to arsenic samples prior to microwave photolysis

For this experiment, 100µl of 2% (w/v) potassium persulphate reagent (prepared as described in Section 3.2) was added to 3mls of solution containing 1µg As/l. The samples were then treated in a similar manner to that described in Section 4.5.2.

Peak height responses for the arsenate and arsenite samples were unchanged, by the photolysis procedure (Table 4.11). Both the MMAA and DMAA were quantitatively converted to 'inorganic' arsenic with no evidence of residual MMA or DMA. The non-hydride reducible organoarsenic species were converted to

'inorganic' arsenic with high efficiency. The addition of persulphate significantly enchanced the breakdown of the organoarsenic compounds to 'inorganic' arsenic.

Arsenic species	% conversion efficiency to 'inorganic' arsenic
arsenate	103±2%
arsenite	95±4%
MMAA	93±4%
DMAA	88±2%
p-arsanilic acid	95±2%
o-arsanilic acid	91±5%
arsenazo(III)	89±2%
arsenazo (I)	96±3%

Table 4.11: Microwave + 100µl persulphate solution photolysis results (n=4)

4.5.4 Discussion

The addition of persulphate solution to the organoarsenic species prior to their microwave photolysis has been found to be beneficial in breaking down a high proportion of organoarsenic compound to 'inorganic' arsenic. As the microwave vessel emitted principally at 254nm, important speciation information was lost due to the photolytic decomposition of the Me-As moieties. The 2.5 minutes required for the effective breakdown of the organoarsenic samples by microwave photolysis is a great improvement on the 18 hours which is necessary with the batch irradiation procedure described in Section 4.2.

For the effective breakdown of the organoarsenic compounds using the microwave excited u.v. lamp, it is necessary to use a persulphate oxidising agent. The mechanisms involved in the breakdown of the organoarsenic species to form 'inorganic' arsenic using persulphate reagent have been discussed previously in Section 4.3.4.

4.5.5 Conclusion

Persulphate induced microwave photolysis is an extremely useful tool for the photodegradation of organoarsenic species to easily measurable hydride reducible arsenic species. With the reduction in pretreatment times necessary for analysis, this method will prove to be attractive in the measurement of estuarine water/sediment porewater samples.

4.6 Dry ashing procedures

4.6.1 Introduction

The decomposition of arsenic compounds by acid digestion using an ashing aid derives from the work of Tam and Conacher [21]. Such methods have been employed to determine the total arsenic levels in plants and animal tissues.

4.6.2 Experimental

The ashing aid was prepared by dissolving 80g magnesium nitrate heptahydrate (GPR, Aldrich) in 200ml deionised water. It was then mixed with 10g magnesium oxide (AnalaR, Aldrich) and shaken well prior to use. 10mls of ashing aid was added to 25ml beakers containing a piece of Whatman number 1 filter paper and 1ml of a solution containing 50µg As/l of the arsenic species to be studied. The beakers were then transferred to a muffle furnace at 100°C for 2 hours to remove any traces of water. After 2 hours operation, the temperature of the furnace was

increased to 500°C and the samples were then left for a further 4 hours at this temperature.

Once the samples had cooled, the ashed samples were each dissolved in 20ml 6mol/l HCl (Fluka, 'Arsenic' free) with the assistance of a hotplate and diluted with deionised water to 50ml. The samples were then analysed by cryogenic trap HG-AAS methods.

4.6.3 Results

The results that were obtained for the arsenic species studied by the above procedure are shown in Table 4.12.

Arsenic species	% conversion efficiency to 'inorganic arsenic
arsenate	108±3%
arsenite	102±5%
MMAA	93±6%
DMAA	91±5%
p-arsanilic acid	93±2%
o-arsanilic acid	96±4%
arsenazo(III)	98±4%
arsenazo (I)	103±5%

Table 4.12: Acid digestion results (n=4)

Dry ashing of the MMAA organoarsenic sample showed no evidence of MMA implying that sample decomposition to 'inorganic' arsenic is in an advanced stage

of completion. The conversion rate to 'inorganic' arsenic of 93% suggests that the sample decomposition of the MMAA sample is near completion. Similar behaviour was observed for the DMAA sample studied.

For the non-hydride reducible arsenic species o- and p-arsanilic acid the conversion rate to 'inorganic' arsenic were very nearly identical at 96% and 93% respectively. With the arsenazo (I) and (III) samples, the 'inorganic' arsenic conversion rates were much higher at 98% and 103%. The results for the non-hydride reducible arsenic species suggest that dry ashing is a very effective tool for the measurement of non-hydride reducible organoarsenic species.

The arsenite and arsenate 'inorganic' arsenic species showed no notable change in 'inorganic' arsenic signal output after dry ashing treatment when compared to the samples that were not treated by the dry ashing procedure. It was noted, however, that for all the arsenic species studied, the acid derived blank values were much higher than expected. This is likely to be due to the high concentrations of acid used in the experiment or the arsenic present in the ashing aids.

4.6.4 Conclusion

The dry ashing procedure appears to be an effective tool in the decomposition of organoarsenic species. Conversion rates of organoarsenic and non-hydride reducible arsenic species to 'inorganic' arsenic were high by the procedure employed.

4.7 Summary

This chapter has given an overview of a number of different techniques that can be used for the determination of both hydride reducible and non-hydride reducible organoarsenic species. Ultraviolet irradiation has been shown to be an effective means of reducing organoarsenic species to measurable hydride-reducible forms

but it is frequently necessary for an additional oxidant to be added. Dry ashing has been shown to be a particularly effective means of converting organoarsenic compounds to a measurable form.

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Chapter 5

A seasonal study of arsenic speciation in the water column of Southampton Water and the estuary of the Itchen River

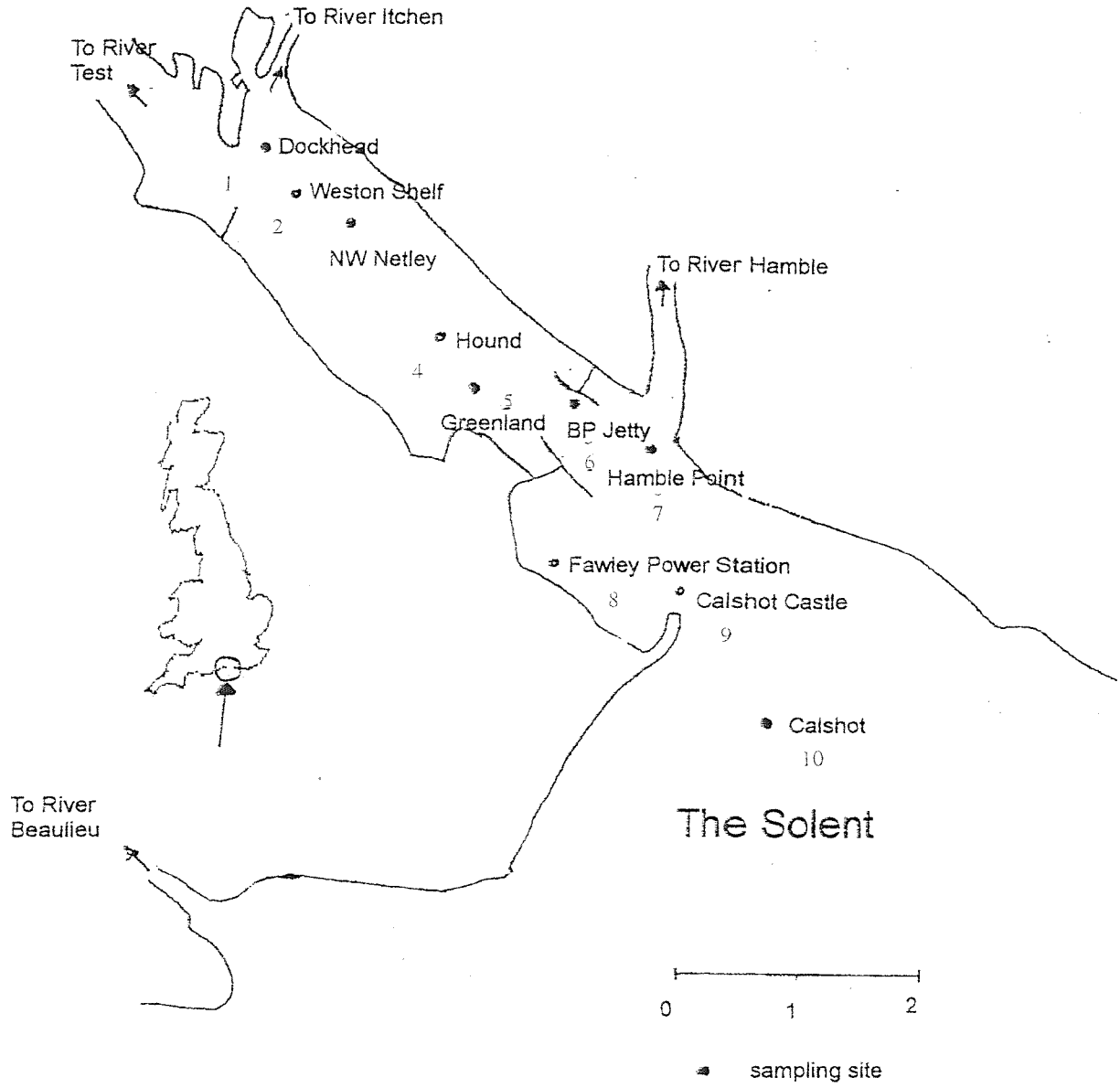
5.1 Introduction

Southampton Water (Hampshire, UK) is an almost linear stretch of water, which forms the northwesterly aperture of the Solent estuary system (Figure 5.1). Approximately 10km long, dredged to a depth of 13m below mean tide level and 2km wide, it is the major shipping channel for the Port of Southampton. On the eastern side of Southampton Water, long stretches of sand and shingle dominate the waterfront scenery, whereas on the western side saltmarsh environments are present [1].

The Test and Itchen estuaries provide the landward means of water entry into Southampton Water. Both estuaries enclose the City of Southampton and meet at Dockhead on the upper fringes of Southampton Water. On the upper areas of the Test and Itchen rivers there are both recreational and intensive agricultural activities on the water and the waterfront areas respectively.

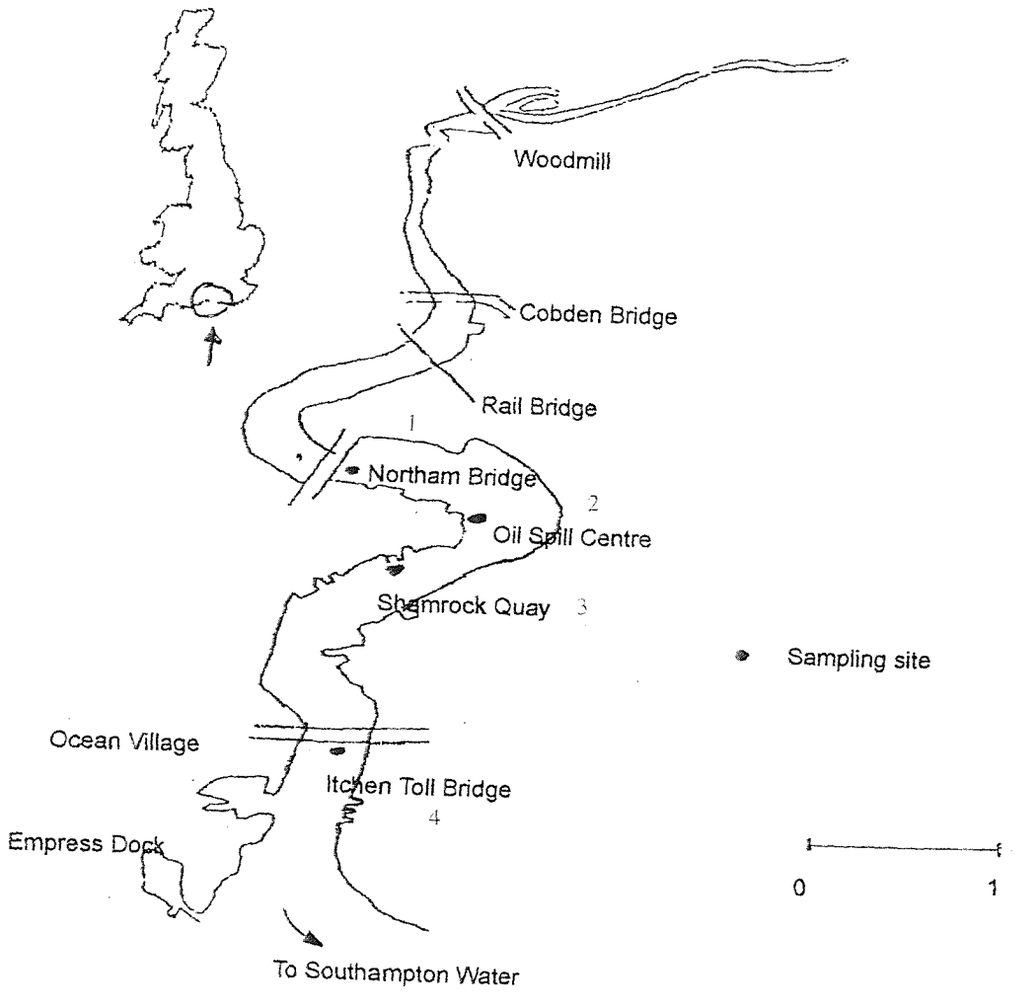
The River Test is the major source of freshwater entry in Southampton Water. Its estuarine environment has been considerably altered by dredging and land reclamation activities for ship docking facilities such as the military port at Marchwood, but marshlands still predominate near the tidal limits at the Southampton Containerbase terminal. The estuary is the recipient of treated effluent from two sewage works, on both sides of the estuary, approximately 3km downstream from Redbridge.

Whilst not as extensively altered in terms of land reclamation and dredging activities, the Itchen estuary has a more 'natural' salinity regime with high levels



Site number	Sampling location	Grid reference number
1	Dockhead	430098
2	Weston Shelf	433092
3	NW Netley	432089
4	Hound	445073
5	Greenland	460068
6	BP Jetty	472058
7	Hamble Point	482045
8	Fawley Power Station	483032
9	Calshot Castle	489024
10	Calshot	522023

Figure 5.1(a): Map illustrating Southampton Water and sampling locations (scale distance is given in miles).



Site number	Sampling location	Grid reference number
1	Northam Bridge	433128
2	Oil Spill Centre	438126
3	Shamrock Quay	432122
4	Itchen Toll Bridge	435112

Figure 5.1(b): Map illustrating the estuary of the River Itchen and sampling locations (scale distance is given in miles).

of stratification at all tidal states and a consistent longitudinal salinity gradient [1]. However, the Itchen Estuary receives a far greater pollution burden than the Test. This is likely to be due to the effluent from two sewage treatment plants, one at Portswood and the other at the mouth of the river, along with the fertiliser runoff from farmland upstream of the river [2]. Improvements in the methods used in the treatment of effluent material discharged from the sewage plants have resulted in a notable improvement in the river water quality [3].

Surrounded by the Itchen and Test estuaries, the City of Southampton is a major European port handling a wide variety of goods including motor vehicles, grain and fruit products [4]. Along the waterfronts of the Itchen River there is considerable evidence of population and industrial activities such as a marina at Ocean Village and a scrapyard at St. Denys. On the western front of Southampton Water there is a large power station at Fawley whereas on the eastern shore, there are extensive marinas at Hamble alongside a large oil transfer/discharge storage facility.

A number of studies have been carried out on arsenic speciation and cycling in the water column of Southampton Water and the Itchen River. They include the work of Howard and Apte [5] where it was revealed that there was a time lag between the detection of dimethylarsenic and the presence of methylarsenic and arsenite in the water column at Northam Bridge. Howard and Apte suggested that this was a consequence of phytoplankton decay where dimethylated arsenic compounds metabolised by the phytoplankton break down to simpler forms such as methylated and inorganic arsenic species. Other studies include the work of Howard and Comber [6] where it was observed that the total dissolved arsenic budget in the Southampton Water estuarine system was underestimated by 25%. This form of arsenic is now known as 'hidden' arsenic. These 'hidden' arsenic compounds were thought to originate from the arsenosugars of marine algae or their related decomposition products.

The main objective of the work described in this chapter was to evaluate the nature and distribution of both 'hidden' and non-'hidden' forms of arsenic in Southampton Water and the Itchen River. Water samples were collected at regular intervals during a one-year period. These water samples were subjected to treatment by the analytical techniques developed (Chapter 4) for the determination of 'hidden' arsenic forms, to determine the most suitable technique for 'hidden' arsenic determination in estuarine waters. Any results gained should, therefore, allow a better understanding of the role, availability and biogeochemical cycling of 'hidden' arsenic in the marine biogoechemical cycle.

5.2 Sampling locations

The surface waters from fourteen sampling locations throughout Southampton Water, the Solent and the Itchen River (Figure 5.1) were sampled on a regular basis (seven surveys in total) between October 1998 and September 1999. Whilst easily reached by the Southampton University School of Ocean and Earth Sciences Research Vessel *Bill Conway*, there were occasions when selected locations were not sampled due to the weather or other operational factors. On those rare occasions, water samples were collected from other locations that were environmentally similar and close to the original sampling site.

Thirteen of the sampling sites were between Northam Bridge and Calshot Castle where surface waters can be influenced by seasonal fluctuations in rainfall. The sheltered nature of these sampling sites provide environments in which phytoplankton that are normally sensitive to weather extremes thrive [2].

The final sampling site at Calshot Buoy lies in the Solent just outside Southampton Water. This is a very consistent salinity environment, which is maintained throughout the year. Due to its exposed location and lower nutrient levels it supports a much more hardier type of phytoplankton species than found in Southampton Water and the Itchen River [2].

5.3 Sampling collection and storage

Surface water samples were collected using the pumped sampling system permanently installed on the Research Vessel *Bill Conway* and sampling 2m below the surface. A temperature/salinity probe monitored the water continuously.

Water samples for arsenic speciation analysis were stored in acid washed 125ml polyethylene bottles. The plastic bottles and caps were rinsed out at least three times with water from the sampling location prior to collection. Once filled, the bottles were transferred to a cool box where the temperature was kept constant at *ca.* 4°C.

After arrival in the laboratory, the water samples were vacuum filtered using Whatman GF/C filter papers. Some samples were kept unfiltered for sonication treatment. Samples that were to be analysed within 24 hours were kept in a refrigerator with the remainder being kept in a freezer [2].

Water was also collected for organic carbon analysis using 125ml brown glass bottles fitted with a PTFE lined cap. The individual sampling bottles were also rinsed out three times with water from the sampling location prior to sample collection. The bottles were stored in a cool box at *ca.* 4°C for the duration of the sampling cruise. Upon arrival in the laboratory, the content of each glass bottle was vacuum filtered using individual acid washed pre-combusted Whatman GF/C filter papers. These filters had been heated in an oven at 450°C for 4 hours to remove any traces of carbon. After vacuum filtration, 0.25ml of a preservative containing 1.6% w/v mercury chloride solution was added to 100ml of filtered water. The remaining filter papers were folded three times and dried overnight at 150°C in an oven. Once cool, the filters were transferred to a dessicator prior to analysis for particulate organic carbon (POC) content.

Water was also collected from the same locations by colleagues from the School of Ocean and Earth Sciences for chlorophyll *a* content, nutrient measurements, as well as the identification and measurement of selected phytoplankton species. Those studies formed parts of separate research projects but results of some of these analyses have been kindly given by Jiang Xiong and Sue Holley to support the arsenic studies reported in this chapter.

5.4 Experimental

5.4.1 Arsenic speciation

The filtered surface water was analysed by the cryogenic trap HG-AAS method described in Chapter 3 for dissolved hydride-reducible 'inorganic' and methylated arsenic content. 'Hidden' arsenic content was measured by three of the methods described in Chapter 4:

1. batch photolysis of the untreated filtered water sample using a 200W medium pressure mercury discharge lamp for 18 hours;
2. 100µl of 2% (w/v) potassium persulphate in 1% (w/v) sodium hydroxide solution (prepared as described in Chapter 3.2) was added to 3mls of each filtered sample before a 4-hour sample irradiation using the 200W medium pressure mercury discharge lamp;
3. the filtered water samples were photolysed using the microwave u.v. lamp (Chapter 4.5) for $2\frac{1}{2}$ minutes before analysis by cryogenic trap HG-AAS;
4. selected unfiltered water samples were sonicated using a MSE sonicator at full power for five minutes. The sample was then vacuum filtered using Whatman GF/C filter papers and analysed using conventional cryogenic trap HG-AAS procedures. When necessary, a fixed quantity of sonicated

sample was photolysed in a quartz test tubes using the 18 hours batch photolysis (no persulphate) method described in (1) above.

5.4.2 Organic carbon analysis

Preserved water samples for dissolved organic carbon determination were analysed using the School of Ocean and Earth Science's u.v.-persulphate dissolved organic carbon (DOC) analyser. Statham and Williams have given a detailed description of the DOC analyser in Grasshoff [7]. For calibration purposes, a stock solution containing 83μmC ml^{-1} (as potassium oxalate mono-hydrate, analytical grade) was prepared in low organic carbon content water (deionised water treated overnight by u.v. irradiation). This stock solution was then diluted appropriately to give working standards in the range 2-5mg/l C. In order to establish a baseline, blanks containing deionised water with a low organic carbon content were used.

The filtered precombusted filter papers were analysed for particulate organic carbon (POC) using an analyser system developed by Williams [8]. This procedure involved the combustion of the particulate material present in the filter paper in the presence of oxygen (500ml/min). The gases formed were passed over copper (II) oxide (heated at 800°C) to ensure complete oxidation and then to a gas-collector unit. When the gas-collector unit was full, the collected gas was held for 1 minute and then moved to the infra-red gas analyser (Analytical Developments Ltd., type 225) by a piston. The signal was recorded using a chart recorder.

The system was calibrated with analytical grade sucrose (BDH). The stock solution was made up to a concentration of 667mgC per 100ml, which was diluted appropriately to give a working standard range of between 1.3 and 6.7mg C/l.

5.5 Results

5.5.1 Water salinity

In general, surface water salinity increases in the Itchen River when moving downstream from Northam Bridge to the Itchen Toll Bridge (Figure 5.2). Salinity values varied on a monthly basis principally due to riverine freshwater inputs which depended upon the rainfall in the catchment area [11]. Within Southampton Water (Figure 5.2), the salinity in general continued to increase while moving downstream towards the Solent. Between Hamble Point and Calshot Castle the salinity remained relatively constant during the period of study with a maxiumum of 34.3 being recorded at Calshot Castle during October 1998. Salinity values between Dockhead and Calshot Castle were variable, again emphasising the relationship between riverine water flow, rainfall and other climatic factors. At Calshot Buoy, however, the salinity values showed very little variation (within 1) throughout the period of study.

5.5.2 Water temperatures

Large variations of water temperatures between sites was not observed although there was evidence of the Itchen site temperatures differing from those at the Southampton Water sites. The average water temperature changed in the manner of a water body influenced by seasonal climatic changes (Figure 5.2) with the lowest water temperature of 7.2°C being measured at Northam Bridge in December 1998. The highest recorded temperature of 20.6°C was measured at Hound in Southampton Water and Northam Bridge in the Itchen Estuary during the September 1999 survey.

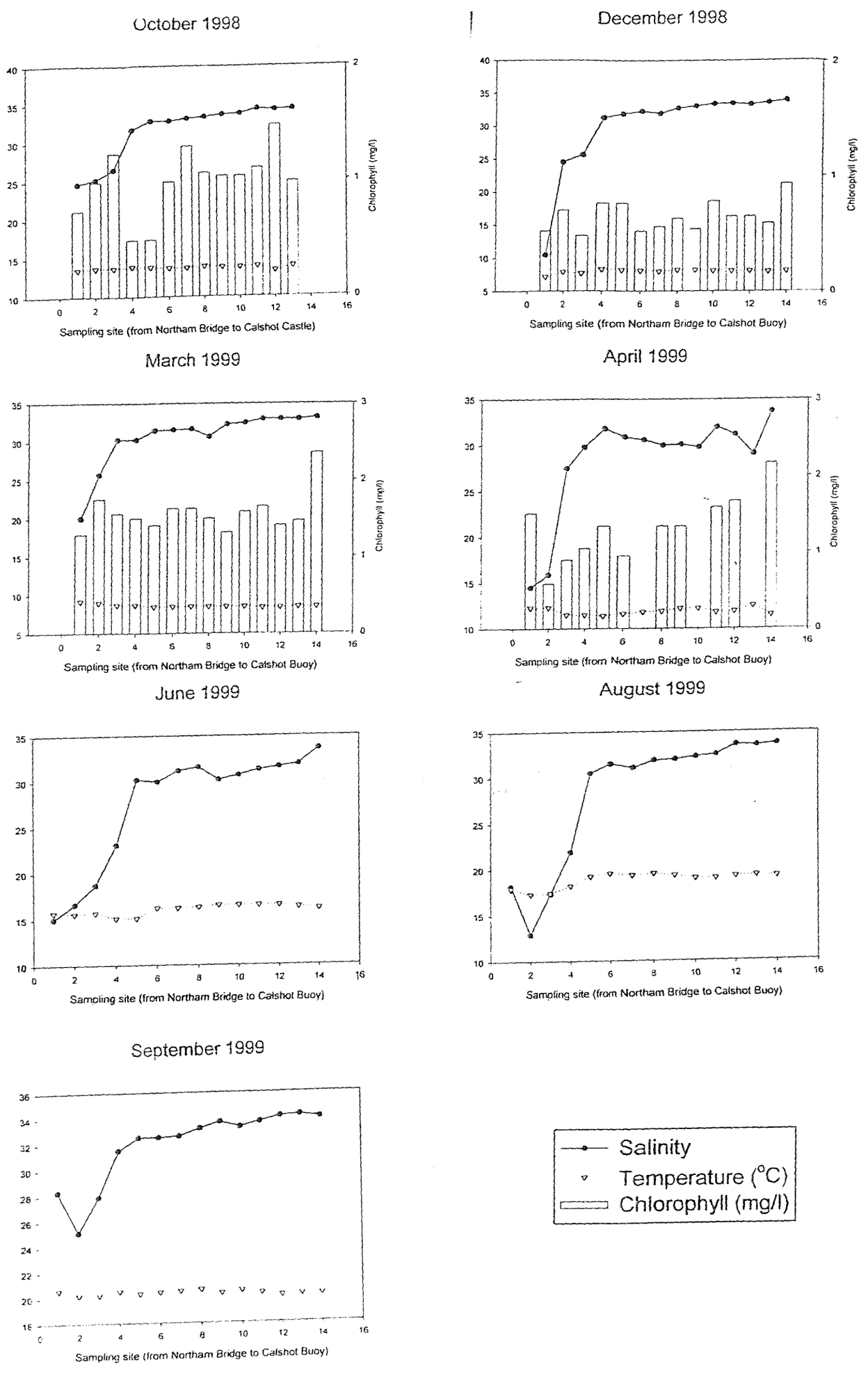


Figure 5.2: Temperature, salinity and chlorophyll *a* values for surface waters in various locations of Southampton Water and the estuary of the River Itchen

5.5.3 Chlorophyll 'a' concentrations

Between December 1998 and April 1999, the chlorophyll *a* concentrations (Figure 5.2) were low at *ca.* 1-2.5µg/l (data courtesy of Sue Holley, School of Ocean and Earth Sciences, University of Southampton).

Late spring/summer levels were expected to be significantly higher [3] but were unfortunately unavailable.

5.5.4 Dissolved hydride-reducible arsenic species

5.5.4.1 Introduction

The results presented in this section are based upon the measurement of hydride-reducible arsenic species found in filtered water samples from the Itchen/Southampton Water system.

5.5.4.2 'Inorganic' arsenic

For the majority of the sampling sites (Figure 5.3), dissolved 'inorganic' arsenic concentrations were at their maximum during the winter months. A significant drop in dissolved 'inorganic' arsenic occurs during the spring presumably due to bio-utilization. These depleted levels are maintained during the summer months.

With the Northam Bridge samples, deviations from the above trends were noted. For example, in the Northam Bridge sample the 'inorganic' arsenic concentration decreased significantly between the October and December 1998 surveys before increasing to a maximum between the December 1998 and March 1999 surveys. This behaviour is believed to result from 'inorganic' arsenic being diluted by

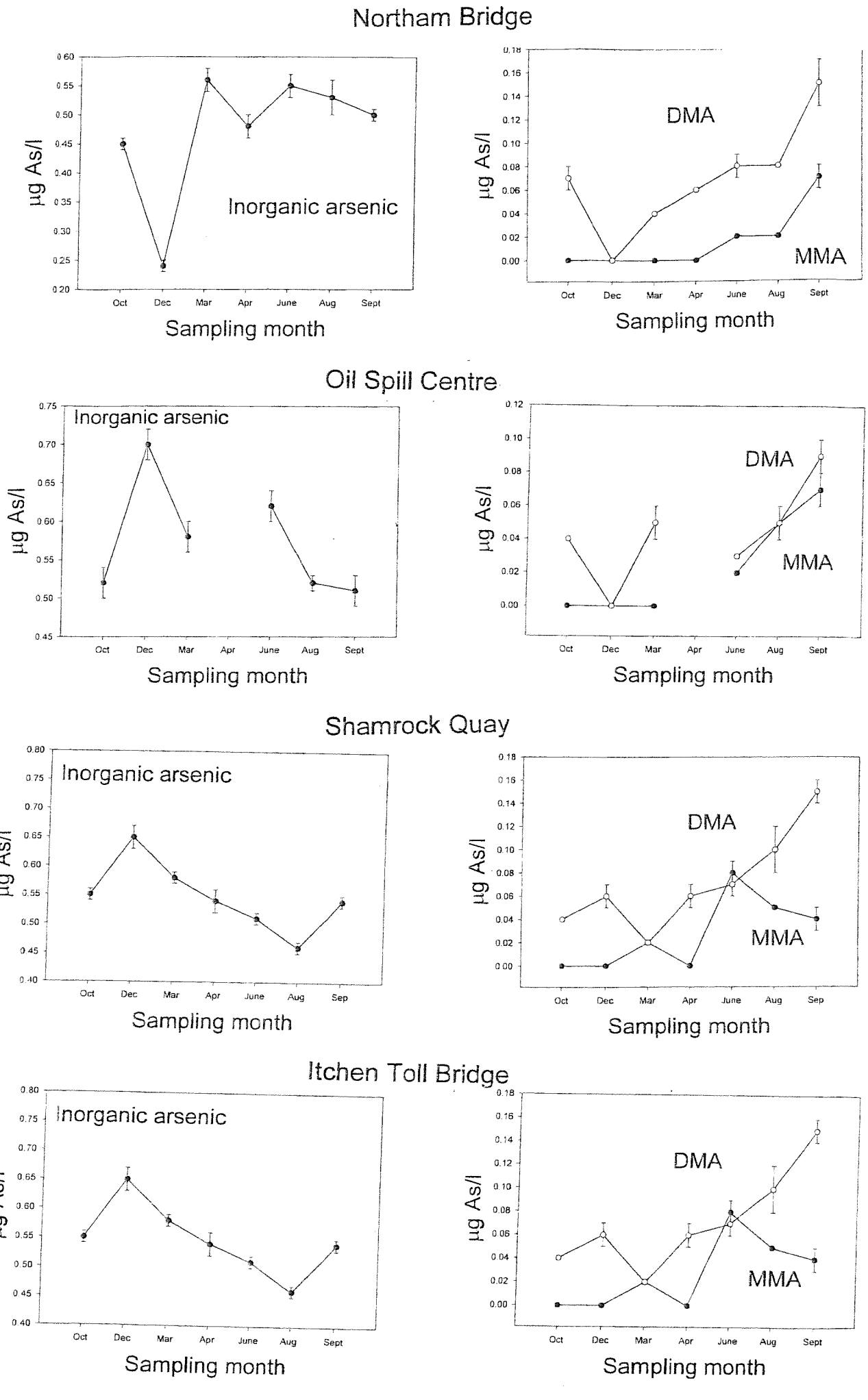


Figure 5.3a: Disssolved 'inorganic' and methylated arsenic content

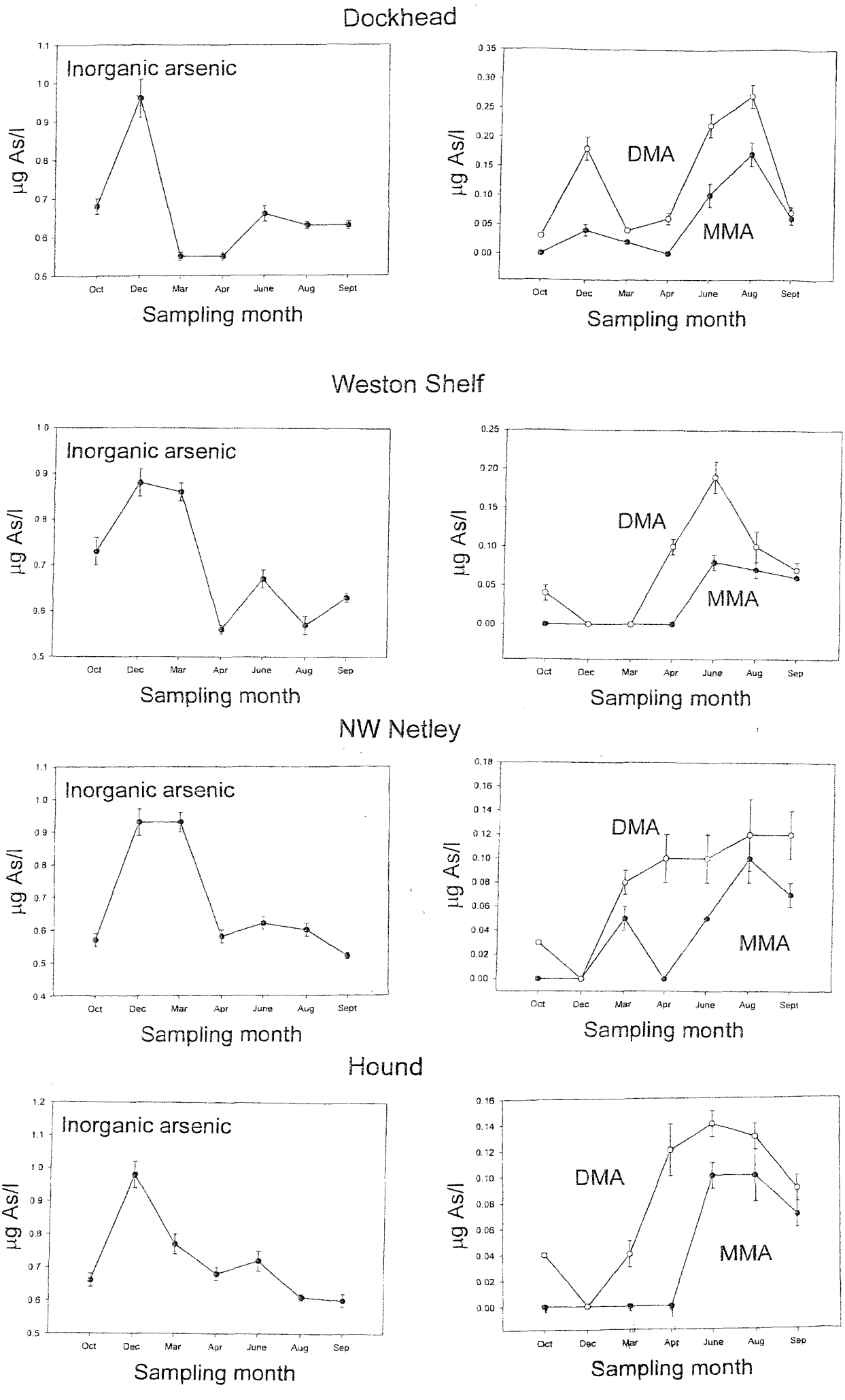


Figure 5.3b: Disssolved 'inorganic' and methylated arsenic content

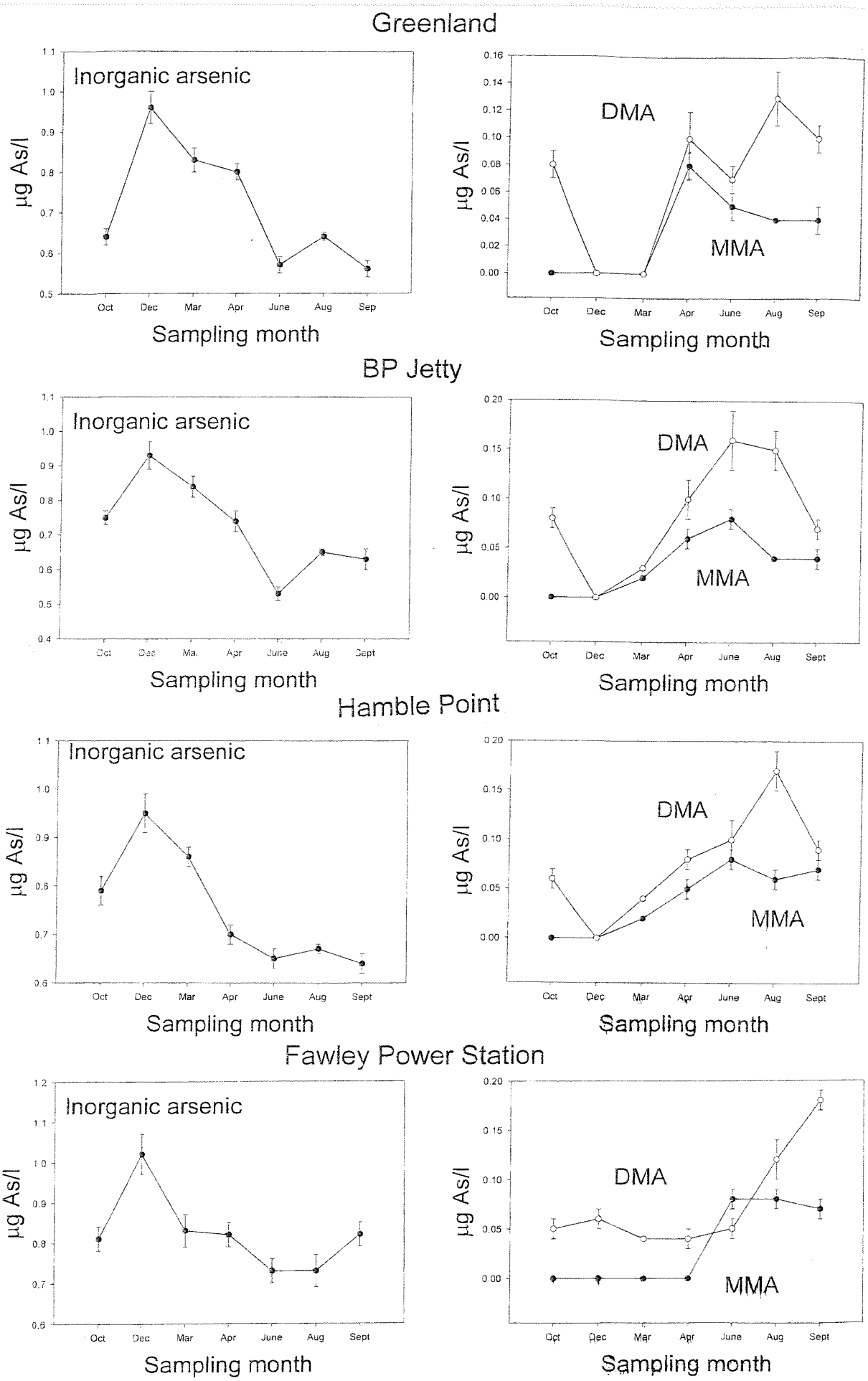


Figure 5.3c: Disssolved 'inorganic' and methylated arsenic content

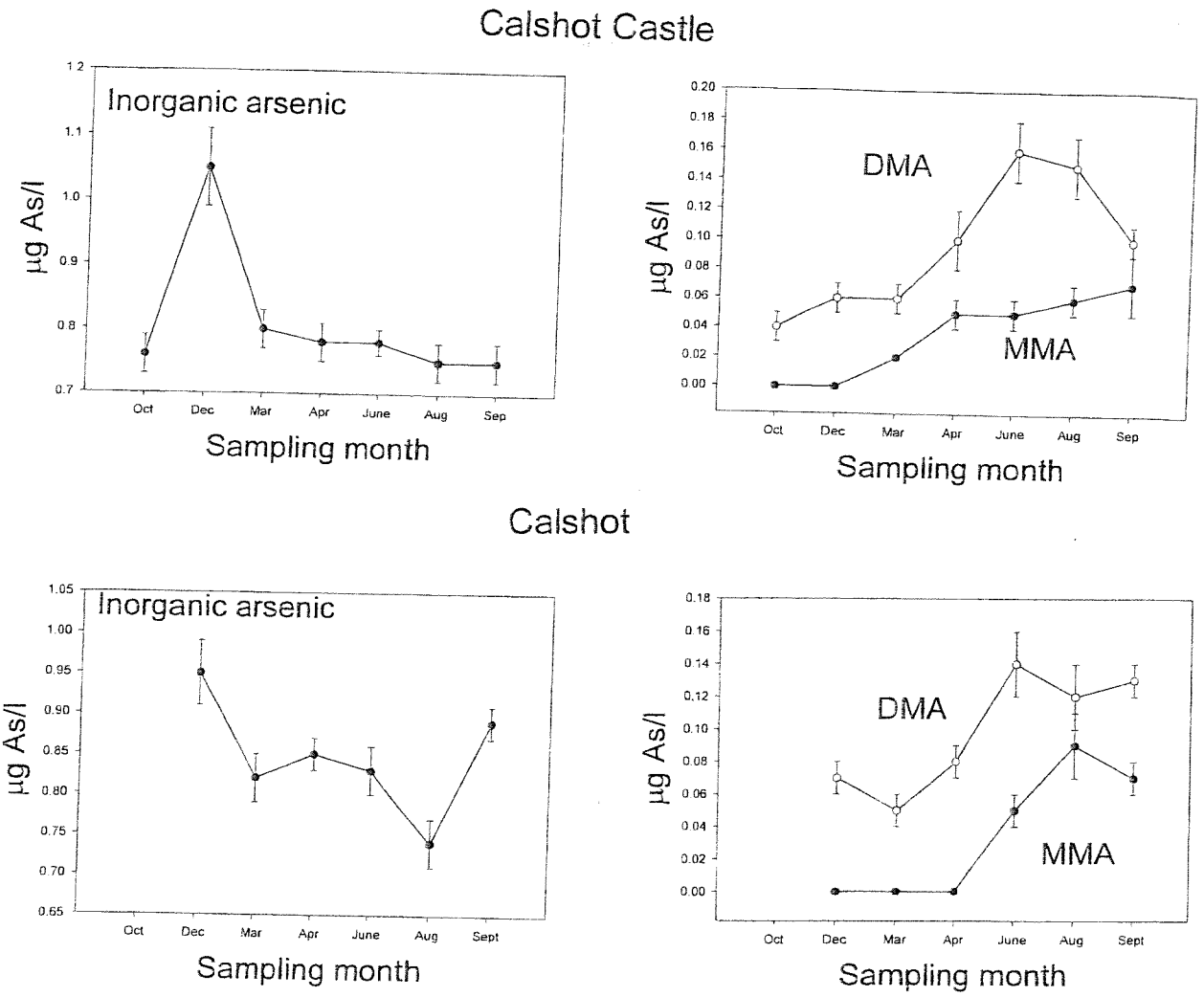


Figure 5.3d: Disssolved 'inorganic' and methylated arsenic content

freshwater riverine inputs as the salinity varied from between 24.6 (October) to 10.5 (December) before increasing to 20 during March.

In order to interpret the relationship between dissolved 'inorganic' arsenic and salinity in the Southampton Water/Itchen River system, it is necessary to separate the Itchen River and Southampton Water components due to the input of Test water at Dockhead (5). Whilst this results in the availability of only limited data sets for each component, tentative conservative dilution lines can be frequently drawn (Figure 5.4)

Extrapolation of the Itchen River mixing line to zero salinity and the Southampton Water line to a token salinity of 34.5 give estimates of the dissolved 'inorganic arsenic in the Itchen River and Solent respectively (Table 5.1). Whilst dissolved 'inorganic' arsenic levels in the Itchen approach expected low levels in the September survey, the elevated levels recorded during the other monthly surveys may reflect inputs into the low salinity end of the estuary from anthropogenic inputs especially at Portswood Sewage Works.

Sampling month	**Arsenic concentration after extrapolation**	
	When salinity = 0	**When salinity = 34.5**
October 1998	0.21	0.75
December 1998	0.33	0.81
March 1999	not determined	not determined
April 1999	0.26	0.74
June 1999	0.39	0.71
August 1999	0.30	0.68
September 1999	0.03	0.73

Table 5.1: 'Inorganic' arsenic extrapolation data (units are in μg As/l)

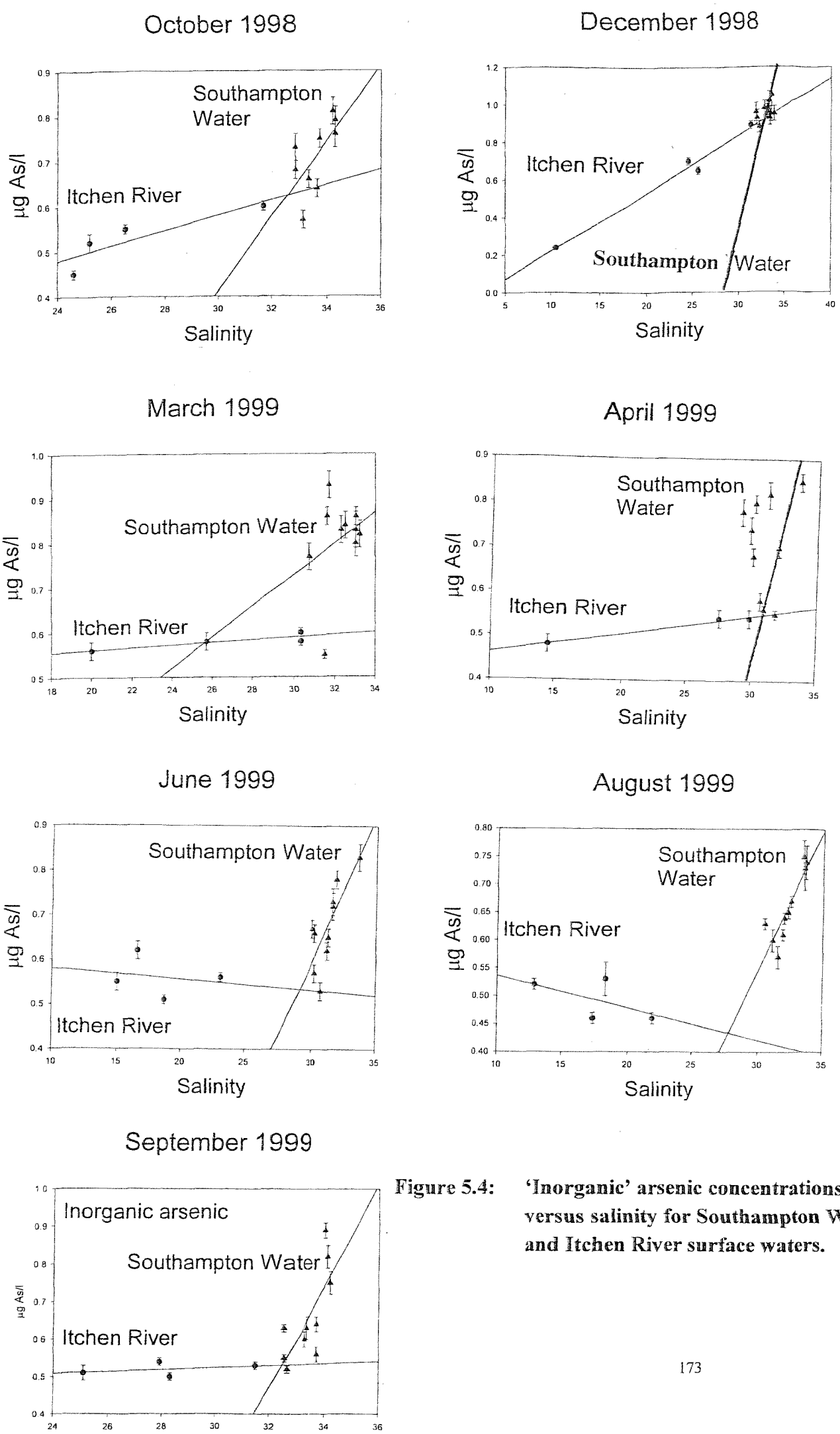


Figure 5.4: **'Inorganic' arsenic concentrations versus salinity for Southampton Water and Itchen River surface waters.**

5.5.4.3 Methylated arsenic

The appearance of dissolved hydride-reducible methylated arsenic species in the water column is consistent with little biological activity in the winter months and increased levels of methylated arsenic being produced biologically during the warmer spring and summer months. The highest value of 0.21μg As/l was recorded at Calshot during the August 1999 survey. The majority of dissolved methylated arsenic was present as DMA. DMA concentrations decreased between October and December 1998 before showing a gradual increase after March 1999. MMA concentrations on the other hand were low between October and March but increased significantly after the March survey. There was no evidence of dissolved TMA in any of the sampling locations studied. Similar behaviour was observed by Comber [3] in his studies of the Southampton Water estuarine system.

In Itchen sites near to Dockhead, there was evidence of significant quantities of dissolved hydride-reducible methylated MMA and DMA during the December survey. This behaviour is unusual as very little detectable dissolved methylated arsenic is generally found when the water temperatures are below 12°C.

Dissolved methylated arsenic concentrations were significant in the surface waters between the Oil Spill Centre and Itchen Toll Bridge as well as between Fawley Power Station and Calshot Castle during the time period when phytoplankton activity is known to be low (December 1998). It is interesting to note that both these areas are regions of turbulent mixing activity resulting from the input of the Itchen into Southampton Water and Southampton Water into the Solent respectively. It is therefore possible that the release of dissolved methylated arsenic into these two areas indicates tidal disturbance of sediment-bound arsenic in these areas.

For the sampling excursions where dissolved MMA concentrations were measurable, graphs of MMA concentrations against salinity were drawn. Whether

conservative behaviour (Figure 5.5) occurred in the Southampton Water/Itchen River system could not be determined.

Graphs of DMA concentrations against salinity reveal that for the Itchen sampling sites (Figure 5.6), the DMA concentration varies according to the salinity of the site concerned for the majority of sampling excursions. However, the data sets for the Southampton Water samples were not suitable for regression analysis.

5.5.5 Hidden 'arsenic' determination after batch photolysis pretreatment

5.5.5.1 Introduction

It is now well established that there are dissolved arsenic species present in estuarine waters which are not detected by conventional hydride generation techniques alone. 'Hidden' arsenic species were first discovered by irradiating samples by u.v. irradiation using a 200W medium pressure mercury arc lamp. The results reported in this section follow that procedure to identify the 'hidden' arsenic species [3].

5.5.5.2 'Inorganic' arsenic

With the exception of a few samples, the percentage increase in dissolved 'inorganic' arsenic resulting from irradiation was low (Figure 5.7). Although very little 'hidden' arsenic is in the form of 'inorganic' arsenic species, there were some cases, when a slight increase in the measured 'inorganic' arsenic content was observed after photolysis. This occurred especially between June and September. at Calshot Buoy, NW Netley, Shamrock Quay, Calshot Buoy, Hound and Greenland samples. In contrast, at Northam Bridge, Itchen Toll Bridge, Weston Shelf and BP Jetty, there was an appreciable increase in 'hidden' 'inorganic' arsenic after photolysis between October and June.

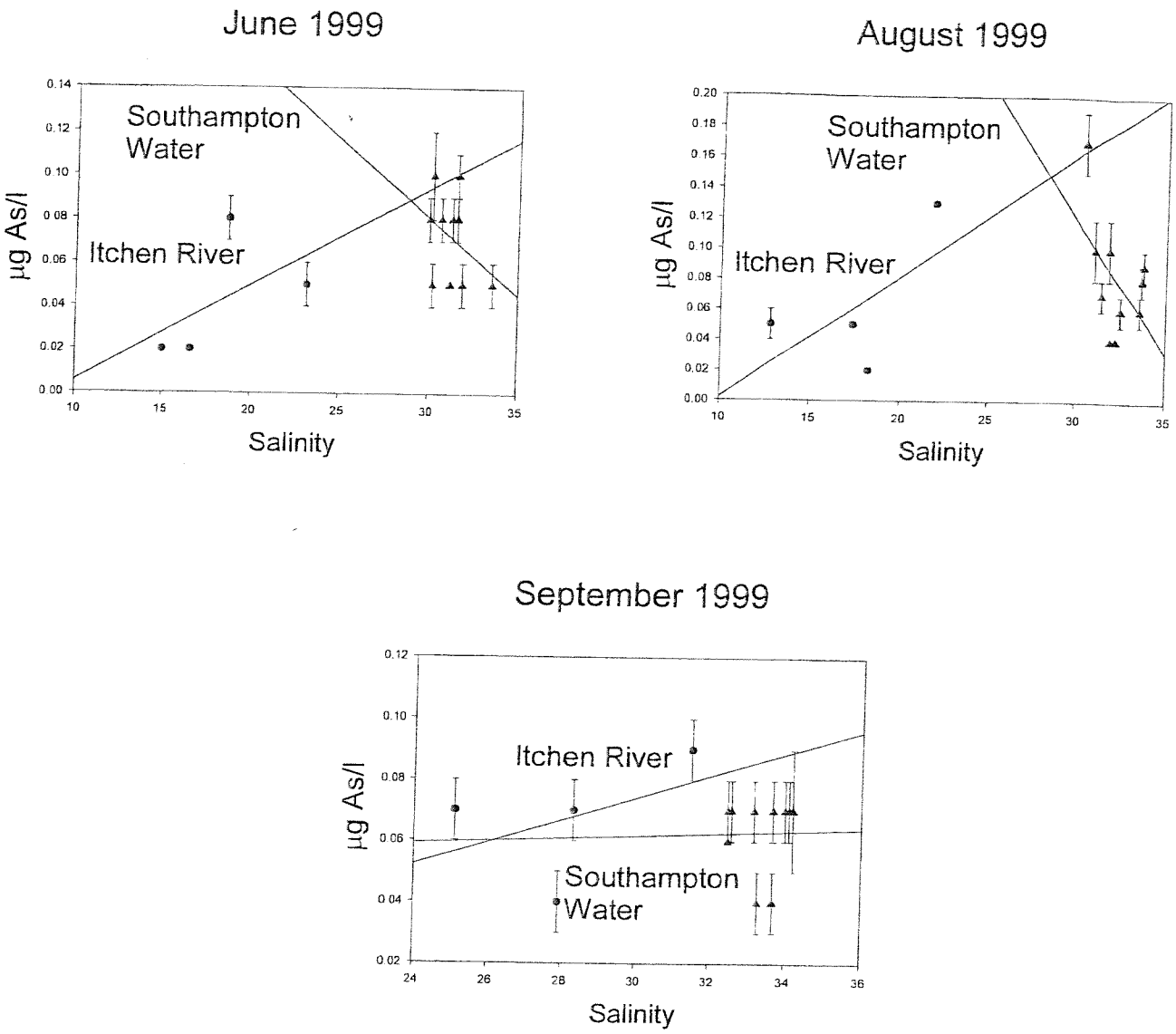


Figure 5.5: MMA concentrations versus salinity for Southampton Water and Itchen River surface waters.

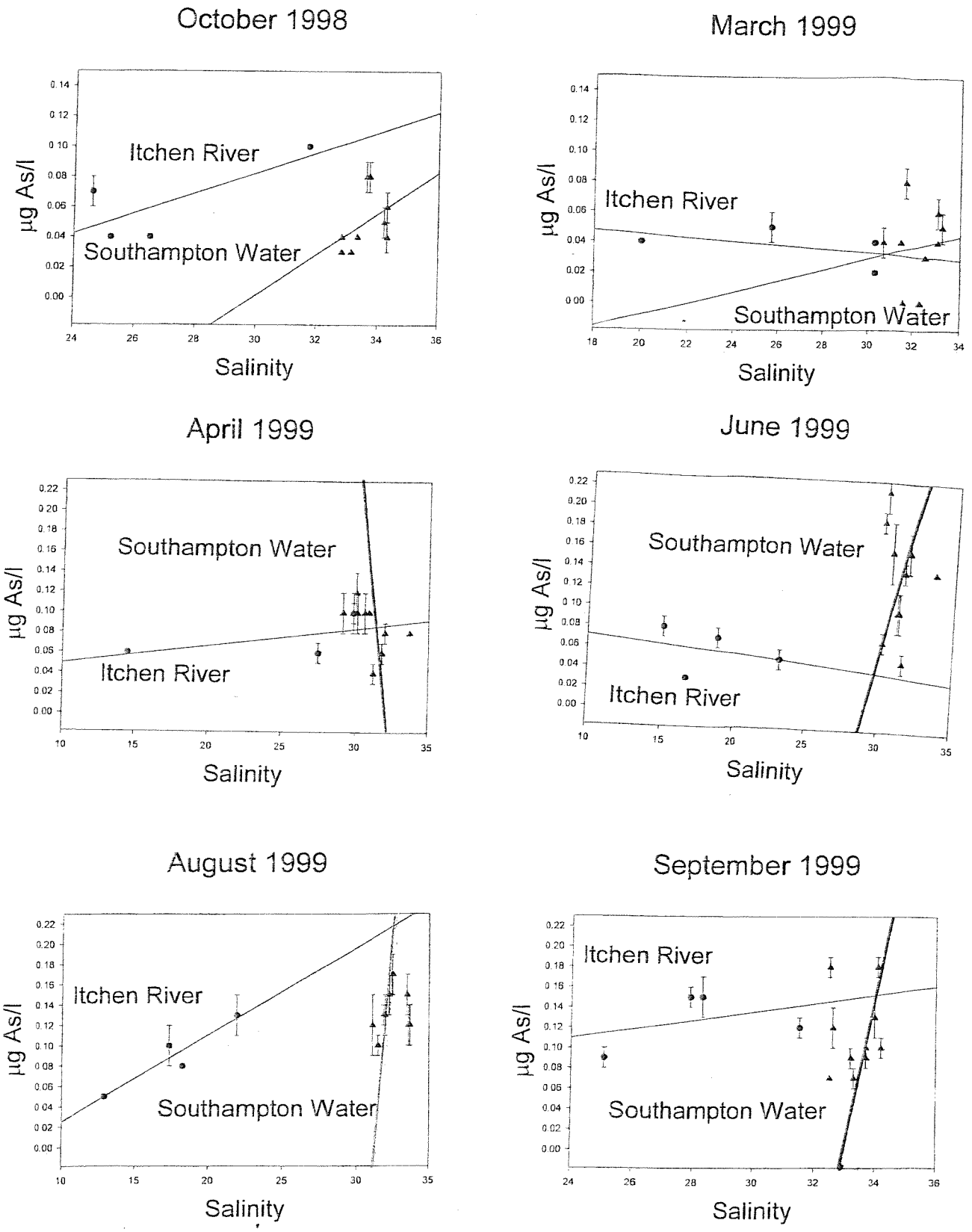
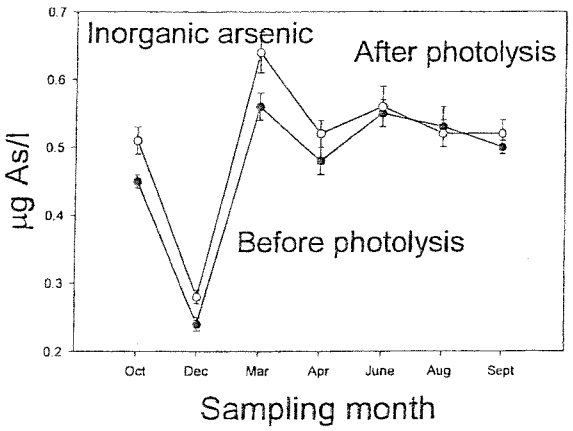
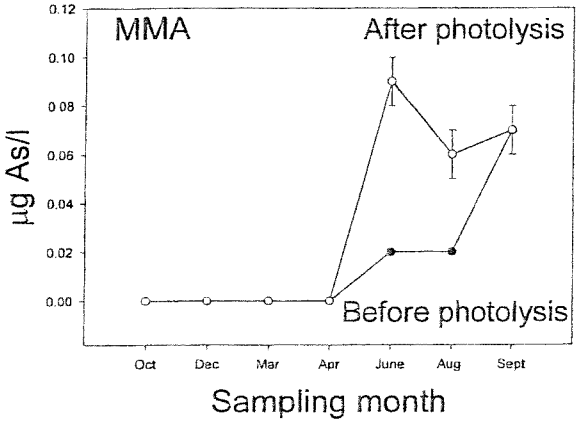
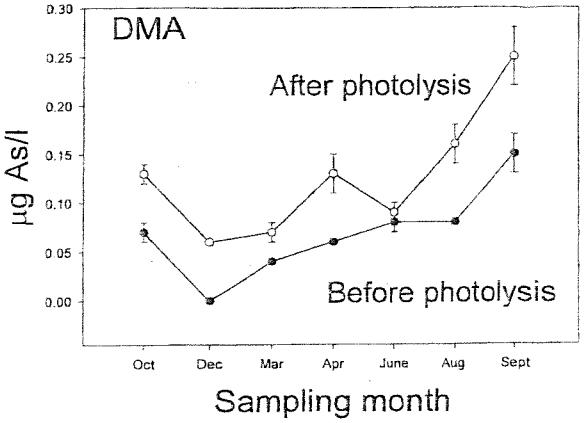


Figure 5.6: DMA concentrations versus salinity for Southampton Water and Itchen River surface waters

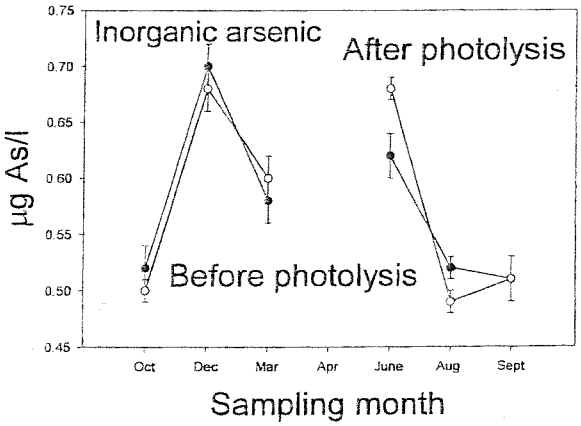
Northam Bridge

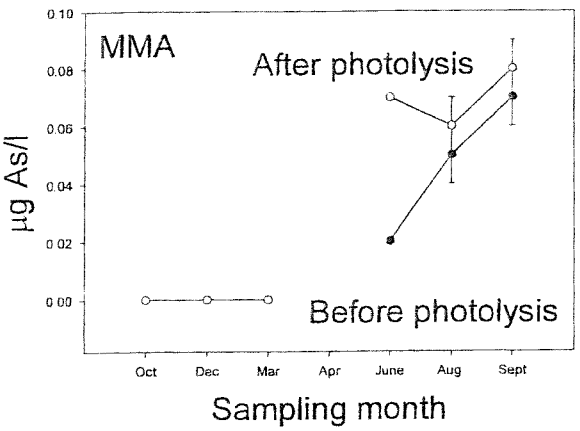






Oil Spill Centre





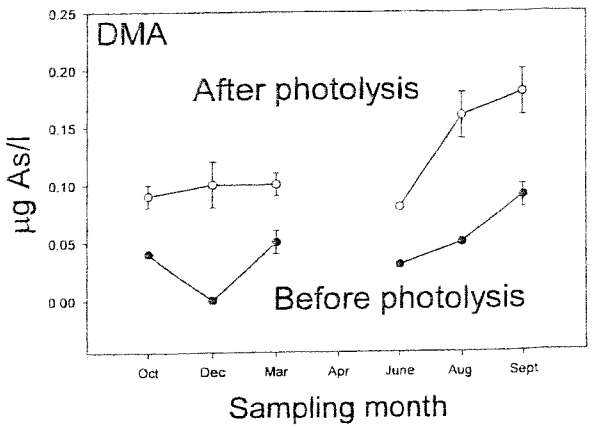


Figure 5.7a: Dissolved 'inorganic' and methylated arsenic content before and after sample photolysis

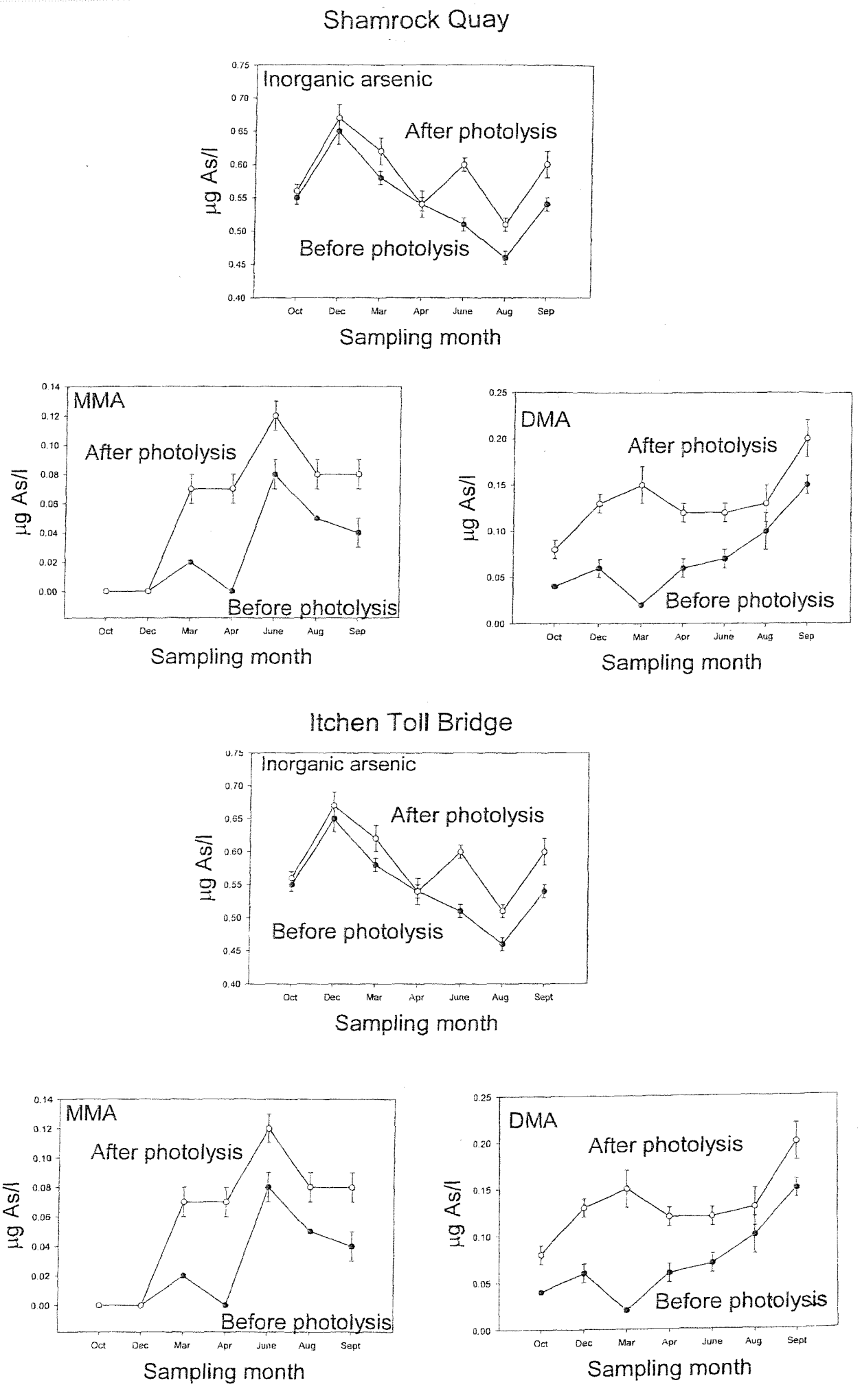
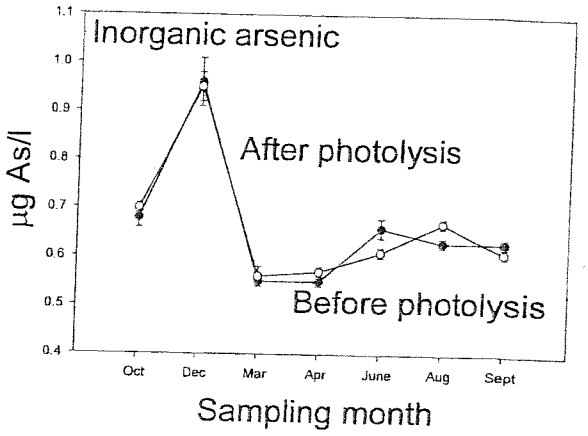
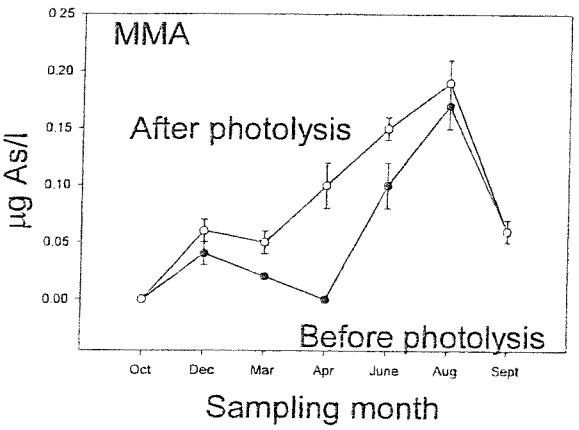
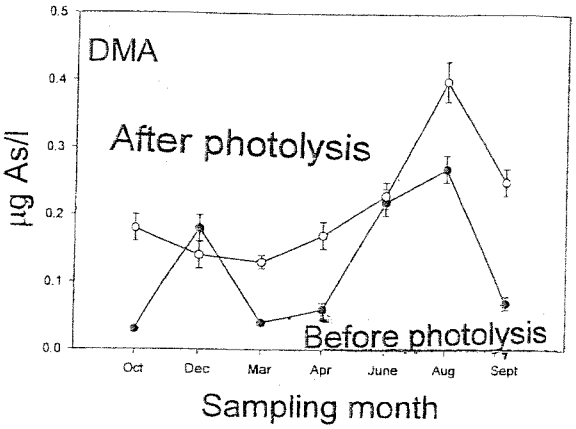


Figure 5.7b: Dissolved 'inorganic' and methylated arsenic content before and after sample photolysis.

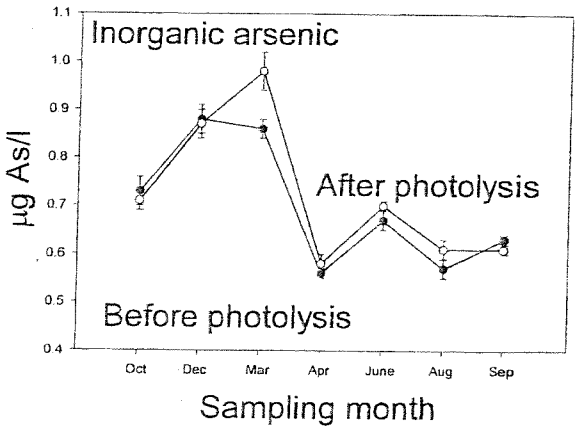
Dockhead

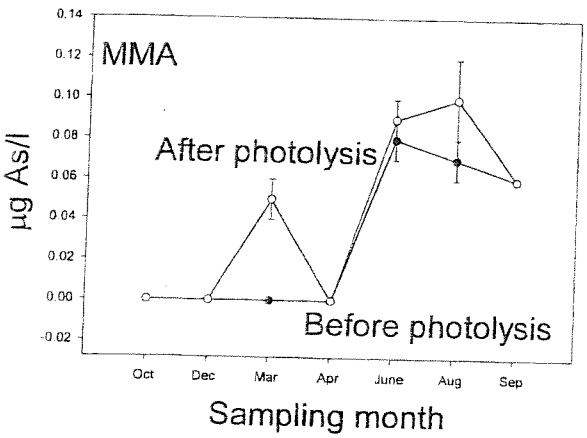






Weston Shelf





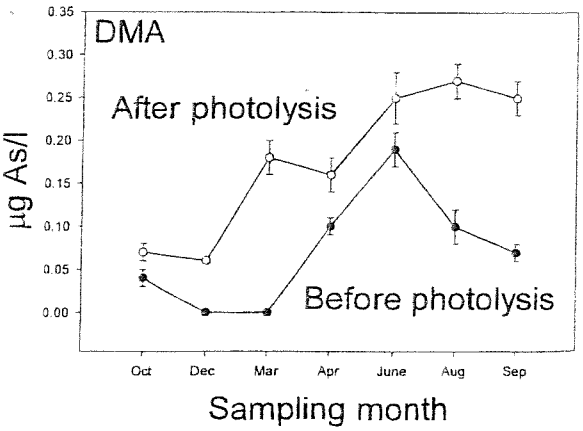
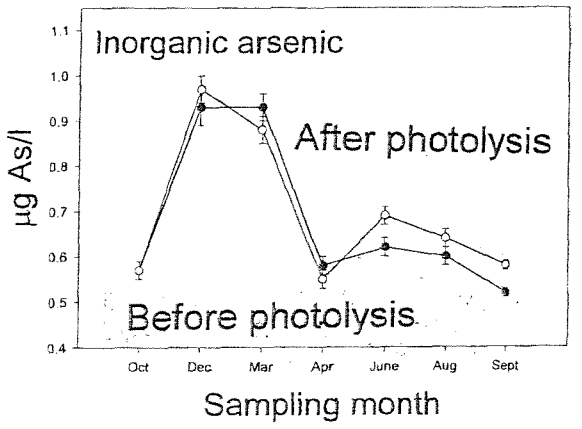
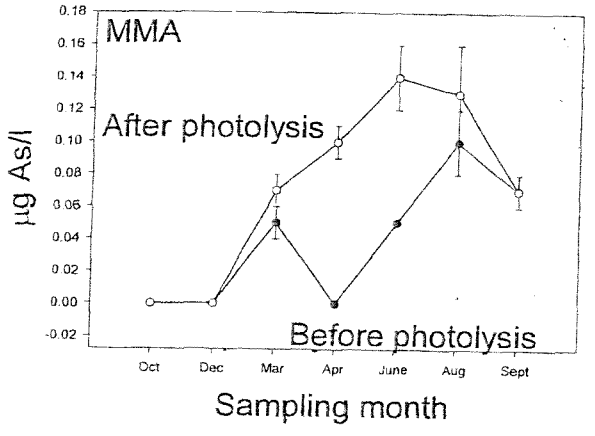
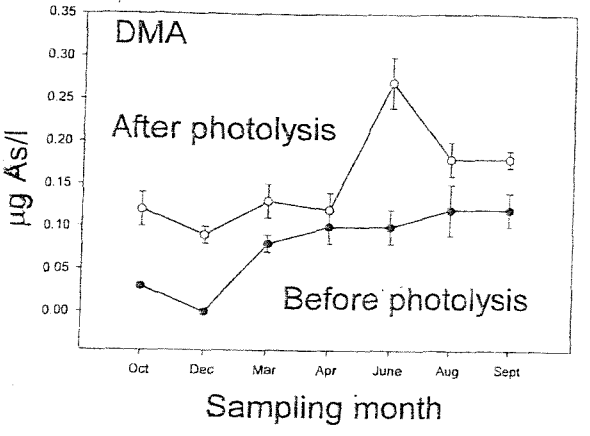


Figure 5.7c: Dissolved 'inorganic' and methylated arsenic content before and after sample photolysis .

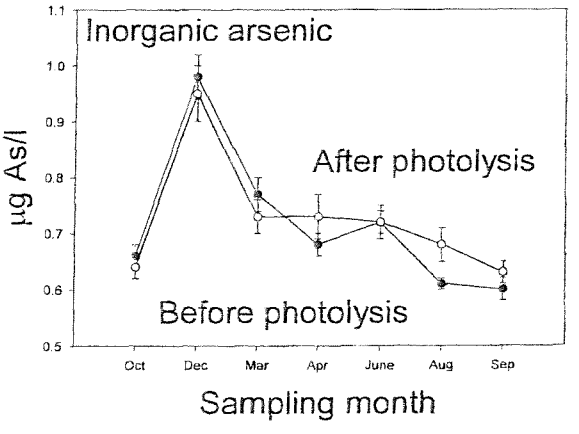
NW Netley

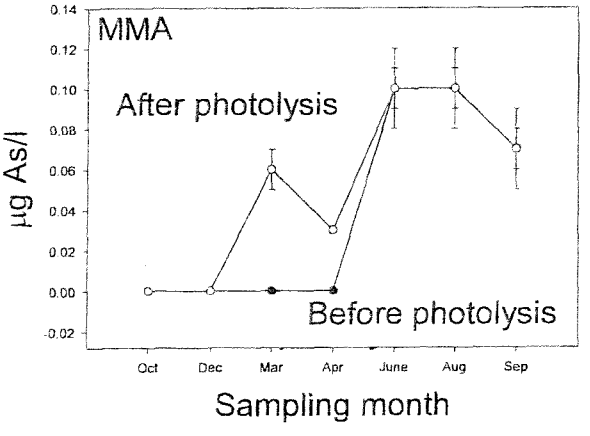






Hound





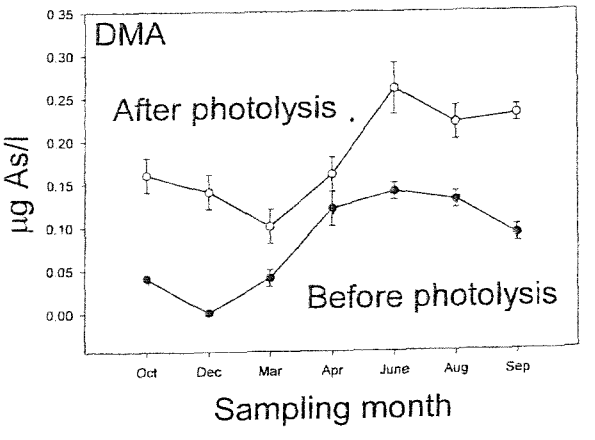
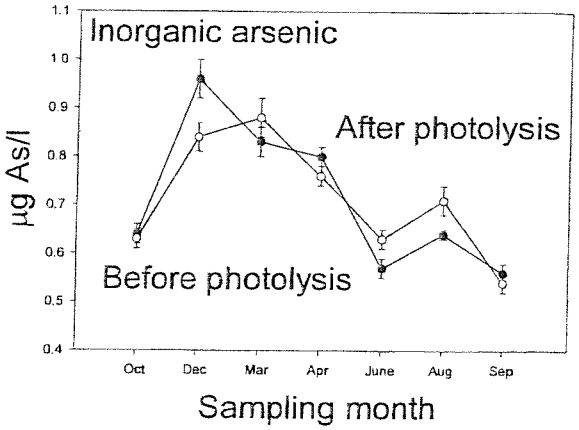
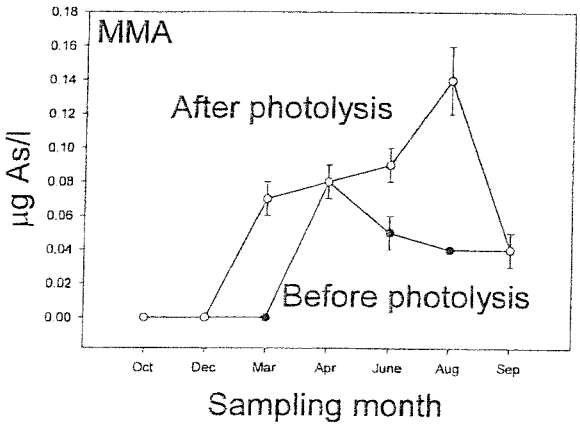
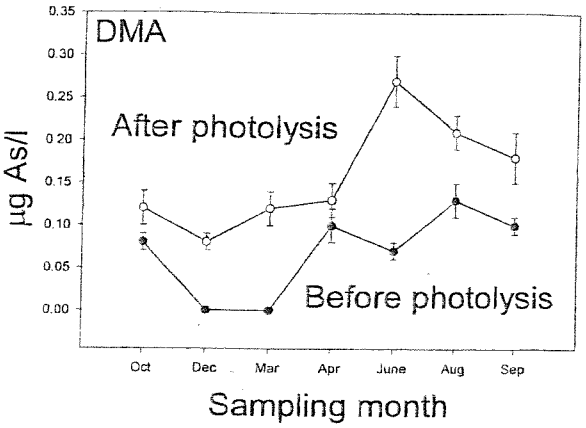


Figure 5.7d: Disssolved 'inorganic' and methylated arsenic content before and after sample photolysis.

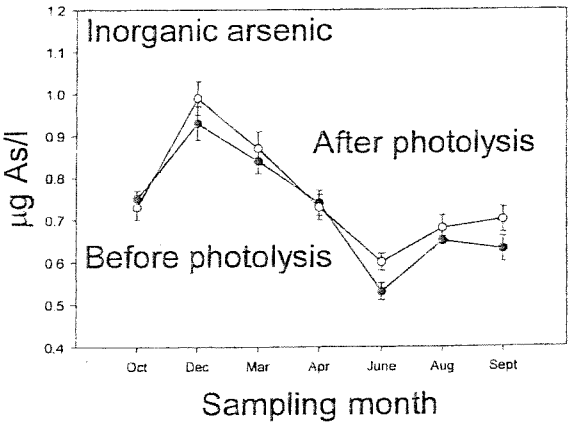
Greenland

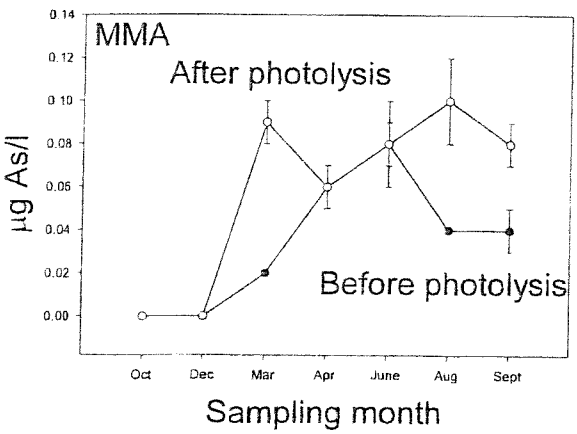






BP Jetty





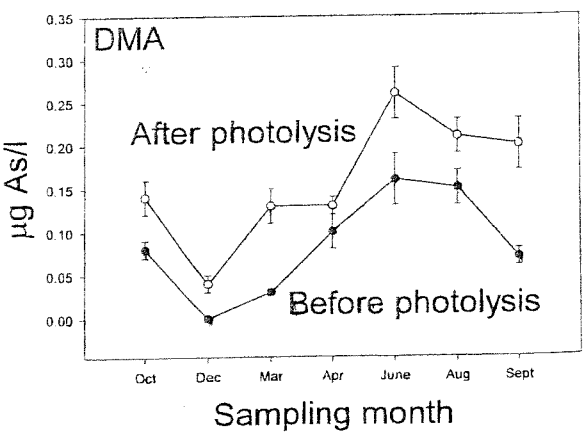


Figure 5.7e: Disssolved 'inorganic' and methylated arsenic content before and after sample photolysis.

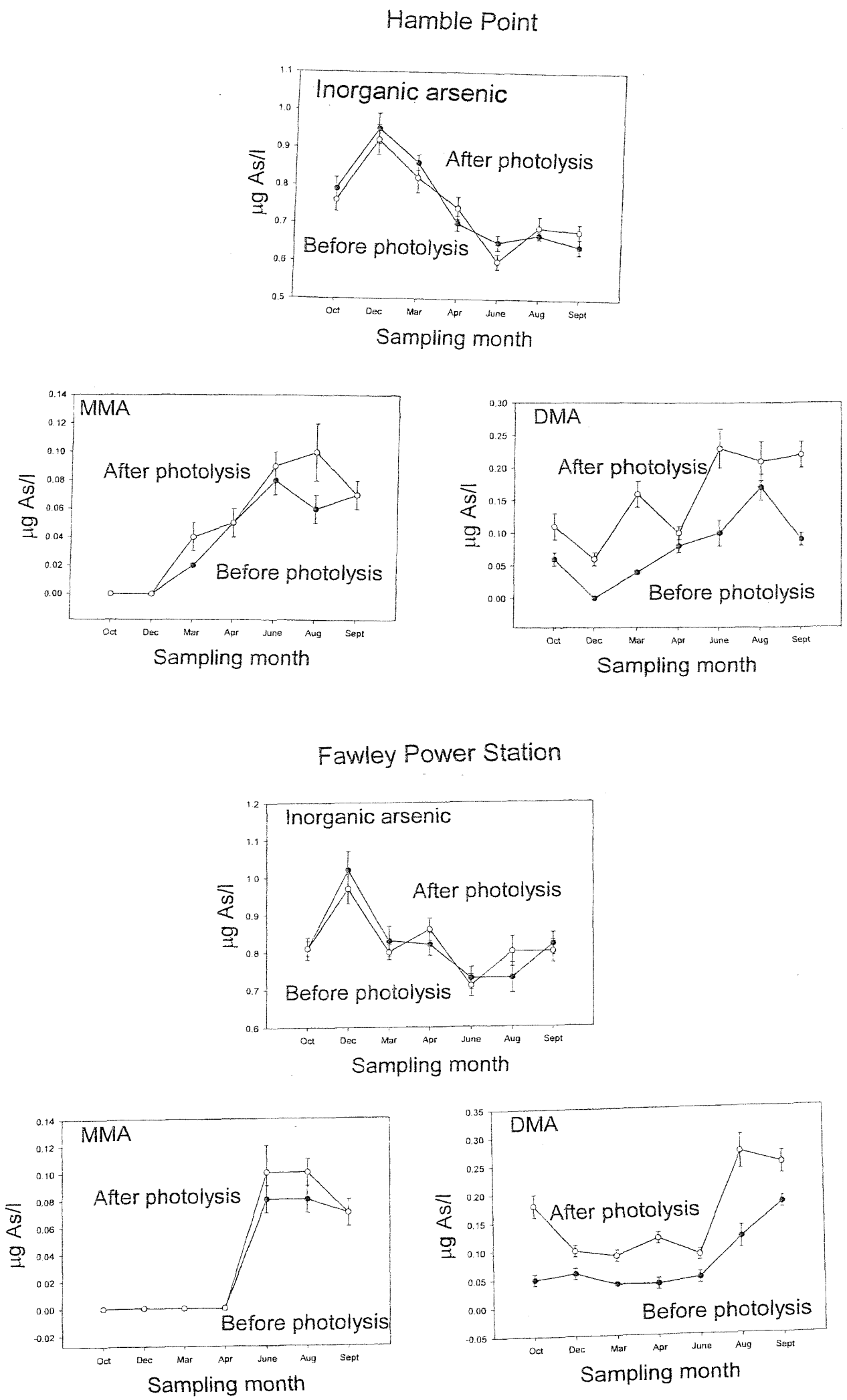


Figure 5.7f: Disssolved 'inorganic' and methylated arsenic content before and after sample photolysis.

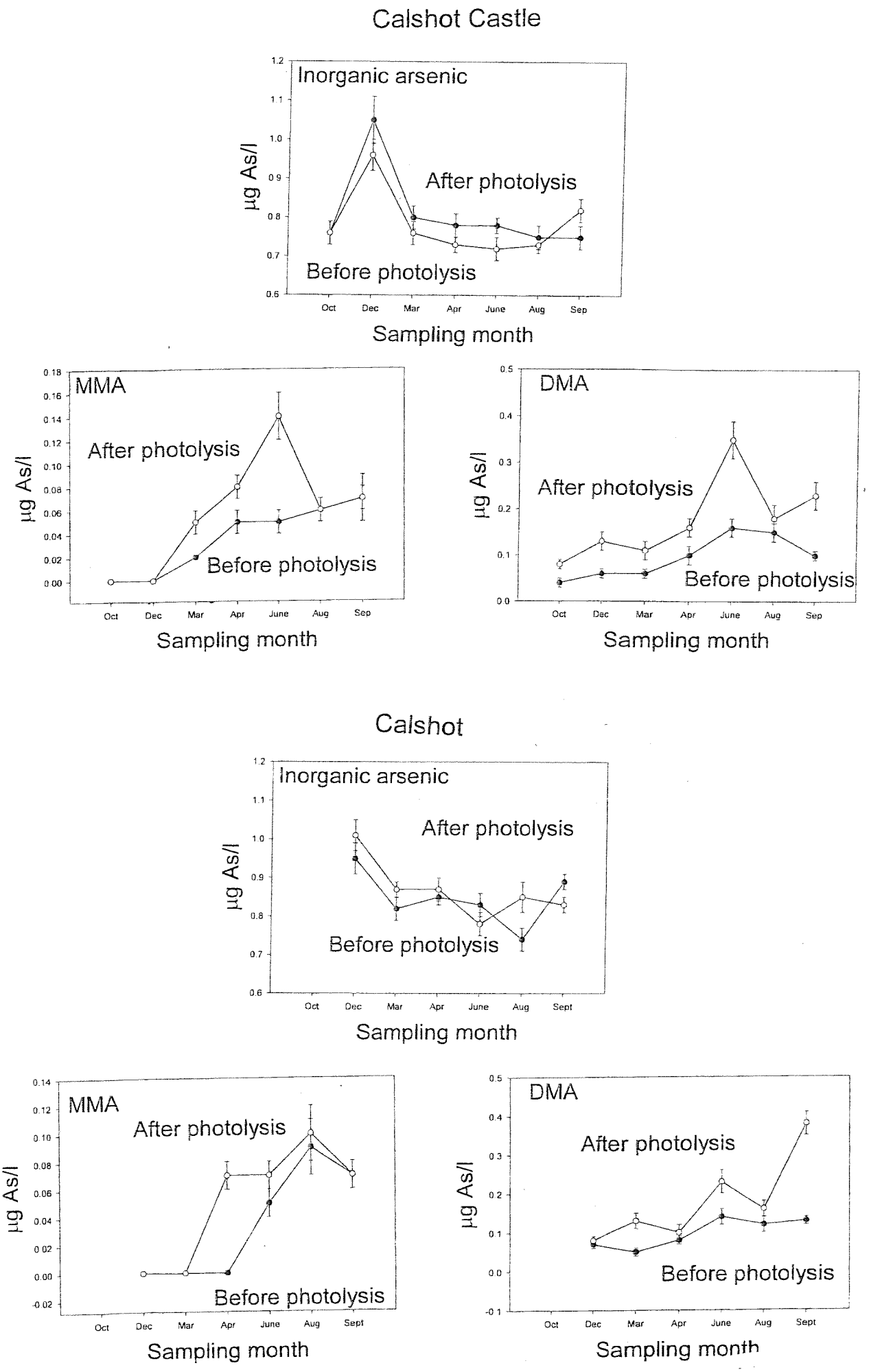


Figure 5.7g: Disssolved 'inorganic' and methylated arsenic content before and after sample photolysis.

5.5.5.3 Methylated arsenic

'Hidden' methylated arsenic was present as either MMA, DMA or both. There was no evidence of any 'hidden' TMA (Figure 5.7).

MMA

For the majority of the sampling locations there was no evidence of 'hidden' MMA during the October and December surveys. The Itchen Toll Bridge sample was an exception to this trend with 0.06µg As/l of 'hidden' MMA was detected in October. This was not accompanied by detectable dissolved hydride-reducible MMA.

From March onwards, 'hidden' MMA was detected in the majority of the samples. This behaviour coincides with increased biological activity. After June-August, 'hidden' MMA concentrations begin to decrease. There were some sites such as Fawley Power Station and the Oil Spill Centre where 'hidden' MMA was not detected until after March/April.

Overall the data indicates that the presence of 'hidden' MMA is cyclical with high concentrations being obtained during the mid-summer months due to biological activity before decreasing to below detectable levels during the winter season.

DMA

Significantly, during the course of the sampling survey, 'hidden' DMA was present at the majority of sampling locations, even during the winter months when dissolved hydride-reducible DMA content and phytoplankton activity are at their lowest. Between the October and March surveys, 'hidden' DMA concentrations remained relatively constant (range 0.05-0.18µg As/l) in the majority of samples

studied. No major general trend could be identified that linked the presence of dissolved 'hidden' DMA to the time of sampling.

In summary 'hidden' DMA is present throughout the year with its concentrations remaining within a relatively narrow band irrespective of the time of year or phytoplankton activity. The proportion of dissolved DMA that is present as 'hidden' DMA is significant, ranging from 0 to 66%.

5.5.6 'Hidden' arsenic determination by microwave photolysis

When samples were treated by microwave photolysis, it was noted that there was a substantial loss of sample and a white solid was deposited on the top of the quartz test tube. With deionised water there was no evidence of water loss.

After photolysis the saline samples were hotter than the deionised water samples, by an average of 20°C. This suggests that microwaves are better coupled to saline samples giving more rapid heating.

Due to substantial loss of sample during irradiation, the microwave photolysis pretreatment method was not investigated further.

5.5.7 'Hidden' arsenic determination by persulphate batch photolysis and inline photooxidation methods

The water samples from the Oil Spill Centre, Greenland and Calshot Castle were selected for further investigation in order to compare the effectiveness of the batch persulphate and inline photooxidation methods investigated in Chapter 4 for the determination of 'hidden' arsenic content and speciation. Results obtained for each method were compared to data obtained by conventional cryogenic trap HG-AAS and batch photolysis pretreatment.

Comparison of the four analysis methods on samples of unknown composition can be based solely on which arsenic species are found. The general trends that were evident are as follows (Figures 5.8-5.10):

1. all three photolysis/photochemical treatment procedures reveal the presence of methylated arsenic species which were not evident by the conventional HG-AAS procedure;
2. the batch persulphate photolysis yields results that are generally very similar to those obtained by the batch photolysis method which has been used in recent years to reveal 'hidden' arsenic content and speciation;
3. the inline photooxidation procedure produces inconsistent results. These results are often indistinguishable from those obtained with the batch persulphate photolysis but with other samples very low levels of methylated arsenic are obtained (in comparison to the other three methods). When low levels of methylated arsenic are obtained there may be some evidence of elevated 'inorganic' arsenic levels indicating demethylation by the inline photooxidation system. This may reflect the presence of a variety of methylated arsenic species, only some of which are suspectible to photochemical demethylation. If this were the case however more evidence of demethylation might be expected with the batch persulphate photolysis procedure.

All three photolysis/photochemical methods give information on the presence of methylated arsenic that can not be found by conventional HG-AAS methods but further work will be necessary to optimize the procedures and to better understand the selectivities of the methods.

5.5.8 Sonication treatment of selected arsenic samples

Sonication of unfiltered samples is an efficient means of disrupting cells and cellular fragments potentially releasing methylated arsenic species into the water.

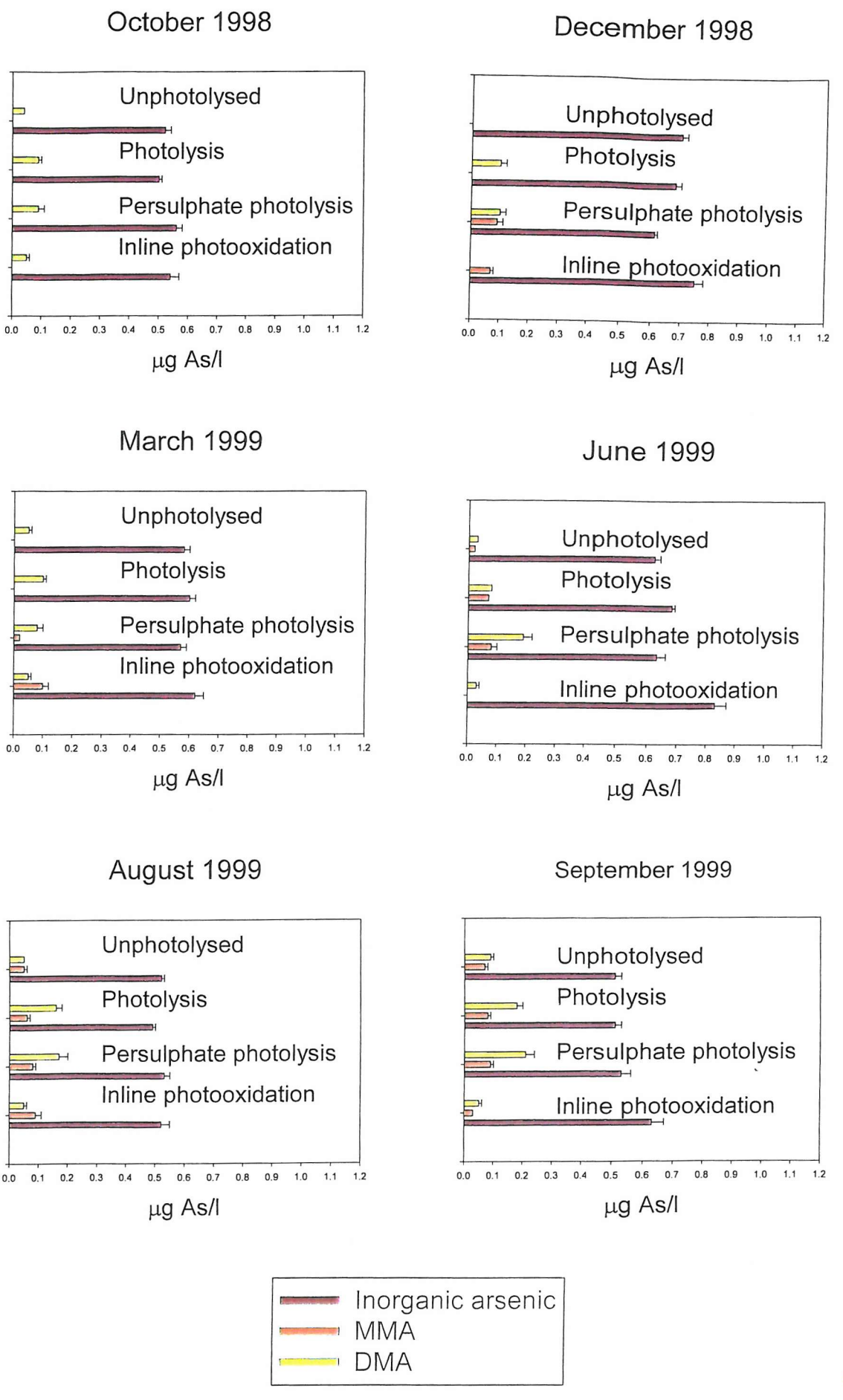


Figure 5.8: Arsenic content and speciation of Oil Spill Centre surface waters after photochemical/photolysis treatment

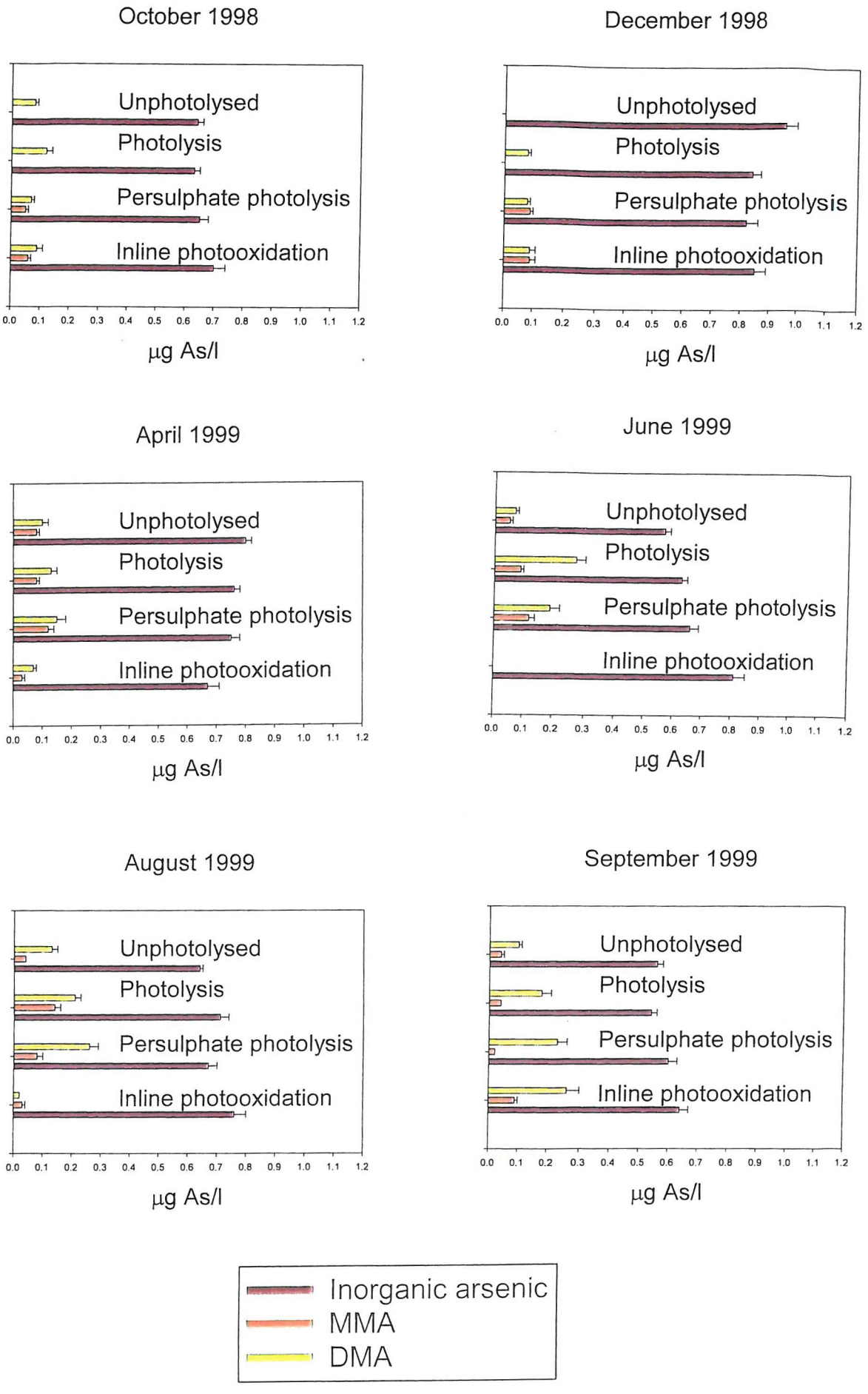


Figure 5.9: Arsenic content and speciation of Greenland surface waters after photochemical/photolysis treatment

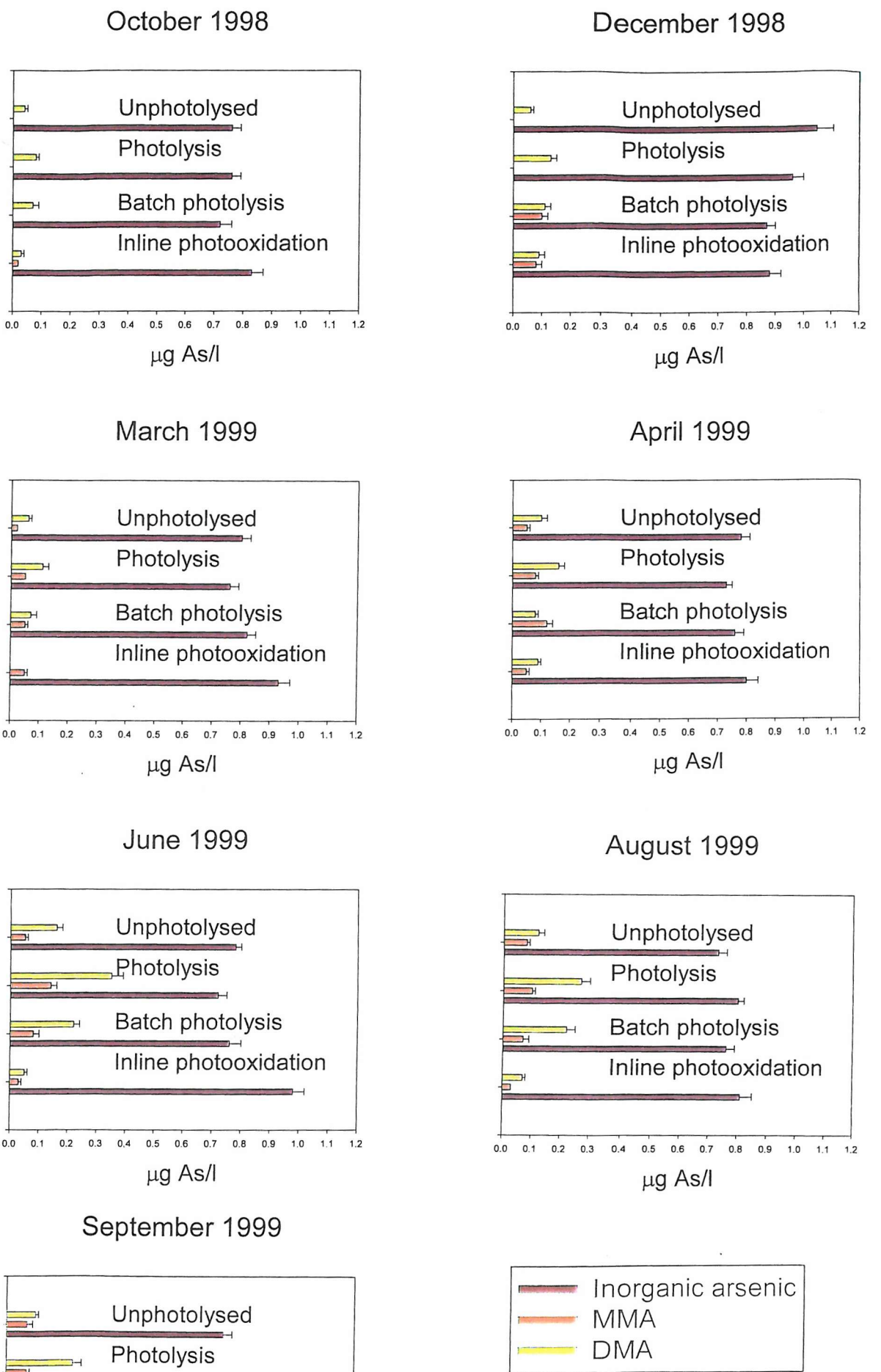


Figure 5.10: Arsenic content and speciation of Calshot Castle surface waters after photochemical/photolysis treatment

Thus sonication may provide a useful approach to investigating the release of 'hidden' arsenic by physical breakdown and to an lesser extent the release of such species during grazing of phytoplankton by zooplankton.

In this work samples from the Oil Spill Centre, Greenland and Calshot Castle were analysed directly after sonication and vacuum filtration to identify hydride reducible species and then after the sonicated and filtered sample had been photolysed for 18 hours by the batch (no persulphate) photolysis method.

The overall trends observed in this study were (Figures 5.11-5.13):

1. from March to September, both the sonication and sonication/photolysis samples released only comparatively low levels of additional hydride-reducible arsenic species. This may be considered to be unexpected as it is during this time period that phytoplankton populations, which have been assumed to contain methylated arsenic species, are at their greatest;
2. the most obvious cases of sonication resulting in the release of significant 'hidden' arsenic were in December when samples from the Oil Spill Centre, Greenland and Calshot Castle all demonstrated significant release of methylated arsenic species on sonication. At this time, very little methylated arsenic was evident by direct HG-AAS analysis but particulate-derived methylated arsenic species were released.

5.5.9 The availability of nutrients

5.5.9.1 Introduction

Colleagues in the Oceanography Department recorded measurements of both dissolved inorganic phosphate and inorganic nitrate. The availability of these nutrients plays a vital role in the growth and sustainability of phytoplankton and bacteria in estuarine waters. The depletion of these nutrients could lead to the

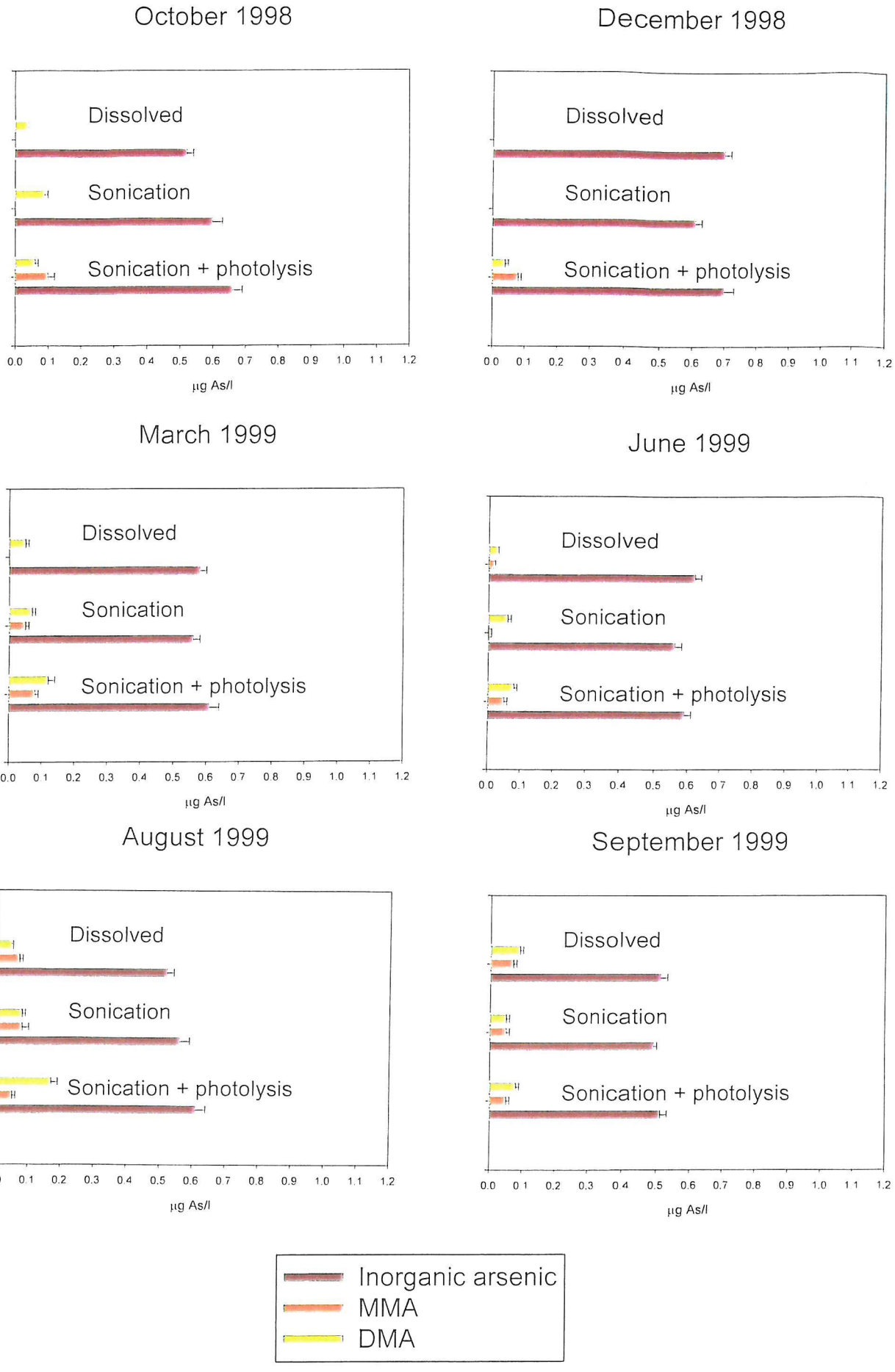


Figure 5.11: Arsenic content and speciation of Oil Spill Centre surface waters after sonication and sonication + photolysis treatment

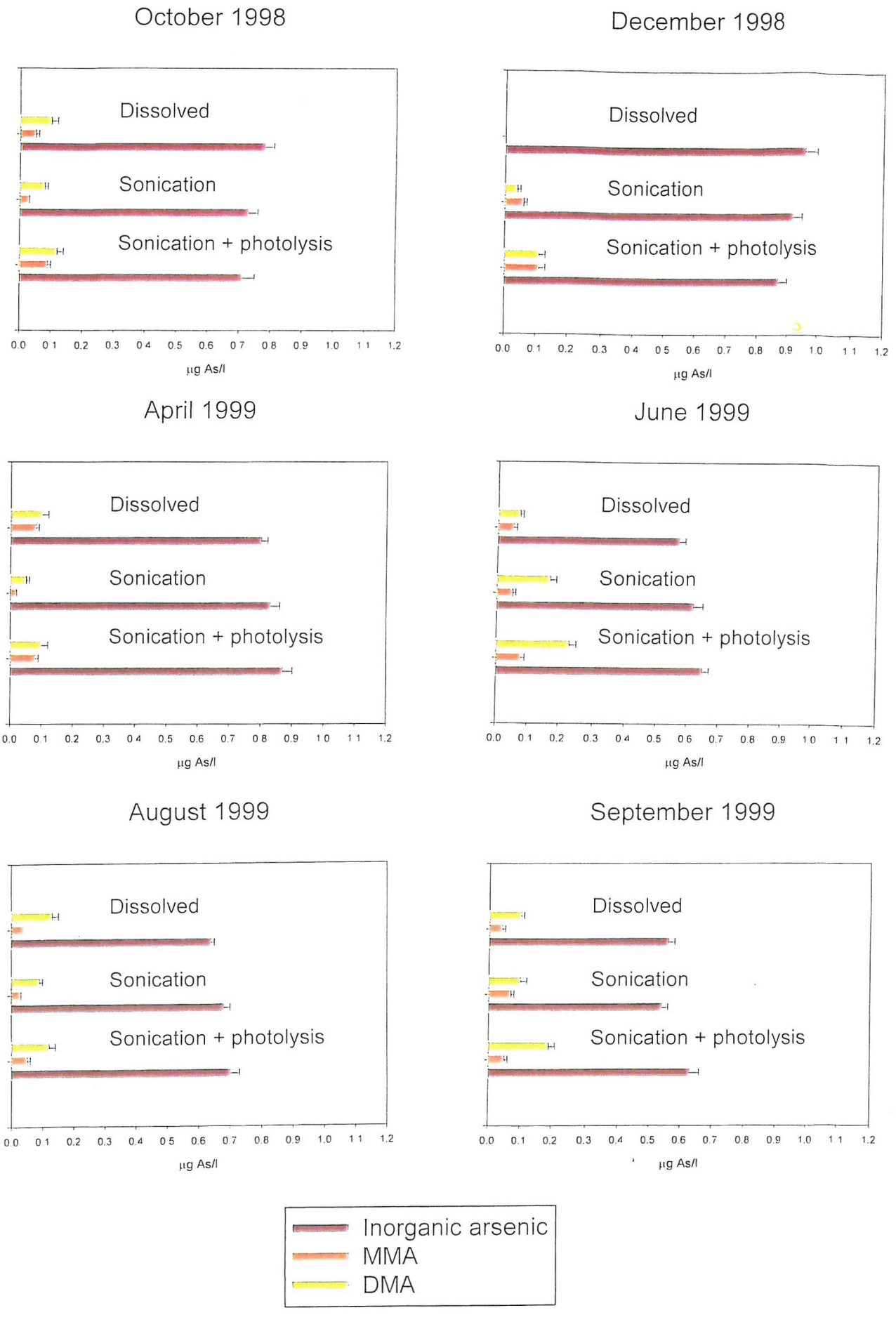


Figure 5.12: Arsenic content and speciation of Greenland surface waters after sonication and sonication + photolysis treatment

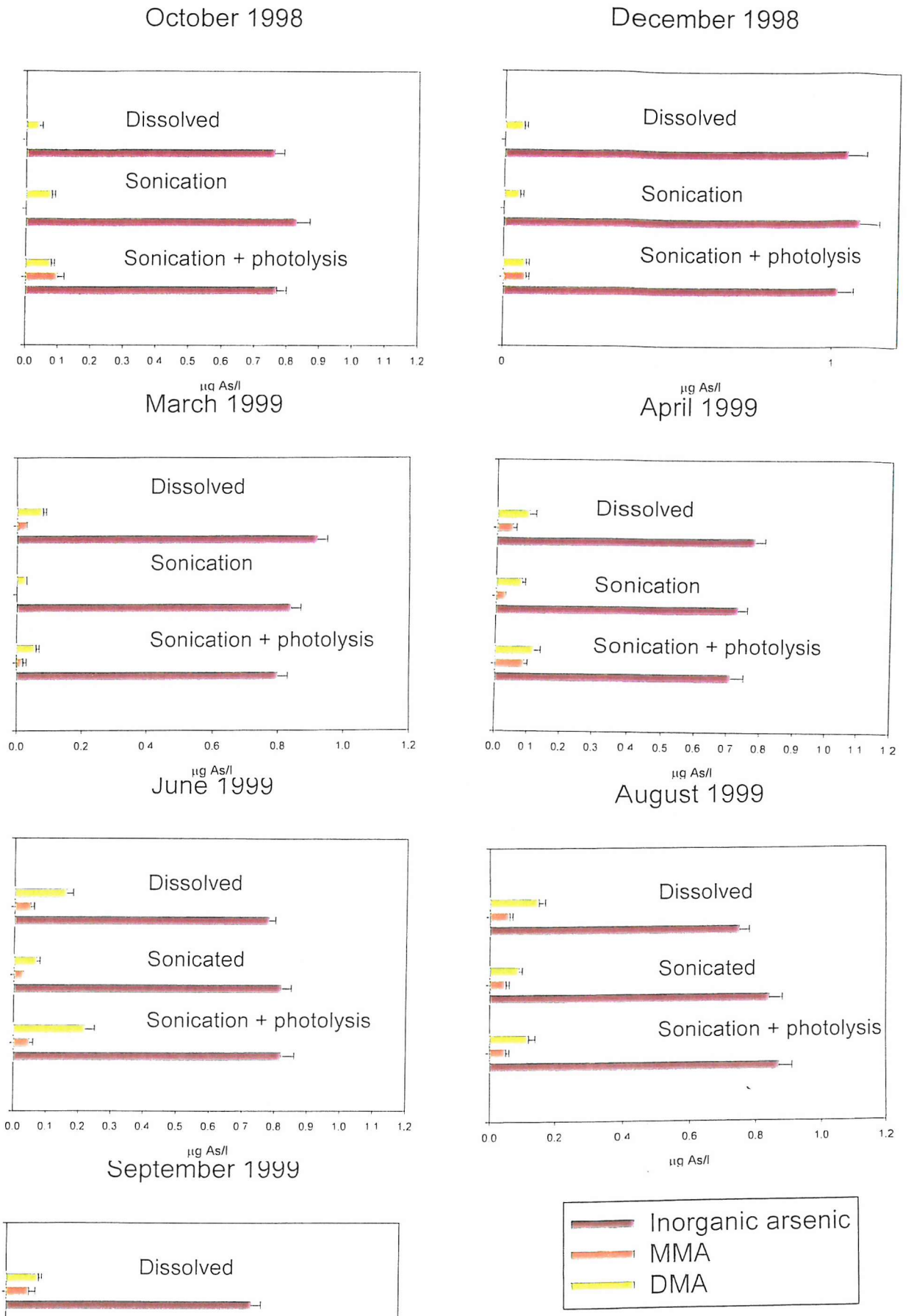


Figure 5.13: Arsenic content and speciation of Calshot Castle surface waters after sonication and sonication + photolysis treatment

eventual death of any algal blooms. When there is a plentiful supply of phosphate in the water column, certain marine organisms take up arsenate indiscriminately [9]. This is thought to be due to the organism being unable to differentiate between the chemically similar arsenate and phosphate. However, other workers have reported that in areas where phosphate concentrations are limited, certain marine organisms ingest arsenate to make up for the shortfall in phosphate [10].

The data collected only covers the first four sampling excursions. However, data collected for June and August 1998 were used for comparative purposes as an indicator of the nutrient concentrations that may be present during the summer months.

If depletion of a nutrient such as phosphate results in the uptake of increased arsenate, then the levels of methylated arsenic released into the water column might be expected to increase due to increased arsenic throughput and as a result of the decay of algal material. The form of this released arsenic might be expected to be 'hidden' arsenic.

5.5.9.2 Dissolved inorganic phosphate

In general, dissolved inorganic phosphate levels (Figure 5.14) decreased with increasing water salinity with each sampling survey. For example, the dissolved inorganic phosphate concentration levels in the sites in the River Itchen (average 4.64mM-P) were much higher than those recorded at Calshot Buoy (0.5mM-P) during the October survey. Comparison of this data with those obtained during June and August 1998 revealed that the summer values were much lower than the October data in the River Itchen (average June 3.25mM-P August 2.81nM-P) and in Calshot Buoy (June < 0.02nM-P, August 0.15nM-P). This will result from increased bio-utilisation and decreased land run-off activity.

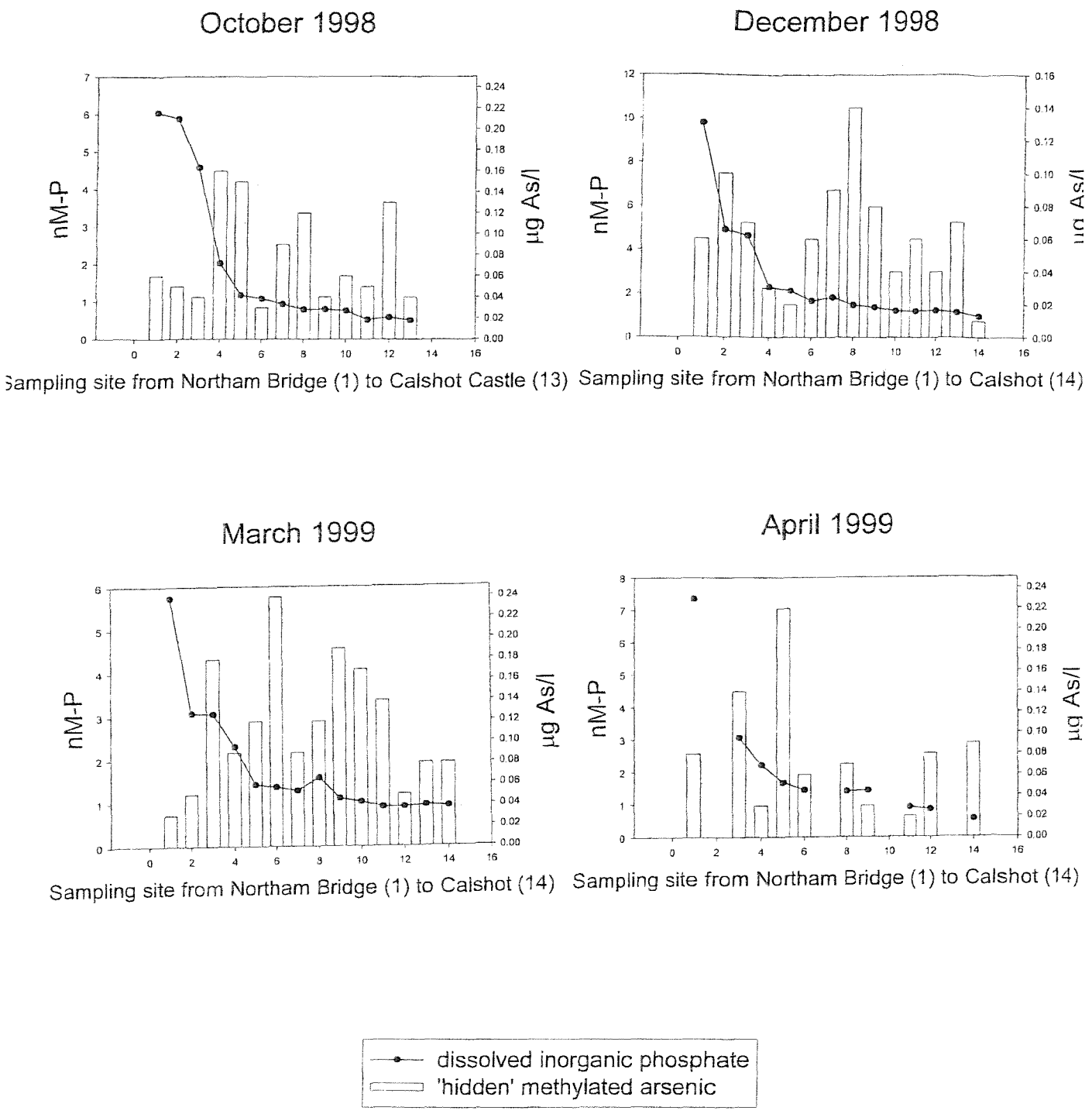


Figure 5.14: Dissolved inorganic phosphate and 'hidden' methylated arsenic content

There appears to be no correlation between 'hidden' methylated arsenic and dissolved inorganic phosphate content.

5.5.9.3 Dissolved inorganic nitrate

For October (Figure 5.15), the average values for Southampton Water were 20.6mM-N (August 16.2mM-N), Calshot Buoy 13.7mM-N (August 2.5mM-N) and the Itchen River 86.8mM-N (August 34.5mM-N). By December, the dissolved inorganic nitrate values increased (from 23.1mM-N for Calshot Buoy, 34.6mM-N for Southampton Water and 150.1mM-N for the River Itchen). Further increases were noted in Southampton Water whilst the Itchen River values remained similar to those recorded during December (average values ranged from 33.7mM-N for Calshot Buoy, 48.7mM-N for Southampton Water and 112.8mM-N for the Itchen River).

During April, there were slight decreases in dissolved inorganic nitrate levels for Calshot Buoy (22.1mM-N) and Southampton Water (47.8mM-N). However for the Itchen River there was an increase in dissolved inorganic nitrate levels (137.6mM-N) compared to March. This, along with the increase in phosphate levels for the Itchen River suggests that any elevated levels of dissolved inorganic nitrate in estuarine waters are very likely to be due to man's activities such as fertiliser effluent runoff or the disposal of sewerage material.

There was no evidence of any relationship between 'hidden' methylated arsenic and dissolved inorganic nitrate content.

5.5.10 Organic carbon analysis

Hasegawa reported that there was a correlation between dissolved organic carbon and the presence of 'hidden' methylated arsenic [11]. Graphs showing dissolved

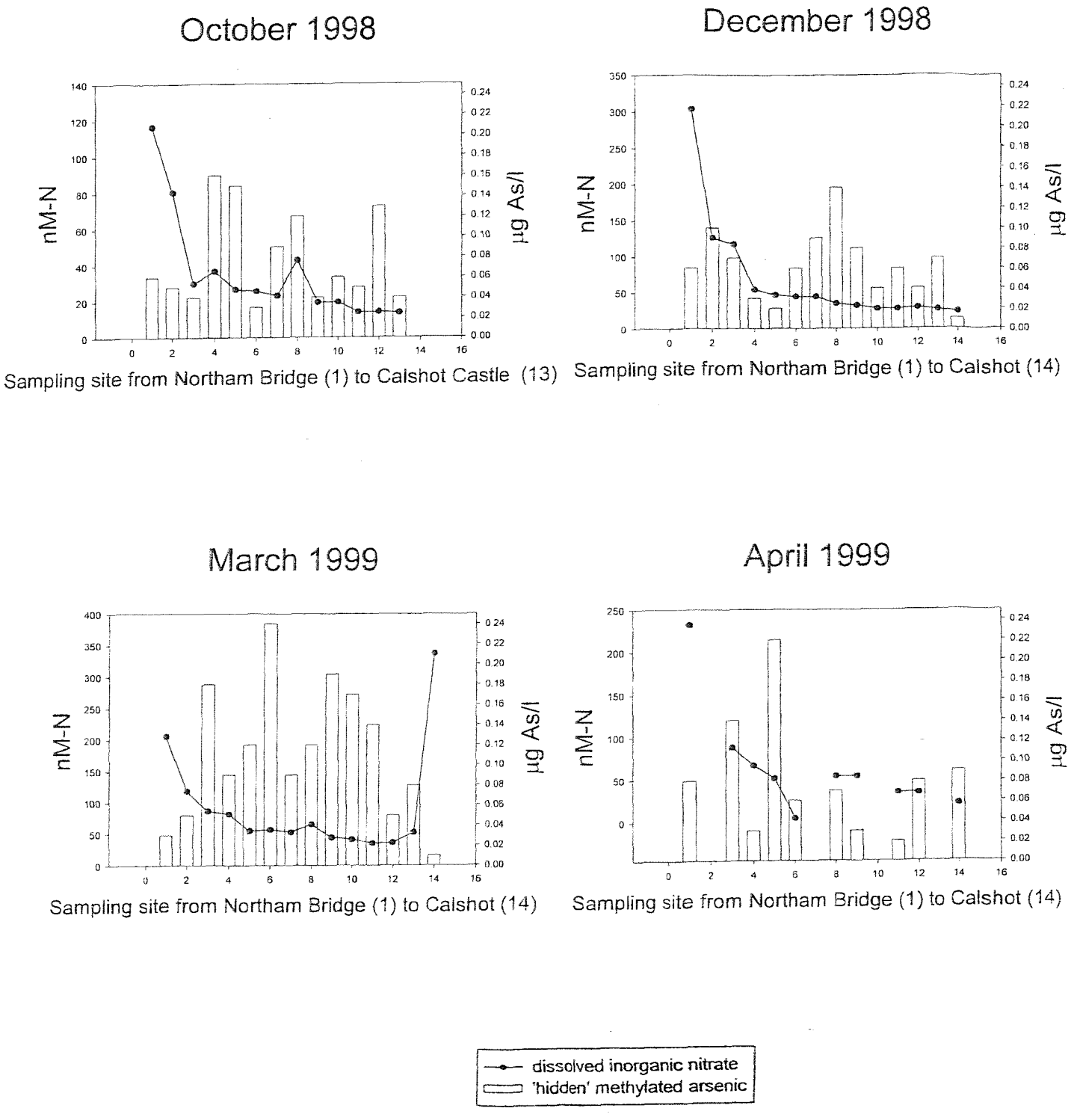


Figure 5.15: Dissolved inorganic nitrate and 'hidden' methylated arsenic content

arsenic concentrations were therefore constructed and any trends or comparisons made.

With the limited data available for the October (Figure 5.16) survey there is a conflicting picture between DOC content and 'hidden' methylated arsenic. For example between Fawley Power Station (12) and Calshot Castle (13), DOC decreases with increasing 'hidden' methylated arsenic content whilst between the Itchen Toll Bridge (4) and Dockhead (5) as well as Greenland (8) and Hound (9), DOC increases with increased methylated arsenic content. POC also shows conflicting behaviour with regard to 'hidden' methylated arsenic content with POC levels increasing when 'hidden' arsenic content decreases as well as increasing when 'hidden' arsenic content increases. This type of behaviour repeats frequently throughout the sampling survey and no relationship can therefore be found between dissolved and particulate organic carbon levels and the presence of 'hidden' arsenic in the Southampton Water/Itchen River estuarine system.

5.6 Discussion

5.6.1 Introduction

This fieldwork study was the first investigation of the seasonal nature of 'hidden' arsenic speciation in Southampton Water and the Itchen River. Factors which may influence 'hidden' arsenic speciation or might be indicators of mechanisms responsible for its production, such as nutrient availability and organic carbon levels, were included in this investigation.

Dissolved arsenic speciation observed in Southampton Water and the Itchen River showed identical trends to those observed in other environmentally similar estuarine water system studies [12-14]. In the winter months, the majority of the dissolved arsenic was present as dissolved 'inorganic' arsenic with very little

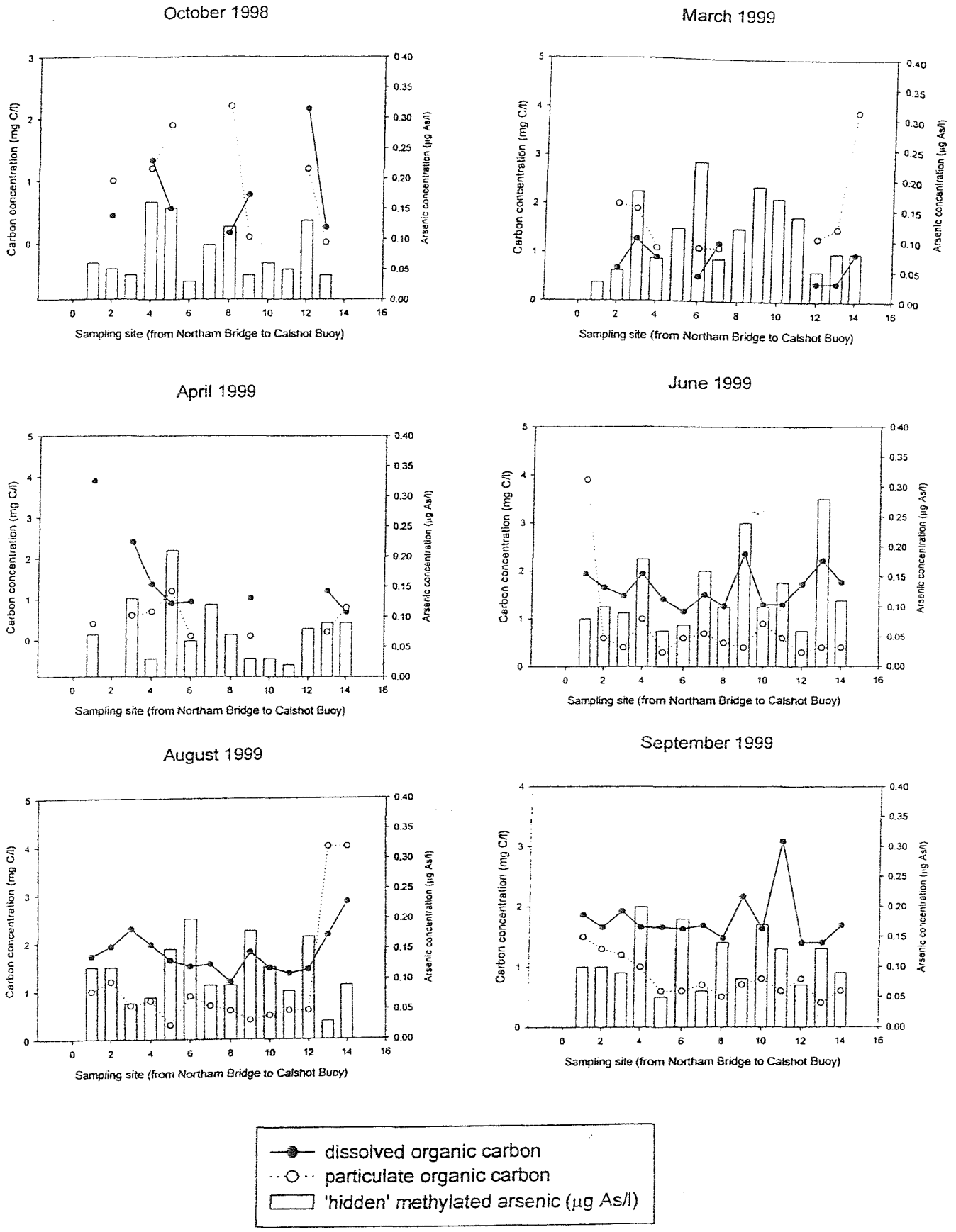


Figure 5.16: Dissolved and particulate organic carbon content with 'hidden' methylated arsenic concentrations

methylated arsenic being present at isolated locations. During the summer months, with increased water temperatures, light levels and biological activity, methylated arsenic concentrations increased. The increase in methylated arsenic is generally accompanied by reduced dissolved 'inorganic' arsenic levels. This is consistent with dissolved 'inorganic' arsenic being assimilated by marine organisms for it to be incorporated into their structures as compounds such as arsenosugars. The biological activity of these systems is at its greatest during the spring and summer months resulting in the active release of methylated arsenic species into the water column together with their release during grazing, breakdown during senescence and death. The release of compounds such as arsenosugars, which are 'hidden' methylated arsenic species, and their subsequent breakdown to simpler hydride reducible species, can account for the observed arsenic behaviour. This overview of the production and breakdown of arsenic species is supported by the generation of 'hidden' arsenic.

'Hidden' arsenic concentrations increase during the summer months. In December 1998 'hidden' arsenic only comprised a small proportion (average 5%) of the total dissolved arsenic present in the Calshot Buoy surface waters. However, during September 1999 its concentration had increased fourfold.

All natural water samples are heterogeneous with the particulate phase being a potential source (or sink) of dissolved phase species. Sonication of water samples was therefore carried out to break down cells and other particulate material. This resulted in the release of dissolved methylated arsenic principally as 'hidden' methylated species from water samples. These methylated arsenic forms are therefore likely to have originated from the release of larger and complex organoarsenic species as particulate matter was broken open by sonication.

5.6.2 Dissolved arsenic speciation

The Southampton Water and Itchen River estuary systems are not highly polluted by arsenic when compared to many other estuarine systems. Consistent with an unpolluted river mixing with seawater containing *ca.* 1μg As/l, dissolved 'inorganic' arsenic concentrations, increased with increasing salinity in the Itchen River estuary. In Southampton Water three major water masses meet as riverwater from the Test and Itchen Rivers mix with seawater from the Solent. The dissolved 'inorganic' arsenic-salinity profiles along the transect from the Itchen Estuary into Southampton Water therefore changes in the area of Dockhead. No specific trend could be identified between dissolved methylated arsenic species and salinity in the Southampton Water/Itchen River estuarine system. This indicates that these species are more dependent on local conditions due to differing populations of phytoplankton and bacteria which assimilate 'inorganic' arsenic through differing metabolic processes. The bioavailability of nutrients differing from one site to another may also influence the uptake of 'inorganic' arsenic. Phytoplankton in particular are known to preferentially populate certain estuarine areas due to favourable nutrient and turbulence conditions [15].

During the winter months the dissolved 'inorganic' arsenic levels were at their highest and decreased gradually during the biologically productive summer months. In the winter months nutrient levels were at their highest due to very low levels of phytoplankton and/or increased nutrient inputs. With the advent of summer weather conditions with increased water temperatures, light levels and decreased rainfall, nutrient concentrations decreased due to increased biological activity and reduced inputs from land run-off. This increase in biological activity also corresponds to an increase in the dissolved methylated arsenic content of the water. This behaviour was observed in the majority of locations throughout Southampton Water and the Itchen River.

With a small number of exceptions, DMA was the predominant hydride-reducible methylated arsenic species present in the water samples indicating that a possible origin could be from the dimethylated arsenosugars from microalgae present in the water column. As no evidence was presented for TMA in the Southampton Water/Itchen River estuarine system this suggests that trimethylated arsenosugars are a very minor end product formed by microalgae. This is consistent with the finding of only very small concentrations of trimethylated arsenosugars in macroalgae [16].

There is a long established association between the presence of methylated arsenic species in the water column and biomethylation processes by marine phytoplankton [17,18]. Work by Howard [14] on the estuary of the River Tamar (Devon/Cornwall border, UK) revealed that the highest levels of dissolved methylated arsenic species were recorded in the higher salinity region of the estuary. In this study, however, the highest concentration levels of dissolved methylated arsenic were not always recorded within the highest salinity region of the estuary.

Interestingly, during December, the highest levels of methylated arsenic were recorded at Dockhead, at the conjunction of Southampton Water and the Itchen River estuary. This area is one in which turbulent mixing or dredging may, especially during the winter months, result in the disturbance of organic matter that had, during the productive summer months, been deposited into the sediments. Winter remobilisation and disturbance of these sediments may have resulted in the release of dissolved methylated arsenic that had previously been buried in the sediments of this area. An alternative means of methylated arsenic entry from the sediments to the water column could originate from bioturbation processes involving sediment-burrowing organisms as a five fold increase in arsenic dissolution from sediment-bound matter to the water column was reported by Sanders in the Chesapeake Bay system [19].

It must be remembered that the levels of dissolved methylated arsenic present in the water column result from the balance between the supply of new methylated arsenic and its flushing from the estuary. Assuming that methylated arsenic levels reached a maximum during the late summer, and were then deposited into sediments on the death of the plankton in late summer, the bottom sediments are expected to be a major reservoir of methylated arsenic slowly releasing arsenicals into the water column either as 'hidden' or hydride reducible arsenic species from cell lysis and/or bacterial breakdown.

5.6.3 Dissolved 'hidden' arsenic speciation

One objective of the sampling programme was to ascertain whether the four pretreatment procedures reported in Chapter 4 would be effective in the determination of 'hidden' arsenic species. Overall, the conventional batch photolysis with a 200W medium pressure mercury arc lamp and persulphate assisted batch photolysis methods were effective but it was unfortunately not possible to utilise the microwave photolysis technique. With this last approach, the analysis of estuarine water samples resulted in rapid volatilisation with a significant loss of analyte. The online photooxidation method, whilst in some cases, giving identical 'hidden' arsenic concentration levels to those obtained by both the persulphate-assisted photolysis and conventional photolysis, unfortunately had the side effect of losing speciation information for methylated arsenic in some samples.

When using the persulphate-assisted photolysis technique, it was observed that additional 'hidden' MMA was present in some water samples. This was particulary the case during the winter season, suggesting that this MMA could be derived from previously undetected complex methylarsenic forms or from the demethylation of dimethylarsenic compounds.

The conventional photolysis method proved to be the most effective technique for the determination of 'hidden' arsenic as there was very little loss of speciation information of the original sample. In the majority of samples that were photolysed, there was an increase in the arsenic concentration levels. The majority of 'hidden' arsenic was in the form of DMA as previous surveys have revealed [3]. There was very little evidence of any notable increase in dissolved inorganic arsenic concentrations after batch photolysis treatment, suggesting that the majority of the 'inorganic' arsenic is present as hydride-reducible forms in the Southampton Water/Itchen River estuary system.

A possible source of the additional dissolved DMA levels is thought to be from the presence of arsenosugars that have been identified as being present in marine algae (**Chapter 1**). When Hunt [20] photolysed the water from a culture of the marine macroalgae species, a large increase in the dissolved DMA concentration was found. In addition, Edmonds [21] has revealed the presence of arsenosugars in the diatom *Chaetoceros concavivornis* based upon HPLC retention times. Estuarine and coastal water environments host large communities of marine micro-algae during the summer months, with very high cell densities in the water column [6]. The release of dimethylarsenoribosides [21] or similar species by such marine algae is likely to contribute significantly towards a large increase in the total ('hidden' and hydride reducible) arsenic budget of the water column.

During winter weather conditions, biological activity decreases which would result in the dimethylarsenicals being diluted by coastal inputs or demethylated to simpler forms by bacteria [18] to levels below detection limits. However, it was observed that 'hidden' DMA was detectable in the majority of samples studied, even during the winter months.

'Hidden' arsenic exhibited seasonality in terms of concentration levels throughout the year. For example, during the winter 'hidden' arsenic levels are at their lowest while during the summer 'hidden arsenic levels increase significantly. This

behaviour is not unexpected as the majority of biological activity, which involves the bioaccumulation of arsenic, takes place during the summer months. During the spring/summer months, nutrient levels decrease possibly leading to the phytoplankton uptake of arsenate in place of phosphate. Once ingested the arsenate is methylated eventually leading to the formation of complex methylarsenic forms such as arsenosugars [16]. Those complex forms of arsenic can be released into the dissolved phase by excretion or death. Release is to be particularly expected during grazing of phytoplankton by zooplankton marine micro fauna. Continual replacement of phytoplankton populations in a succession is known to occur in this area [22]. The release of arsenic will be as both 'hidden' and non-'hidden' forms and 'hidden' arsenic will decompose to non-'hidden' forms by bacterial breakdown and demethylation.

Hunt reported that the photochemical treatment of arsenobetaine and arsenocholine [20] results in the formation of a mixture of 'inorganic' arsenic, mono, di and trimethylated arsenic species. The lack of any detectable 'hidden' trimethylated arsenic (TMA) suggests that if arsenobetaine or arsenocholine is present in the water column, it is present in very small quantities below the detection limit of the cryogenic trap system.

5.6.4 Arsenic speciation after sonication treatment

Sonication treatment of unfiltered water samples is thought to break open any cellular structures that may be present. It is well established that methylated arsenic is present in the cellular structures of phytoplankton and bacteria but they cannot be detected by conventional HG-AAS techniques as they do not form a volatile hydride following HG-AAS treatment. However, by comparing the concentration and speciation of arsenic in sonicated and untreated samples, any additional methylated arsenic present in the sonicated samples may be assumed to have originated from previously undetected methylated arsenic compounds such as dimethylarsenosugars present within the particulate phase.

In the majority of water samples that were sonicated, an additional level of previously undetected methylated arsenic was observed indicating that cell lysis through natural decay or grazing is a potential source of dissolved 'hidden' methylated arsenic in the water column.

5.6.5 Organic carbon and 'hidden' methylated arsenic content

Dissolved and particulate organic carbon measurements can provde an indication of biological activity in the water column. High levels of DOC can also be an useful indicator of the release of organic matter from bottom sediments. It was not, however, possible to identify any correlation between DOC and POC levels and the presence of any of the 'hidden' methylated arsenic species studied in this work.

5.7 Conclusion

Dissolved hydride-reducible arsenic speciation in the Southampton Water/Itchen River estuarine system showed trends similar to those observed in other temperate estuarine systems. Hydride-reducible 'inorganic' arsenic concentrations reached a maximum during the winter season before decreasing in the summer months when biological activity is at its highest. Dissolved methylated arsenic concentrations were at their highest during the biologically productive summer season. In the Southampton Water/Itchen River estuarine system, it was observed that dissolved hydride-reducible methylated arsenic was present in significant quantities in locations where the surface water temperatures were below 12°C.

A number of novel approaches have been investigated for the study of 'hidden' methylated arsenic species in the Southampton Water/Itchen River estuarine environment. The utilisation of these techniques in a sampling survey has for the

first time revealed the presence of 'hidden' DMA throughout the year in a temperate estuary.

Sonication of unfiltered water has revealed that at least one potential source of 'hidden' methylated arsenic originates from suspended particulate matter. Utilisation of the sonication process revealed that methylated arsenic release was significant during the winter period in the sampling locations studied.

The presence of dissolved hydride-reducible methylated arsenic in selected locations during the winter months where water temperatures were below 12°C and the continued presence of detectable 'hidden' DMA during the winter months of the sampling survey suggests that the sediments are a major means of dissolved methylated arsenic entry during the winter months. Towards the end of the summer season when water temperatures drop and light levels are reduced, the microalgae present on the surface waters die and are deposited into the sediment surface. In the surface layers, the microalgae decompose slowly to release methylated arsenic into the sediment interstitial waters. During periods of storms, dredging or, less probably, bioturbation, this methylated arsenic may be released into the water column either as dissolved hydride-reducible or 'hidden' arsenic.

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Chapter 6

Arsenic speciation of aqueous extracts from sediments

6.1 Introduction

In this chapter a number of the analytical methods reported in Chapter 4 for the determination of 'hidden' arsenic will be applied to measure the arsenic content and speciation of aqueous extracts from sediments. The sediments were collected from two different locations, one of which is at the mouth of an estuary where the dissolved arsenic content is within a concentration range of between 1-2μg As/l [1] (River Test, Hampshire). Other sediment samples were collected from an area of past intense arsenic mining activity [2] (River Tamar, Devon/Cornwall).

6.2 Sample collection and treatment

Two custom-built corers were used to sample sediments. The sediment was removed from the corer, measured and divided into sections of between 2-5cm in depth. Each individual section was transferred to a polyethylene bag and stored in a cool box at *ca.* 4°C until delivered to the laboratory.

Each individual sediment section was divided again into at least four pieces, weighed accurately, inserted into a centrifuge tube and centrifuged using a Centaur 2 centrifuge at 3000g for 1 hour. The water obtained by the centrifugation was filtered (Whatman GF/C, acid washed filter paper), measured and diluted using deionised water. Any sediment sections not analysed immediately were transferred to a freezer pending analysis.

6.3 Analysis of River Test sediment water extracts

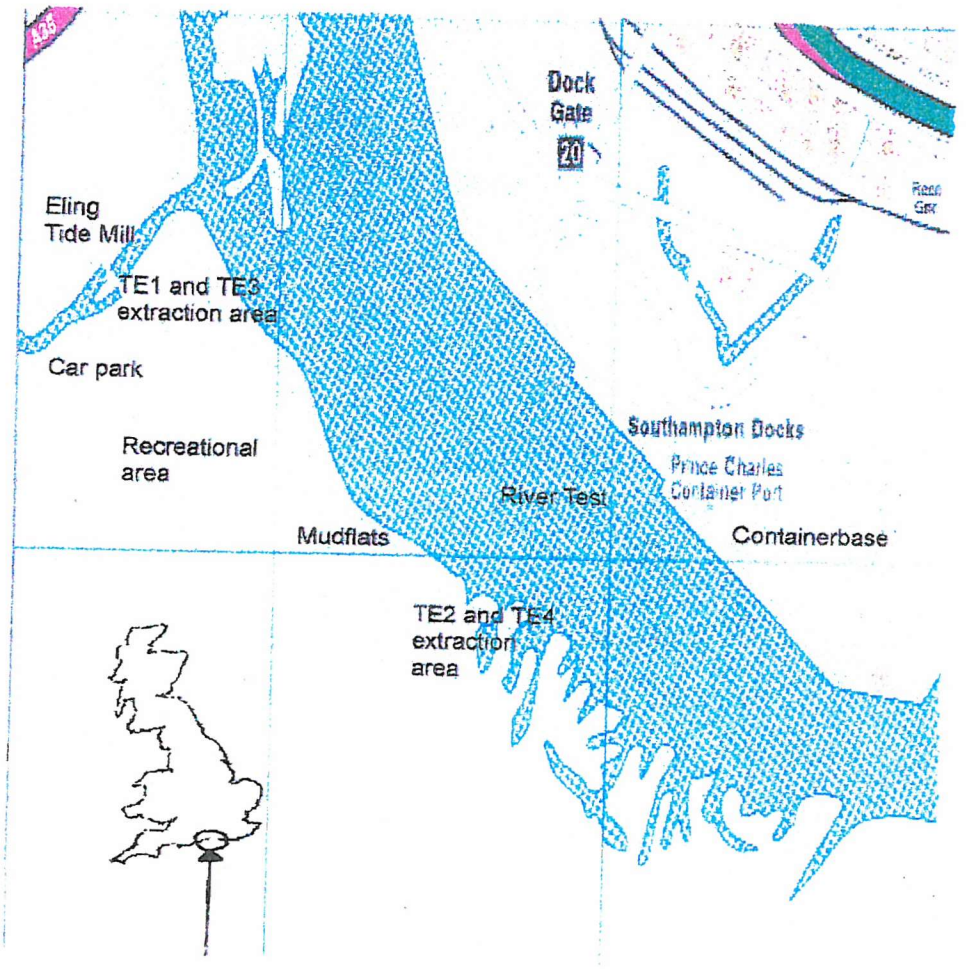
6.3.1 Introduction and sample collection

A brief overview of the location and environments from which the Test estuary samples were obtained is given in Chapter 5.1. Sediments were collected from two locations (Figure 6.1), one facing Eling Tide Mill (sediments TE1 and TE3) (Totton, Hampshire) and the other opposite Southampton Containerbase Terminal (sediments TE2 and TE4) (near Totton, Hampshire). All samples were collected during low tide on two occasions during February 1998. A 10cm (in length) sediment corer (diameter *ca.* 4cm) was used for the collection of samples TE1 and TE2 (10th February) whilst a 45cm corer (diameter *ca.* 10cm) was used for the TE3 and TE4 (27th February) samples.

6.3.2 Experimental

The filtered water extracts collected from the above sediment samples were first analysed by the cryogenic trap HG-AAS method (Chapter 3) for their dissolved hydride-reducible 'inorganic' and methylated arsenic contents. Three methods were then used to determine their 'hidden' arsenic contents:

1. Water was photolysed using a 250W Wotan medium pressure mercury lamp for 18 hours before analysis by cryogenic trap HG-AAS methods;
2. 100µl of persulphate solution (prepared as described in Chapter 3.2) was added to each quartz tube containing 3mls of water extract, before a 4-hour photolysis using the 250W Wotan mercury lamp. Cryogenic trap HG-AAS analysis was then carried out on the photolysed sample;
3. the inline photo-oxidation method (Chapter 4.4) was also employed.



Code	Sampling location	Grid reference number
TE1	Eling Tide Mill	365124
TE2	Opposite Southampton Containerbase terminal	373122
TE3	Eling Tide Mill	365124
TE4	Opposite Southampton Containerbase terminal	373122

Figure 6.1: Map of the River Test indicating sediment extraction points. Adapted from the 2000 Southampton Tourist Guide.

6.3.3 Results

'Inorganic' arsenic was the only form of arsenic detected by the HG technique in the untreated water extracted from both sediments (Table 6.1).

A significant increase in the dissolved 'inorganic' arsenic content of sample TE1 is observed after batch irradiation (no persulphate) and using the in-line persulphate photolysis. Sample TE1 therefore appears to contain significant levels of 'hidden' arsenic. When sample TE1 was analysed after batch photolysis with persulphate, the measured levels of 'inorganic' arsenic were depressed suggesting the presence of an interferent.

6.3.4 Interference study

6.3.4.1 Introduction

A standard addition experiment was carried out which involved the stepwise addition of known concentrations of arsenic to the sediment extract and its subsequent analysis.

6.3.4.2 Experimental

Fresh sediment was extracted from two areas: one extracted from the riverbed opposite Eling Tide Mill (Sediment TE3) and the other collected from mudflats opposite Southampton Containerbase terminal (Sediment TE4). Both extracts were collected using the 45cm sediment corer. The aqueous solution was extracted from the sediment using the procedure described in Section 6.2.

1ml aliquots of sediment water were placed into 10 different 10ml volumetric flasks, which had been 'spiked' with arsenite to increase the concentrations by between 0 and 10µg As/l. The samples were made up to the mark with deionised

Sediment extract	Depth(cm)	'Inorganic' arsenic concentration (μg As/l)			
		Untreated	After batch photolysis	After S_2O_8 photolysis	Inline photooxidation
TE1	0-2	22.3±1.1	32.3±1.3	16.5±0.3	33.1±1.3
	2-4	29.8±0.8	37.9±1.5	18.2±1.0	46.2±2.3
(Eling Tide Mill)	4-6	34.8±0.6	75.4±3.8	18.5±0.7	79.8±3.1
	> 6	19.6±0.4	59.3±3.0	12.4±0.2	66.9±2.7
TE2	0-2	34.7±1.8			
	2-4	45.2±2.3	not determined	not determined	not determined
(Containerbase)	4-6	49.8±3.0			
	> 6	35.3±1.1			

All units are in μg As/l

Table 6.1: Concentrations of arsenic in sediment aqueous extracts from the River Test before and after sample pretreatment for 'hidden' arsenic.

water and shaken well. This experiment was repeated with arsenate, MMAA and DMAA taking the place of arsenite. The resulting solutions were analysed by the cryogenic trap HG-AAS method. Appropriate diluted solutions of arsenate, arsenite, MMAA and DMAA in deionised water were also analysed for reference purposes.

For the analysis of As(III) and (V), concentrations were measured using sodium acetate buffer solution adjusted to pH 5. Under such conditions arsenic (III) forms are measured but not arsenic(V). As(V) concentrations were obtained by difference from total 'inorganic' arsenic measured at pH 1.

6.3.4.3 Results

With sediment TE3, it was observed (Table 6.2) that for all the arsenic species studied, the concentrations obtained by direct analysis and the extrapolation of standard addition were almost identical. This implies that the other species present in the sediment water extract did not affect the generated arsenic signal significantly. It must be noted that the magnitude of the error associated with the standard addition results restricts the interpretation of this data.

Analysis method	Arsenic species			
	As(III)	As(V)	MMA	DMA
Direct analysis	11.5±0.6	10.6±0.4	< 0.01	< 0.01
Extrapolation of standard addition	12.1±3.5	9.8±3.6	0.04±0.1	0.06±0.2

All units are in µg As/l

Table 6.2: Results obtained for the TE3 sediment aqueous extracts by direct analysis and the extrapolation of standard addition.

For sediment TE4, (Table 6.3) the concentrations obtained by direct analysis and by standard addition of As(V) and the methylated arsenic species (MMA and DMA) did not differ significantly, implying that interferents did not affect methylated arsenic signal output drastically. For As(III), however, the concentrations obtained by direct analysis and the extrapolation of standard addition are not identical. This indicates that there are interfering species, which affected the measured As(III) concentrations.

Analysis method	Arsenic species			
	As(III)	As(V)	MMA	DMA
Direct analysis	11.4±0.6	25.2±0.7	< 0.01	< 0.01
Extrapolation of standard addition	18.8±9.7	23.2±7.8	0.06±0.1	0.03±0.1
Extrapolation of standard addition + L-cysteine	10.3±3.0	26.0±6.3	0.07±0.1	0.03±0.1

All units are in µg As/l

Table 6.3: Results obtained for the TE4 sediment aqueous extracts by direct analysis and the extrapolation of standard addition.

The masking agents L-cysteine and ethylenediaminetetracetic acid have been suggested as ways by which interference effects can be reduced [3]. An investigation of the effectiveness of L-cysteine in overcoming interferences present in the sediment water extracts was therefore carried out.

6.3.4.4 L-cysteine as a masking agent in the analysis of sediment aqueous extracts

200mg of L-cysteine was added to each of 9 10ml volumetric flasks, which already contained water extracted from sediment (TE4) and 'spiked' with arsenite, arsenate, MMAA and DMAA (Section 6.3.4.2). Solutions were shaken thoroughly and left to stand for approximately 10 minutes before analysis by cryogenic trap HG-AAS methods.

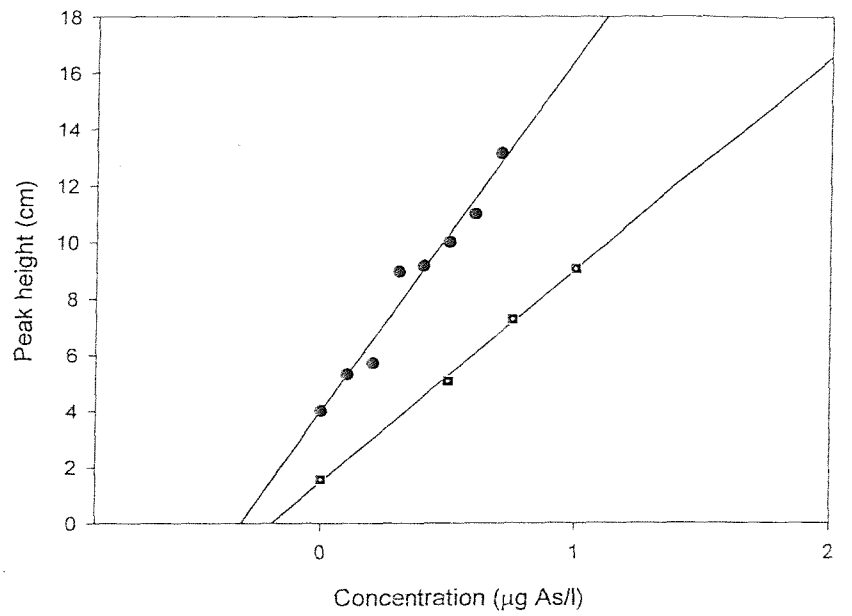
There were no significant interference effects observed for As(V), MMAA or DMAA 'spiked' sediment solutions pretreated with L-cysteine. For the As(III) samples pretreated with L-cysteine, however, the gradients of the standard addition and calibration were almost parallel to each other (Figure 6.2). It was also noted that the As(III) concentrations obtained by direct analysis and the extrapolation of standard addition were almost identical (Table 6.3). This suggests that the addition of L-cysteine to the sediment solution overcame the As(III) interferance problem that was present in the untreated sample.

6.3.5 Discussion

6.3.5.1 Arsenic speciation

Dissolved 'inorganic' arsenic

The dissolved 'inorganic' arsenic concentrations in the sediment aqueous extracts collected from the different layers of the TE1 and TE2 sediments varied through the sediment core. Within the surface layer, the dissolved 'inorganic' arsenic concentrations are low compared to the middle layers of the sediment core suggesting that the arsenic is mobilised from the top layer of the core into the water column. Below the surface layer, the dissolved 'inorganic' arsenic concentrations increase steadily (2-6cm) before decreasing in the lower layer of sediment (>6cm) to concentration levels almost identical to those found in the surface layer. These trends observed are almost in agreement with those obtained by Millward in the Thames Plume [4]. Millward has suggested that the increases in 'inorganic' arsenic content in the middle layers (2-6cm) of the sediment core are a consequence of Fe(III) reduction which permits sediment-bound arsenic to be released into the aqueous layers of the sediment. In our study, however, as the increases in arsenic concentrations in the middle layers from those on the sediment surface are smaller than these observed by Millward which indicates



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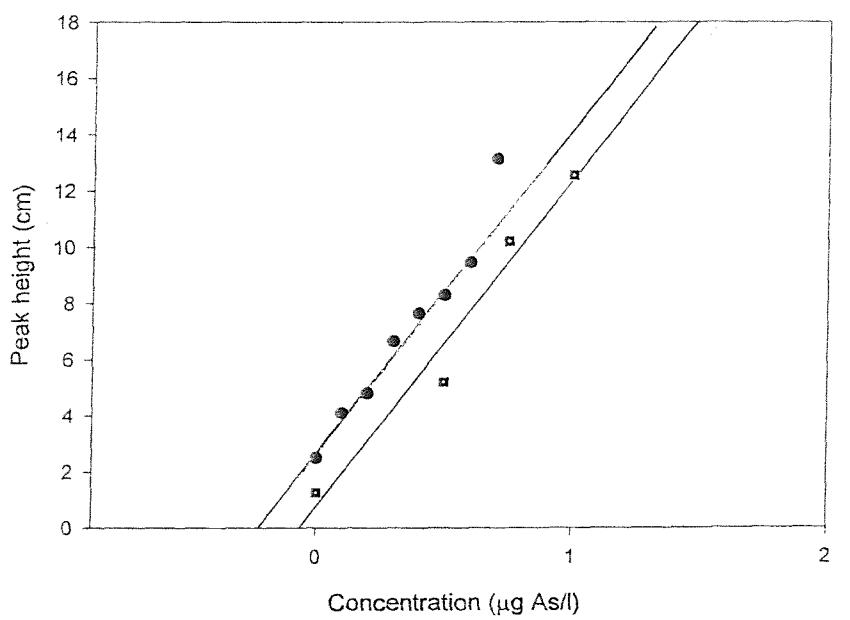

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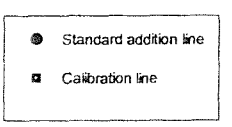


Figure 6.2: TE4 arsenite **standard addition with and without L-cysteine**

(a)- no L-cysteine added (b)- L-cysteine added

that any processes which release sediment-bound arsenic into the aqueous layers of the sediments are less significant or that the arsenic concentrations in the Test sediments themselves are low.

Dissolved methylated arsenic

There was very little detectable dissolved methylated arsenic in the water extracts extracted from the sediment cores collected from the Test indicating restricted breakdown and bacterial decomposition of complex organoarsenic compounds deposited by marine algae onto and into the sediment surface [5,6]. This behaviour also implied that microbial methylation of sediment 'inorganic' arsenic was low or not present. Millward, incubated Tamar Estuary sediments, at 15°C and found evidence of MMA and trace amounts of DMA in the aqueous layers of sediment after 5 days [7]. Other studies performed by Millward [8] on the temperate Humber Estuary revealed that there was evidence of a significant quantity of MMA in the sediment aqueous layers of an area (Silver Pit) where the average water temperature was 11°C and high nutrient concentrations were present. The River Test samples were collected at a time when microbial activity would be expected to be low due to winter conditions and the lack of any detectable dissolved methylated arsenic can probably therefore be related to a low level of microbial activity.

'Hidden' arsenic

'Hidden' arsenic, in the form of 'inorganic' arsenic, is present in the TE1 sediment aqueous layers with significant quantities in the lower layers of the sediment. One possible source of this additional 'inorganic' arsenic could originate from the dissolution of previously unidentified 'inorganic' arsenic compounds which were tightly bound to dissolved or colloidal organic debris that was present in the sediment aqueous extracts [9]. Alternatively, some non-hydride reducible non-methylated arsenic compounds, such as p-arsanilic acid and

arsenazo(I), bond break down directly to 'inorganic' arsenic with high recoveries on batch photolysis (no persulphate). The significant increase (average 98%) in the measured 'inorganic' arsenic after irradiation could have arisen from either of these routes.

The irradiation of iron(III) oxides can result in their photo-reduction releasing iron(II) into solution [10]. Any 'inorganic' arsenic attached to colloidal iron(III) oxide (which would pass through the filter after gravity filtration treatment) could be released by such photo-reduction.

The detection of 'hidden' arsenic principally as 'inorganic' arsenic on the sediment surface suggests that the decomposition of complex organoarsenic species such as algal arsenosugars and arsenobetaine is at an advanced stage or that there is very little decomposition activity [6]. It is possible that there are 'hidden' methylated arsenic forms present in the TE1 sediment aqueous extracts but their concentrations are too low to permit detection by cryogenic trap HG-AAS procedures.

Of the three procedures employed for the determination of 'hidden' arsenic in the TE1 sediment aqueous extracts sample, the S_2O_8 batch procedure did not perform well. It was initially thought that the S_2O_8 batch procedure gave erratic results due to the presence of an interferent but no evidence of interferands were revealed by the standard addition experiments in environmentally similar sediment aqueous extracts (sample TE3). As the S_2O_8 batch photolysis experiment was the last experiment to be carried out on the TE1 samples, it may just be possible that the samples had decomposed to an extent which affected the arsenic speciation and content of the sample.

6.3.5.2 Interferences study

It was observed that water extracts from the two cores, when assessed for interferands behaved differently. Whilst the TE3 sample was relatively free from interference effects, the TE4 arsenate signal was significantly affected. This could be due to the different environments of the areas where the sediments were collected. The area surrounding Eling Tide Mill does not contain any significant industrial activity and is mainly used for recreational purposes. Around the Southampton Containerbase terminal area however there is a significant disturbance of sediments by continual dredging activity. It is, therefore, important to carry out standard addition experiments on all sediment aqueous extracts to assess possible interference effects.

Studies by Howard and Salou [3] on interferences affecting the cryogenic-trap HG-AAS procedures have revealed that Fe(II) and Mn(II) can interfere with the signal output. In order to overcome these interference effects, the use of L-cysteine as a masking agent was recommended due to its ability to form metal complexes with many interfering metal ions that may be present in the sample matrix. Samples containing oxidants, that can also suppress the arsenic signal can also be pretreated with L-cysteine to remove the interference effect. In this study, the addition of L-cysteine overcame the interference effects observed in the measurement of arsenate in the TE2 sample.

6.3.6 Conclusion

Cryogenic trap HG-AAS procedures have proven to be useful in the determination of arsenic species in sediment aqueous extracts. Some evidence has been presented that there may be 'hidden' 'inorganic' arsenic present in the sediment aqueous extracts of the River Test sediments but further experiments will be necessary to quantify the 'hidden' arsenic that is present.

The analysis of sediment aqueous extracts taken from near Southampton Containerbase was influenced by the presence of interferands affecting arsenate measurements. The pretreatment of sediment aqueous extracts with L-cysteine reduced the interference effect significantly.

6.4 Arsenic speciation studies of the estuary of the River Tamar

6.4.1 Introduction

Located on the Devon/Cornwall border in South West England, the Tamar Estuary has been the focus of much scientific research in recent decades. For example, research has been carried out on the cycling and distribution of trace metals [11,12], nutrient fluxes [13], sediment dynamics [14], turbidity behaviour [15] and biological processes [16].

The Tamar Estuary has proven to be an ideal candidate for the investigation of the behaviour of arsenic in an anthropogenically perturbed environment. This is due to the drainage of arsenic from disused mines, which results in increased levels of dissolved arsenic entering the water column. Dissolved arsenic levels can be as high as 6µg As/l [17] in the upper estuary. Extensive studies have been made on the origin and biogeochemistry of arsenic in the water column [12], sediments [18] and vegetation of a number of areas within the estuary. A 1997 study of the Devon Great Consols Mine in the southern part of the Tamar valley area of the estuary revealed that the soils were highly contaminated, with arsenic concentrations in the range 120-52600µg/g As [19].

A review of the literature has revealed that no attempts have been made to ascertain whether 'hidden' arsenic is present in the Tamar Estuary surface waters and sediment aqueous extracts. Hence, preliminary experiments were devised to determine whether 'hidden' arsenic is present in the surface and sediment aqueous

extracts of the upper Tamar Estuary and to study whether arsenic transport to and from the sediment aqueous extracts to the water surface is significant.

6.4.2 Experimental

Surface water samples were collected from four locations along the upper region of the Tamar Estuary between the New Bridge at Gunnislake and Cargreen landing place (Figure 6.3) on 24th May 1998, using 125ml polyethylene bottles. Plastic bottles and caps were rinsed out at least three times with water from the sampling location prior to collection. Once filled, the bottles were transferred to a cool box where the temperature was kept constant at *ca.* 4°C. After arrival in the laboratory, the water samples were filtered using Whatman GF/C acid washed filter papers and analysed by cryogenic-trap HG-AAS procedures.

Sediment was collected from Halton Quay using the 45cm sediment corer. The procedure described in Section 6.2 was followed for the collection of sediment aqueous extracts. The volume of the aqueous extract was measured and diluted appropriately using deionised water. The resulting solution was then analysed using cryogenic trap HG-AAS methods.

Samples were analysed for 'hidden arsenic by the procedures described in Section 6.3.2.

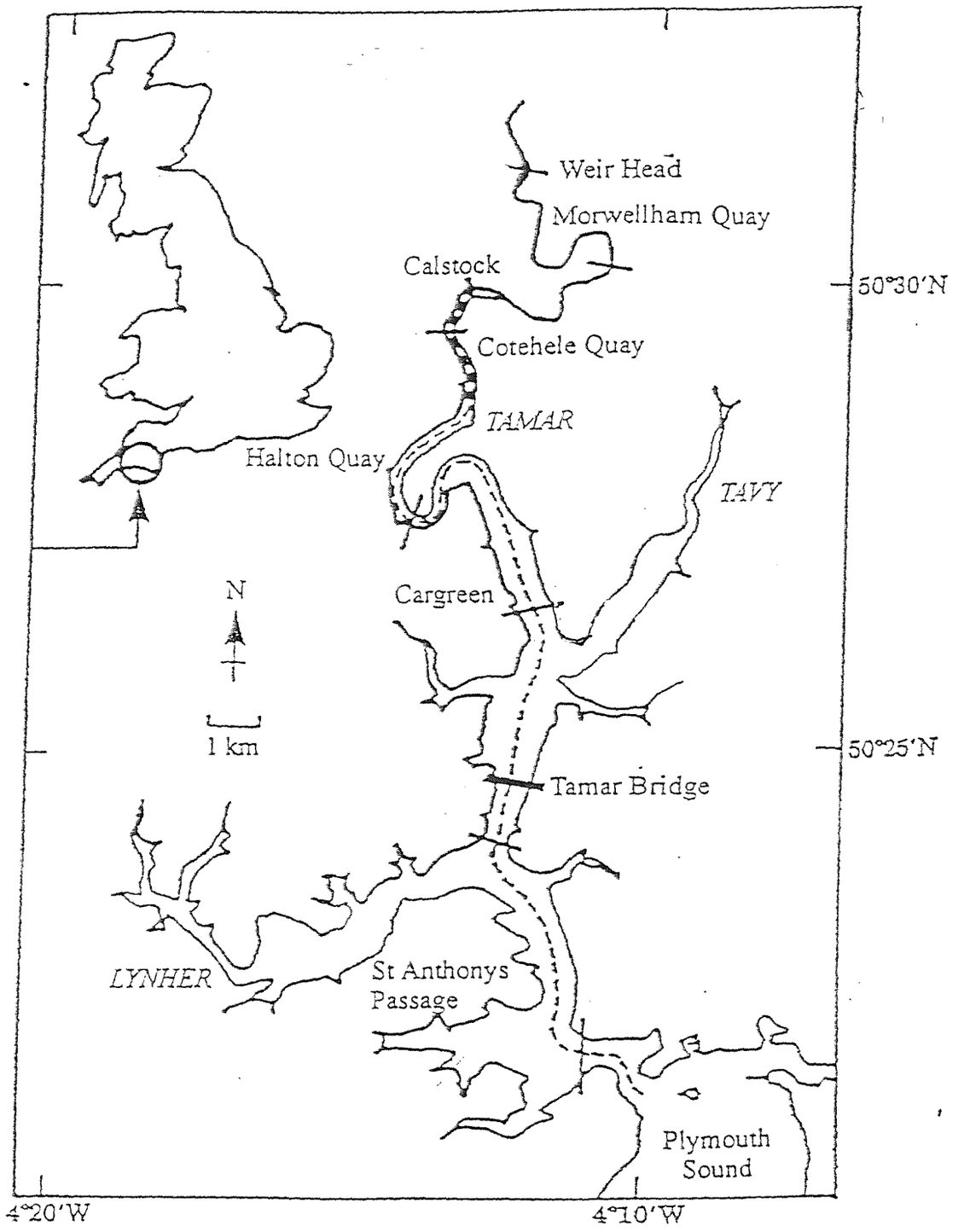


Figure 6.3: **Map of the Tamar Estuary. Adapted from Newton [M.M-Y. Newton, *Ph.D. thesis*, University of Southampton, 1998)**

6.4.3 Results

6.4.3.1 Analysis of surface water samples

Dissolved arsenic concentration and speciation

In addition to 'inorganic' arsenic, both dissolved MMA and DMA were present at all four locations (Table 6.4). Concentration levels for the methylated arsenic species were within a narrow band of between 0.06-0.12µg As/l.

After pretreatment using batch photolysis methods

An increase in the measured dissolved arsenic concentration was found after batch photolysis. On average, for all four sampling locations, the dissolved 'inorganic' arsenic, MMA and DMA concentrations increased by 29%, 61% and 41% (Table 6.4) respectively. These increases in dissolved arsenic concentration confirm the presence of dissolved 'hidden' arsenic.

6.4.3.2 Analysis of Halton Quay sediment aqueous extracts

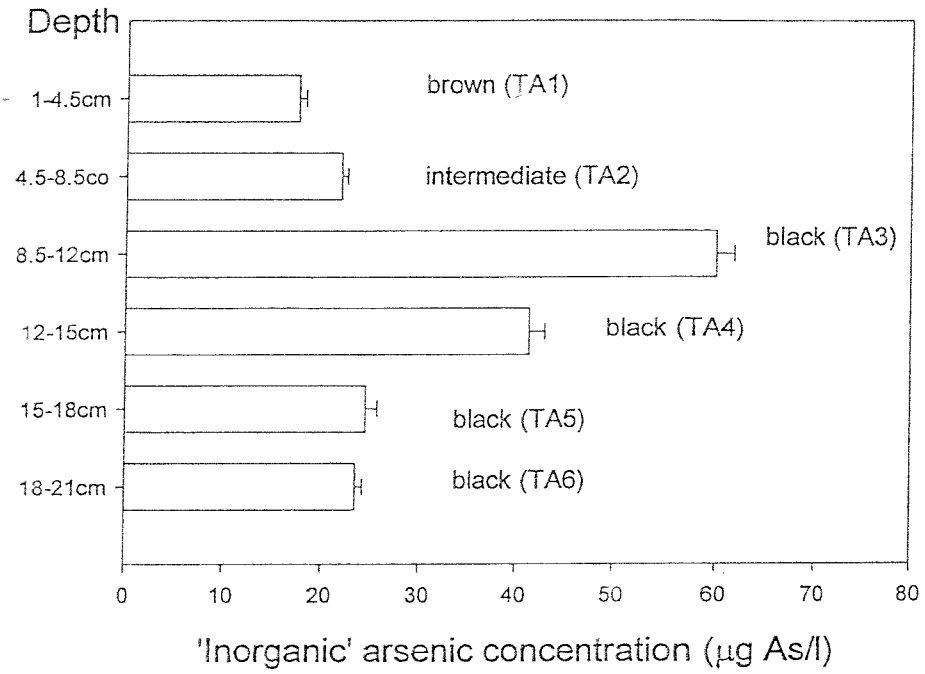
Dissolved arsenic speciation and concentration

There was no evidence of any detectable dissolved methylated arsenic (Figure 6.4) in any of the sediment aqueous extracts after analysis by cryogenic trap HG-AAS procedures. From the water surface layer downwards, the dissolved 'inorganic' arsenic concentration gradually increased (from 23.5-41.3µg As/l) to a maximum in the middle layer (56.0µg As/l) of the sediment before decreasing to 23.5µg As/l.

Location	Dissolved (untreated)			After batch photolysis			% increase after batch photolysis		
	As_{IN}	MMA	DMA	As_{IN}	MMA	DMA	As_{IN}	MMA	DMA
Gunnislake	2.62±0.10	0.08±0.02	0.06±0.01	3.47±0.14	0.09±0.02	0.09±0.02	32.4	12.5	50.0
Cotehele	3.43±0.14	0.08±0.01	0.11±0.02	3.88±0.08	0.14±0.02	0.13±0.01	13.1	75.0	36.3
Halton Quay	3.21±0.06	0.07±0.01	0.11±0.01	3.77±0.15	0.11±0.01	0.14±0.02	17.4	57.1	27.2
Cargreen	2.11±0.06	0.07±0.01	0.12±0.01	3.24±0.13	0.14±0.02	0.18±0.03	54.0	100.0	50.0

As_{IN} represent the 'inorganic' arsenic signal output
All units are in µg As/l

Table 6.4: Dissolved arsenic speciation in the surface waters of the Upper Tamar Estuary



Colours represent physical appearance of sediment extract

Figure 6.4: Arsenic speciation and content of Halton Quay interstitial waters

Interferences study

In order to ascertain whether any interferences affected the measured arsenic levels, standard addition measurements were employed for the Halton Quay sediment aqueous extracts TA1, TA4 and TA6.

For the 'spiked' sediment solutions that were not treated with L-cysteine, the standard addition lines and calibration lines were almost parallel to each other for MMA and DMA implying that interferands were not affecting MMA and DMA measurements. It was also noted that the concentrations obtained from both direct analysis and extrapolation of standard addition were almost identical (Table 6.5).

With arsenite, the standard addition and calibration lines were not parallel for the (presumed) anoxic samples TA3 and TA6, indicating the presence of an interferand. No evidence of an As(III) interference was found for the surface sediment extract TA1. As(V) showed interferent effects with TA4 and TA6.

Analysis of the sediment aqueous extracts containing L-cysteine revealed that the gradients of both the standard addition and calibration lines were unchanged for the TA1 As(III) sample and all of the MMA and DMA sediment aqueous extracts. For TA3 and TA6 samples 'spiked' with As(III) the interference effect decreased slightly with the addition of L-cysteine but not enough to remove effect totally. For As(V), the interference effect reduced slightly for TA1 and TA3. With the TA6 As(V) sample, however, the interfering species remained a problem as there was no notable change in the gradients of both standard addition and calibration lines treated for samples with and without added L-cysteine.

'Hidden' arsenic studies

Table 6.6 shows that widely differing data was obtained for each sediment aqueous extract by two established methods for the determination of 'hidden'

Sediment extract	Analysis method	Arsenic species			
		As(III)	As(V)	MMA	DMA
TA1	Direct analysis	13.6±1.8	9.3±0.5	<0.01	<0.01
	Extrapolation of standard addition	14.6±4.4	9.6±5.3	0.06±0.1	0.03±0.1
	Extrapolation of standard addition + L-cysteine	13.2±3.7	8.9±4.2	0.03±0.1	0.03±0.1
TA3	Direct analysis	31.8±0.6	18.2±0.9	<0.01	<0.01
	Extrapolation of standard addition	26.9±10.9	14.7±7.2	0.08±0.1	0.03±0.1
	Extrapolation of standard addition + L-cysteine	33.9±8.2	16.8±6.4	0.06±0.2	0.02±0.1
TA6	Direct analysis	13.4±2.4	12.1±0.7	<0.01	<0.01
	Extrapolation of standard addition	23.3±3.9	8.5±2.3	0.01±0.1	0.03±0.1
	Extrapolation of standard addition + L-cysteine	18.9±4.8	9.0±1.6	0.02±0.2	0.02±0.1

All units are in µg As/l
n.d. = not detected

Table 6.5: Arsenic speciation and content of sediment aqueous extracts collected from Halton Quay

Sediment extract	'Inorganic' arsenic concentration (μg As/l)		
	Untreated	Batch photolysis	Inline photooxidation
TA1	17.5±0.7	90.6±3.6	17.5±0.5
TA2	26.9±0.8	4.3±0.2	22.1±0.6
TA3	56.0±2.2	10.7±0.3	40.8±2.0
TA4	41.2±1.7	10.1±0.4	29.7±0.6
TA5	25.0±0.8	3.9±0.2	18.4±1.1
TA6	23.5±0.5	4.2±0.1	19.1±0.8

No evidence of methylated arsenic species was found in any of the samples analysed

Table 6.6: 'Hidden' arsenic speciation and concentrations in Halton Quay sediment aqueous extracts

arsenic in estuarine waters. As the sediment aqueous extracts are complex matrices containing elevated levels of trace elements (when compared to the concentration levels found in surface waters), it may be possible that the photolysis/photochemical treatment of the aqueous extracts resulted in the formation of interfering species which affected the ability of the photolysis/photochemical process to breakdown any 'hidden' arsenic species that may be present in the aqueous extract effectively.

6.4.4 Discussion

Dissolved arsenic speciation analysis of the Halton Quay surface waters revealed the presence of significantly more methylated arsenic than was found in the sediment aqueous extracts. This suggests that the sediments did not contribute significantly towards the presence of methylated arsenic in the surface waters. The majority of the methylated arsenic in the surface waters are therefore likely to have originated from other sources such as the release of methylated arsenic from dimethylarsenosugars present in marine microorganisms or the methylation of 'inorganic' arsenic by bacteria.

'Hidden' methylated arsenic has been found to be present in the Tamar surface waters and it is therefore likely that the dissolved methylated arsenic budget in the area has previously underestimated the role of methylated arsenic in the system. Studies by Comber [12] of the Tamar surface waters in June 1987, before the advent of 'hidden' arsenic techniques, revealed that the dissolved methylated arsenic concentrations were similar to those reported in this section. When the samples were analysed for 'hidden' arsenic content, however, there was an average 51% increase in dissolved methylated arsenic concentrations. As the batch irradiation procedure is not capable of breaking down MMAA and DMAA, the additional level of arsenic present in dissolved Tamar water must come from methylated arsenic species which do not form a volatile hydride upon HG-AAS treatment. As marine microorganisms are known to metabolise 'inorganic' arsenic

to complex methylated arsenic compounds, it is possible that some of the 'hidden' methylated arsenic could have originated from the release of these methylated arsenic forms into the surface waters.

Water column dissolved 'inorganic' arsenic concentrations are lower than the values supplied by Comber [12] by 1-2µg As/l but these values vary significantly with the tidal state and such differences are therefore not unexpected. After sample treatment to reveal 'hidden' arsenic, the dissolved 'inorganic' arsenic increased, on average, by 29%. This 'hidden' dissolved 'inorganic' arsenic is likely to have originated from the breakdown of dissolved complex organoarsenic species which do not have any methyl groups attached to the arsenic. Unfortunately, it was not possible to ascertain whether 'hidden' inorganic arsenic is present in the sediment aqueous extracts of Halton Quay due to the conflicting results obtained by the photolysis/photochemical methods employed.

Studies on the sediment aqueous extracts from Halton Quay revealed the presence of interfering species, which affected the As(III) and As(V) measurements. The addition of L-cysteine only managed to reduce slightly these interference effects. This may imply that insufficient L-cysteine had been employed to reduce the interferent effect or that L-cysteine was not a suitable masking agent.

6.4.5 Conclusion

Dissolved 'hidden' arsenic is present in the Tamar water column between the New Bridge at Gunnislake and Cargreen landing place. The majority of 'hidden' arsenic was in the form of MMA and DMA after sample pretreatment. The contribution of methylated arsenic species from the aqueous layers of the sediment to the water column appears to be low as no evidence of detectable methylated arsenic species could be found in the sediment aqueous extracts.

Studies of Halton Quay sediment aqueous extracts revealed that 'inorganic' arsenic is the dominant arsenic species present in the aqueous extracts. However due to the presence of severe interference effects on the As(III) and As(V) measurements it was not possible to gain a true picture of arsenic speciation and concentration even with the utilisation of L-cysteine as a masking agent. These difficulties meant that an accurate picture of arsenic speciation and concentrations in Halton Quay sediment aqueous extracts could not be made.

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Chapter 7

Conclusions and suggestions for future work

7.1 Introduction

Estuarine systems are complex water bodies where dissolved and particulate arsenic forms undergo frequent changes in physical and chemical behaviour. This behaviour results in a wide and diverse variety of arsenic species, most of which can be present at very low concentrations. For the detection of dissolved arsenic species, cryogenic-trap hydride-generation techniques were developed, the first of which was reported in 1973 by Braman and Foreback [1]. Such techniques operate on the principle that the arsenic species form a volatile arsine upon reaction with sodium borohydride, which can be condensed in an cryogenic trap prior to their release to an arsenic specific detector. These arsenic species are now referred to as the hydride-reducible forms. In the past decade however, Howard [2] and de Bettencourt [3] have revealed that there were arsenic species present in marine waters which were not capable of forming a volatile arsine upon reaction with sodium borohydride. These types of non-hydride reducible arsenic species are known as the 'hidden' or 'refractory' forms of arsenic respectively. These compounds are currently believed to include arsenosugars, arsenobetaine and to a lesser extent arsenocholine [4]. The discovery of these 'hidden' and 'refractory' arsenic species has necessitated a reappraisal of the analytical techniques used to determine arsenic speciation and to assess the role of these arsenic species in the estuarine biogeochemical cycle of arsenic. Whilst alternative analytical systems such as HPLC-ICP-MS [5] provide a powerful means of detecting arsenic species, cryogenic trap HG-AAS techniques remain an attractive option for the detection and speciation of arsenic in estuarine systems. These methods offer high levels of sensitivity, rapid sample throughput and relative freedom from interferences. The main objective of this study was therefore to develop analytical techniques,

utilising cryogenic trap HG-AAS methods, which would permit the detection and characterisation of 'hidden' arsenic in estuarine systems.

7.2 Development of analytical techniques for 'hidden' arsenic determination

As 'hidden' arsenic concentrations can be much lower than the concentrations of the hydride-reducible arsenic species, initial approaches to this work involved a study of agents for the pre-derivisation of arsenic species, to improve the peak sensitivity of the hydride generation technique. The addition of L-cysteine to a sample resulted in a notable improvement in peak sensitivity and reduced the acid levels required for each analysis. This behaviour was in accordance with the results obtained by Howard and Salou [6]. The slow rate of reaction between two of the arsenic species and L-cysteine precluded its incorporation into an inline cryogenic trap-HG-AAS system. Thioglycolic acid offered better reaction rates than L-cysteine, but it is unpleasant to use and toxic.

Howard [2] was able to convert 'hidden' arsenic in estuarine water samples to hydride-reducible forms by batch irradiation of samples utilising a 200W medium pressure mercury arc lamp. This mercury lamp delivers very little radiation at 254nm (the main line is at 365nm) and preserves the Me-As bond present in the hydride-reducible MMAA and DMAA species. Any additional levels of MMA and DMA present after pretreatment and analysis cannot, therefore be attributed to MMAA or DMAA but to 'hidden' arsenic forms. One disadvantage, however, of the batch irradiation process was the long times (18 hours) required for effective decomposition of the 'hidden' arsenic species. Addition of persulphate solution to the sample matrix prior to batch irradiation resulted in the near complete decomposition of MMAA, DMAA and selected non-hydride reducible arsenic species to 'inorganic' arsenic. The high efficiencies with which the species were converted is believed to result from the hydroxyl radicals generated by the persulphate solution. This had the benefit of reducing the times required for the effective decomposition of 'hidden' arsenic to hydride-reducible forms from

18 hours to between 4-8 hours. An unfortunate side-effect of the persulphate batch irradiation process was the loss of important speciation information as many organoarsenic species decomposed directly to 'inorganic' arsenic.

In order to reduce further the times required for the effective sample decomposition, u.v. irradiation of water samples was carried out in a microwave-driven lamp shaped as a beaker. This technique had been successfully employed by Labonne [7] for the conversion of organoselenium compounds to 'inorganic' selenium. As the beaker emitted principally at 254nm, important speciation information was lost due to the photolytic decomposition of the Me-As moieties. Lower than expected conversion efficiencies to 'inorganic' arsenic were achieved for both hydride-reducible and 'hidden' arsenic species. However, with the addition of persulphate solution to the sample prior to the irradiation treatment, high conversion efficiencies to 'inorganic' arsenic were obtained for both hydride reducible and 'hidden' arsenic species within a short timeframe (2.5 minutes).

An inline method to convert 'hidden' arsenic content to hydride-reducible forms for cryogenic trap HG-AAS is an attractive goal as it would reduce sample loss during sample pretreatment and would speed the analysis. An inline photo-oxidation system was therefore developed utilising a 4W low pressure mercury lamp in the cryogenic trap HG-AAS system. In the presence of persulphate a high conversion efficiency to 'inorganic' arsenic was obtained for both hydride reducible and 'hidden' arsenic species.

'Hidden' arsenic in estuarine waters was assessed using the analytical tools studied in this thesis. Both the batch photolysis (with and without persulphate) yielded similar results. The inline photooxidation method yielded results when compared that differed from these obtained by the batch irradiation process. This may be due to the higher levels of persulphate interfering with other species present within the complex estuarine water matrix. Microwave-assisted photolysis had the side-effect of losing substantial quantities of estuarine water, thus making

it unsuitable for the determination of 'hidden' arsenic content in estuarine systems.

In conclusion, a number of new approaches to the determination of 'hidden' arsenic have been developed utilising cryogenic trap HG-AAS methods. With the persulphate batch photolysis, microwave assisted photolysis and inline photo-oxidation methods, however, it is imperative that the hydride-reducible arsenic content is assessed prior to carrying out the pretreatment procedure to ensure that the hydride-reducible organoarsenic content is accounted for. These methods whilst revealing the presence of 'hidden' arsenic species do so by more extensive breakdown of the 'hidden' species, in some cases, resulting in complete conversion to 'inorganic' arsenic, thereby losing the important speciation obtainable by the use of the 200W medium pressure mercury lamp.

7.3 'Hidden' arsenic in the estuarine environment

In the Southampton Water/Itchen River temperate estuary system, part of the dissolved hydride-reducible 'inorganic' arsenic present during the winter months is taken up by algae when the water temperatures and light levels increase. Whilst the dissolved 'inorganic' arsenic concentrations decrease, the proportions of hydride reducible methylated (MMA and DMA) arsenic compounds increase during the spring/summer season. Similar behaviour has been observed in the Thames [8] and Humber [9] estuaries.

The reduction in the dissolved 'inorganic' arsenic levels in the water column can be attributed to the uptake of arsenate, a hydride reducible 'inorganic' arsenic form by algae. Due to the chemical similarities between arsenate and the phosphate nutrient, high rates of arsenate incorporation into the cellular structures of some algae can occur when phosphate concentrations are low [10] whereas in others discrimination between arsenate and phosphate only occurs when phosphate concentrations are in plentiful supply [11]. Once arsenate is

incorporated into the phosphate metabolic cycle of the alga, the arsenic species are thought to substitute for nitrogen in the pathways of phospholipid biosynthesis leading to the formation of 'hidden' arsenic species such as arsenosugars and arsenolipids [12].

The arsenolipids are poorly understood components of some marine macroalgae and animals. Some evidence has been presented to imply that there is a link between arsenolipids and their conversion to other arsenic species as the hydrolysis of arsenolipids present in a lobster gland (*Panulirus cygnus*) [13] resulted in the formation of arsenocholine. Arsenocholine, under simulated conditions has been shown to degrade to arsenobetaine in the presence of marine microorganisms but the transformations involved are as yet unclear [14].

Arsenosugars have only been isolated from a few macroalgae species [15,16] but have recently been identified based on HPLC retention times as being present in the diatom *Chaetoceros concavivornis* [5]. Edmonds and Francesconi have suggested that arsenosugar formation originates from the methylation of arsenate with the assistance of adenosylmethionine (Ado-Met) which acts both as a methyl and ribosyl species donator [4]. Ado-Met then transfers its adenosyl component to form an arsenic-containing ribosyl nucleoside intermediate. Glycosidation of this nucleoside intermediate results in the formation of the wide variety of arsenosugars found in the estuarine environment. It is possible that the dimethylarsenosugars are methylated further to form trimethylarsenosugar forms [4].

The major means of arsenosugar release into the dissolved phase of the estuarine environment is likely to occur by the grazing of phytoplankton by zooplankton marine microfauna or as a product of microalgal excretion or death [2]. As the majority of arsenosugars contain dimethylated arsenic components, it was not surprising that 'hidden' DMA was the major 'hidden' methylated arsenic species detected in our estuarine water studies. Upon entry into the water column, the

'hidden' dimethylarsenosugars undergo both chemical and/or microbial mediated decomposition reactions to form simpler 'hidden' arsenic forms such as dimethyloxarsylethanol (DMAOE) [17]. DMAOE eventually breaks down to the hydride-reducible dimethylarsinate (DMAA) form. As DMAA is relatively stable under the oxidising conditions commonly found in the estuarine water column it is difficult to demethylate unless specific microbial activity is present [18].

The lack of any detectable hydride-reducible or 'hidden' TMA in the surface waters of the Southampton Water/Itchen River estuarine system is consistent with the observation that trimethylated arsenosugars make a very small contribution towards the arsenic budget in the temperate estuarine environment [4]. This behaviour is consistent with low concentrations of trimethylarsenosugars in macroalgae but it is not yet known whether microalgae contain trimethylarsenosugars.

The uptake of arsenosugars by marine animals *via* the digestion of phytoplankton or the presence of symbiotic organisms is believed to result in the formation of arsenobetaine, but the mechanisms involved are not yet clear [19]. It has been postulated that the dimethylarsenosugars are transformed to dimethylarsenoylacetic acid. Subsequent reduction of dimethylarsenoylacetic acid results in the formation of dimethylarsenoylethanol (DMAE) which upon oxidation and methylation forms arsenobetaine [20]. Edmonds also suggested that bacterial conversion of trimethylarsenosugars to arsenobetaine could occur *via* their conversion to arsenocholine under anaerobic conditions [14]. No evidence has yet been presented to suggest the presence of arsenobetaine in the water column but it can account for up to 95% [21] of the arsenic present in marine animals.

The sediments are believed to play a crucial role in the cycling of arsenic in estuarine environments. When light levels are reduced, nutrient concentrations depleted and water temperatures decrease in temperate estuarine environments,

phytoplankton species die rapidly and are deposited onto the sediment surface where they decompose slowly to release hydride reducible methylated arsenic species into the sediment interstitial waters and the overlying water column [22]. Marine microfauna, and with a less generalised distribution marine animals, will undergo decomposition within the sediments releasing water soluble arsenic into the sediment interstitial waters. Assuming that a high proportion of the arsenic present in the marine animal is arsenobetaine and that the surface sediments are aerobic, arsenobetaine undergoes rapid decomposition to trimethylarsine oxide (TMAO). Hanaoka has reported that TMAO undergoes further decomposition to hydride-reducible arsenic forms under aerobic conditions [23]. In contrast, in the presence of anaerobic sedimentary bacteria, very little degradation of arsenobetaine is observed. This is thought to be due either to the activities of aerobic bacteria or that oxygen is required for the effective breakdown of 'hidden' arsenobetaine to its eventual hydride reducible products.

The discovery of hydride reducible MMA and DMA in the Dockhead surface waters during the December survey was unusual and may indicate that the sediment surface, which was in the process of degrading biological material containing arsenic, was disturbed and released MMAA and DMAA. Such activity could have occurred for a number of reasons including storms, dredging or, less probably at this time of year, bioturbation [24].

It is possible that the lack of hydride-reducible methylated arsenic in water extracts collected from the surface layers sediments towards the start of the spring season in the River Test is an indicator that the decomposition of 'hidden' arsenic has reached a stage where very little or no further decomposition occurs. However, the presence of a significant quantity of 'hidden' 'inorganic' arsenic in the water collected from the middle layers of the sediment extracted from the River Test indicates that this 'hidden' arsenic may originate from tightly bound colloidal material containing sediment bound 'inorganic' arsenic which was released following photochemical treatment [2].

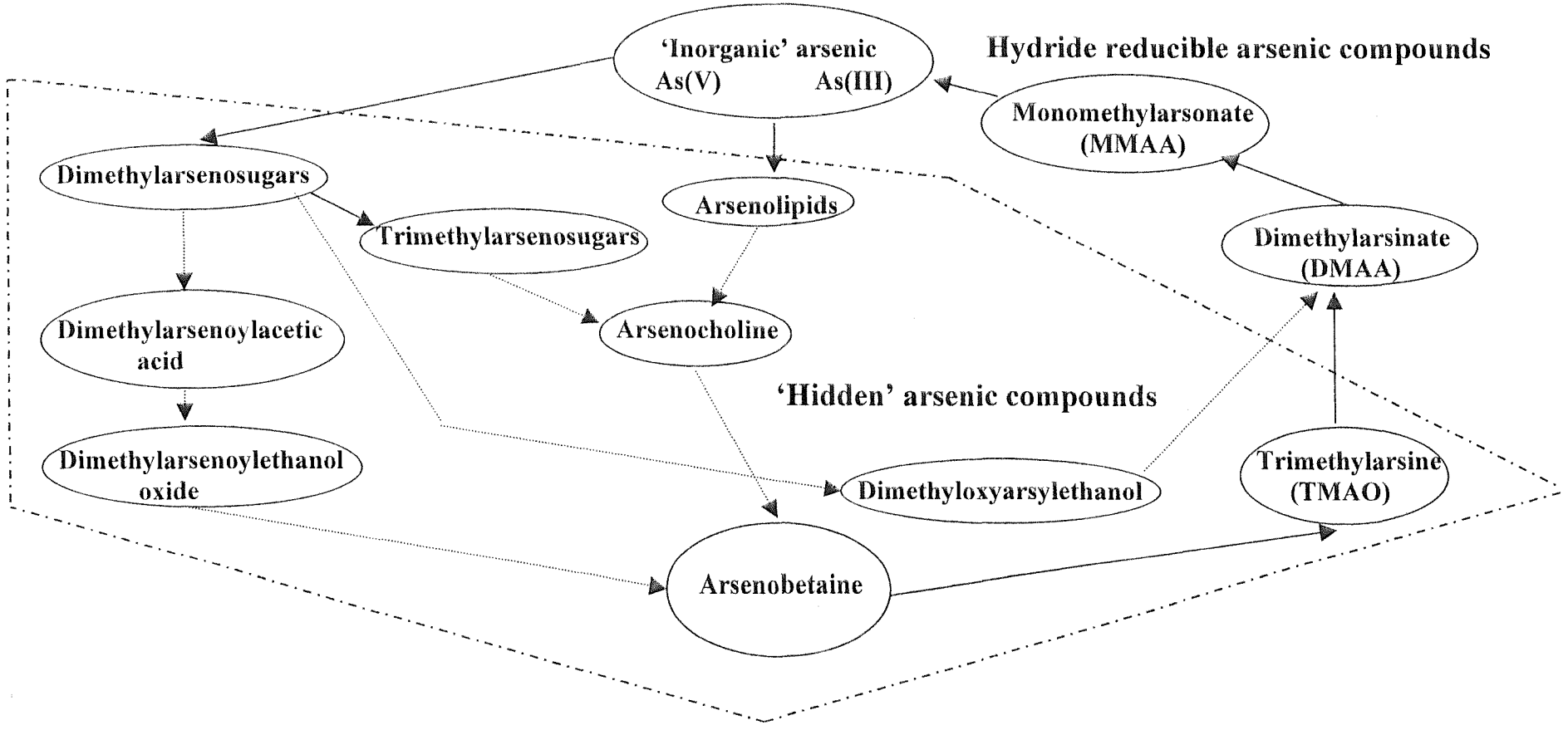
In conclusion, based upon the data given in this thesis and literature sources, a tentative diagram for the cycling of arsenic species in estuarine environments showing how arsenic species convert from one form to another is given in Figure 7.1.

7.4 Suggestions for future work

Our current understanding on the presence of 'hidden' arsenic in the water column is based upon a number of assumptions which require clarification and confirmation. The main established 'hidden' arsenic inputs are currently believed to be the arsenosugars, arsenobetaine and to a lesser extent arsenocholine. The relative abundances of these compounds must be firmly established and source organisms identified. The role of arsenolipids has received very little attention. Whilst algal arsenosugars are currently believed to be the major sources of 'hidden' arsenic, the only evidence of such compounds being present is based upon studies of macroalgae and a limited number of HPLC-based identification studies in microalgae. Further work must, therefore, be carried out to establish the presence, distribution and quantity of arsenosugars in phytoplankton.

Once the major sources of organoarsenicals have been better established, it will be necessary to study the abiotic and bacterial breakdown of the source organisms and compounds within model systems. Isolation and full characterization of each individual breakdown will, therefore, be necessary. Any new developments with this approach will probably result from improved sensitivity with MS identification. The radiolabelling of synthetic source compounds would be a potentially fruitful approach.

Having identified the structures of 'hidden' arsenic species, attention could then turn again to the use of chromatographic techniques such as HPLC-ICP-MS for the identification of the newly identified organoarsenicals in the water column.



Established pathways — Yet to be established pathways

Boundary between 'hidden' and hydride reducible arsenic species

Figure 7.1: Proposed cycling of arsenic in the estuarine environment

7.4 References

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Appendix
Southampton Water and estuary of the River Itchen sampling data

October 1998 (Part 1)

	Salinity	Water temp	Chlorophyll (ug/l)	Inorganic arsenic	MMA	DMA	Inorganic arsenic	MMA	DMA	Inorganic arsenic	MMA	DMA
Northam Bridge	24.6	13.5	0.74	0.45	<0.01	0.07	0.51	<0.01	0.13			
Oil Spill Centre	25.2	13.7	0.99	0.52	<0.01	0.04	0.5	<0.01	0.09	0.56	<0.01	0.09
Shamrock Quay	26.5	13.7	1.24	0.55	<0.01	0.04	0.56	<0.01	0.08			
Itchen Toll Bridge	31.7	13.9	0.49	0.6	<0.01	0.1	0.63	0.06	0.2	0.57	0.07	0.13
Dockhead	32.8	13.8	0.49	0.68	<0.01	0.03	0.7	<0.01	0.18	0.64	0.1	0.21
Weston Shelf	32.8	13.7	0.99	0.73	<0.01	0.04	0.71	<0.01	0.07			
NW Netley	33.1	13.7	1.3	0.57	<0.01	0.03	0.57	<0.01	0.12			
Hound	33.3	13.9	1.07	0.66	<0.01	0.04	0.64	<0.01	0.16	0.64	0.07	0.16
Greenland	33.6	13.8	1.04	0.64	<0.01	0.08	0.63	<0.01	0.12	0.65	0.05	0.07
BP Jetty	33.7	13.8	1.04	0.75	<0.01	0.08	0.73	<0.01	0.14			
Hamble Point	34.3	13.9	1.11	0.79	<0.01	0.06	0.76	<0.01	0.11			
Fawley PS	34.2	13.3	1.48	0.81	<0.01	0.05	0.81	<0.01	0.18	0.73	<0.01	0.07
Calshot Castle	34.3	13.9	0.99	0.76	<0.01	0.04	0.76	<0.01	0.08	0.72	<0.01	0.07
	Salinity	Water temp		Dissolved arsenic content			Photolysis samples			S2O8 photolysis		

October 1998 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.54	<0.01	0.05	0.6	<0.01	0.09	0.66	0.1	0.06
Oil Spill Centre									
Shamrock Quay	0.58	0.06	0.1	0.73	<0.01	0.09	0.65	0.08	0.17
Itchen Toll Bridge	0.7	0.06	0.09	0.75	<0.01	0.07	0.67	0.1	0.06
Dockhead									
Weston Shelf									
NW Netley	0.83	0.04	0.1	0.53	<0.01	0.05	0.53	0.1	0.1
Hound	0.7	0.06	0.09	0.69	<0.01	0.05	0.68	0.1	0.08
Greenland									
BP Jetty									
Hamble Point	0.82	0.05	0.04	0.83	<0.01	0.08	0.77	0.1	0.08
Fawley PS	0.83	0.02	0.03	0.88	<0.01	0.09	0.89	0.1	0.13
Calshot Castle									
	Online photooxidation			Sonication----------→			Sonication + photolysis ---------->		

December 1998 (Part 1)

	Salinity	Water temp	Chlorophyll (ug/l)	Inorganic arsenic	MMA	DMA	Inorganic As	MMA	DMA
				Conventional HG-AAS			Photolysis		
Northam Bridge	10.5	7.2	0.52	0.24	<0.01	<0.01	0.28	<0.01	0.06
Oil Spill Centre	24.5	7.9	0.7	0.7	<0.01	<0.01	0.68	<0.01	0.1
Shamrock Quay	25.6	7.7	0.48	0.65	<0.01	0.06	0.67	<0.01	0.13
Itchen Toll Bridge	31.3	8.3	0.76	0.89	<0.01	0.07	0.86	<0.01	0.1
Dockhead	31.9	8.2	0.76	0.96	0.04	0.18	0.95	0.06	0.14
Weston Shelf	32.3	8.1	0.52	0.88	<0.01	<0.01	0.87	<0.01	0.06
NW Netley	32	8	0.56	0.93	<0.01	<0.01	0.97	<0.01	0.09
Hound	32.8	8.2	0.63	0.98	<0.01	<0.01	0.95	<0.01	0.14
Greenland	33.1	8.2	0.54	0.96	<0.01	<0.01	0.84	<0.01	0.08
BP Jetty	33.4	8.1	0.78	0.93	<0.01	<0.01	0.99	<0.01	0.04
Hamble Point	33.5	8.1	0.65	0.95	<0.01	<0.01	0.92	<0.01	0.06
Fawley PS	33.3	8.1	0.65	1.02	<0.01	0.06	0.97	<0.01	0.1
Calshot Castle	33.6	8	0.59	1.05	<0.01	0.06	0.96	<0.01	0.13
Calshot	33.9	8.1	0.93	0.95	<0.01	0.07	1.01	<0.01	0.08

December 1998 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.26	<0.01	0.08	0.32	0.05	<0.01	0.35	0.07	0.08	0.32	0.04	0.05
Oil Spill Centre	0.61	0.09	0.1	0.75	0.07	<0.01	0.61	<0.01	<0.01	0.7	0.08	0.04
Shamrock Quay												
Itchen Toll Bridge	0.55	0.12	0.08	0.81	0.05	<0.01	0.81	0.04	0.05	0.78	0.15	0.11
Dockhead	0.97	0.09	0.1	0.93	0.05	0.04	1.04	0.08	0.17	0.97	0.1	0.21
Weston Shelf												
NW Netley												
Hound												
Greenland	0.82	0.09	0.08	0.85	0.09	0.09	0.92	0.06	0.04	0.87	0.11	0.11
BP Jetty												
Hamble Point												
Fawley PS	1.02	0.12	0.09	1.03	0.09	0.1	1.09	<0.01	0.05	1.02	0.07	0.017
Calshot Castle	0.87	0.1	0.11	0.88	0.08	0.09	1.02	<0.01	0.05	0.96	0.15	0.14
Calshot	0.83	0.07	0.09	0.87	0.1	0.07	1.02	<0.01	0.07	0.96	0.04	0.12
	S2O8 photolysis ---------->			Online photo-oxidation ---------->			Sonication---------->			Sonication + photolysis ---------->		

March 1999 (Part 1)

	Salinity	Water temp	Chlorophyll (ug/l)	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	20	9.2	1.29	0.56	<0.01	0.04	0.64	<0.01	0.07			
Oil Spill Centre	25.7	9	1.75	0.58	<0.01	0.05	0.6	<0.01	0.1	0.57	0.02	0.08
Shamrock Quay	30.3	8.7	1.56	0.58	0.02	0.02	0.62	0.07	0.15	0.57	0.08	0.1
Itchen Toll Bridge	30.3	8.7	1.5	0.6	0.02	0.04	0.64	0.03	0.1	0.62	0.05	0.07
Dockhead	31.5	8.5	1.41	0.55	0.02	0.04	0.56	0.05	0.13			
Weston Shelf	31.6	8.5	1.63	0.86	<0.01	<0.01	0.98	0.05	0.18	0.83	0.08	0.07
NW Netley	31.7	8.5	1.63	0.93	0.05	0.08	0.88	0.07	0.13	0.96	0.09	0.14
Hound	30.7	8.5	1.5	0.77	<0.01	0.04	0.73	0.06	0.1			
Greenland	32.3	8.5	1.32	0.83	<0.01	<0.01	0.88	0.07	0.12			
BP Jetty	32.5	8.5	1.59	0.84	0.02	0.03	0.87	0.09	0.13			
Hamble Point	33	8.4	1.66	0.86	0.02	0.04	0.82	0.04	0.16			
Fawley PS	33	8.4	1.41	0.83	<0.01	0.04	0.8	<0.01	0.09	0.76	0.03	0.1
Calshot Castle	33	8.5	1.47	0.8	0.02	0.06	0.76	0.05	0.11	0.82	0.05	0.07
Calshot	33.2	8.5	2.36	0.82	<0.01	0.05	0.87	<0.01	0.13	0.84	0.09	0.09
				dissolved arsenic			hidden arsenic			S2O8 photolysis ---------->		

March 1999 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge									
Oil Spill Centre	0.62	0.1	0.05	0.56	0.05	0.07	0.61	0.08	0.12
Shamrock Quay	0.74	0.08	0.05	0.65	0.03	0.05	0.67	0.07	0.16
Itchen Toll Bridge	0.75	0.03	<0.01	0.6	<0.01	0.07	0.63	0.03	0.07
Dockhead									
Weston Shelf	0.7	0.04	<0.01	0.8	<0.01	0.03	0.85	0.02	0.05
NW Netley	0.9	0.05	<0.01	0.76	0.03	0.07	0.83	0.07	0.1
Hound									
Greenland									
BP Jetty									
Hamble Point									
Fawley PS	0.94	0.04	0.09	0.84	<0.01	0.03	0.8	0.07	0.07
Calshot Castle	0.93	0.05	<0.01	0.87	<0.01	0.03	0.92	0.03	0.08
Calshot	0.9	0.06	<0.01	0.88	<0.01	0.05	0.92	0.02	0.08
	online photo			Sonication---------->			Sonication + photolysis ---------->		

April 1999 (Part 1)

	Salinity	Water temp	Chlorophyll (ug/L)	Asin	MMA	DMA	Asin	MMA	DMA	AsIn	MMA	DMA
Northam Bridge	14.5	12.3	1.51	0.48	<0.01	0.06	0.52	<0.01	0.13	0.44	<0.01	0.1
Oil Spill Centre	15.9	12.3	0.59	not determ	not determ	not determ	not determ	not determ	not determ	not determ	not determ	not determ
Shamrock Quay	27.5	11.5	0.9	0.54	<0.01	0.06	0.54	0.07	0.12	0.55	0.07	0.1
Itchen Toll Bridge	29.8	11.5	1.05	0.54	0.06	0.1	0.59	0.06	0.13	0.54	0.09	0.13
Dockhead	31.8	11.4	1.34	0.55	<0.01	0.06	0.57	0.1	0.17	0.57	0.12	0.13
Weston Shelf	30.9	11.6	0.95	0.56	<0.01	0.1	0.58	<0.01	0.16	0.57	<0.01	0.14
NW Netley	30.6	11.8	not determ	0.58	<0.01	0.1	0.55	0.1	0.12			
Hound	30	11.9	1.34	0.68	<0.01	0.12	0.73	0.03	0.16			
Greenland	30.1	12.2	1.34	0.8	0.08	0.1	0.76	0.08	0.13	0.75	0.12	0.15
BP Jetty	29.8	12.2	not determ	0.74	0.06	0.1	0.73	0.06	0.13			
Hamble Point	32	11.8	1.59	0.7	0.05	0.08	0.74	0.05	0.1			
Fawley PS	31.2	11.9	1.67	0.82	<0.01	0.04	0.86	<0.01	0.12			
Calshot Castle	29.1	12.5	not determ	0.78	0.05	0.1	0.73	0.08	0.16	0.76	0.12	0.08
Calshot	33.7	11.5	2.17	0.85	<0.01	0.08	0.87	0.07	0.1	0.77	0.09	0.08
				dissolved arsenic ---------->			photolysis ---------->			S2O8 photolysis ---------->		

April 1999 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.48	0.08	<0.01	0.47	<0.01	0.03	0.54	0.05	0.07
Oil Spill Centre	not determ	not determ	not determ	not determ	not determ	not determ	not determ	not determ	not determ
Shamrock Quay	0.52	0.09	0.05	0.58	<0.01	0.3	0.55	0.02	0.07
Itchen Toll Bridge	0.63	0.05	0.09	0.57	<0.01	0.05	0.6	0.2	0.09
Dockhead	0.67	0.05	0.07	0.59	0.05	0.03	0.56	0.09	0.1
Weston Shelf	0.64	0.03	0.07	0.53	0.02	0.03	0.57	0.05	0.12
NW Netley									
Hound									
Greenland	0.67	0.03	0.07	0.83	0.02	0.05	0.87	0.05	0.1
BP Jetty									
Hamble Point									
Fawley PS									
Calshot Castle	0.8	0.05	0.09	0.73	0.03	0.08	0.71	0.09	0.12
Calshot	0.87	0.05	0.09	0.81	<0.01	0.05	0.87	0.12	0.09
	Online photooxidation ---------->			Sonication---------->			Sonication + photolysis ---------->		

June 1999 (Part 1)

	Salinity	Water temp	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	15	15.7	0.55	0.02	0.08	0.56	0.09	0.09	0.6	0.08	0.17
Oil Spill Centre	16.6	15.6	0.62	0.02	0.03	0.68	0.07	0.08	0.63	0.08	0.19
Shamrock Quay	18.7	15.7	0.51	0.08	0.07	0.6	0.12	0.12	0.59	0.1	0.22
Itchen Toll Bridge	23.1	15.1	0.56	0.05	0.05	0.6	0.09	0.19	0.63	0.14	0.24
Dockhead	30.2	15.1	0.66	0.1	0.22	0.61	0.15	0.23	0.63	0.17	0.26
Weston Shelf	30	16.2	0.67	0.08	0.19	0.7	0.09	0.25	0.66	0.17	0.24
NW Netley	31.2	16.2	0.62	0.05	0.1	0.69	0.14	0.27	0.65	0.24	0.28
Hound	31.6	16.3	0.72	0.1	0.14	0.72	0.1	0.26	0.76	0.08	0.22
Greenland	30.2	16.5	0.57	0.05	0.07	0.63	0.09	0.27	0.66	0.12	0.19
BP Jetty	30.7	16.5	0.53	0.08	0.16	0.6	0.08	0.26	0.59	0.16	0.26
Hamble Point	31.3	16.5	0.65	0.08	0.1	0.6	0.09	0.23	0.63	0.09	0.26
Fawley PS	31.6	16.5	0.73	0.08	0.05	0.71	0.1	0.09	0.64	0.14	0.2
Calshot Castle	31.9	16.3	0.78	0.05	0.16	0.72	0.14	0.35	0.76	0.08	0.22
Calshot	33.6	16.1	0.83	0.05	0.14	0.78	0.07	0.23	0.81	0.12	0.28
			dissolved arsenic ---------->			Photolysis ----------			S2O8 photolysis ---------->		

June 1999 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.72	0.08	0.03	0.53	<0.01	0.05	0.54	0.08	0.12
Oil Spill Centre	0.83	<0.01	0.03	0.56	0.01	0.06	0.59	0.05	0.08
Shamrock Quay	0.81	<0.01	0.05	0.54	0.09	0.12	0.57	0.14	0.14
Itchen Toll Bridge	0.78	<0.01	0.03	0.58	0.09	0.12	0.62	0.12	0.28
Dockhead	0.76	0.05	0.07	0.63	0.03	0.07	0.67	0.12	0.09
Weston Shelf	0.79	0.05	0.03	0.68	0.09	0.14	0.73	0.14	0.28
NW Netley	0.78	0.05	0.07	0.66	0.1	0.22	0.63	0.1	0.23
Hound	0.91	<0.01	0.09	0.7	0.07	0.05	0.72	0.12	0.24
Greenland	0.81	<0.01	<0.01	0.62	0.05	0.17	0.65	0.08	0.23
BP Jetty	0.82	<0.01	0.09	0.57	0.07	0.05	0.59	0.09	0.09
Hamble Point	0.79	<0.01	0.09	0.69	0.09	0.12	0.73	0.05	0.12
Fawley PS	0.84	0.05	0.08	0.75	0.05	0.17	0.79	0.08	0.26
Calshot Castle	0.98	0.03	0.05	0.82	0.03	0.07	0.82	0.05	0.22
Calshot	0.93	0.02	0.04	0.89	0.07	0.14	0.92	0.17	0.24
	Online photooxidation ---------->			Sonication---------->			Sonication + photolysis ---------->		

August 1999 (Part 1)

	Salinity	Water temp	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	18.2	17.9	0.53	0.02	0.08	0.52	0.06	0.16	0.58	0.08	0.17
Oil Spill Centre	12.9	17.3	0.52	0.05	0.05	0.49	0.06	0.16	0.53	0.08	0.17
Shamrock Quay	17.3	17.4	0.46	0.05	0.1	0.51	0.08	0.13	0.55	0.12	0.19
Itchen Toll Bridge	21.9	18.2	0.46	0.13	0.13	0.54	0.14	0.19	0.51	0.16	0.17
Dockhead	30.5	19.2	0.63	0.17	0.27	0.67	0.19	0.4	0.64	0.17	0.35
Weston Shelf	31.5	19.5	0.57	0.07	0.1	0.61	0.1	0.27	0.6	0.12	0.26
NW Netley	31.1	19.3	0.6	0.1	0.12	0.64	0.13	0.18	0.61	0.08	0.17
Hound	31.9	19.5	0.61	0.1	0.13	0.68	0.1	0.22	0.6	0.08	0.24
Greenland	32	19.3	0.64	0.04	0.13	0.71	0.14	0.21	0.67	0.08	0.26
BP Jetty	32.3	19	0.65	0.04	0.15	0.68	0.1	0.21	0.71	0.1	0.21
Hamble Point	32.5	19	0.67	0.06	0.17	0.69	0.1	0.21	0.8	0.1	0.19
Fawley PS	33.6	19.2	0.73	0.08	0.12	0.8	0.1	0.27	0.76	0.07	0.22
Calshot Castle	33.5	19.3	0.75	0.06	0.15	0.73	0.06	0.18	0.82	0.1	0.25
Calshot	33.7	19.2	0.74	0.09	0.12	0.85	0.1	0.16	0.89	0.09	0.17
			dissolved arsenic ---------->			photolysis ---------->			S2O8 photolysis ---------->		

August 1999 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.56	<0.01	0.05	0.57	0.03	0.1	0.54	0.06	0.14
Oil Spill Centre	0.52	0.09	0.05	0.56	0.08	0.08	0.61	0.05	0.17
Shamrock Quay	0.56	0.03	0.17	0.53	0.08	0.07	0.56	0.08	0.17
Itchen Toll Bridge	0.65	0.05	0.09	0.61	0.05	0.1	0.64	0.08	0.12
Dockhead	0.77	0.05	0.09	0.66	0.07	0.12	0.63	0.09	0.14
Weston Shelf	0.54	0.09	0.12	0.6	0.03	0.08	0.62	0.12	0.22
NW Netley	0.66	0.03	0.07	0.64	0.07	0.12	0.68	0.09	0.16
Hound	0.7	0.17	0.12	0.63	0.02	0.12	0.65	0.07	0.17
Greenland	0.76	0.03	0.02	0.68	0.03	0.09	0.7	0.05	0.12
BP Jetty	0.68	0.05	0.12	0.63	0.02	0.05	0.67	0.09	0.09
Hamble Point	0.6	0.05	0.03	0.7	0.05	0.12	0.72	0.06	0.17
Fawley PS	0.81	0.03	0.07	0.72	0.05	0.19	0.79	0.09	0.17
Calshot Castle	0.94	0.03	0.05	0.84	0.05	0.09	0.87	0.05	0.12
Calshot	0.98	0.09	0.03	0.86	0.08	0.12	0.89	0.07	0.34
	Online photo-oxidation ---------->			Sonication---------->			Sonication + photolysis ---------->		

September 1999 (Part 1)

	Salinity	Water temp	Inorganic arsenic	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	28.3	20.6	0.5	0.07	0.15	0.52	0.07	0.25	0.54	0.12	0.21
Oil Spill Centre	25.1	20.2	0.51	0.07	0.09	0.51	0.08	0.18	0.53	0.09	0.21
Shamrock Quay	27.9	20.2	0.54	0.04	0.15	0.6	0.08	0.2	0.57	0.13	0.35
Itchen Toll Bridge	31.5	20.5	0.53	0.09	0.12	0.55	0.09	0.32	0.48	0.09	0.29
Dockhead	32.5	20.3	0.55	0.07	0.18	0.54	0.07	0.23	0.53	0.09	0.3
Weston Shelf	32.5	20.4	0.63	0.06	0.07	0.61	0.06	0.25	0.57	0.09	0.27
NW Netley	32.6	20.5	0.52	0.07	0.12	0.58	0.07	0.18	0.54	0.09	0.23
Hound	33.2	20.6	0.6	0.07	0.09	0.63	0.07	0.23	0.65	0.07	0.35
Greenland	33.7	20.3	0.56	0.04	0.1	0.54	0.04	0.18	0.6	0.02	0.23
BP Jetty	33.3	20.5	0.63	0.04	0.07	0.7	0.08	0.2	0.67	0.11	0.18
Hamble Point	33.7	20.3	0.64	0.07	0.09	0.68	0.07	0.22	0.71	0.12	0.35
Fawley PS	34.1	20.1	0.82	0.07	0.18	0.8	0.07	0.25	0.8	0.12	0.3
Calshot Castle	34.2	20.2	0.75	0.07	0.1	0.82	0.07	0.23	0.74	0.12	0.24
Calshot	34	20.2	0.89	0.07	0.13	0.83	0.07	0.38	0.92	0.11	0.35
			Dissolved arsenic content			photolysis ---------->			S2O8 photolysis ---------->		

September 1999 (Part 2)

	Asin	MMA	DMA	Asin	MMA	DMA	Asin	MMA	DMA
Northam Bridge	0.62	0.02	0.03	0.5	0.03	0.07	0.58	0.05	0.1
Oil Spill Centre	0.63	0.03	0.05	0.49	0.05	0.05	0.51	0.05	0.08
Shamrock Quay	0.86	0.04	0.09	0.53	0.02	0.07	0.54	0.07	0.1
Itchen Toll Bridge	0.66	0.03	0.04	0.61	0.03	0.05	0.65	0.05	0.1
Dockhead	0.76	0.03	0.03	0.67	0.05	0.14	0.73	0.09	0.18
Weston Shelf	0.7	0.05	0.07	0.57	0.03	0.1	0.62	0.07	0.19
NW Netley	0.82	0.05	0.05	0.63	0.07	0.09	0.67	0.09	0.18
Hound	0.84	0.03	0.09	0.59	0.1	0.1	0.66	0.17	0.19
Greenland	0.64	0.09	0.26	0.54	0.07	0.1	0.63	0.05	0.19
BP Jetty	0.76	0.03	0.05	0.61	0.07	0.07	0.63	0.05	0.16
Hamble Point	0.93	0.05	0.07	0.6	0.05	0.1	0.64	0.09	0.19
Fawley PS	0.58	0.09	0.33	0.69	0.05	0.09	0.7	0.05	0.16
Calshot Castle	0.9	0.03	0.09	0.77	0.05	0.09	0.8	0.09	0.12
Calshot	0.9	0.03	0.07	0.87	0.02	0.05	0.94	0.09	0.12
	Online photooxidation ---------->			Sonication---------->			Sonication + photolysis ---------->		