2003

IN SITU MEASUREMENT OF TRACE METALS IN COASTAL WATERS

Howell, Kate Ann

http://hdl.handle.net/10026.1/1120

http://dx.doi.org/10.24382/4343
University of Plymouth

IN SITU MEASUREMENT OF TRACE METALS IN COASTAL WATERS

KATE A. HOWELL

Ph.D. 2003

7.

Copyright Statement

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that it's copyright rests with the author and that no quotation from the thesis and no information derived from it may be published without the author's prior consent.

Signed......KAHowell..........................

Date.......29/07/03...........................

IN SITU MEASUREMENT OF TRACE METALS IN COASTAL WATERS

by

Kate Ann Howell

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Environmental Sciences
Faculty of Science

June 2003

ABSTRACT

In situ Measurement of Trace Metals in Coastal waters

Kate Ann Howell

This thesis describes the use of *in situ* and laboratory techniques for monitoring of trace metals and master variables in the environment. Chapter 1 presents a general overview of trace metals, their sources, sinks and roles in estuaries and near coastal zones, their general characteristics and the importance of their behaviour in estuaries. Filtration and commonly used analytical methods for trace metals determinations in natural waters are also discussed.

Chapter 2 highlights the importance of colloids (in the size range 0.2 to 0.4 μm) to membrane clogging and concludes that colloids account for a significant part of clogging of membrane filters. The aim of this study was to extend earlier studies by examining the trends of filtrate concentrations as a function of filtration volume in riverine and estuarine waters with contrasting matrix composition, over two different seasons. Based on the findings presented in this chapter some metal specific filtration protocols are suggested.

Chapter 3 provides an overview of developments in instrument automation that have led to miniaturisation and resulting in manufacture of portable electrochemical instrumentation. Such instrumentation has been used for continuous trace metal measurements from the banks of estuaries and on-board ships. The most recent developments in flow-through cells with a gel-coated Iridium (Ir) micro-electrode array have resulted in submersible *in situ* voltammetric probes which allow long-term trace metal measurements at sub-nanomolar concentrations in coastal waters. Such *in situ* probes hold great promise for water quality monitoring.

Chapter 4 presents the optimisation and validation of a Voltammetric *In situ* Profiling (VIP) system for the simultaneous determinations of Cd(II), Cu(II) and Pb(II) in estuarine and coastal waters. The trace metal faction measured by the VIP system is termed 'dynamic' and includes and includes these free ions and small complexes with size smaller than a few nm. Systematic studies in $NaNO_3$ and seawater were conducted and these showed that variations in ionic strength, pH and dissolved oxygen did not affect the response of the instrument, whereas an Arrhenius type temperature response was observed. During the work, physical and voltammetric settings of the VIP system were considered and tested in detail in order to achieve the analytical requirements to perform measurements in the laboratory and during in *situ* deployments. The VIP instrument has a detection limit (defined as 3σ) in seawater for Cd(II) of 23 pM, Cu(II) 1.13 nM, and Pb(II) 23 pM. The accuracy of the VIP system was determined with certified reference material (CRM), showing good agreement.

Chapter 5 discusses the results of an international laboratory inter-comparison exercise, in order to verify precision and accuracy of measurements made by 4 laboratories that operate VIP systems located in Plymouth, U.K; Geneva, Switzerland and Ancona, Italy, using laboratory reference methods as a comparison. Inter-comparison included analysis of Tamar Estuary samples and *in situ* VIP system measurements in the coastal waters of the Gullmar Fjord, Sweden.

Chapter 6 presents the results of field deployment of the automated Voltammetric *In situ* Profiling (VIP) system for the simultaneous determinations of dynamic Cd(II), Cu(II) and Pb(II) in three estuaries located in the Southwest of England, UK. The VIP instrument allowed the *in situ* determination of 2-4 samples h^{-1}. In addition, the VIP system measured total filterable (0.4 μm pore size filter) concentrations of Cd(II) and Pb(II) in discrete estuarine water samples. Through a series of tidal surveys lasting 7-12 h each and totalling 7 surveys a high resolution data set including speciation information was built up that enabled an interpretation of geochemical processes in the studied estuaries.

Chapter 7 shows the results of *in situ* determination of VIP dynamic metal concentrations in the Po plume and Adriatic Sea. Concentrations were similar to those previously reported for this coastal sea system. The correlation of the metals with salinity was presented and highlighted the importance of local effluent inputs close to the river mouth for Cd(II). The data demonstrated that the Adriatic was not heavily contaminated with trace metals.

Chapter 8 presents the overall conclusions from the research and makes suggestions for future work.

ACKNOWLEDGEMENTS

I would like send my sincere gratitude to my immensely patient and understanding supervisors, Dr Eric Achterberg, Dr Alan Tappin, and Prof. Paul Worsfold whose advice and suggestions have proved both helpful and constructive throughout my Ph.D. I apologise for the occasional tears.

I am especially grateful to Prof David R. Turner, principal scientist of the KEYCOP and IMTEC (1) Cruises not only for the opportunity to take part in the work but also for the lengthy 'loan' of essential instrumentation and support in other matters.

I would like to acknowledge the co-operation Dr Charlie Braungardt not only for her scientific collaboration but also for her practical, sailor's knowledge during surveys of the Fal ...ey ey capt'n.

A huge 'Thank You' to all the technical support of past and present who include Ian, Andrew (next year, you'll be first mate!), Sally, Andy (who's gonna chat to me 'bout music now??), Jeremy, Adrian, Derek and Dr Andy Fisher (the most patient man......ever), all of whom have made my work easier and my laughs louder. Thanks to ginger Chris for fixing all computer stuff. Thanks also to the School of Environmental Science office based staff, Tamsin, Donella, Lee, Debbie, Gemma and Elaine, -maybe this year I'll get a card with lipstick???

Thanks a lot to all those who made our extended research group so enjoyable, best wishes to Veronique (for her fantastic support in all matters), members of the Complex (T, Vinnie, Phil, Cyril, Paulo and Grady)...you are brilliant people. All the past ones-thanks for everything: Andy 'Perfect' Bowie (I promise I'll wear my Hawaiian shirt), Sophie soph, Cathy R., Matt, James, Toby, Ana, Raquel, Manolo, Rosa, Antonio, Rian, Montse, Denise-Denise, Agathe, Gregor, Cristina, Luis (Dr No), Paula (so cool) and Stephan (and his system)

To all those who remain here, good luck with everything to Jose (so generous), Turki, Sayed, Ange (keep at it), Jason, Paul Mc, Sylvia, Laura, Si (random man), Rich (thanks for listening mate!), 'karma' Utra, Orif, Yaqoob, Omaka, Nui, Emma, Paul Sutton, Ben, Andy B., Andy C., Rob, Pippa, Sarah, Elena (s), Tony (cool housemate), and my one of my bessie mates, Rebe (I promise to stop whingeing to you one day).

Special thanks to the Brazilian community (esp. Claudia) - Vôces tem corações muito grande, muito obrigada e lembrem-se, da proximo copa no Natal!!

Thanks to all my Cardiff, Warwick and Brum buddies for their tireless efforts to keep in touch with me over the years.

All my love to Paulinho, who has made me realise my dreams. We are so sorted babe.

...and finally, a huge debt of gratitude to my Mum and Dad and the rest of my family, who's unconditional love, support and generosity have helped me to achieve everything I dreamed of. THANK YOU ALL.

...Tested by time and fate, but strong in will
To strive, to seek, to find, and not to yield.

- 'Ulysses', Alfred Tennyson, 1842

AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

The work was financed with the aid of a studentship from the Natural Environmental Research Council and carried out in collaboration (in part) with Plymouth Marine Laboratory Ltd.

A programme of advanced study was undertaken, which included participation in three scientific cruises [KEYCOP Cruise; IMTEC cruise (1) and IMTEC cruise (2)]; research skills and methods course; atomic spectroscopy course and a laboratory demonstrating course.

Relevant scientific seminars and conferences were regularly attended, at which work was presented, and two papers and one book chapter prepared for publication.

The work presented in Chapter 5 was prepared with the collaboration of Dr M.-L. Tercier-Waeber, (University of Geneva); Dr C. Braungardt, (University of Plymouth); and Mr L. Lambertutcci, (University of Ancona), and the work presented in Chapter 6 was performed in collaboration with Dr C. Braungardt. All other data presented in this thesis were prepared by the author, whom the ownership rests with. Before using this data in any presentation or printed publication, please contact the author and include full acknowledgements.

Signed..........

Date.....29/07/03.....

PUBLICATIONS (in Appendix B)

Achterberg, E. P., Braungardt, C. B., and **Howell, K. A.** (2002) *The Application of High-Resolution In-Situ Monitoring Using Voltammetric Techniques to Study Trace Metal Distributions in Dynamic Estuarine and Coastal Waters.* In: Electrochemical Methods for the Environmental Analysis of Trace Element Biogeochemistry, T. F. Rozan and M. Taillefert, Eds., American Chemical Society Symposium Series # 811, pp 73-101.

Howell, K. A., Achterberg, E. P., Tappin, A. D., Braungardt, C. B., Worsfold, P. J. and Turner, D. R. (2003) *The Determination of Trace Metals in Estuarine and Coastal Waters Using a Voltammetric In Situ Profiling System.,* Analyst, **128,** 734-741.

Howell, K. A., Achterberg, E. P., Tappin, A. D., Braungardt, C. B., Worsfold, P. J. and Turner, D. R. (2003) *Voltammetric In situ Measurements of Trace Metals in Coastal Waters,* Tren. Anal. Chem., *in press.*

PRESENTATIONS AND CONFERENCES ATTENDED

PICO III - Progress in Chemical Oceanography, University of Plymouth, UK. September 1999.

Royal Society of Chemistry Analytical Research Forum - Incorporating R&D Topics, University of Manchester Institute of Science and Technology, Manchester, UK. Poster presentation, '*Membrane Filter Clogging - Variation in the Trace Metal Concentrations of Sequential Filtrate Aliquots of Estuarine Waters*', July 2000.

6 th International Symposium on Applied Bioinorganic Chemistry (ISABC-6), University of Wales College Cardiff, Cardiff, UK, Young Researchers Day. Poster Presentation, '*The Application of a VIP (Voltammetric In-situ Profiling) System in an Estuary for the Quantification and Speciation of Cu, Pb, and Cd Trace Metals*', June 2001, *Awarded prize for best communication*.

Royal Society of Chemistry Analytical Research Forum - Incorporating R&D Topics, University of East Anglia, Norwich, UK. Poster presentation: '*The Application of a VIP (Voltammetric In-situ Profiling) System in an Estuary for the Quantification and Speciation of Cu, Pb, and Cd Trace Metals*. June 2001.

PICO IV - Progress in Chemical Oceanography, Bangor - Wales. University of Wales. Oral presentation. '*The Use of a VIP (Voltammetric In-situ Profiling) System In An Estuary For The Measurement Of Dissolved Cu, Pb, and Cd Species.*' September 2001, *Awarded prize for best oral presentation*.

Royal Society of Chemistry Analytical Research Forum - Incorporating R&D Topics, Kingston University, Kingston-upon-Thames, UK. Oral presentation, '*The Application of a VIP (Voltammetric In-situ Profiling) System in Estuaries for the Quantification and Speciation of Cu, Pb,and Cd Trace Metals*', July 2002.

SeaSense 'Marine Measurement Forum Meeting', University of Plymouth, UK. Poster presentation, '*The Application of a VIP (Voltammetric In-situ Profiling) System in an Estuary for the Quantification and Speciation of Cu, Pb, and Cd Trace Metals*', July 2002.

Challenger Centenary Conference: Marine Science 2002, University of Plymouth, Plymouth, UK. Poster presentation, '*The Application of a VIP (Voltammetric In-situ Profiling) System in Estuaries for the Quantification and Speciation of Cu, Pb,and Cd Trace Metals*', September 2002.

TABLE OF CONTENTS

Chapter 1 - Introduction

Chapter 2 - The Influence of Membrane Filtration on the Determination of Dissolved Trace Metal Concentrations in Turbid Estuarine Waters

Chapter 3 - Voltammetric In *Situ* Measurements of Trace Metals in Coastal Waters

Chapter 4 - Optimisation of the Voltammetric *In situ* Profiling system for Use in Estuarine and Coastal Systems.

Chapter 5 - Inter-comparison of VIP Systems and Reference Methods for the Determination of Trace Metals in Estuarine and Fjord Waters

Chapter 6 - Application of the Voltammetric Profiling System to Estuaries of the South West of England, U.K

Chapter 7 – Determination of VIP Dynamic Concentrations of Cd (II), Pb(II) and Cu(II) in the Po Estuary and Adriatic Sea

Chapter 8 - Conclusions and Future work

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

LIST OF ABBREVIATIONS

AdCSV	Adsorptive cathodic stripping voltammetry
AMD	Acid mine drainage
APDC	Ammonium pyrrolidine dithiocarbamate
ASV	Anodic stripping voltammetry
CL	Chemiluminescence
CRM	Certified reference material
CTD	Conductivity-Temperature-Depth
DGT	Diffusive gradient thin films
DMG	Dimethyl glyoxime
DO	Dissolved oxygen
ETAAS	Electrothermal atomic absorption spectrometry
FFF	Field flow fractionation
FSI	Freshwater/seawater interface
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
GC	Glassy carbon
GFAAS	Graphite furnace-atomic absorption spectrometry
HDPE	High density polyethylene
HEPES	n-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HMDE	Hanging mercury drop electrode
IMTEC	*In Situ* automated monitoring of trace metal speciation in estuaries and coastal zones in relation with biogeochemical processes.
MES	2-(N-morpholino)-ethanesulfonic acid
MFE	Mercury film electrodes
MPCP	Multi physical-chemical profiler
OSPAR	Oslo-Paris
RDE	Rotating disc electrode
SPM	Suspended particulate matter
SW	Square wave
$TF_{0.4}$	Total filterable (0.4 μm pore size filter)
TMZ	Turbidity maximum zone
UV	Ultra violet
VIP	Voltammetric *In situ* profiling system
WFD	Water Framework Directive

CHAPTER 1

Introduction

Abstract

This chapter gives an overview of the speciation of trace metals in estuaries and the techniques used to separate trace metals into *operationally* defined fractions. The sources, sinks and roles of trace metals in estuaries and near coastal waters are discussed together with the estuarine processes affecting the distribution of these metals in estuaries. General characteristics of the physical classification of estuaries are highlighted.

Processes affecting distributions of trace metals in the oceans are described and analytical methods for determination of trace metals in estuaries and coastal waters are discussed.

1.1 Trace Elements in Natural Waters

Over the past century, elements required in very small amounts in the diet of a living organism have been described as 'trace' elements. In natural waters, Drever (1988) defined a trace element as 'an element present in plants and animals in low concentrations (< 1 mg L^{-1}, which for most elements is equal to molar concentrations of 10^{-4} M or less)'. Elements typically found in natural waters at this trace concentration include non-metals (*e.g.* Se, I), metals (*e.g.* Fe, Mn, Cu).

1.2 Role of Trace Metals in Estuaries and Coastal Waters

Most trace metals are micronutrients that play an important role in biological processes, with a range of metals (including Co, Cu, Fe, Zn) being essential for efficient growth and functioning of aquatic organisms (Butler, 1998). In molluscs and crustaceans for example, the blood pigment, heamocyanin requires Cu (Taylor *et al.*, 1999) and Brand *et al.*, (1983) found that depletion in 'bioactive' metals such as Mn, Fe, Co, Ni, Cu and Zn limited primary production in oceanic waters. At concentrations higher than background, however, trace metals inhibit the growth of organisms such as phytoplankton (Sunda, 1989; Morel and Price, 2003). The availability of a trace metal for biological uptake depends on its speciation and on various environmental parameters that are described in section 1.3

1.3 Speciation of Trace Metals

The speciation of an element describes the individual physico-chemical forms of an element, which together make up the total concentration in a sample. Trace metals occur in rivers, estuaries, seawater and oceans as aqueous metal ions, preferentially complexed with inorganic anions and organic ligands (van den Berg *et al.*, 1987), and associated with colloids and particulate matter (Panko, 1991; Figure 1.1). In natural

waters, chemical speciation plays a significant role in the interaction and reactivity of trace metals with biotic material (bioavailability) and toxicity (Sunda and Guillard, 1976), rate and extent of transport to the sediment and overall mobility within the environment (Millward, 1995).

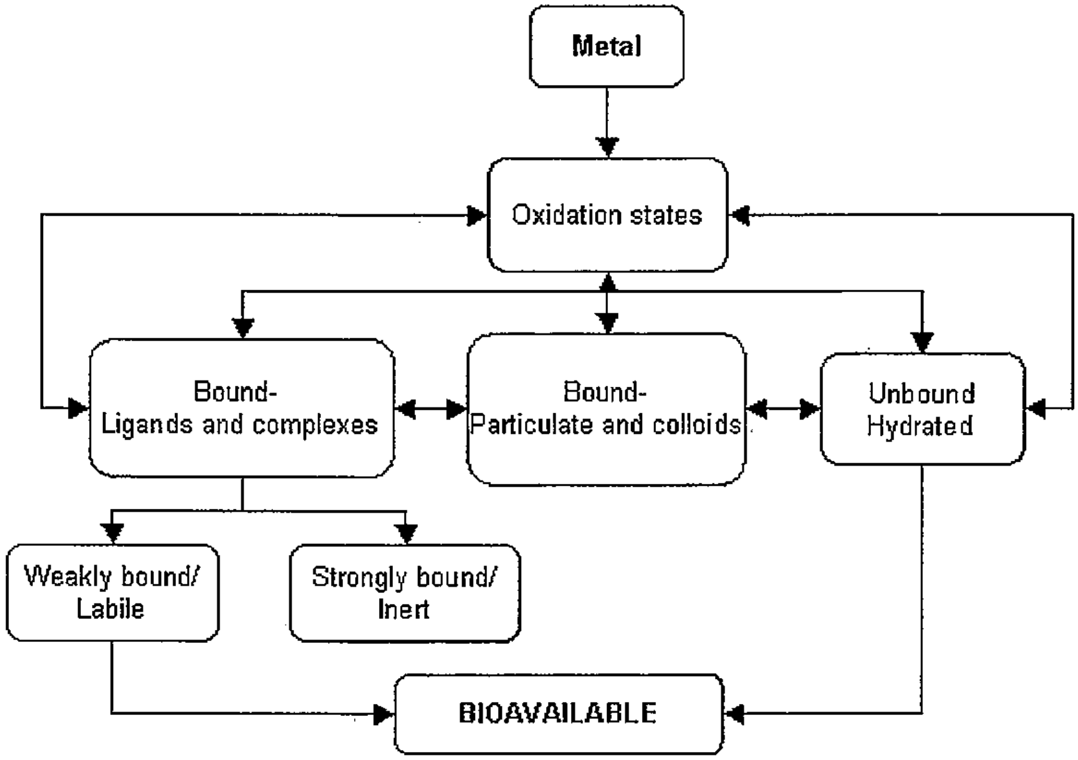


Figure 1. 1 The speciation of trace metals in natural waters.

For most metals it is usually the aqueous metal ion that is available for assimilation by biota such as phytoplankton and macroalgae, due to its capability of passing through cell membranes (Luoma, 1983) [complexed forms of metal are generally prevented from passage (Gledhill *et al.,* 1999)]. It is essential, therefore, to characterise total trace metal concentrations together with the proportions of the specific forms (*e.g.* dissolved and particulate phases) in order to gain greater comprehension of the biogeochemical processes that take place within natural waters including estuaries.

1.4 Separation Techniques

Various techniques exist for the direct physical separation of species of trace metals including sieving; filtration; ultrafiltration; sedimentation and centrifugation and field flow fractionation (FFF; Buffle and Leeuwen, 1992). Filtration however, remains the most widely used technique for separating dissolved and particulate phases of trace metals in natural waters. The first commonly accepted definition of 'dissolved' species, introduced by Goldberg *et al.*, (1952) was based upon filtration through a 0.5 μm pore filter. This pore size prevented passage of particulate matter (of density >1 to 3 g cm^{-3}) and most biological particles – with the exception of bacteria and viruses – into the filtrate (Lerman, 1979). Nowadays (Horowitz *et al.*, 1996), the term dissolved refers to a trace metal species able to pass through a filter of 0.40 μm or 0.2 μm pore size and particulate trace metals are defined as those retained on the filter. There is no *actual* nominal cut-off point separating species into dissolved and particulate phases, so the definition of a dissolved species is purely *operational.* The size ranges of constituents found in natural waters are shown in Figure 1.2.

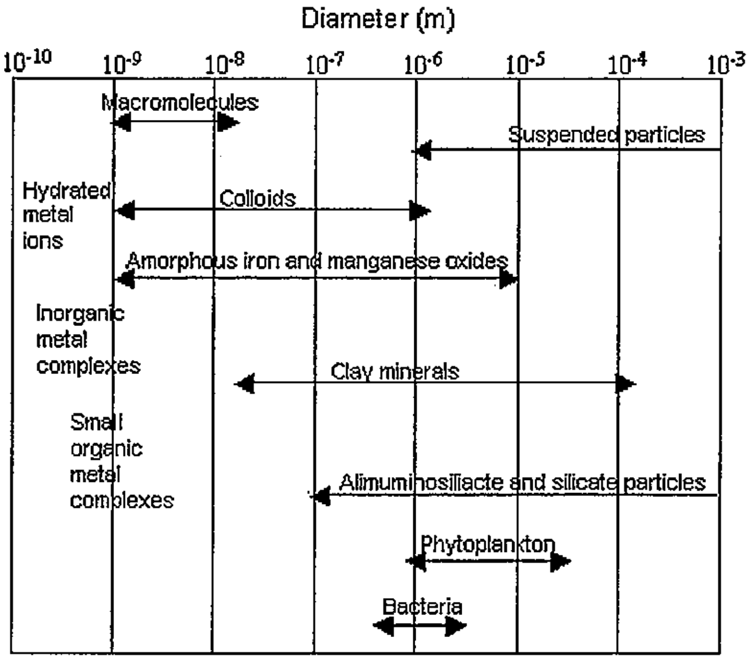


Figure 1. 2 The size ranges of particles found in natural waters. (Adapted from Hassellöv, 1999)

It is noted from Figure 1.2 that colloidal material, Fe and Mn oxides and macromolecules can pass through a 0.40 μm filter even though they are not truly dissolved and not biologically available. The passage of colloidal material, less than 0.40 μm in size, may result in the overestimation of dissolved concentrations (Danielsson *et al.*,1982). Filter loading and clogging of pores with fine particles may also occur, reducing the nominal size of the filter (Danielsson, 1982; Morrison and Benoit 2001). For these reasons, filtration should be viewed as only one approach for determining the 'dissolved' phase concentrations and others such as diffusive gradient thin films (DGT, Zhang and Davison, 1995; Zhang and Davison, 2001) and centrifugation (Ledin *et al.*, 1995)] should be explored.

1.5 Sources of Trace Metals

The world's oceans cover about 70 % of the surface of the Earth and rivers and estuaries provide the main pathway of water between land and ocean. The major source of trace metals to estuaries and coastal waters is through rivers (via surface or subsurface flow), which contain enhanced concentrations of metals, mainly adsorbed to particles, derived as a result of physical, chemical and biological weathering in the catchment area. The other major source of trace metals in estuaries and coastal waters is from sedimentary regeneration (Millward *et al.*, 1996) and pore water infusions (Chiffoleau *et al.,* 1994).

Anthropogenic inputs (including road run off, industrial and municipal effluents, some agricultural inputs, burning of fossil fuels, ore smelting and mining activities) into rivers, estuaries and coastal waters can enhance trace metal concentrations to exceed natural, baseline concentrations albeit to differing degrees, depending upon location. In open oceanic waters, other factors play a part in sources of trace metals into the hydrosphere. These include the airborne transport and surface wet (*e.g.* rain) and dry (*e.g.* volcanic dust, sand storms) deposition of continental material derived from weathering in arid regions of the world (Spokes *et al.,* 2001); vertical mixing during upwelling of deep ocean waters (Loscher, 1999), and the addition of large amounts trace metals, including Fe, Zn, Cu and Pb, into the base of the water column during hydrothermal venting (Dando *et al.*, 2000).

1.6 Estuarine Processes

Estuaries form the important interface between rivers and oceans. They are highly dynamic regions where chemical, biological and physical processes modify the composition of river waters. A great deal of the particulate discharge (and associated trace metals) from rivers that is received by estuaries will become trapped via chemical, biological and physical processes and settle in the estuarine or coastal zone, rather than reaching the open sea. As such, estuaries can be thought of as filters that modify the chemical signal received from the rivers, so that the form of the exiting signal is considerably different from that which entered the estuary (Chester, 2000). For some trace metals, the simple dilution by mixing of river and seawater leads to a uniform (conservative) behaviour along the salinity gradient, *e.g.* Cd and Ni in the Rhone (Elbaz-Poulichet *et al.,*1996). Deviation from conservative behaviour occurs when trace metals undergo dissolved-particulate reactions, leading to addition to [*e.g.* Mn in the Tay estuary (Owens and Balls, 1997)] or removal from [*e.g.* Zn in English coastal waters (Achterberg *et al.*, 1999)] the dissolved phase (non-conservative behaviour). The processes by which the estuarine filter act upon dissolved trace metals are exclusive for the metal and particular estuarine conditions. Estuarine processes are discussed in sections 1.6.1 to 1.6.4.

1.6.1 *Particle Water Interactions*

Within estuaries, over the length of the salinity gradient there is a development of a freshwater/seawater interface (FSI; Millward, 1995). The FSI typically contains elevated concentrations of suspended particulate matter (SPM), caused and maintained by tidal energies (Bale *et al.*, 1985). The turbidity maximum zone (TMZ) is a zone of high concentration of SPM (compared to that of landward and seaward sources; Festa and Handson, 1978), located at or near to the head of the salt intrusion and is a result of the seaward transport of river particles and the net movement of sediment up estuary due to tidal currents.

There can be uptake of dissolved metals onto particles, leading to removal from the water column if the particle settles, and likewise there can be desorption from particulate matter into the dissolved phase. It is in the FSI and TMZ regions that particle-water interactions are most prominent, thereby significantly contributing to the internal cycling and retention of trace metals within the estuary (Morris *et al.*, 1986).

1.6.2 *Flocculation*

Flocculation is a process that causes smaller particles (colloids or semi-colloids) to increase in size to form larger units (Chester, 2000A). The aggregation of these small particles is mediated by the changes in ionic strength encountered in the estuary during mixing, when electrical and physical forces that keep the colloids in solution are exceeded by other electronic and physical forces. Flocculation can affect trace metals (*e.g.* Fe, Cu and Cd) that are associated with colloidal species, clay mineral suspensions and humic acids (Sholkovitz and Copland, 1981), and can limit the extent to which some river-derived solutes enter the sea.

1.6.3 Redox Cycling

Redox processes typically occur at land-water interfaces and can play a part in the internal processes of estuaries. In low salinity, anoxic conditions, dissolved trace metals may be precipitated as sulphides, which upon entering higher salinity regions become re-oxidised and free the metal back into the dissolved phase *e.g.* Mn (Ackroyd *et al.*, 1986)

Some elements such as Cr can exist in estuaries in more than one oxidation state *e.g.* Cr (IV), Cr (VI). The thermodynamically unstable species of these elements are most likely formed through biological processes, for example bacteria can oxidise Mn(II) on the surface of particles to Mn(IV) (Vojak *et al.*, 1985), and can lead to mobilisation.

1.6.4 Biologically Mediated Processes

The majority of estuarine biomass is generated from primary production within the estuary. Particles produced as a result of this can actively uptake trace metals by adsorption onto surfaces. In addition, phytoplankton and marine organisms can alter the concentrations and speciation of many trace metals, through the release of high affinity specific complexing ligands, in order to assist uptake, *e.g.* Fe-binding ligands in the form of siderophores (for bacteria; Macrellis *et al.*, 2001) or plasma membrane reduction of Fe(III) followed by Fe(II) uptake by phytoplankton (Morel and Price, 2003). These are extracellular biomolecules that sequester iron, making it more bioavailable to the bacterium producer.

The physical and chemical mixing of the water masses within an estuary will lead to changes in speciation, and each species may vary as a function of salinity and pH, and to a lesser extent, light; mixing conditions; redox chemistry; suspended particulate matter concentrations; temperature; concentration of competing ligands and inorganic complexation processes.

1.7 Sinks of Trace Metals

The major sink for trace metals is by removal into the underlying sediment through trapping or by direct deposition of particulate matter. Processes such as flocculation of colloidal material, adsorption and precipitation result in the removal of dissolved metals into the particulate phase (Chester, 2000). This will often lead to sinking and removal out of the upper mixed layer into deeper waters and sediments. Additionally, metal can be removed through uptake by biological organisms such as phytoplankton, followed by breakdown of the phytoplankton and settling (Millero, 1996). Furthermore, surface-active trace metals such as lead are often scavenged and removed from the water column by sinking particulate material (Carvalho, 1997). However the ultimate removal mechanism in estuaries is transport out into surrounding coastal waters. Figure 1.3 summarises the sources, sinks and processes governing the cycling and speciation of trace metals in the hydrosphere.

1.8 The Physical Classification of Estuaries

From a physical and chemical perspective, Cameron and Pritchard (1963) defined an estuary as 'a semi-enclosed coastal body of water having a free connection with the open sea and, within which, seawater is measurably diluted with fresh water associated with land drainage'. This dilution of seawater with river water, leads to a longitudinal salinity gradient and the estuary can be segmented into three distinct regions that include an upper part, characterised by freshwater, but subject to daily

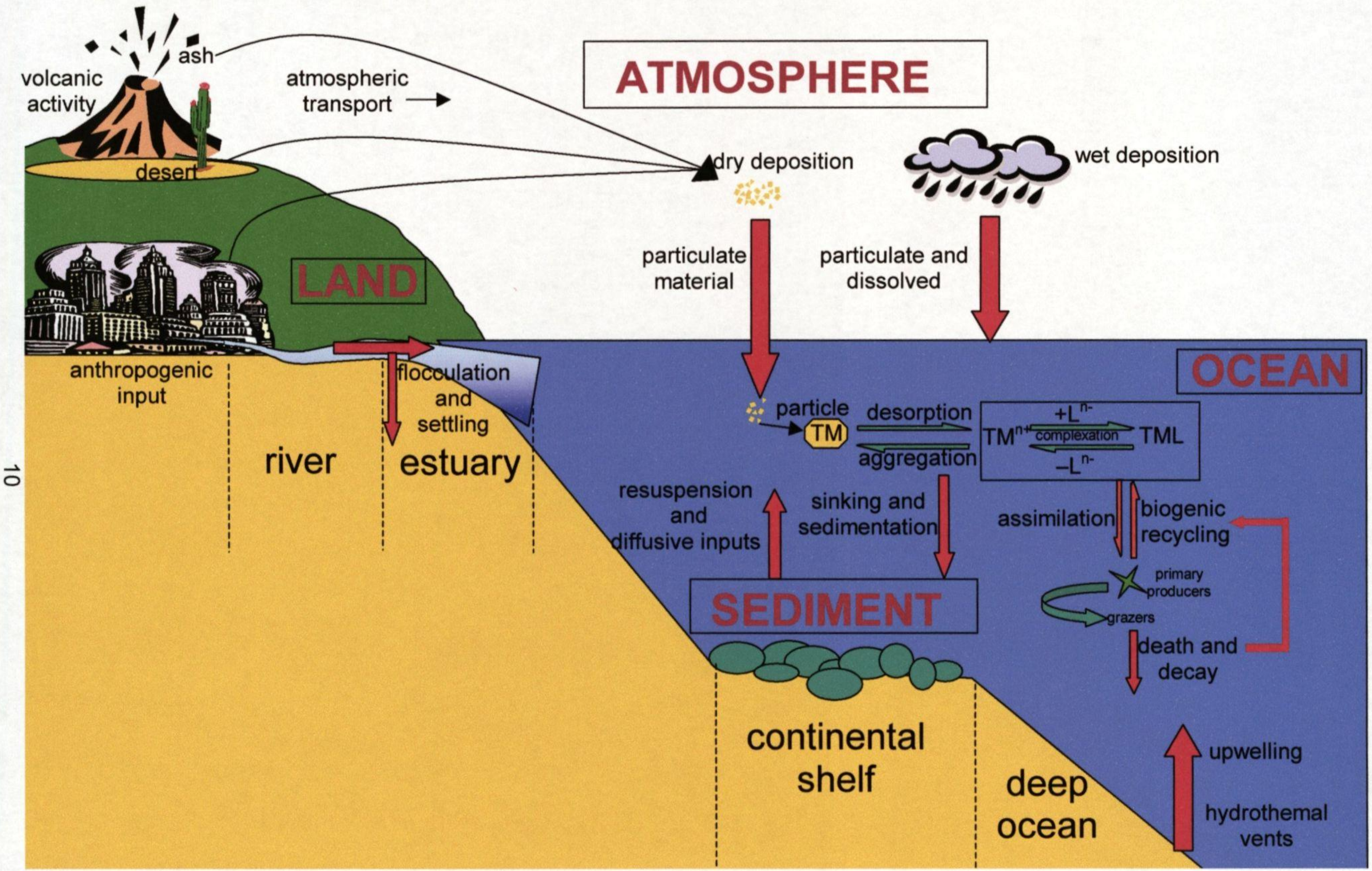


Figure 1.3 Schematic diagram of trace metal biogeochemistry. TM= trace metal; L= ligand.

tidal actions; a middle part, subject to strong fresh and saltwater mixing and a lower part that is in connection with the open sea (Fairbridge, 1980). Each estuary has its own distinct characteristics according to which it can be classified. Various schemes have been developed to aid the recognition of different estuary types, simplify their description and group estuaries of similar characteristics together.

Estuaries can be classified in several ways: by their tidal action; morphology; topography and salinity structure (Millero, 1996). These characteristics directly influence estuarine suspended particle cycling, ecology, seasonal and inter-annual variations, tidal limits and residence time. Table 1.1 briefly presents the main characteristics of each type of estuary in each classification. Further details of estuary classification can be found in the work of Dyer (2000) and Dyer *et al.* (2000).

1.9 Distributions of Trace Metals in Oceans

In oceans, the vertical distributions and removal of dissolved trace metals may be controlled by so called ‘conservative type’, ‘scavenged-type’ or ‘nutrient-type’ mechanisms, or by a hybrid of these mechanisms, for example, nutrient and scavenged type (*e.g.* Cu, Ga; Li, 1991). Conservative trace metals (*e.g.* Ba) have a more or less constant vertical distribution with concentrations determined by salinity distributions. Trace metals that exhibit scavenged-type vertical distributions include Pb and Al (Bruland *et al.*, 1994) and profiles indicate enrichment of the trace metals at the surface of the ocean (up to 5 m depth), probably from atmospheric inputs such as the deposition of aeolian dust (Spokes *et al.*, 2001). The profiles mostly show depletion in down column concentrations due to removal by particles that continue to scavenge the dissolved trace metals in intermediate and deep waters (Chester, 2000A). These particles sink and hence ultimately remove the trace metals.

Table 1. 1 Classification of estuaries according to their main characteristics and with examples of each type

Class base	*Type*	*Main characteristics*	*Example*
Tidal (Davis, 1964)	Microtidal	Tidal range < 2 m	York, U.S.A.
	Mesotidal	Tidal range < 4 m, > 2 m	Hamble, U.K
	Macrotidal	Tidal range < 6 m, > 4 m	Mersey, U.K.
	Hypertidal	Tidal range > 6 m	Severn, U.K.
Morphological (Dalrymple *et al.* 1992)	Wave dominated	Waves at mouth are significant and sediment eroded from coastline forms a spit. Tidal currents decrease, and fluvial processes increase. Marshes and mudflats in mid-estuary. Likely to occur in micro- or meso-tidal esuaries.	Exe, U.K.
	Tide dominated	Result of the large tidal currents relative to the wave effects, mouth contains sandbanks aligned to the current flow, salt marshes occur in macro-tidal areas.	Severn, U.K.
Topography (Pritchard, 1952)	Coastal plain	Topography similar to river valley with shallow depths (<30 m) and large width-depth ratio. Extensive mud flats. River flow is normally small and central channel is often sinuous. Usually found in temperate latitudes.	Chesapeake bay, U.S.A.
	Fjords	Bars or sills in place at mouth of fjord with sill depths, ranging from 4 m (in Norway) to 150 m (British Columbia) Can reach 100 km length. Due to over-deepening, small width-depth ratio. Generally found in high latitudes and mountainous areas	Sogne Fjord, Norway
	Bar-built	Characteristic bar across mouths. High SPM conc. Generally only a few meters deep and often have extensive lagoons and shallow waterways just inside the mouth, Large, seasonal river flow and bar can reposition and hence mouth position can vary over the years. Generally found in tropical areas.	Vellar Estuary, India
	Others	Estuaries that do not conform to other types, encompassing those formed by tectonic movements that include faulting, landslides and volcanic eruptions.	San Francisco Bay, U.S.A.
Salinity Structure (Pritchard, 1952; Cameron and Pritchard, 1963; Millero, 1996)	Highly stratified: salt wedge	Interface between layers of different salinity (halocline) is thin. Strong horizontal salinity gradient at the bottom and strong vertical gradient. Large ratio of river to tidal flow. Small width to depth ratio and generally micro-tidal.	Mississippi, U.S.A.
	Highly stratified: fjord	Deep water has almost uniform salinity. Net inflow in deeper water and outflow in surface layer. Strong halocline. Circulation dependant on sill depth and river runoff and sills can promote mixing, reducing stratification. River flow usually dominant over tidal flow. If sill depth is shallow, bottom waters often become stagnant and anoxic.	Alberni Inlet, British Columbia
	Partially mixed	Shallow, salinity increasing head to mouth. Water in two distinct layers- upper layer less saline, with net seaward flow and deep layer, more saline with net inward flow. Turbulent eddies mix salt water up and fresh water down give overall vertical mixing.	Tamar, U.K.
	Vertically homogeneous	Shallow basin with well-mixed water. Similar salinity from deep to surface waters. Large tidal range relative to the depth producing turbulence. Tidal flow dominates river flow. Usually found in macrotidal conditions	Delaware, U.S.A.

Nutrient-type vertical distributions show surface water depletion and sub-surface enrichment, and are generally encountered by those trace metals involved in internal biogeochemical cycles and biological cycles. The involvement results in removal of trace metals at the surface and transportation to depths, by biogenic carriers (Chester, 2000A), where they are remineralised. This, together with physical mixing and circulation patterns, controls the distributions of nutrient-type metals in oceans, and examples include Cd and Zn (Bruland *et al.*, 1994). These metals often show depleted concentrations in surface waters, where productivity is high (*e.g.* phytoplankton blooms; Bruland *et al.*, 1991; Butler, 1998).

1.10 Analytical Methods for the Determination of Trace Metals in Natural Waters

Detailed systematic geochemical studies have made data available on the concentrations, speciation and distribution of trace metals in natural water systems, thereby providing valuable information for interpretation of aquatic processes and enhancing our understanding of biogeochemical cycling in a variety of natural water matrices, such as rivers, *e.g.* Cauvery River (Vaithiyanathan *et al.*, 1993), estuaries, *e.g.* Tamar estuary (van den Berg *et al.*,1991), coastal seas, *e.g.* North Sea (Tappin *et al.*, 1995) and oceans, *e.g.* Atlantic Ocean (Saager *et al.*,1997). However, in contrast to oceans, the processes controlling the concentration and distribution of trace metals in estuaries and coastal waters are more complex and still poorly understood (Kremling, 1999). Consequently, there is a need to develop analytical instrumentation for the determination of trace metal concentrations in order to understand the cycling and function in these aquatic systems. Such instrumentation is required to be both sensitive and selective, whilst providing the necessary limit of detection and wide linear ranges for its utilisation in most environmental locations.

Also, the instrument required should be preferably inexpensive and easy to use so that several monitors can be employed and can be operated by unskilled personnel. In addition estuarine and coastal waters are dynamic in nature and consequently, high frequency monitoring is required to detect marked spatial and temporal changes. This is best achieved with the use of shipboard instrumentation. It allows minimal sample handling and facilitates high spatial and temporal resolution that enables more thorough interpretation of biogeochemical cycles.

The main challenge to most analytical methods, applied to estuarine and coastal waters, is the typically high concentration of dissolved salts (up to 3.5 % by weight). However, over the past two decades, modern developments in laboratory techniques and sensitive instrumentation have made reliable measurements possible. Determinations of trace metals in estuarine and coastal waters have been carried out by a variety of methods, with the main ones summarised in Table 1.2 and described in depth in section 1.10.1 to 1.10.4.

Table 1. 2 Methods used the determination of trace metals in estuaries and coastal waters.

Technique	Metal	LOD (nM/ pM)	Precision (at stated conc [nM/pM])	Linear range	Reference
ICP-MS and AES	Cu	0.03 nM	3.1 % (at 1.6 nM)	0.03 nM to 3nM	Wu and Boyle,1997
	Cd	5.0 pM	1.1 % (at 0.35 nM)	5.0 pM to ≥ 1800 pM	Wu and Boyle,1997
	Pb	1.3 pM	2.0 % (at 50 pM)	1.3 pM to ≥ 140 pM	Wu and Boyle,1997
ETAAS	Cu	0.90 nM	< 7.0 % (not stated)	Not stated	Chan and Huang, 2000
	Cd	0.42 nM	3.2 % (at 0.2 nM)	Not stated	Colbert *et al.*, 1998
	Pb	0.02 nM	Not stated	Not stated	Liu and Huang, 1995
CL	Cu	0.40 nM	7.3 % (at 1.6 nM)	0.40 nM to 700 nM	Coale *et al.*, 1992
	Fe	0.04 nM	3.2 % (at 1.0 nM)	0.04 nM to 10 nM	Bowie *et al.*, 1998
Voltammetry	Cu	0.24 nM	3.5 % (at 0.5 nM)	Not Stated	van den Berg, 1986
	Cd	8 pM	1.4 % (at 0.15 nM)	0.008 nM to 1000 nM	Fisher and van den Berg,1999
	Pb	5 pM	4.6 % (at 65 pM)	0.008 to 1000 nM	Fisher and van den Berg,1999

ICP= Inductively Coupled Plasma; MS= Mass Spectrometry; AES=Atomic Absorption Spectromeryy; ETAAS= Electrothermal Atomic Absorption Spectrometry.

1.10.1 Inductively Coupled Plasma (ICP) Spectrometric Methods

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and ICP- Atomic Emission Spectrometry (AES) offer low detection limits (pM concentrations), wide linear dynamic ranges (10^6), high resolution, simultaneous multi-element determination and short analysis times (Ebdon *et al.*, 1998). A major drawback of ICP-MS is spectroscopic polyatomic interference from the seawater matrix, in addition to inferences from atomic or molecular ions of the plasma gas. This difficulty can often be overcome by use of isotope dilution ICP-MS (McLaren *et al.* 1987). However, in ICP-methods, samples must contain less than 0.1% (by weight) of dissolved solids, in order to prevent build up of solids on the nickel cones and/or deposition of solids on the ion lenses (Ebdon *et al.*, 1998). Simple dilution of sample prior to ICP-MS analysis can minimise these effects, but is problematic for trace metals that typically have very low concentrations in seawater. Methods have been developed that can chemically separate and concentrate analytes from the matrix, reducing polyatomic interferences, preventing deposition of matrix on the cones and improve detection limits. The use of an iminodiacetate resin, Metpac CC-1 (Dionex), has been reported for the preconcentration of trace metals that include Cu and Pb (Bloxham *et al.*, 1994) and Cd (Nicolai *et al.*, 1999;). A further example is the preconcentration of trace metals by complexation with 8-hydroxyquinoline and adsorption on C-18 bonded silica-gel, prior to analysis (Watanabe *et al.,* 1981).

Disadvantages of ICP methods include the high cost of the instrument and its maintenance and running costs, together with its clear unsuitability for shipboard analyses. In addition, it is a destructive technique, which makes speciation measurements difficult, consequently the use of extra techniques coupled to the instrument may be needed, making the technique even more complex, *e.g.* methods based on electrospray ICP-MS (Kebarle and Tang, 1993), and a few methods have been applied to seawater (*e.g.* Bloxham *et al.* 1994)

1.10.2 Electrothermal Atomic Absorption Spectrometry (ETAAS)

The mostly frequently used type of ETAAS is the graphite furnace-atomic absorption spectrometry (GFAAS). GFAAS has several advantages that include high sensitivity, ability to handle small volumes (0.5-10 μL) and low electronic noise from the furnace. The method is more sensitive than flame AAS owing to long residence time and a high degree of atomisation. The analysis of estuarine and coastal samples requires some form of preconcentration, including extraction (by organic solvents; Danielsson *et al.*, 1982; Statham, 1985; Apte and Gunn, 1987; Ohta *et al.*, 1988) or sorption onto columns (Sarzanini *et al.* 2001) in order to separate the analyte/s from major seawater ions (Na^+, Mg^{2+}, Ca^{2+} and Cl^-) to avoid spectroscopic interferences. The numerous sample-handling stages lead to increased risk of sample contamination (Apte and Gunn, 1987).

1.10.3 Chemiluminescence (CL) Methods

CL methods are flow injection analysis methods and are rapid, require small sample volumes, have very low detection limits for example, 0.04 nM for Fe (Cannizzaro *et al.,* 2000) and long linear ranges for example, 0.04 to 10 nM for Fe (Bowie *et al.*, 1998). In addition to methods described previously, the instrumentation can be miniaturised, allowing field portability. Several CL methods have been developed for the determination of Cu in natural waters (Zamzow *et al.,* 1998; Achterberg *et al.,* 2001) using 1,10-phenanthroline (Coale *et al.,* 1992), and with low limits of detection (pM range). The main disadvantage of chemiluminescence methods is that they are often specific to one analyte, *i.e,* methods do not allow simultaneous measurements of several metals.

1.10.4 Voltammetric Methods

These methods offer high sensitivity, wide dynamic linear ranges (typically up to 10^5 M; Achterberg and Braungardt, 1999), multi-element capabilities and application in all natural water matrices with little or no interference from major ions. Separate pre-concentration steps are unnecessary, as the technique uniquely allows this to be completed during analysis. These attributes allow direct analysis of dissolved trace metals in natural waters at low concentrations (limits of detection (LOD), typically pM) lending the technique to application in natural water analysis. The versatile technique can also be used to determine individual metal redox species, *e.g.* Fe (Gledhill and van den Berg, 1995), dissolved organic and inorganic species, *e.g.* using 8-hydroyqinoline (van den Berg, 1986) and salicylaldoxime (Campos and van den Berg, 1994), natural metal ligand concentrations, *e.g.* complexation of Cu (van den Berg, 1982) and total trace metal concentrations (Achterberg and Braungardt, 1999).

1.11 Sampling and Clean Techniques

Conventionally, the determination of trace metal concentrations in estuarine and coastal water samples involves sequential stages, namely, sampling; transportation; storage; handling; pre-treatment and finally, analysis. Each step in the process poses a risk for chemical contamination and sampling procedures should be conducted in such a way that contamination, *e.g.* from the ship's hull, is avoided. The advent of 'clean techniques' (*e.g.* Howard and Statham, 1993) that impose stringently clean environments for sample collection, storage, pre-treatment and handling has reduced contamination problems. Recommendations for cleaning procedures and working conditions, are given by Howard and Statham (1993) and Kremling (1999). These include acid cleaning protocols for sampling apparatus, reagents purifications, through to filtration of laboratory air. Class-1000, clean air working laboratory environments that provide dust-free conditions, have become commonplace, but associated capital cost is often high. Improved working environments, coupled with advances in analytical methods have lowered instrumental LODs, culminating in greater accuracy of reported trace metal concentrations. This is particularly the case for trace metals such as Fe (de Jong *et al.*, 2000).

1.12 Project Aims and Objectives

The overall aim of this research was to optimise analytical techniques for the determination of trace metals in estuarine and coastal waters (both *in situ* and in the laboratory), with emphasis on Cu, Cd and Pb, and to investigate estuarine biogeochemical processes (in association with other master variable data). The objectives of the research were to:

- Investigate sample filtration techniques for separation of trace metals in estuarine waters in samples obtained during different seasons (Chapter 2).

- Discuss the need for development of miniaturised automated *in situ* electrochemical instrumentation for the use in water quality monitoring (Chapter 3).

- Optimise and validate a submersible Voltammetric In *situ* Profiling (VIP) system for *in situ* determinations of Cu(II), Cd(II) and Pb(II), in estuarine and coastal waters and evaluate possible correlations from master variables (Chapter 4)

- Undertake an international laboratory inter-comparison exercise, in order to verify precision and accuracy of measurements made by 4 laboratories that operate VIP systems (in Plymouth, U.K; Geneva, Switzerland and Ancona, Italy) and use laboratory reference methods as a comparison. Inter-comparison to include analysis of Tamar Estuary samples and *in situ* VIP system measurements in the coastal waters of the Gullmar Fjord, Sweden. (Chapter 5)

- Undertake tidal cycle surveys in the Tamar, Plym and Fal estuaries, UK by deployment of the VIP system whilst monitoring master variables. Use laboratory methods to acquire additional trace metal speciation data. Interpret estuarine biogeochemical cycling of Cu(II), Cd(II) and Pb(II) in specified estuaries (Chapter 6)

- Undertake studies using the VIP system in the Adriatic Sea, Italy to determine the influence of the Po estuary on metal distribution. (Chapter 7).

The project was funded by the Natural Environmental Research Council (NERC; GT 4/98/MS/234), but parts of the work presented in this thesis were carried out as part of a European Union funded project (EVK3-CT-2000-00036) entitled '*In Situ* Automated Monitoring of Trace Metal Speciation in Estuaries and Coastal Zones in Relation With The Biogeochemical Processes' (IMTEC) project. This project gave an opportunity to perform laboratory inter-comparisons both in land based and fjord and sea based locations. As a result, joint field testing took place in 3 coastal ecosystems with different characteristics, 1) A fjord system in west Sweden, characterised by small tidal range and influenced by some anthropogenic inputs, 2) macro-tidal estuaries in south west England, characterised by strong variations in water flow and turbidity and subject to enhanced trace metal inputs, and 3) the Po estuary and its coastal plume, characterised by low tidal range and important pollution inputs. The field campaigns were aimed at: a) improving analytical developments for the monitoring for application needs of the VIP system b) harmonising fieldwork and analytical techniques to lead to improved compatibility between EU laboratory data, c) gaining valuable environmental data for the three contrasting environmental locations, d) enhancing the understanding of trace metal behaviour in coastal ecosystems and thus e) improving the capability of coastal managers to assess and predict the risks of contaminant inputs on ecosystems.

Five research partners took part in the project. Partners were from University of Plymouth, UK (2 VIP users); University of Gothenberg, Sweden (1 VIP user); University of Ancona, Italy (1 VIP user) and University of Geneva, Switzerland (1 VIP user). As a result some of the data presented in this thesis were not collected by the author, but data were included by kind permission. All work undertaken by other persons is acknowledged.

In Chapter 2, Mr S. Badr, School of Environmental Sciences, University of Plymouth provided the DOC concentrations for the April campaign.

In Chapter 4, Dr C. Braungardt, School of Environmental Sciences, University of Plymouth provided the results of the pH trials in seawater and LOD's in seawater.

For work included in Chapter 5, Dr A. Tappin, University of Plymouth, carried out preparation, filtration and distribution of samples for the inter-comparison exercises. VIP inter-comparison data were supplied by Dr M.-L. Tercier-Waeber, Analytical and

Biophysical Environmental Chemistry, University of Geneva, Switzerland; Dr C. Braungardt; Mr L. Lambertutcci, Institute of Marine Sciences, University of Ancona, Italy and the author of this Thesis. Prof. D. R. Turner, Department of Analytical and Marine Chemistry, Göteborg, Sweden provided CTD profiles of the Gullmar Fjord.

In Chapter 6, Dr Braungardt provided *in situ* Cd and Pb profiles for Fal estuary samples for the dates 02/08/02 and 05/12/02. Dr Tappin determined all Fal salinities.

In Chapter 7, Mr Lambertutcci provided all CTD data and Dr Tercier-Waeber; Dr Braungardt, and Mr Lambertutcci provided additional trace metal data.

Reference List

Ackroyd, D. R., Bale, A. J., Howland, R. J. M., Knox, S., Millward, G. E., and Morris, A. W. (1986) *Distributions and Behavior of Dissolved Cu, Zn and Mn in the Tamar Estuary.* Estuar. Coast. Shelf Sci., **23**, 621-640.

Achterberg, E. P., Braungardt, C. B., Sandford, R. C. and Worsfold, P. J. (2001) *UV Digestion of Seawater Samples Prior to the Determination of Copper Using Flow Injection with Chemiluminescence Detection.* Anal. Chim. Acta, **440**, 27-36

Achterberg, E. P., Colombo, C. and van den Berg, C. M.G. (1999) *The Distribution of Dissolved Cu, Zn, Ni, Co and Cr in English Coastal Surface Waters.* Cont. Shelf Res., **19**, 537-558

Achterberg E. P. and Braungardt, C. (1999) *Stripping Voltammetry for the Determination of Trace Metal Speciation and In-situ Measurements of Trace Metal Distributions in Marine Waters.* Anal. Chim. Acta, **400**, 381-397

Apte, S. C., Gunn, A. M. (1987) *Rapid-Determination of Copper, Nickel, Lead and Cadmium in Small Samples of Estuarine and Coastal Waters by Liquid-Liquid-Extraction and Electrothermal Atomic-Absorption Spectrometry.* Anal. Chim. Acta, **193** ,147-156

Bale, A. J., Morris, A. W. and Howland, R. J. M. (1985) *Seasonal Sediment Movement in the Tamar Estuary.* Oceanol. Acta, **8**, 1-6

Bowie, A. R., Achterberg, E. P., Mantoura, R. F. C. and Worsfold, P. J. (1998) *Determination of Sub-Nanomolar Levels of Iron in Seawater Using Flow Injection with Chemiluminescence Detection.* Anal. Chim. Acta, **361**, 189-200

Bloxham, M. J., Hill, S. J. and Worsfold, P. J. (1994) *Determination of Trace-Metals in Sea-Water and the Online Removal of Matrix Interferences by Flow-Injection with Inductively-Coupled Plasma-Mass Spectrometric Detection,* J. Anal. Atom. Spec., **9**, 935-938

Butler, A., (1998) *Acquisition And Utilization of Transition Metal Ions by Marine Organisms,* Science, **281**, 207.

Brand, L. E., Sunda, W. G. and Guillard, R. R. L. (1983) *Limitation of Marine-Phytoplankton Reproductive Rates by Zinc, Manganese, and Iron.* Limnol. Oceanogr., **28**, 1182-1198

Bruland, K. W., Orians, K. J. and Cowen, J. P. (1994) *Reactive Trace-Metals in the Stratified Central North Pacific.* Geochim. Cosmochim. Acta, **58**, 3171-3182

Bruland, K. W., Donat, J. R., Hutchins, D. A. (1991) *Interactive Influences Of Bioactive Trace-Metals On Biological Production In Oceanic Waters.* Limnol. Oceanogr., **36**, 1555-1577

Buffle, J. and Leeuwen, (1992) *Environmental Particles* in: Environmental Analytical and Physical Chemistry Series, C. Buffle, J., Perret, D. and Newman, M., (Eds.) Lewis Publishers, London, pp171-320

Cameron, W. M. and Pritchard, D. W. (1963) *Estuaries.* In: The Sea, Hill, M. N., (Ed.) 2nd Ed., Wiley, New York, pp 306-324.

Campos, M. L. A. M and van den Berg, C. M. G (1994) *Determination of Copper Complexation in Sea-Water by Cathodic Stripping Voltammetry and Ligand Competition with Salicylaldoxime.* Anal. Chim. Acta, **284,** 481-496

Carvalho, F. P. (1997) *Distribution, Cycling and Mean Residence Time of Ra-226, Pb-210 And Po-210 in the Tagus Estuary.* Sci. Total Environ., **196**, 151-161

Chan, M. S., Huang, S. D. (2000) *Direct Determination of Cadmium and Copper in Seawater Using a Transversely Heated Graphite Furnace Atomic Absorption Spectrometer with Zeeman-Effect Background Corrector.* Talanta, **51**, 373-380

Chester, R. (2000) *The Transport of Material to the Oceans; the River Pathway.* In: Marine Geochemistry, Blackwell Science, 4th Ed, pp 11-51

Chester, R. (2000A) *Trace Elements in the Oceans.* In: Marine Geochemistry, Blackwell Science, 4th Ed, pp 258-310

Chiffoleau, J. F., Cossa, D., Auger, D. and Truquet, I. (1994) *Trace-Metal Distribution, Partition and Fluxes in the Seine Estuary (France) in Low Discharge Regime.* Mar. Chem., **47**, 145-158

Coale, K. H., Johnson, K. S., Stout, P. M., Sakamoto, C. M. *Determination of Copper in Sea-water using a Flow-Injection Method with Chemiluminescence Detection.* Anal. Chim. Acta, **266**, 1992

Colbert, D., Johnson, K. S., Coale, K. H. (1998) *Determination of Cadmium in Seawater using Automated On-Line Preconcentration and Direct Injection Graphite Furnace Atomic Absorption Spectrometry.* Anal. Chim. Acta, **377**, 255-262

Dalrymple, R. W., Zaitlin, B. A., Boyd, R. (1992) *Estuarine Facies Models - Conceptual Basis and Stratigraphic Implications.* J. Sediment. Petrol., **62**, 1130-1146

Dando, P. R., Aliani, S., Arab, H., Bianchi, C. N., Brehmer, M., Cocito, S., Fowler, S. W., Gundersen, J., Hooper, L. E., Kolbl, R., Kuever, J., Linke, P., Makropoulos, K. C., Meloni, R., Miquel, J. C., Morri, C., Muller, S., Robinson, C., Schlesner, H., Sievert, S., Stohr, R., Stuben, D., Thomm, M., Varnavas, S. P. and Ziebis, W. (2000) *Hydrothermal Studies in the Aegean Sea.* Phys. Chem. Earth Pt B, **25**, 1-8

Danielsson, L. G., Magnusson, B., Westerlund, S. and Zhang, K. (1982) *Trace-Metal Determinations in Estuarine Waters by Electrothermal Atomic-Absorption Spectrometry after Extraction of Dithiocarbamate Complexes into Freon.* Anal. Chim. Acta, **144**, 183-188

Danielsson, L. G. (1982) *On the Use of Filters for Distinguishing Between Dissolved and Particulate Fractions in Natural-Waters.* Water Res.,**16**, 179-182.

Davies, J. H. (1964) *A Morphogenetic Approach to World Shorelines.* Z. Geomorphol. **8**, 127-142

de Jong, J. T. M, Boye, M., Schoemann, V. F., Nolting, R. F. and de Baar, H. J. W. (2000) *Shipboard Techniques Based on Flow Injection Analysis for Measuring Dissolved Fe, Mn and Al in Seawater.* J. Environ. Monitor., **2**, 496-502

Drever, J.I. (1988) The Geochemistry of Natural Waters, 2nd. Ed., Prentice Hall, New Jersey, p 437

Dyer, K. R. (2000) *Estuaries: A Physical Introduction,* 2nd Ed., John Wiley and Sons, Chichester, pp195

Dyer, K. R., Christie, M. C. and Wright, E. W . (2000) *The Classification of Intertidal Mudflats.* Cont. Shelf Res. **20**, 1039-1060

Ebdon, L., Evans, E. H., Fisher, A. and Hill, S. J. (1998) *An Introduction to Analytical Atomic Spectrometry,* 1st Ed.; Wiley: Chichester.

ElbazPoulichet, F., Garnier, J. M., Guan, D. M., Martin, J. M. and Thomas, A. J. (1996) *The Conservative Behaviour of Trace Metals (Cd, Cu, Ni And Pb) and as in the Surface Plume of Stratified Estuaries: Example of the Rhone River (France).* Estuar. Coastal Shelf Sci., **42**, 289-310

Fairbridge, R. W. (1980) *The Estuary: Its Definition and Geodynamic Cycle.* In: Chemistry and Biogeochemistry of Estuaries. John Wiley and Sons, 1st Ed., pp 1-35

Festa, J. F. and Hansen, D. V., (1978) *Turbidity Maxima in Partially Mixed Estuaries: a Two Dimensional Numerical Model,* Est. Coast. Shelf Sci., **7**, 347-359

Fischer, E. and van den Berg, C. M. G. (1999) *Anodic Stripping Voltammetry of Lead and Cadmium Using a Mercury Film Electrode and Thiocyanate.* Anal. Chim. Acta, **385**, 273-280

Gledhill, M., Nimmo, M. and Hill, S. J. (1999) *The Release of Copper-Complexing Ligands by the Brown Alga Fucus Vesiculosus (phaeophyceae) in Response to Increasing Total Copper Levels.* J. Phycol., **35**, 501-509

Gledhill, M. and van den Berg, C. M. G (1995) *Measurement of the Redox Speciation of Iron in Seawater by Catalytic Cathodic Stripping Voltammetry.* Mar. Chem. **50**, 51-61

Goldberg, E. D. Baker M. and Fox, D. L. (1952) *J. Mar. Res.* **11**, 197-202.

Hassellöv, M. (1999) *FFF-ICPMS: Development of a New Technique for Determination of Colloidal Trace Element Size Distributions in Natural Waters.* PhD Thesis, Göteborg University, Sweden.

Horowitz, A. J., Lum, K. R., Garbarino, J. R., Hall, G. E. M, Lemieux, C. and Demas, C. R. (1996) *Problems Associated with using Filtration to Define Dissolved Trace Element Concentrations in Natural Water Samples.* Environ. Sci. Technol. **30**, 3398-3400

Howard, G. and Statham, P. J. (1993) Inorganic Trace Analysis: Philosophy and Practice, A. G. Howard and P. J. Statham (Eds), J. Wiley & Sons, Chichester, pp. 182.

Kebarle, P. and Tang, L. (1993) *From Ions in Solution to Ions in the Gas-Phase -The Mechanism of Electrospray Mass-Spectrometry.* Anal. Chem., **65**, 972-986

Kremling, K. (1999) Determination of Trace Elements. In *Methods of Seawater Analysis*, 3rd Ed. Grasshoff K. Kremling K. Ehrhardt M. (Eds.), Wiley- VCH Publishing, NY.,pp256-360

Ledin, A., Karlsson, S., Duker, A. and Allard, B. (1995) *Characterization of the Submicrometer Phase in Surface Waters - A Review.* Analyst, **120**, 603-608

Lerman, A. (1979) Geochemical Processes, Wiley, New York.

Li, 1991: Li, Y. H. (1991) *Distribution Patterns of the Elements in the Ocean: A Synthesis*, Geochim. Cosmochim. Acta, **55** , 3223-3240.

Liu, Z. S. and Huang, S. D. (1995) *Determination of Lead in Sea-Water with a Graphite-Furnace Atomic-Absorption Spectrometer and an Improved Automatic Online Preconcentration System.* Spectrochim Acta B Atomic Spec., **50**, 197-203

Loscher, B. M. (1999) *Relationships Among Ni, Cu, Zn, and Major Nutrients in the Southern Ocean.* Mar. Chem., **67,** 67-102

Luoma, S. N. (1983) *Bioavailability of Trace-Metals to Aquatic Organisms - a Review.* Sci. Tot. Environ., **28**, 1-22

Macrellis, H. M., Trick, C. G., Rue, E. L., Smith, G. and Bruland, K. W. (2001) *Collection and Detection of Natural Iron-Binding Ligands from Seawater.* Mar. Chem., **76**, 175-187

Millero, F. J. (1996) Descriptive Oceanography in *Chemical Oceanography,* Kennish, M. J. and Lutz, P. L. (Series Eds), CRC Marine Science Series, CRC Press, Inc., Florida, 2nd Ed., pp 1-50

McLaren, J.W., Beauchemin, D. and Berman, S. S. (1987) *Application Of Isotope-Dilution Inductively Coupled Plasma Mass-Spectrometry To The Analysis Of Marine-Sediments.* Anal. Chem., **59**, 610-613

Millward, G. E., Allen, J. I., Morris, A. W. and Turner, A. (1996) *Distributions and Fluxes of Non-Detrital Particulate Fe,Mn,Cu,Zn in the Humber Coastal Zone, UK.* Cont. Shelf Res., **16**, 967-993

Millward, G. E. (1995) *Processes Affecting Trace-Element Speciation In Estuaries - A Review.* Analyst, **120**, 609-614

Morel, F. M. M. and Price, N. M. (2003) *The Biogeochemical Cycles of Trace Metals in the Oceans.* Science, **300**, 944-947

Morris, A. W., Bale, A. J., Howland, R. J. M., Millward, G. E., Ackroyd, D. R., Loring, D. H. and Rantala, R. T. T. (1986) *Sediment Mobility and its Contribution to Trace-Metal Cycling and Retention in a Macrotidal Estuary.* Water Sci. Technol. **18** 111-119

Morrison, M. A. and Benoit, G. (2001) *Filtration Artifacts Caused by Overloading Membrane Filters.* Environ. Sci. Technol., **35**, 3774-3779

Nicolai, M., Rosin, C., Tousset, N. and Nicolai, Y. (1999) *Trace Metals Analysis in Estuarine and Seawater by ICP-MS Using on line Preconcentration and Matrix Elimination with Chelating Resin.* Talanta, **50**, 433-444

Ohta, K., Aoki, W. and Mizuno, T. (1988) *Elimination Of Interferences In Electrothermal-Atomization Atomic-Absorption Spectrometry Of Cadmium.* Talanta, **35**, 831-836

Owens, R. E. and Balls, P. W. (1997) *Dissolved Trace Metals In The Tay Estuary.* Estuar. Coast. Shelf Sci., **44**, 421-434

Panko, J. F. (1991) Aquatic Chemistry Concepts, Lewis Publishers, Chelsea, Michigan.

Pritchard, D. W. (1952) *Salinity Distribution and Circulation in the Chesapeake Bay Estuarine System.* J. Mar. Res., **11**, 106-123.

Saager, P. M., debar, H. J. W., deJong, J. T. M., Nolting, R. F. and Schijf, J. (1997) *Hydrography And Local Sources of Dissolved Trace Metals Mn, Ni, Cu, and Cd In the Northeast Atlantic Ocean.* Mar. Chem., **57**, 195-216

Sarzanini, C., Abollino, O. and Mentasti, E., (2001) *Column Flow-Injection Preconcentration and Electrothermal Atomic Absorption Spectrometry Determination of Manganese in Seawater.* Anal. Chim. Acta, **435**, 343-350

Sholkovitz, E. R. and Copland, D. (1981) *The Coagulation, Solubility and Adsorption Properties of Fe, Mn, Cu, Ni, Cd, Co and Humic Acids in a River Water.* Geochim. Cosmochim. Acta, **45**, 181-189

Spokes, L., Jickells, T. and Jarvis, K. (2001) *Atmospheric Inputs of Trace Metals to the Northeast Atlantic Ocean: The Importance of Southeasterly Flow.* Mar. Chem., 6, 319-330

Statham, P. J. (1985) *The Determination of Dissolved Manganese and Cadmium in Seawater at Low nmol* L^{-1} *Concentrations by Chelation and Extraction Followed by Electrothermal Atomic-Absorption Spectrometry.* Anal. Chim. Acta, **169**, 149-159

Sunda, W.G. (1989) *Trace Metal Interactions with Marine Phytoplankton.* Biol Oceanogr., **6**, 411- 442

Sunda, W.G. and Guillard, R. R. L. (1976) *The Relationship between Cupric Ion Activity and the Toxicity of Copper to Phytoplankton.* J. Mar. Res., **34**, 511-529.

Tappin, A. D., Millward, G. E., Statham, P. J., Burton, J. D. and Morris, A. W. (1995) *Trace-Metals In The Central And Southern North-Sea.* Estuar. Coast. Shelf Sci., **41**, 275-323

Taylor, H. H., Anstiss, J. M. (1999) *Copper and Haemocyanin Dynamics in Aquatic Invertebrates,* Mar. Fresh. Res., **50**, 907-931

Vaithiyanathan, P., Ramanathan, A. and Subramanian, V. (1993) *Transport And Distribution Of Heavy-Metals In Cauvery River.* Water Air Soil Poll. **71**, 13-28

van den Berg, C. M. G., Khan, S.H., Daly, P. J., Riley, J. P., Turner, D. R. (1991) *An Electrochemical Study Of Ni, Sb, Se, Sn, U And V In The Estuary Of The Tamar.* Estear. Coast. Shelf Sci., **33**, 309-322

van den Berg, C. M. G., Merks, A. G. A. and Duursma, E. K. (1987) *Organic Complexation and its Control of the Dissolved Concentrations of Copper and Zinc in the Scheldt Estuary.* Estuar. Coast. Shelf Sci., **24**, 785-797 van den Berg, C. M. G (1986) *Determination of Copper, Cadmium and Lead in Seawater by Cathodic Stripping Voltammetry of Complexes with 8-Hydroxyquinoline.* J. Electroanal. Chem., **215**, 111-121

van den Berg, C. M. G. (1982) *Determination of Copper Complexation with Natural Organic-Ligands in Sea-Water by Equilibration with MnO_2 .1. Theory.* Mar. Chem., **11**, 307-322

Vojak, P. W. L., Edwards, C. and Jones, M. V. (1985) *Evidence For Microbiological Manganese Oxidation in the River Tamar Estuary, South-West England.* Estuar. Coast. Shelf Sci., **20**, 661-671

Watanabe, H., Goto, K., Taguchi, S., Mclaren, J. W., Berman, S. S And Russell, D. S. (1981) *Preconcentration of Trace-Elements in Seawater by Complexation with 8-Hydroxyquinoline and Adsorption on C-18 Bonded Silica-Gel.* Anal. Chem., **53**, 738-739

Wu, J. F. and Boyle, E. A. (1997) *Low Blank Preconcentration Technique for the Determination of Lead, Copper, and Cadmium in Small-Volume Seawater Samples by Isotope Dilution ICPMS.* Anal. Chem., **69**, 2464-2470

Zamzow, H., Coale, K. H, Johnson, K. S. and Sakamoto C. M. (1998) *Determination of Copper Complexation in Seawater Using Flow Injection Analysis with Chemiluminescence Detection.* Anal. Chim. Acta, **377**, 133-144

Zhang, H. and Davison, W. (2001) *In Situ Speciation Measurements. Using Diffusive Gradients in Thin Films (DGT) to Determine Inorganically and Organically Complexed Metals.* Pure App. Chem., **73**, 9-15.

Zhang, H. and Davison, W. (1995) *Performance Characteristics of Diffusion Gradients in Thin-Films for the In-Situ Measurement of Trace Metals in Aqueous Solution.* Anal. Chem., **67**, 3391-3400.

CHAPTER 2

The Influence of Membrane Filtration on the Determination of Dissolved Trace Metal Concentrations in Turbid Estuarine Waters

Abstract

The aim of this work was to extend previous studies on lakes and rivers by examining the trends of metal filtrate concentrations as a function of filtration volume. Riverine and estuarine waters with contrasting matrix composition, as well as waters from different seasons were investigated. In particular, this study examined the importance of colloids (in the size range 0.2 to 0.4 μm) to membrane clogging and concludes that colloids account for a significant part of clogging of membrane filters during filtration of relatively turbid estuarine waters. Samples were collected from three sites on the lower River Tamar and its estuary (salinity range 0-32) and filtered through two 47 mm diameter hydrophilic polycarbonate filter membranes of differing pore size: Cyclopore™ 0.4 μm and 0.2 μm. Trace metal (Al, Cd, Co, Cr, Cu, Mn, Pb, U and Zn) filtrate concentrations as a function of filtered volume were determined, with analysis performed by ICP-MS and voltammetry. Based on the findings presented in this chapter some metal specific filtration protocols are recommended. The most effective filtration protocol for Al, Cd and Pb is to filter low volumes through the filter (≤ 100 mL), replacing the filter regularly. For Mn, the first 50 mL of filtrate should be discarded. Co, Cr, Cu, U and Zn remain relatively unperturbed by filter clogging and the filter can therefore be used even after the onset of clogging.

2.1 Introduction

Trace metals contribute to the ecological health of natural waters, playing an essential role in biological processes with a range of metals vital for growth and functioning of aquatic organisms (Butler *et al,* 1998). The partitioning and distribution of metals between dissolved and particulate phases is key to their bioavailability and biogeochemical cycling (Shiller and Taylor, 1996; Turner and Millward, 2002). Concentrations of dissolved trace metals are generally determined in the filtrate of sample waters that pass through a 0.4 or 0.45 µm pore diameter membrane filter, with particulate matter (of density >1 g cm^{-3}) and most biological particles remaining trapped on the filter surface or within the membrane itself (Laxen and Chandler, 1982). This method, however, reflects an arbitrary separation, as the dissolved phase as so defined includes not only free ions, but also readily dissociable (labile) metal complexes and metals bound to colloidal material [of size 1-400 nm in at least one dimension (Mantoura and Morris, 1983; Greenamoyer and Moran, 1997)]. The physical and biogeochemical properties of marine colloids remain largely uncharacterised (Wells, 1998). Recently, Guo *et al.* (2000) reported that metal:carbon ratios in colloidal material (1 kDa-0.2 µm) collected from Galveston Bay were similar to those in humic substances and marine plankton, suggesting that marine colloids, at least in this environment are derived largely from plankton and are mostly organic in composition.

The potential modifying effects of filtration on resultant filtrate concentrations of trace metals, particularly the influence arising from filter clogging, has received increasing attention in recent years. Laxen and Chandler (1982) and Danielsson (1982) filtered lake water and found that concentrations of Fe in the filtrate decreased as the sample volume filtered increased. They concluded that this was due to the increased trapping of particles smaller than the nominal pore size of the filter as filtration proceeded. In a landmark study, Horowitz *et al.* (1992) demonstrated that filtrate concentrations of Fe and Al in river water samples could be significantly affected by the filter type used, filter diameter, filtration method, suspended particulate matter (SPM) concentration, SPM grain size distribution, colloid and colloidal metal concentrations, and volume of sample filtered. Subsequent studies (Horowitz *et al.*, 1996; Hall *et al.*, 1996; Morrison and Benoit, 2001) extended this work to include a wider range of minor and major elements and fresh water matrices, and found results in line with the earlier reports. Filter clogging, leading to increased retention of colloidal metals on the filter cake, was again cited as a major factor in the observed decline in filtrate metal concentrations with volume filtered. Horowitz *et al.* (1996) and Morrison and Benoit (2001) were surprised to find these effects even in samples with SPM concentrations as low as 4 to 39 mg L^{-1}.

In rivers, Al is predominantly in the solid phase [as aluminium oxides (Al_2O_3)] and associated with colloids (Morris *et al.*, 1986B; Hill and Aplin, 2001). Hassellöv (1999) raised the question whether Al could be a colloidal carrier for trace metals in its own right, rather than adsorbed to Fe colloids but concluded that sorption by Al colloids was insignificant in comparison to the Fe and C based colloids. Nevertheless the study highlighted the importance of the colloidal fraction in riverine systems. In estuaries, much of the colloidally associated Al is destabilised due to increasing ionic strength leading to subsequent flocculation and coagulation (Chester, 2000). Much of the Mn delivered by rivers is supplied as Mn oxyhydroxides or as aqueous ions and simple ion pairs and possibly in the reduced Mn(II) form (Chester, 2000). Under oxic environmental conditions, Mn(II) can be oxidised to Mn(IV) a reaction catalysed by the presence of microbes, as previously demonstrated in the Tamar by Knox *et al.*,

(1981) who reported a higher concentration of colloidally associated Mn (in particular in the turbidity maximum zone; TMZ) in comparison to the river, with very little free Mn(II) present. Hart and Davies (1981) reported most (80 %) of the Cd supplied by the Yarra river was in the aqueous Cd(II) species and was similar to the estuarine colloidal fraction (97 %). Similar results were also found for Cu (75 % and 74 % aqueous ions in river and estuary respectively). Pb showed high concentrations of colloidally associated metals (53 %) in the river as did Zn (52 %) and similar colloidal concentrations were found in the estuarine water. Elbaz-Poulichet *et al.*, (1984) reported that in the Gironde Estaury, dissolved Pb was removed by adsorption onto particles (due to turbidity) in the TMZ and in the lower estuary. Pb mobilisation occurred simultaneously with coagulation of organically and Mn/ Fe oxide associated Pb. Co forms strong organic complexes and is associated with colloids in both river (Pham and Garnier, 1998) and estuarine water and Wen *et al.* (1999) reported 19 % colloidal Co in the Galvestan Bay Estuary. U has a strong affinity for carbonate ions (Turner *et al.,* 1981) and often binds to carbonate groups in humic substances and may also be associated with organic colloids in river waters (Hassellöv, 1999). However, van den Berg *et al.* (1991) reported that U occurred as stable anionic complexes of cations and were not readily complexed by dissolved organic material (that include colloids) in the Tamar Estuary.

The aim of this work was to extend the earlier studies to examine any effects of filter clogging on 'dissolved' metal concentrations in relatively turbid estuarine waters of different salinities. Filter membranes chosen for the study were Cyclopore^{TM} hydrophilic polycarbonate 'sieve'-type filters [Laxen and Chandler (1982)] of 47 mm diameter. Both 0.2 and 0.4 µm pore diameter membranes were used, as these are most frequently employed to discriminate the dissolved metal fraction (Horowitz *et al.,* 1996; Greenamoyer and Moran, 1997; Guo *et al.,* 2000). Samples were collected from the lower River Tamar (at the tidal limit) and its estuary. The Tamar Estuary is a typical, temperate, macro-tidal system, with mean river flows of 19 m^3 s^{-1}, a maximum neap-spring tidal range of 2.2. to 4.7 m, dissolved oxygen concentrations of 5-10 mg L^{-1}, pH 6.8-8.5 and SPM concentrations <5-1000 mg L^{-1} (Millward, 1995). At low river flows, there is a pronounced turbidity maximum in the low salinity zone (Uncles *et al.*, 1985). A number of dissolved trace metal studies have been reported for this estuary (*e.g.* Morris *et al.*, 1979; 1982, 1986A,B; Morris and Bale, 1979; Knox *et al.*, 1981; Ackroyd *et al.*, 1986; Toole *et al.*, 1987; van den Berg *et al.,* 1991), thus providing an historical context for this study.

2.2 Experimental

2.2.1 Sampling Procedure

Water samples were taken by hand through immersing a 10 L acid cleaned polypropylene bottle ~ 0.30 m below the surface. The bottle was allowed to purge three times before sampling commenced. Once collected, all samples were stored on ice in the dark and transported to the laboratory. Samples were collected from the River Tamar and its estuary on 05-04-2001 (April campaign) and again on 27-09-2001 (September campaign) from three sampling locations (Figure 2.1; Gunnislake, Calstock and Saltash). Sampling was carried out at high water for both sampling campaigns for ease of access and to ensure the same tidal state. Both high and low river flow periods (56.4 m^3 s^{-1} and 2.8 m^3 s^{-1}; April and September, respectively) were sampled in order to examine whether potential seasonal changes in the water matrix would influence the results of the filtration experiment. (Supporting master variable data characterising these water samples are given in Table 2.1).

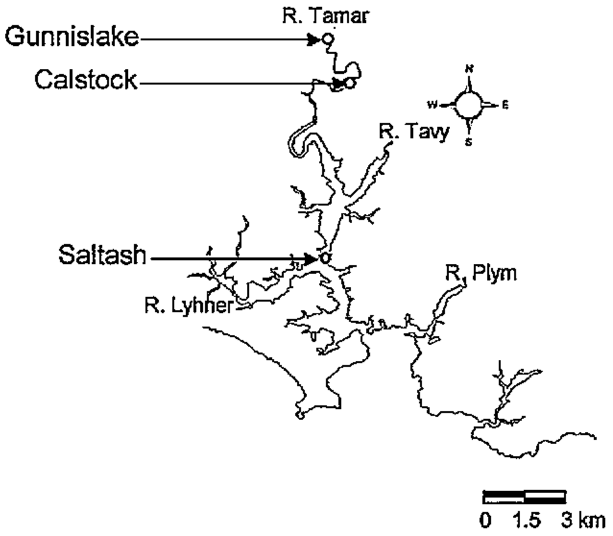


Figure 2.1 Tamar Estuary with the sampling locations marked in red.

2.2.2 Reagent Cleanup Procedures

Purified Water

Water was purified by reverse osmosis (Milli-RO, Millipore, Bedford, USA) followed by ion exchange (MQ water) (Millipore, 18.2 MΩ cm^{-1}).

Purification of ammonia

Ultrapure aqueous ammonia (iso-NH_4OH) was prepared by isothermal distillation of impure NH_4OH into MQ water. A schematic of the procedure is given in Figure 2.2.

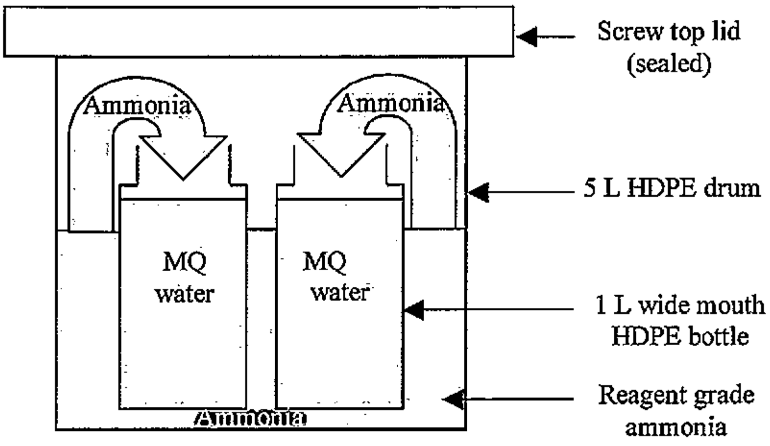


Figure 2.2 Schematic diagram of the procedure for the purification of ammonia

Two acid cleaned 1 L wide mouth, high density polyethylene (HDPE; Nalgene, Rochester, NY, USA) containers, each containing 800 mL of MQ water were sealed in a 5 L acid cleaned HDPE drum containing ammonia (AnalaR; VWR, dorset, UK). The unit was sealed and left at ambient temperature for 5 days allowing ammonia gas to dissolve into the MQ water and equilibrate between the aqueous solutions, producing iso-NH_4OH. Concentration of the ammonia determined by titration with hydrochloric acid of known concentration and was found to be 5.9 M.

Purification of acids

Ultrapure nitric (sub-HNO_3), hydrochloric (sub-HCl) and acetic (sub-CH_3COOH) acids were prepared by sub-boiling quartz distillation (Howard and Statham, 1993) using an in-house distillation unit. A schematic diagram of the still is shown in Figure 2.3.

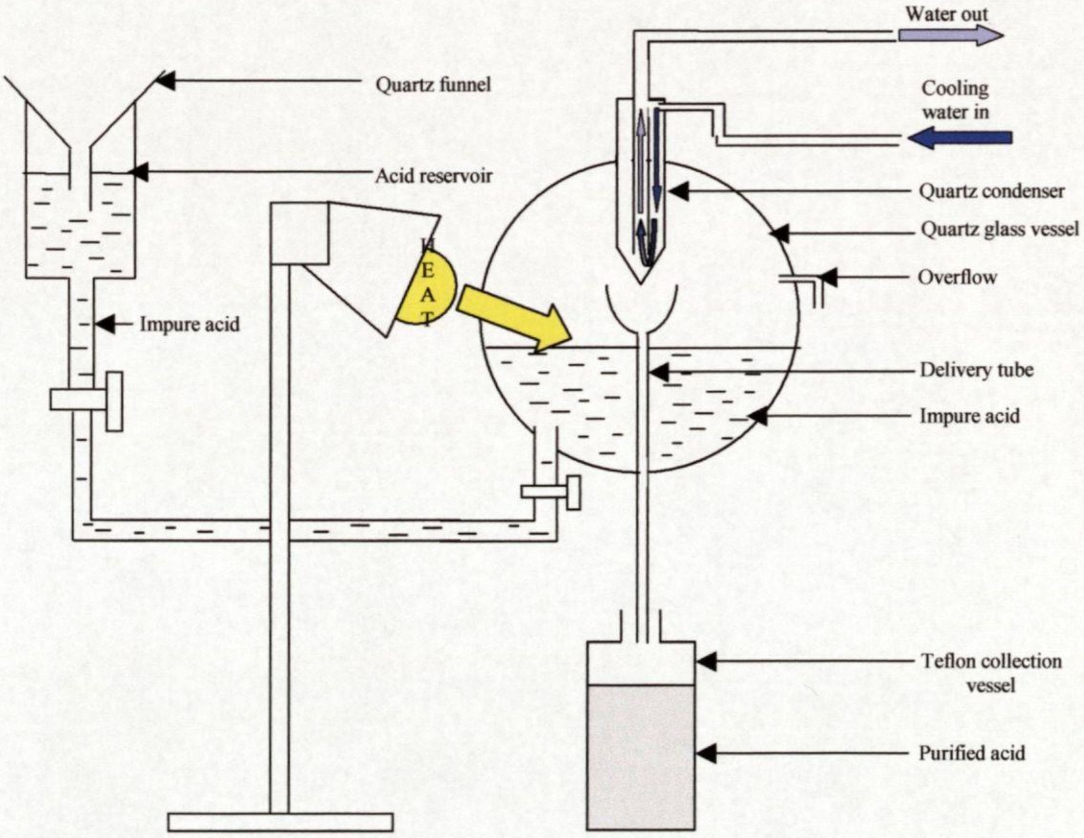


Figure 2. 3 Schematic diagram of procedure for the purification of acids.

AnalaR grade acids (VWR) were introduced into the still from a reservoir *via* a delivery tube. A heating lamp was directed onto the surface of the impure acid contained within the quartz still. The purified acid evaporated and condensed on the cold finger leaving behind involatile impurities such as trace metals. After condensing, the pure acid travelled to the receptacle (1 L Teflon acid washed HDPE bottle) *via* a delivery tube and the first 100 mL collected was discarded before collection was started. The purified acids were titrated against sodium hydroxide of known concentration, and concentrations were measured at 11.9 M for sub-HNO_3 and sub-HCl and ~ 17 M for sub-CH_3COOH.

2.2.3 *Acid Cleaning Procedures*

HDPE sample and reagent bottles were cleaned by soaking in 2 % (v/v) Decon 90 in MQ water for 24 h, 0.1 M NaOH for 24 h, 5 M HCl for one week, and finally 2 M HNO_3 for one week. A thorough MQ water rinse was carried out after each step. Bottles were filled with acidified MQ water (1 % sub-HNO_3, pH 2) and stored inside two re-sealable plastic bags.

2.2.4 *Preparation of Solutions*

Laboratory handling of samples, reagents and standards were undertaken in a Class 100 laminar flow cabinet. All reagents were of AnalaR grade and were supplied by VWR (Dorest, UK) unless otherwise stated. Aqueous reagents were prepared using MQ water. Ultrapure HCl was prepared by sub-boiling (sub) quartz distillation (Howard and Statham, 1993), and ultrapure NH_4OH by isothermal distillation (iso) by the methods described in this section. Standard solutions of 1×10^{-4} M were prepared from atomic absorption spectrometry standard solutions (SpectrosoL; 1000 mg L^{-1}), acidified to pH 2 using sub-HNO_3. An aqueous solution of 1.0 M *n*-2-

hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES, pH buffer) was prepared and the pH adjusted to 7.7 using iso-NH_4OH. Ammonium acetate buffer (pH 5.5) was prepared from sub-CH_3COOH and iso-NH_4OH, to give a final concentration of 1 M. Aqueous stocks of 8-hydroxyquinoline (oxine; 0.01 M) and ammonium pyrrolidine dithiocarbamate (APDC; 0.1 M) were prepared by dissolution in MQ water. Aqueous solutions of potassium thiocyanate (KSCN; 1 M) and mercury (II) nitrate [$Hg_2(NO_3)_2$], were prepared by dissolution of the salts in MQ water.

High density polyethylene (HDPE; Nalgene, Rochester, NY, USA) sample, standard and reagent bottles and Teflon® magnetic follower were cleaned by soaking in 2 % (v/v) Decon 90 in MQ water for 24 h, 0.1 M NaOH for 24 h, 5 M HCl for one week, and 2 M HNO_3 for one week. A MQ water rinse was carried out after each step. Bottles were filled with acidified MQ water (1% v/v sub-HNO_3, pH 2) and stored inside two re-sealable plastic bags until required for use. Polycarbonate filters (47 mm filter diameter; 0.4 and 0.2 μm pore diameter; Cyclopore™, Whatman International Ltd, Kent, UK) were cleaned by soaking in sub-HCl (pH 2; 24 h) with a subsequent rinse with MQ water. A Nalgene filtration unit (250 mL; Nalgene Europe Ltd, Neerijse, Belgium) was cleaned by soaking in 2 % (v/v) Decon 90 in MQ water for 24 h, 0.1 M NaOH for 24 h, 1 M HCl (24 h). A thorough MQ water rinse was carried out after each step.

2.2.5 *Laboratory Filtration*

In order to avoid settling and ensure homogeneity, samples were placed in a Class 100 laminar flow cabinet and stirred (10 min.) using a magnetic follower (Teflon®, VWR, Dorset, UK) preceding filtration. Samples were filtered at low pressure (*ca* 0.6 bar) to avoid lysis of any biological cells present. In order to examine sample stability over the 8 h duration of the actual filtration experiment, a 200 mL aliquot was filtered immediately upon return to the laboratory (t = 1 h), and again 8 h later (t=9). Samples for the filtration experiment were filtered and the filtrate collected in sequential four 50 mL aliquots in pre-weighed, acid cleaned bottles. Filtrations were performed in triplicate, with a separate membrane used for each set of four aliquots. Filtrates were acidified (pH 2) with sub-HCl and stored at *ca* 4°C until analysed. The filtration unit was thoroughly rinsed with MQ water in between each filtration. These samples were filtered within 9 h of collection. Procedural blanks were determined using MQ water, and metal concentrations found to be below the detection limits in every case.

2.2.6 Analysis

Total dissolved trace metal concentrations in samples with salinity < 5 (*i.e.* samples 1, 2 and 4) were determined by ICP-MS (VG elemental PQ2 turbo; Winsford, Cheshire) fitted with an Ebdon high solids 'V' groove nebuliser with a fixed Ar plasma gas flow of 1 L min^{-1}. The analyte was ionised at 1350 W with a detection dwell time of 40 ms. An indium internal standard (9.0 nM L^{-1}) was used for instrumental drift correction.

Total filterable trace metal concentrations in the remaining three samples (*i.e.* samples 3, 5 and 6) were determined by adsorptive cathodic stripping voltammetry (AdCSV) with a hanging Hg drop electrode (663 VA stand, Metrohm, Herisau, Switzerland) or a rotating disc electrode (RDE; Glassy carbon; Metrohm) interfaced with a µAutolab (EcoChemie, Utrecht, The Netherlands). All samples were UV-digested (5 h; 400 W medium pressure lamp; Photochemical Reactors, Slough, UK) in 30 mL quartz tubes after the addition of H_2O_2 (8.8 mM, final concentration), prior to analysis (Achterberg and van den Berg, 1994). The acidified samples were adjusted to the appropriate pH by the addition of iso-NH_4OH immediately prior to analysis.

Total dissolved Cu was determined using AdCSV in conjunction with the complexing ligand oxine (25 μM final concentration) in the presence of HEPES (pH 7.7; 0.01 M final concentration) as a pH buffer (van den Berg, 1986). Cd was determined with a RDE in mercury nitrate (30 μM, final concentration) and potassium thiocyanate (5 mM, final concentration), using ammonium acetate (pH 5.5; 0.1 M final concentration) as a pH buffer (Fischer and van den Berg, 1999). Zn was determined using AdCSV with APDC as the complexing ligand (25 mM final concentration) and HEPES (pH 7.7; final concentration 0.1 M) as a pH buffer (van den Berg, 1985).

Voltammetric peaks were quantified by standard addition and repeat measurements were made until <5 % RSD was obtained (n=3). Detection limits for all metals were measured and the accuracy of the trace metal analyses using both ICP-MS and voltammetric methods was assessed using CRM SLRS-3 (National Research Council, Canada). SPM was determined gravimetrically after filtration of 250 mL of sample through a 0.4 μM filter. DOC was analysed using a Shimatzu TOC 5000A analyser, by Mr S. Badr.

2.3 Results and Discussion

2.3.1 *Master Variables*

Table 2.1 presents physico-chemical data for the samples. River flows measured at station 1 were 56.4 m^3sec^{-1} and 2.8 m^3sec^{-1} for the April and September campaigns respectively. The pH and temperature were uniform between sampling sites varying ± 0.5 pH units and ±0.5 °C, respectively. Any changes in apparent metal fractionation in this study may in principle, be caused by variations in salinity, SPM and pH, *inter alia*, at the equivalent salinities.

Table 2.1 Physico-chemical parameters and locations for samples collected on 05/04/01 (sites 1 to 3) and on 27-09-01 (sites 4 to 6) (nd=not determined)

Date	Location	Site #	pH	Salinity	Temp. ^{0}C	Dissolved oxygen (%)	SPM (mg L^{-1})	DOC (µM)
05-04-01	Gunnislake	1	7.2	0	10.1	96.8	261	780
	Calstock	2	7.2	5	9.7	97.1	171	480
	Saltash	3	8.1	25	10.6	94.4	241	233
27-09-01	Gunnislake	4	8.8	~0	15.0	86.8	132	nd
	Calstock	5	8.6	14	15.7	97.2	130	nd
	Saltash	6	8.6	32	15.6	93.9	72	nd

SPM concentrations and salinity showed the general trends that are associated with the Tamar Estuary and all variables were within ranges observed by Morris *et al.* (1982). Concentrations of DOC were consistent with those reported by Miller (1999) for the Tamar Estuary.

2.3.2 Analytical Figures of Merit for Trace Metal Analysis by ICP-MS and AdCSV

The lower and upper limits of analysis, together with the precision at the lower limit and correlation coefficient for the calibration, are shown in Table 2.2.

Table 2.2 Analytical figures of merit for analyses (n=3). N/A= not applicable

Metal	*Method*	*Precision at lower limit (%)*	*Linear Range (nM)*	*Correlation (R^2)*
Al	ICP-MS	3.3	5 to ≥ 1300	0.999
Cd	ICP-MS	3.9	0.05 to ≥ 100	0.999
	AdCSV	3.7	0.02 to ≥ 17.6	N/A
Co	ICP-MS	4.5	0.25 to ≥ 17.0	0.998
Cr	ICP-MS	4.6	0.20 to ≥ 9.60	0.999
Cu	ICP-MS	1.5	0.30 to ≥ 170	0.999
	AdCSV	2.8	0.20 to ≥ 100	N/A
Mn	ICP-MS	4.1	0.50 to ≥ 120	0.999
Mo	ICP-MS	4.3	0.50 to ≥ 50	0.998
Pb	ICP-MS	5.0	0.05 to ≥ 2.00	0.997
U	ICP-MS	4.7	0.01 to ≥ 4.20	0.998
Zn	ICP-MS	3.9	0.30 to ≥ 240	0.998
	AdCSV	4.2	0.30 to ≥ 170	N/A

The results indicate that the both ICP-MS and voltammetry were ideally suited for total dissolved trace metal measurements in both the river and estuarine samples respectively. The accuracy of trace metal analyses using both ICP-MS and voltammetric methods were assessed using CRM SLRS-3 (National Research Council, Ottawa, Canada). CRM was UV-digested prior to voltammetric analysis (Achterberg and van den Berg, 1994).

Table 2. 3 Results of analysis of SLRS-3 CRM using ICP-MS and voltammetry, with confidence intervals, for measurements quoted as $\pm 2\ \sigma$ ($n \geq 3$).

Metal	*Method*	*Certified Value*	*Measured Value*
Al	ICP-MS	1.33 ± 0.11 (μM)	1.32± 0.04 (μM)
Cd	ICP-MS	0.171± 0.018 (nM)	0.175 ± 0.015 (nM)
	AdCSV	0.171± 0.018 (nM)	0.182 ± 0.010 (nM)
Co	ICP-MS	0.458 ± 0.052 nM)	0.470 ± 0.022 (nM)
Cr	ICP-MS	5.77± 0.30 (nM)	5.87 ± 0.32 (nM)
Cu	ICP-MS	20.7 ± 1.8 (nM)	20.5 ± 0.7(nM)
	AdCSV	20.7 ± 1.8 (nM)	20.3 ± 0.4 (nM)
Pb	ICP-MS	0.417 ± 0.034 (nM)	0.408 ± 0.055 (nM)
Mn	ICP-MS	70.9 ± 5.5 (nM)	71.2 ± 1.8(nM)
U	ICP-MS	0.189 ± 0.017 (nM)	0.189 ± 0.023 (nM)
Zn	ICP-MS	16.0 ± 1.4 (nM)	17.3 ± 0.5(nM)
	AdCSV	16.0 ± 1.4 (nM)	15.9 ± 0.5 (nM)

The results in Table 2.3 indicate a good agreement between the observed and certified values, with confidence intervals for measurements quoted as $\pm 2\ \sigma$.

2.3.3 Changes in Filtration Rate

The extent of membrane clogging was followed through changes in filtration rate (mL min^{-1}). Figure 2.4 shows filtration rates for both 0.4 and 0.2 μm pore filters for each sample collected during the April (sites 1, 2 and 3) and September campaigns (sites 4, 5 and 6).

Whilst the concentrations of SPM between the two months were not wildly dissimilar (particularly samples 2, 4 and 5; Table 2.1), the overall flow rates were quite different, with much higher flow rates exhibited by the September samples. Furthermore, the most marked relative reductions in flow rate were observed in the April samples, particularly between the first and second aliquots. This implies that the characteristics of the suspended particles, with respect to filter clogging, were distinct between the two seasons. Absolute flow rates were generally higher with the 0.4 µm filters, relative to the 0.2 µm fitlers, as might be anticipated.

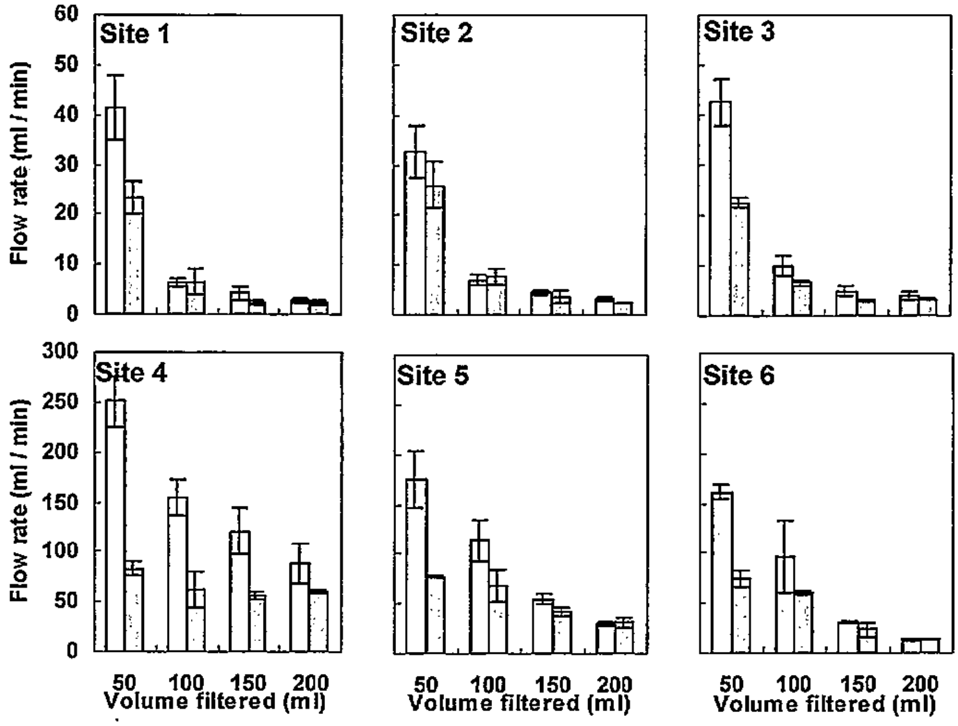


Figure 2.4 Flow rate (mL min^{-1}) of sample through 0.4 µm (white bar) and 0.2 µm (grey bar) filters *versus* volume of sample filtered on during April sites, 1, 2 and 3 and during September, sites 4, 5 and 6. Note change in y- axis scale. (n=2 for each filter)

2.3.4 Sample Stability

A *t*-test showed that the concentrations of metals in filtrates from the 200 mL aliquot samples filtered 1 and 9 h after collection were not significantly different (99 % confidence interval) for all metals, indicating no measurable change in partitioning over this time period. Samples filtered within 1 h of collection gave combined filtration and measurement errors of ≤ 15 % and ≤ 14 % (99 % confidence level; n=6) for the 0.4 µm and 0.2 µm filter, respectively, for all metals. For the t = 9 h samples, errors were ≤ 12 % and ≤ 11 % for the 0.4 and 0.2 µm filters respectively. Mean total dissolved trace metal concentrations in the 200 mL aliquot samples (both 0.2 and 0.4 µm filtered) are presented in Table 2.4.

Table 2. 4 Concentrations of metals in the filtrate of the 200 mL samples for both the April and September campaigns following filtration through 0.2 and 0.4 μm pore size filters. Mean concentrations ± 3 σ (n=12) are shown

Site	*Filter pore (μM)*	*Concentration of Metal (nM)*								
		Al	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Pb**	**U**	**Zn**
1	0.4	101± 3	0.206 ± 0.009	2.64 ± 0.30	3.99 ± 0.33	83.9 ± 3.6	34.0 ± 0.3	0.442 ± 0.078	0.162 ± 0.012	158 ± 7
	0.2	65.6 ± 4.8	0.149 ± 0.045	2.08 ± 0.31	3.72 ± 0.05	76.0 ± 2.7	29.7 ± 0.9	0.290 ± 0.081	0.132 ± 0.012	155 ± 10
2	0.4	41.6 ± 3.1	0.257 ± 0.016	0.78 ± 0.03	4.36 ± 0.33	48.8 ± 1.0	4.62 ± 0.11	0.108 ± 0.015	0.134 ± 0.007	46.7 ± 0.3
	0.2	22.9 ± 2.7	0.257 ± 0.009	0.69 ± 0.05	4.28 ± 0.06	48.8 ± 1.2	3.97 ± 0.24	0.090 ± 0.015	0.135 ± 0.002	44.5 ± 0.7
3	0.4	nd	0.248 ± 0.024	nd	nd	28.8 ± 1.8	nd	nd	nd	43.9 ± 0.6
	0.2	nd	0.239 ± 0.045	nd	nd	20.9 ± 1.2	nd	nd	nd	39.8 ± 2.4
4	0.4	32.3 ± 2.4	0.173 ± 0.021	4.57 ± 0.05	3.33 ± 0.31	67.2 ± 1.8	8.80 ± 0.10	0.260 ± 0.030	0.137 ± 0.012	32.7 ± 0.6
	0.2	21.5 ± 1.2	0.171 ± 0.036	3.90 ± 0.40	3.12 ± 0.31	55.1 ± 5.1	8.83 ± 0.81	0.245 ± 0.024	0.136 ± 0.009	27.9 ± 0.6
5	0.4	nd	0.222 ± 0.018	nd	nd	43.9 ± 0.9	nd	nd	nd	146 ± 4
	0.2	nd	0.197 ± 0.024	nd	nd	37.3 ± 2.7	nd	nd	nd	129 ± 5
6	0.4	nd	0.309 ± 0.027	nd	nd	30.3 ± 1.6	nd	nd	nd	53.2 ± 3.3
	0.2	nd	0.230 ± 0.033	nd	nd	17.9 ± 0.1	nd	nd	nd	41.3 ± 0.1

If the amount of colloidal metal is defined as the difference in metal concentrations between the 0.4 µm and 0.2 µm filtrations [following Wu and Luther (1996)], then it can be seen that this fraction is highly variable for each metal, both spatially and temporally (Table 2.5). Whilst this variability reflects the actual concentrations of colloidally-associated metal in these waters, it may also be due, in part, to the differential retention of various colloidal fractions as filtration progressed (Hassellöv, 1999; Hassellöv *et al.*, 1999; Morrison and Benoit, 2001). For all metals examined, except Cr, the colloidal fraction is significant in at least one sample: a finding consistent with Greenamoyer and Moran (1997), who concluded that the colloidal fraction of trace metals, as a proportion of the total 'dissolved' concentration, becomes more important in estuarine and nearshore regions of relatively high SPM loading.

Table 2.5 The percentage of colloidal metal (defined as the difference in metal concentration in filtrate passing through a 0.4 and 0.2 µm pore diameter membrane as a percentage of total concentration passing through 0.4µm). nd= not determined.

	Metal % Colloidal fraction								
Site	Al	Cd	Co	Cr	Cu	Mn	Pb	U	Zn
1	35	28	21	7	9	13	34	19	2
2	45	0	12	2	0	14	17	0	5
3	nd	4	nd	nd	27	nd	nd	nd	9
4	33	1	15	6	18	0	6	0	15
5	nd	11	nd	nd	15	nd	nd	nd	12
6	nd	26	nd	nd	41	nd	nd	nd	22

The amount of DOC (Table 2.1) at sites 1, 2 and 3 did not correlate with the proportions of colloidally associated metals in all cases. This has been previously reported by Hill and Aplin (2001) and could be due to the differential association of metals with colloids over a range of physical and chemical characteristics.

2.3.5 Comparison of this Study in Relation to Earlier Tamar Studies

In all cases the concentrations of Al, Cd, Cu, Mn, and U (Table 2.4) are slightly lower but generally consistent with previously reported dissolved trace metal concentrations in the Tamar for Al (74 to 2000 nM; Morris *et al.*,1986A), Cd (0.3 to 1.1 nM; Morris *et al.*, 1986B), Cu (3 to 183 nM; Ackroyd *et al.*,1986); Mn (9 to31 nM; Knox *et al.*, 1981), U (1 to 15 nM; van den Berg *et al.*,1991) and Zn (15 to 446 nM; Ackroyd *et al.*,1986). The concentrations of Co, Cr, Pb are consistent with concentrations reported for other European estuaries for Co (Mersey Estuary, UK; 0.5 to 40 nM; Martino *et al.*, 2002), Cr (Po Estuary, Italy; 3 to 4 nM, Pettine *et al.*, 1997), and Pb (Mersey, UK; 0.4 to 6 nM; Martino *et al.*, 2002). Non-conservative behaviour has been reported for Cd, Cu and Zn in the Tamar Estuary (Millward, 1995) and this is found to be indicated by this study (Table 2.4), but only three points per survey were available. Zn showed concentration maxima at site 2, probably due to pore water infusions from sediment mobilisation (Ackroyd *et al.*, 1981; Morris *et al.*, 1981).

2.3.6 Variations in Metal Filtrate Concentrations with Sample Volume Filtered

April Campaign

Al and Pb metals demonstrated the largest colloidal fraction (35 to 45 % for Al and 17 to 34 % for Pb; Table 2.5). These metals are particle reactive elements that often undergo salinity-induced flocculation and coagulation with colloids (Elbaz-Poulichet *et al.*, 1984). Concentration profiles for consecutive aliquots of Al, Pb and Cd, presented in Figure 2.5, were characterised by rapid decline in trace metal concentration after the first or second aliquot and values fell outside the analytical errors given in Table 2.4. Trace metal concentration profiles obtained with both pore size filters at all sites examined showed concentrations of Al, Pb and Cd reduce by ≥10 % (n=4), ≥19 % (n=6) and ≥18 % (n=4) respectively from the first (0 to 50 mL) aliquot to the fourth (150 to 200 mL) aliquot. At site 1 the colloidal fraction was similar for Al (35 %; Table 2.5) and Pb (34 %) and both elements showed decreasing

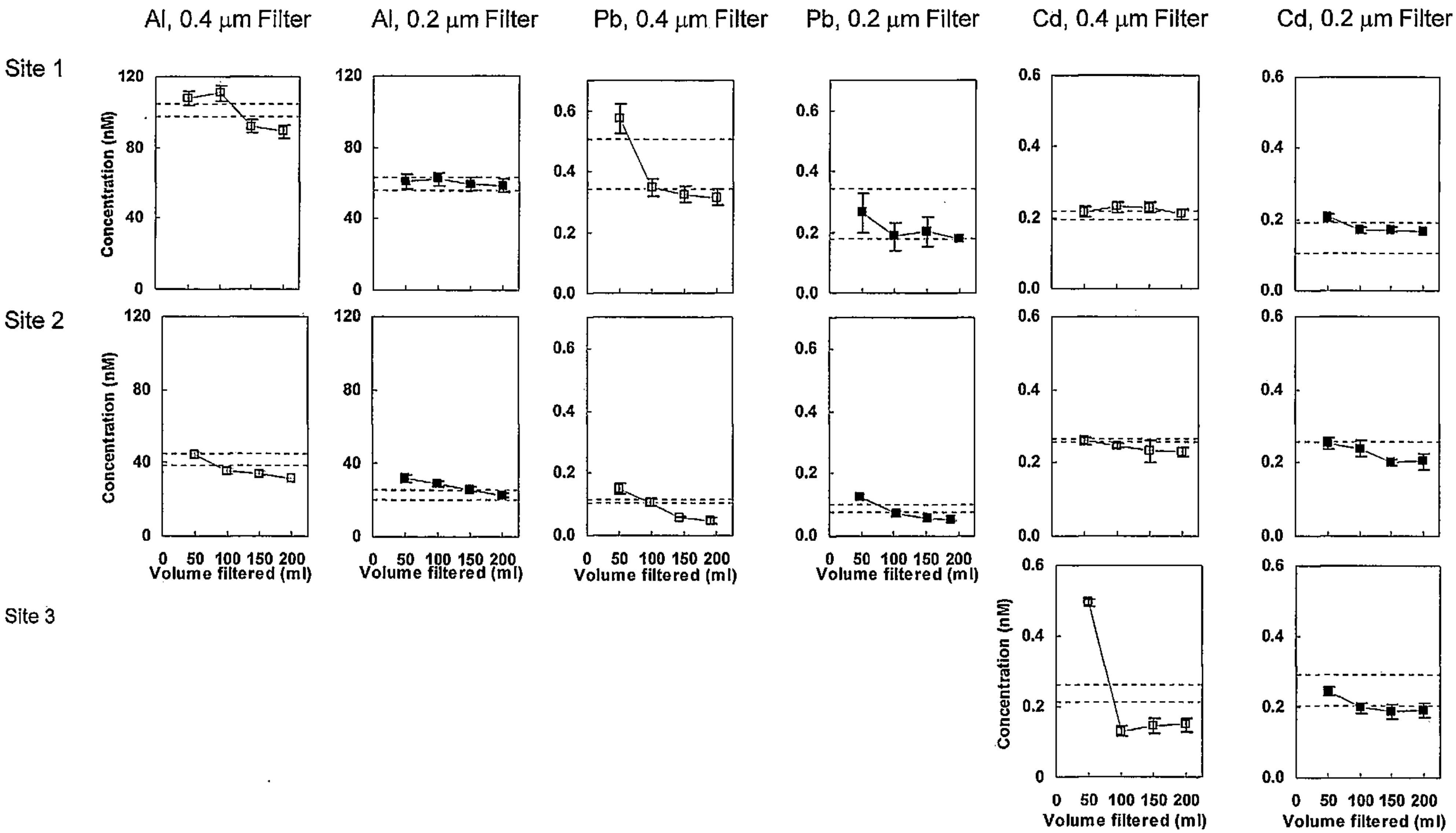


Figure 2. 1 Concentrations (nM) of metals (Al, Pb, Cd, Co, Cu, U, Cr, Zn and Mn,) in filtrate versus filtered volume (mL) on 05-04-01. Data reported for site 1 and 2. Error bars represent ± 3 s (n=3) of analysis of individual aliquots. Dotted lines represent ± 3 s (n=12) around the mean concentration of the of 200 mL aliquot sample. Open squares represent filtration though 0.4 μm pore size filter. Filled Squares represent filtration though 0.2 μm pore size filter.

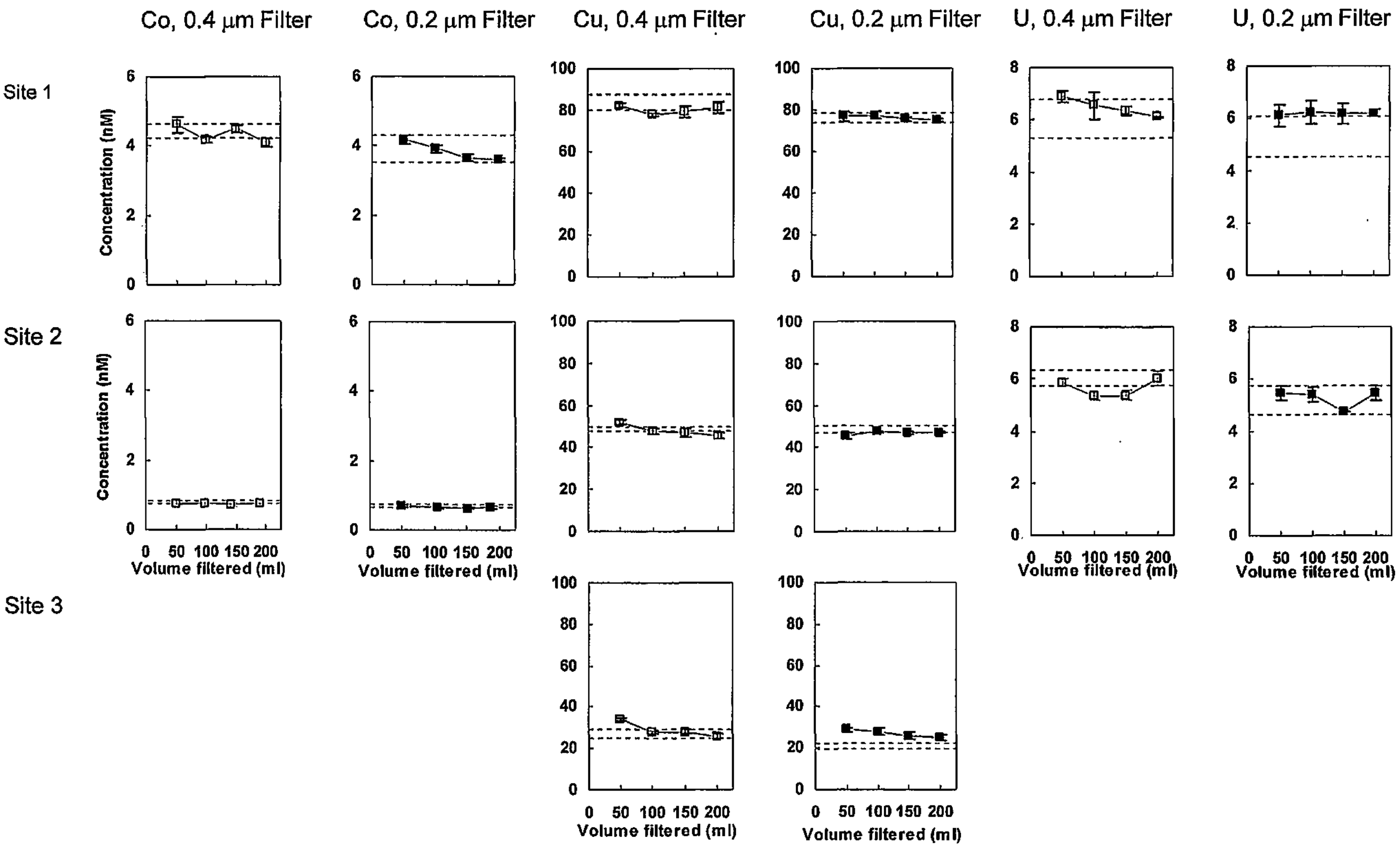


Figure 2. 5 Continued...

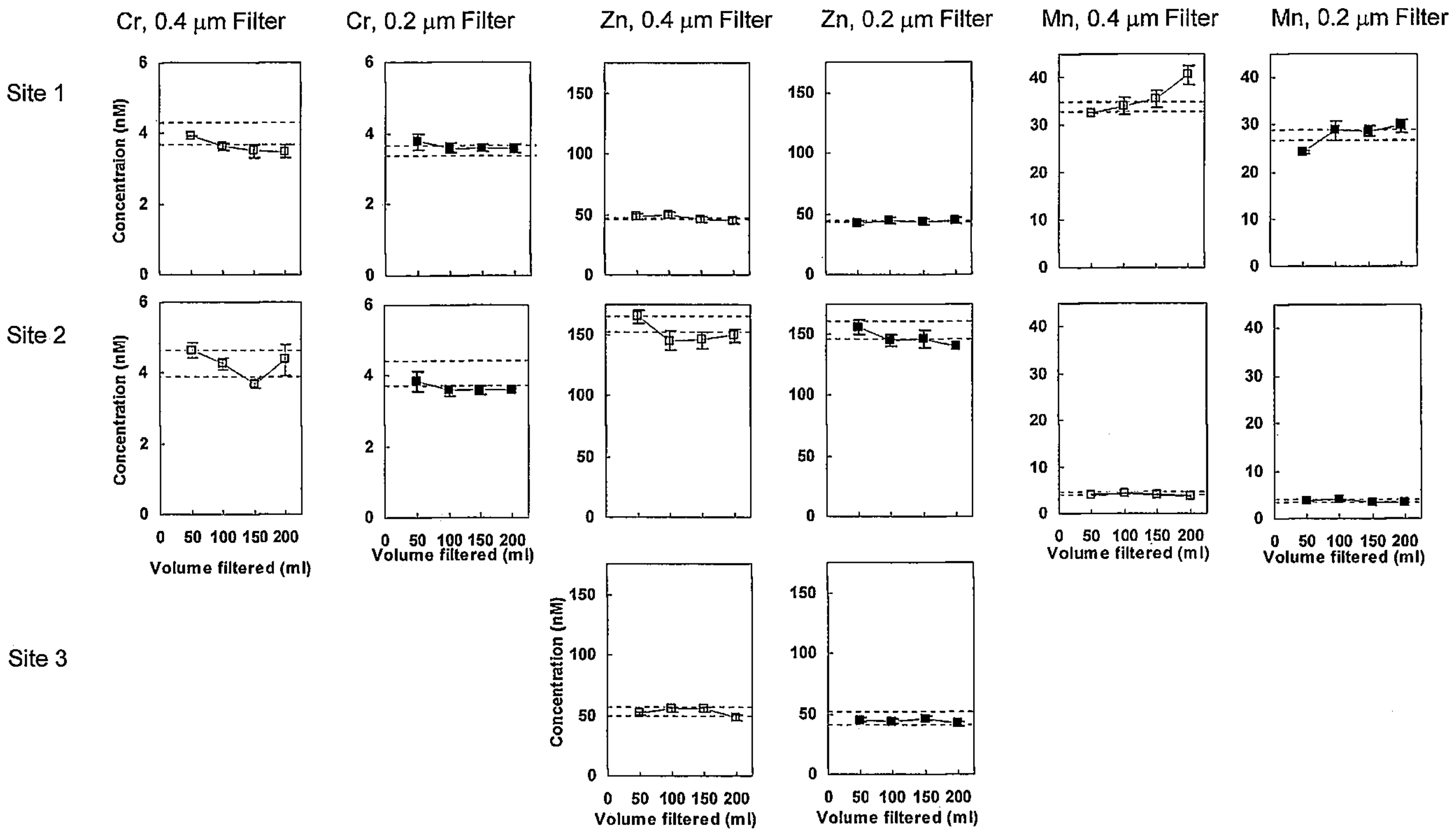


Figure 2. 5 Continued...

concentration profiles with the amount of sample filtered through the 0.4 µm filter. These profiles were not observed for the 0.2 µm filter, implying that the filtration volume affected fractions < 0.4 µm and > 0.2 µm. At site 2 colloidal fractions remained high (17 % for Pb and 45 % for Al; Table 2.5) and the filtration profile indicted changes in concentration with filtration volume for the < 0.4µm fraction. Site 1 shows the highest colloidal fraction, for Cd (28 %; Table 2.5) and is similar to the Yarra Estuary colloid concentrations (20 %) reported by Hart and Davies (1981). However for the 0.4 µm filter at this site, no change in concentration in the filtration profile was observed whilst changes were observed for the 0.2 µm pore filter, indicating species > 0.2 µm and < 0.4 µm were affected by filtration volume. Sites 2 and 3 had low colloidal fraction (≤ 4 %; Table 2.5) and showed changes in concentration in the filtration profile.

The reduction is believed to be due to two reasons. Firstly, the rapid clogging of the membrane through the accumulation of colloidal and particulate matter on the filter surface and thus the reduced porosity caused retention of colloids that would normally pass through the filter with ease (Danielsson, 1982; Laxen and Chandler, 1982). Secondly, as the filtration proceeded the concentration of particulate matter on the filter surface increased relative to particulate matter concentration in the raw sample. This perturbed speciation equilibrium, by providing binding sites for trace metals, and hence prevented their passage through the filter. Both Sharp (1973) and Benes and Steinnes (1974) demonstrated this effect but neither study suggested alternative methods to circumvent this problem.

In addition, Hassellöv (1999) and Hassellöv *et al.* (1999) used flow field flow fraction (flFFF) coupled to ICP-MS to investigate the competition between iron and carbon based colloidal carriers for trace metals in river water. In the study, it was clear that Fe colloids had a larger size distribution than carbon colloids and Pb and Al appeared to show a strong association with the relatively large Fe colloid spectra (Cd was not investigated). It is possible in our study that the interaction of Pb and Al with the larger Fe based colloids led to retention of these metals on the filter as it became clogged. Other metals such as Cu and U that may have been associated with smaller carbon based colloids, were not retained. Co showed varying colloidal fractions at site 1 (21 %) and 2 (12 %). However, filtration profiles for Co were similar for both sites and both size filters. Profiles for U were unchanging with the exception of the samples filtered through the 0.4 μm pore size at site 2. Here there was a decrease in concentration with volume of sample filtered followed by an increase.

Cu showed varying colloid concentration at the three sites. At site 1 (9 % colloidal fraction; Table 2.5) and site 2 (0 % colloidal fraction) filtrate profiles fell outside the analytical errors [calculated from 1σ (n=6) for the 200 mL filtered aliquot; represented by dashed lines on Figure 2.5] for the 0.4 μm filter but not for the 0.2 μm filter indicating that the filtration volume affected species < 0.4 μm and > 0.2 μm. At site 3 (with the highest colloid concentration for this element of 27 %; Table 2.5) profiles fell outside the analytical errors (Table 2.4) and in the case of the 0.4 μm filter, a clear decrease was observed in the first aliquot compared to subsequent aliquots and showed that filtration volume affected species < 0.4 μm. For the 0.2 μm filtration, the profile probably reflects contamination of the sample, rather than filtration effects since all concentrations for the profile are higher than the analytical error for all profiles (Table 2.4).

Cr and Zn were found to be mainly unassociated with colloids demonstrating the lowest colloidal fractions of Cr ≤7 %, (n=2) and Zn ≤9 %, (n=3) (Table 2.5) at each site. Both elements show similar colloid concentration in the river water compared to estuarine water, demonstrating the trend observed by Hart and Davies (1981) for Zn in the Yarra. Accordingly, a relatively unchanging concentration profile for these metals was observed at each site, indicating that clogging had little effect on filtrate concentrations. Mn demonstrated a filtration profile that was distinct from that of other metals by showing an increase in concentration after the first 50 mL aliquot that subsequently remained relatively uniform. No other metals exhibited this behaviour, which suggests a colloidal association that was distinct for Mn compared to the other metals examined. A possible reason is that even though trace metal adsorption to polycarbonate filters is reported to be low (Laxen and Chandler, 1982; Buffle and Leeuwen, 1992), upon commencement of filtration, Mn had a high affinity towards to adsorption sites on the filter and was adsorbed and retained. The number of sites decreased with filtration and rising concentrations of Mn appeared in the filtrate after the first 50 mL aliquot. This Mn filtration profile has been reported previously (Horowitz *et al.*, 1996; Morrison and Benoit, 2001) for tortuous path filters, but not by Tappin *et al.*, (2001) for the Trent Estuary, a markedly more turbid system than the Tamar.

September Campaign

The profiles were similar to those of the April campaign samples (Cd, Cu and Zn profiles are presented in Figure 2.6 at sites 4, 5 and 6). Filtrate concentrations, decline with volume filtered, though the reduction is less pronounced than that observed in the April, 2001 samples. Concentrations in 200 mL aliquot filtered samples (Table 2.4) are generally higher in the April study than the September study.

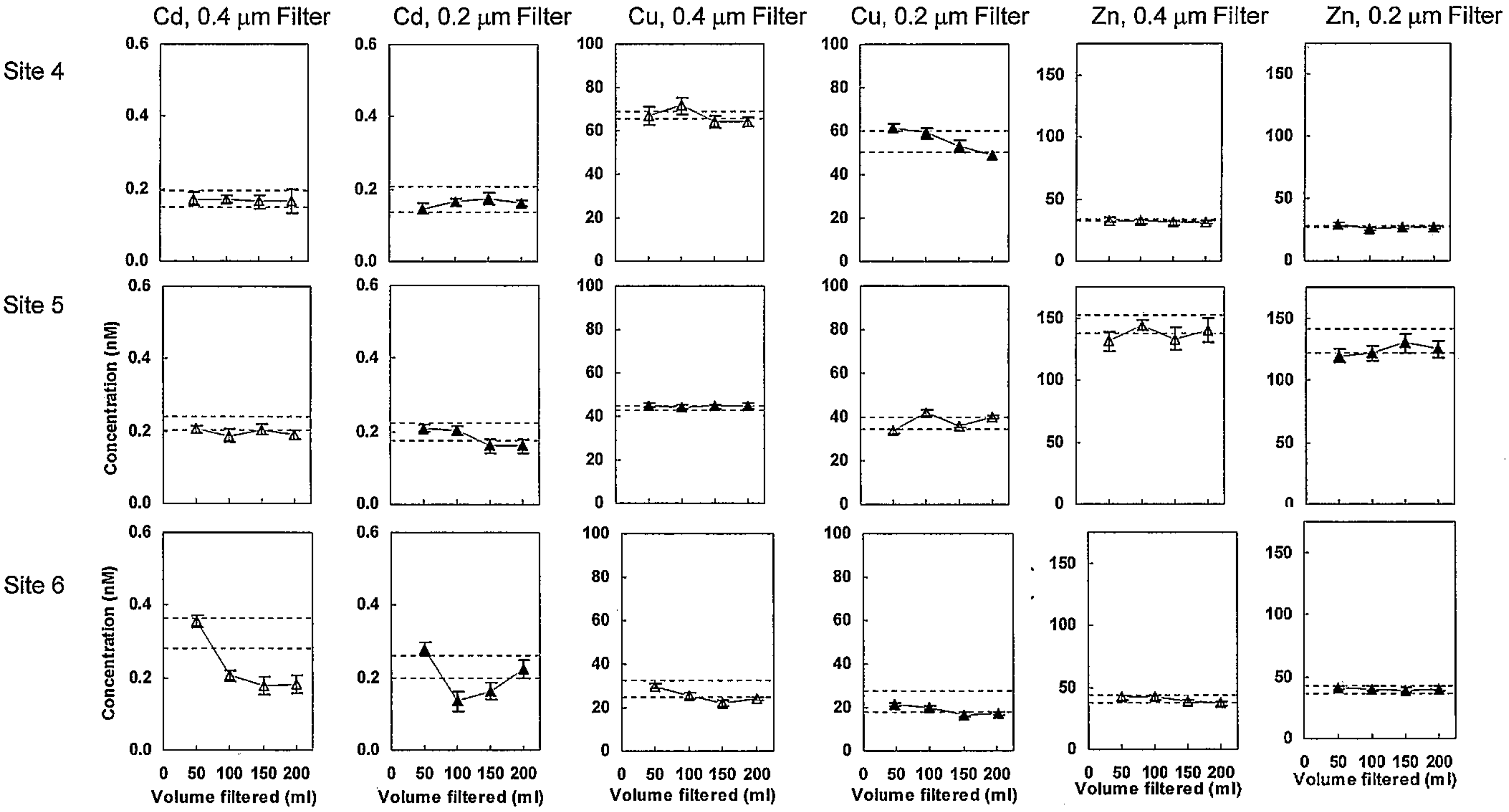


Figure 2. 1 Concentrations (nM) of metals (Cd, Cu, and Zn) in filtrate versus filtered volume (mL) on 27-09-01. Data reported for sites 4, 5 and 6. Error bars represent ± 3 σ (n=3) of analysis of individual aliquots. Dotted lines represent ± 3 σ (n=12) of 200 mL aliquot sample around the mean concentration of the bulk sample. Open triangles represent filtration though 0.4 μm pore size filter. Filled triangles represent filtration though 0.2 μm pore size filter.

All profiles remain within the analytical error given in Table 2.4 at all sites with the exception of Cd (0.2 and 0.4 μm pore filter) and Cu (0.2 μm pore filter) at site 6, indicating a significant amount of clogging occurred with samples at this site. The colloidal phase concentrations were typically higher in the most saline samples compared to the riverine samples (Table 2.4) and concentration profiles as a function of volume filtered reflect this (Figure 2.6) and suggests that factors other than salinity and SPM may be influencing the colloidal speciation in the samples from this site.

2.4 Conclusions

Trace metal concentrations are equal to or for the most part higher in filtrates of samples filtered through 0.4 compared to 0.2 μm pore polycarbonate filters and is indicative of the presence of colloids in samples in the size range 0.2 to 0.4 μm. Of the metals investigated Al, Cd, Pb and Mn, often found to be associated with colloids, exhibited the most pronounced differences at all sites, and for Cd, with change in season. The difference between 0.4 and 0.2 μm filtrate concentration was labelled 'the colloidal phase'. It is likely that the colloidal phase variations observed in this study are caused by changes in salinity and SPM concentrations. Because of the marked variations in these master variables, the selection of one reliable filtration protocol suitable for all metals in all matrices appears inherently difficult for estuarine work. This study highlights the importance of colloids (in the size range 0.2 to 0.4 μm) to membrane clogging and suggests that colloids themselves could account for a significant part of clogging of membrane filters.

In the filtration experiment, Al, Cd, Pb and Mn exhibited changes in trace metal filtrate concentrations as a function of volume filtered. At most sites, Pb showed a large decrease in metal concentrations after the filtration of 50 mL, whereas Al and

Cd concentrations decreased after the filtration of larger volumes (≥ 100 mL). Mn concentrations increased with filtration volume. The concentration profiles for Al, Pb and Cd were probably due to retention of colloidally associated metals that were prevented from passage through the filter due to reduced porosity through particulate matter accumulation on the filter surface may be due to aqueous ion adsorption to this particulate matter. The lower concentrations observed for Mn in the first 50 mL of filtration were probably a consequence of binding to adsorption sites on the filter that became filled as filtration progressed leading to an increase in Mn concentration in the filtrate. The concentrations of other metals (Co, Cr, Cu, Zn and U) were relatively uniform, unperturbed by filtration.

Concentrations of colloidally associated metal showed variability between riverine and estuarine waters. Higher colloidally associated Al concentrations were observed in estuarine water compared to river water. Mid estuarine inputs of Al have been previously reported in the Tamar Estuary by Morris *et al.,* (1986B), who attributed inputs to desorption and or/dissolution from tidally resuspended sediment. It is possible that this occurred in our study and the Al became associated with colloidal material, thus increasing the colloidal fraction. Similarly, Mn showed an increase in colloid concentration in the estuarine sample. Morris *et al.,* (1982) reported mid estuarine enrichment of Mn into the Tamar Estuary and attributed it to reductive solubilisation of Mn oxides from upper sediment combined with upward diffusion of reduced Mn(II) from the permanently deposited sediment. Mn(II) is reactive in sufficiently oxic environments and is therefore likely to form Mn oxyhydroxides or associate with other colloidal material, increasing the colloidal Mn concentrations. The variation of colloidal concentrations for Cd during April agree with results from the River Yarra and its estuary, reported by Hart and Davies (1981), who measured higher colloid concentrations in the river compared to the estuary. However, in September, Cd was higher in the estuary than in the river, indicating seasonal behaviour. Colloid concentrations of Co were similar to those reported by Wen *et al.* (1999), for an estuarine system, and concentrations decreased from river to estuary, probably due to flocculation and settling of colloids upon entering the estuary. Cu showed a significantly larger colloidal fraction at site 3 and 6 with respect to sites 1 and 2, and sites 4 and 5 respectively but overall the colloidally associated Cu concentrations were similar to those observed in the Yarra system (≤ 26 % colloidal fraction). In saline estuarine waters, dissolved Cu is extensively complexed with dissolved organic ligands. A significant proportion of this fraction may be colloidal (van den Berg *et al.,* 1981) and the data from sites 3 and 6 are consistent with these findings. The origin of the colloidal Cu is unknown at these sites. U showed a large riverine colloidal concentration for the April sample, which decreased upon entering the estuary. These findings are in contrast to van den berg (1991) for the Tamar, but are consistent with the more extensive study carried out by Hassellöv (1999). Pb colloid concentrations were in contrast to the River Yarra and estuary concentrations, reported by Hart and Davies (1981), and showed a decrease in colloidal fraction with salinity. This was probably due to flocculation and settling in the TMZ. Colloidally associated Zn concentrations increased with salinity, in contrast to the literature; nevertheless the concentration of Zn associated colloids was low (≤ 9 %) at all sites. A similar trend was observed for September samples although at this time, colloidal Zn was in the range 12-22 %. Colloidal Cr was < 10 % at all sites. However there is little information on the colloidal association of Cr in rivers and estuaries against which the data can be compared.

For optimum filtration protocols, it is essential to design an appropriate site-specific, metal specific protocol before undertaking any sampling programme for the determination of trace metals in estuarine waters. Based on the findings presented in this paper, such a protocol should take into account the following general guidelines:

1. A rigorous cleaning procedure should be followed in order to avoid trace metal contamination.
2. Filtration should be performed with a low vacuum membrane pressure (<1 atm across membrane) to avoid sheering of colloidal material and lyses of biological cells that release trace metals.
3. The diverse ways that trace metals behave during the filtration procedure leads to suggestions for individual filtration protocols for each metal. It would appear that the most effective filtration protocol for Al, Cd and Pb would be to filter low volumes through the filter (≤ 100 mL), replacing the filter regularly. For Mn, the first 50 mL of filtrate should be discarded as this contains an under representation of subsequent filtrate. Co, Cr, Cu, U and Zn appear to remain relatively unperturbed by filtration artefacts and therefore can be filtered to exhaustion.
4. Although the shape of the filtrate profiles varied considerably with salinity and season, a correlation between flow rate and filtrate profiles was evident. As such, filtration rate can be used as a qualitative parameter to judge when the onset of membrane clogging is occurring and hence when the filter should be discarded and replaced.
5. The term 'dissolved' should not be used for metals that are able to pass 0.4 µm, but rather total filterable (TF) concentrations should be quoted. A suggestion would be to use TF followed by pore size of filter (µm) as a subscript *e.g.* $TF_{0.4}$.

6. As the range in SPM (and presumable colloid) concentration is large within estuaries, both spatially and temporally, a single filtration protocol for a given estuary may not be adequate.

Reference List

Achterberg, E. P. and van den Berg C. M. G (1994) *In-Line Ultraviolet-Digestion of Natural-Water Samples for Trace-Metal Determination Using an Automated Voltammetric System,* Anal. Chim. Acta, **291**, 213-232

Ackroyd, D. R., Bale, A. J., Howland, R. J. M., Knox, S., Millward, G. E. and Morris, A. W. (1986) *Distributions and Behaviour of Dissolved Cu, Zn and Mn in the Tamar Estuary.* Estuar, Coast. Shelf Sci. **23**, 621-640

Benes, P and Steinnes, E. (1974) *In Situ Dialysis for The Determination of the State of Trace Metals in Natural Waters.* Water Res., **8**, 947-953.

Buffle, J. and Leeuwen, C. (1992) *Environmental Particles* in: Environmental Analytical and Physical Chemistry Series, Eds Buffle, J., Perret, D. and Newman, M., Lewis Publishers, London, pp171-320

Butler, A. (1998) *Acquisition and Utilization of Transition Metal Ions by Marine Organisms.* Science, **281**, 207-120.

Chester, R. (2000) *The Transport of Material to the Oceans; the River Pathway,* in: Marine Geochemistry, Blackwell Science, 4th Ed, pp 11-51

Danielsson L. G. (1982) *On the Use of Filters for Distinguishing Between Dissolved and Particulate Fractions in Natural-Waters.* Water Res.,**16** 179-182.

Elbaz-Poulichet, F., Holliger, P., Huang, W. W., and Martin, J.-M. (1984) *Lead Cycling in Estuaries, Illustrated by the Gironde Estuary, France,* Nature, **308,** 409-414

Fischer, E. and van den Berg, C. M. G. (1999) *Anodic Stripping Voltammetry of Lead and Cadmium Using a Mercury Film Electrode and Thiocyanate.* Anal. Chim. Acta, **385,** 273-280

Greenamoyer, J. M. and Moran, S. B. (1997) *Investigation Of Cd, Cu, Ni and Th-234 in the Colloidal Size Range in the Gulf of Maine.* Mar. Chem., **57**, 217-226

Guo, L. D., Santschi, P. H. and Warnken, K. W. (2000) *Trace Metal Composition of Colloidal Organic Material in Marine Environments.* Mar. Chem. **70**, 257-275

Hall, G. E. M, Bonham-Carter, G. F., Horowitz, A. J., Lum, K., Lemieux, C., Quemerais, B. and Garbarino, J. R. (1996) *The Effect of using Different 0.45 µM Filter Membranes on 'Dissolved' Element Concentrations in Natural Waters.* App. Geochem., **11**, 243-249

Hart, B. T. and Davies, S. H. R. (1981) *Trace-Metal Speciation in the Fresh-Water and Estuarine Regions of the Yarra River, Victoria.* Estuar. Coast. Shelf Sci., **12**, 353-374

Hassellöv, M. (1999) *FFF-ICPMS: Development of a New Technique for Determination of Colloidal Trace Element Size Distributions in Natural Waters.* PhD Thesis, Göteborg University, Sweden.

Hassellöv, M., Lyvén, B, Haraldsson, C. and Sirinawin, W. (1999A) *Determination of Continuous Size and Trace Element Distribution of Colloidal Material in Natural Water by On-line Coupling of Flow Field-Flow Fractionation with ICPMS.* Anal. Chem., **71**, 3497-3502.

Hill, D. M. and Aplin, A. C. (2001) *Role of Colloids and Fine Particles in the Transport of Metals in Rivers Draining Carbonate and Silicate Terrains.* Limnol. Oceanogr., **46**, 331-344

Horowitz, A. J., Lum, K. R., Garbarino, J. R., Hall, G. E. M, Lemieux, C. and Demas, C. R. (1996) *Problems Associated with using Filtration to Define Dissolved Trace Element Concentrations in Natural Water Samples.* Environ. Sci. Technol., **30**, 3398-3400

Horowitz, A. J., Elrick, K. A. and Colberg, M. R. (1992) *The Effect Of Membrane Filtration Artefacts on Dissolved Trace-Element Concentrations.* Water Res., **26**, 753-763

Howard, G. and Statham, P. J. (1993) *Inorganic Trace Analysis: Philosophy and Practice*, Eds. A. G. Howard and P. J. Statham, J. Wiley & Sons, Chichester, pp. 182.

Knox, S., Turner, D. R., Dickson, A. G., Liddicoat, M. I., Whitfield, M. and Butler, E. I. (1981) *Statistical-Analysis of Estuarine Profiles - Application to Mn and Ammonium In the Tamar Estuary.* Estuar. Coast. Shelf Sci., **13**, 357-371

Laxen D. P. H. and Chandler, I. M. (1982) *Comparison of Filtration Techniques for Size Distribution in Fresh-Waters.* Anal. Chem., **54**, 1350-1355

Mantoura, R.F.C. and Morris, A.W. (1983) *Measurement of Chemical Distributions and Processes.* In: Practical Procedures For Estuarine Studies. Natural Environment Research Council, UK, pp. 55-100.

Martino, M., Turner, A., Nimmo, A. and Millward, G. E., (2002) *Resuspension, Reactivity and Recycling of Trace Metals in the Mersey Estuary, UK* Mar. Chem., **77**, 171-186

Miller, A.E.J. (1999) *Seasonal Investigations Of Dissolved Organic Carbon Dynamics In The Tamar Estuary, UK.* Estuar. Coast. Shelf Sci., **49**, 891-908

Millward, G. E. (1995) *Processes Affecting Trace-Element Speciation In Estuaries - A Review.* Analyst, **120**, 609-614

Morris, A. W., Bale, A. J., Howland, R. J. M., Millward, G. E., Ackroyd, D. R., Loring, D. H. and Rantala, R. T. T. (1986A) *Sediment Mobility and its Contribution to Trace-Metal Cycling and Retention in a Macrotidal Estuary.* Wat. Sci. Technol., **18**, 111-119

Morris, A. W., Howland, R. J. M. and Bale, A. J. (1986B) *Dissolved Aluminum in the Tamar Estuary, Southwest England.* Geochim. Cosmochim. Acta. **50**, 189-197

Morris, A. W., Bale, A. J. and Howland, R. J. M., (1982) *The Dynamics of Estuarine Manganese Cycling.* Estuar. Coast. Shelf Sci. **14**, 175-192

Morris, A. W. and Bale, A.J. (1979) *Effect of Rapid Precipitation of Dissolved Mn in River Water on Estuarine Mn Distributions.* Nature, **279**, 218-219.

Morrison, M. A. and Benoit, G. (2001) *Filtration Artefacts Caused By Overloading Membrane Filters.* Environ. Sci. Technol., **35**, 3774-3779.

Pettine, M., Mastroianni, D., Camusso, M., Guzzi, L., and Martinotti, W. (1997) *Distribution of As, Cr and V Species in the Po-Adriatic Mixing Area, (Italy).* Mar. Chem., **58**, 335-349

Pham, M. K. and Garnier, J. M. (1998) *Distribution of Trace Elements Associated with Dissolved Compounds (< 0.45 μm) in Freshwater Using Coupled (Frontal Cascade) Ultrafiltration and Chromatographic Separations.* Environ. Sci. Technol., **32**, 440-449

Pihlar, B., Valenta, P. and Nurnberg, H. W. (1981) *New High-Performance Analytical Procedure for the Voltammetric Determination of Nickel in Routine Analysis of Waters, Biological-Materials and Food.* Fresenius Z. Fur Anal. Chem., **307,** 337-346

Sharp, J. H. (1973) Size Classes of Organic Carbon in Seawater Limnol. Oceanog., **18**, 441-446.

Shiller, A. M. and Taylor, H. E. (1996) *Problems Associated with Using Filtration to Define Dissolved Trace Element Concentrations in Natural Water Samples.* Environ. Sci. Technol., **30**, 3397-3398

Tappin, A. D., Millward, G. E. and Burton, J. D. (2001) *Estuarine And Coastal Water Chemistry.* In: Land-Ocean Interaction, Eds Huntley, D. Leeks, G. and Walling, D., IWA Publishing, London, pp 241-*279.*

Toole. J., Baxter, M. S. and Thomson, J. (1987) *The Behaviour of Uranium Isotopes with Salinity Change in 3 UK Estuaries.* Estuar. Coast. Shelf Sci., **25**, 283-297

Turner, A. and Millward, G. E. (2002) *Suspended Particles: Their Role in Estuarine Biogeochemical Cycles.* Estuar. Coast. Shelf Sci., **55**, 857-883

Turner, D. R., Whitfield, M. and Dickson A. G., (1981) *The Equilibrium Speciation of Dissolved Components in Fresh Water and Seawater at 25 Degrees C and 1 Atm Pressure*. Geochim. Cosmochim. Acta, **45**, 855-881

Uncles, R. J., Elliot, R. C. A. and Weston, S. A. (1985) *Observed fluxes of Water, Salt and Suspended Sediment in a Partly Mixed Estuary.* Estuar. Coast. Shelf Sci., **20**, 147-168.

van den Berg, C. M. G, Khan, S. H., Daly, P. J, Riley, J. P. and Turner, D. R. (1991) *An Electrochemical Study of Ni, Sb, Se, Sn, U and V in the Estuary of the Tamar. Estuar. Coast. Shelf Sci.*, **33**, 309-322

van den Berg C. M. G., Merks, G. A. and Duursma, E. K. (1987) *Organic Complexation and its Control of the Dissolved Concentration of Cu And Zn in the Scheldt Estuary.* Estuar. Coast. Shelf Sci., **24**, 785-797

van den Berg, C. M. G. (1986) *Determination of Copper, Cadmium and Lead In Seawater by Cathodic Stripping Voltammetry of Complexes with 8-Hydroxyquinoline.* J. Electroanal. Chem., **215**,111-121

van den Berg, C. M. G. (1985) *Determination of the Zinc Complexing Capacity in Seawater by Cathodic Stripping Voltammetry of Zinc APDC Complex-Ions.* Mar. Chem., **16**, 121-1

Wells, M. L. (1998) *Marine colloids - A neglected dimension.* Nature, **391**, 530-531

Wen L. S., Santschi, P., Gil, I.G. and Paternostro, C. (1999) *Estuarine Trace Metal Distributions in Galveston Bay: Importance of Colloidal Forms in the Speciation of the Dissolved Phase.* Mar. Chem., **63**, 185-212

Wu, J. F. and Luther, G. W. (1996) *Spatial and Temporal Distribution of Iron in the Surface Water of the Northwestern Atlantic Ocean.* Geochim. Cosmochim. Acta, **60**, 2729-2741

CHAPTER 3

Voltammetric *In Situ* Measurements of Trace Metals in Coastal Waters

Abstract

The introduction of contaminant monitoring programmes in coastal waters requires sensitive, reliable and robust analytical methods for trace metal measurements. Developments in instrument automation and miniaturisation have resulted in the manufacture of portable electrochemical instrumentation. Such instrumentation has been used for continuous trace metal measurements from the banks of estuaries and on-board ships. The most recent developments in flow-through cells with a gel-coated Iridium (Ir) micro-electrode array have resulted in submersible *in situ* voltammetric probes which allow long-term trace metal measurements at subnanomolar concentrations in coastal waters. Such *in situ* probes hold great promise for water quality monitoring in anthropogenically perturbed coastal ecosystems.

3.1 Introduction

Trace metals play an important role in biological processes, with a range of metals (including Co, Cu, Fe, Zn) being essential for efficient growth and functioning of aquatic organisms (Butler *et al.*, 1998). Anthropogenic inputs of trace metals to coastal waters from domestic and industrial waste waters can substantially increase natural background concentrations. At enhanced concentrations, most trace metals become toxic to aquatic organisms (Brand *et al.*, 1986, Campbell *et al.*, 1995), resulting in perturbations to ecosystem functioning and potentially posing human health risks through seafood consumption.

In Western Europe, legislative measures [Water Framework Directive (WFD) (European Commission, 2002) and the Oslo-Paris (OSPAR) Convention (OSPAR Commission, 2000)] have been implemented to assess and reduce anthropogenic inputs and improve the quality of marine environments. The implementations of the WFD and OSPAR Conventions require long term monitoring programs of pollutants (including trace metals). Generally, monitoring activities in coastal waters involve the collection of discrete samples using survey vessels.

This approach results in a limited number of collected samples. Estuarine and coastal waters are dynamic, with complex current structures and potentially numerous pollutant sources. Consequently, high frequency or more beneficially continuous pollutant monitoring activities are required commensurate with the marked spatial and temporal variability of estuarine and coastal systems, necessitating the use of the reliable, accurate, and sensitive analytical techniques, *e.g.* stripping voltammetry.

Stripping voltammetry is such a technique and allows the direct determination of trace metals in marine waters. The technique is very sensitive (typical detection limits 10^{-9} to 10^{-12} M), has a wide linear dynamic range (typically two orders of magnitude) and can measure several elements simultaneously *e.g.* Cd(II), Pb(II) and Cd(II) (Tercier *et al.*, 1995). Furthermore, the instrumentation can easily be taken into the field as it is small and readily automated.

This chapter highlights recent advances, current trends and applications of stripping voltammetry for the determination of trace metals in coastal waters. The progression of voltammetric instruments and methods from laboratory to shipboard and to proper *in situ* measurements are discussed and the advantages of this electrochemical approach illustrated. Readers are referred to comprehensive text books for detailed information on electrochemical systems (Kissinger and Hieneman, 1995).

3.2 Laboratory Instrumentation and Methods

The monitoring of trace metals in coastal waters typically involves the collection of discrete samples with subsequent land-based laboratory analysis. This process is time consuming and hence expensive. Land-based laboratory techniques for trace metal analyses in seawater include electrothermal atomic absorption spectroscopy (Danielsson *et al.,* 1978) and inductively coupled plasma mass spectrometry (Bloxham *et al.,* 1994), after matrix removal and sample preconcentration. Anodic and cathodic stripping voltammetric techniques have also been applied in the shipboard laboratory, and allow determination of trace metals in seawater with minimal sample handling (Achterberg *et al.,* 1999).

3.3 Automated Voltammetric Systems

The development of automated voltammetric systems has enhanced sample analysis throughput in the laboratory. Voltammetric systems have been described using automated batch methods with a hanging mercury drop electrode (HMDE) as the working electrode (Achterberg *et al.,* 1994). In this approach, using peristaltic and syringe pumps, sample, reagents and standards are pumped in and out of a standard voltammetric cell, which typically has a volume of 10 mL.

The advantage of automated measurements is that each sample is fully calibrated with standard metal additions, thereby enhancing the accuracy of the method. The implementation of flow through cells has resulted in faster sample processing and reduced sample volumes. A flow cell based on a wall jet cell design with an HMDE has been described by Colombo *et al.* (1997), allowing the measurement of *ca* 60 samples h^{-1}.

3.4 Field Instrumentation and Methods

The automation of voltammetric trace metal analyses in land-based laboratories has reduced the risk of sample contamination due to the minimisation of sample handling. Examples of electrodes used in automated flow systems are given in Table 3.1. Even with flow systems, the risk of contamination may still occur during sample collection, handling and storage. An important development has been the use of the continuous sampling of surface waters with subsequent automated voltammetric trace metal determination on the bank of an estuary or bay (Zirino *et al.,* 1978; Whitworth *et al.,* 1998) or on-board ship (Braungardt *et al.,* 1998; Achterberg *et al.,* 1999). The earliest application of automated shore based monitoring, reported by

Table 3.1 Examples of the electrodes used, and the metal ions analysed, in specific voltammetric flow systems for the determination of trace metals species in natural waters. The voltammetric method, LOD and method of organic and oxygen removal are indicated, together with an example of the application of the system

Electrode type	Metal ion/s	Species	Method	Ligand	Samples/ hour	LOD (depostition time)	Organic removal/ Oxygen removal	Application	Reference
MFE	Bi(III), Cd(II), Cu(II), In(III), Pb (II), Zn(II)	Labile	ASV	N/A	3-5	All Sub μM (not stated)	Chelating Resin-Quinolin-8-ol/ Not stated	Coastal seawater	Daih and Huang, (1992)
MFE	U(VI)	Labile	AdCSV	Cupferron	Not stated	60.0 nM (2 min)	Not used/ He purge	Seawater	Economou *et al.*, (1994)
MFE	Cr (VI)	Labile	AdCSV	DPTA	20	0.842 nM (2 min)	Not used/ N_2 Purge	Estuarine water	Dobney and Greenway, (1994)
MFE	Zn(II)	Acid exchangeable metals	ASV	N/A	10	Not stated	Not stated/ N_2 Purge	River water	Wang, (1983)
HMDE	Co(II)	Total Filterable (0.45μm)	AdCSV	Nioxime	60	0.063 nM (not stated)	UV lamp/ N_2 Purge	Ocean Seawater	Daniel *et al.*, (1997)

MFE=Mercury film electrode; HMDE=Hanging mercury drop electrode; ASV=Anodic stripping voltammetry; AdCSV=Adsorptive cathodic stripping voltammetry; Cupferron=N-nitroso-N-phenylhydroxylamine; Nioxime=Cyclohexane-1,2-dioxime; DTPA=Diethylenetriaminepentaacetic acid.

Zirino *et al.*, (1978), who used anodic stripping voltammetry (ASV) on a mercury film electrode (MFE; glassy carbon surface). The continuous sampling approach requires a peristaltic or Teflon bellows pump, acid-cleaned Teflon or PVC tubing, and a float and anchor for positioning the sample inlet during monitoring from an estuarine bank at a fixed point (Whitworth *et al.*, 1998). On a survey vessel, the float and anchor are replaced by a 'fish' which is towed at a depth of *ca* 2-4 m (Braungardt *et al.*, 1998). Figure 3.1 shows the instrumental set-up used during continuous sampling and automated voltammetric analysis.

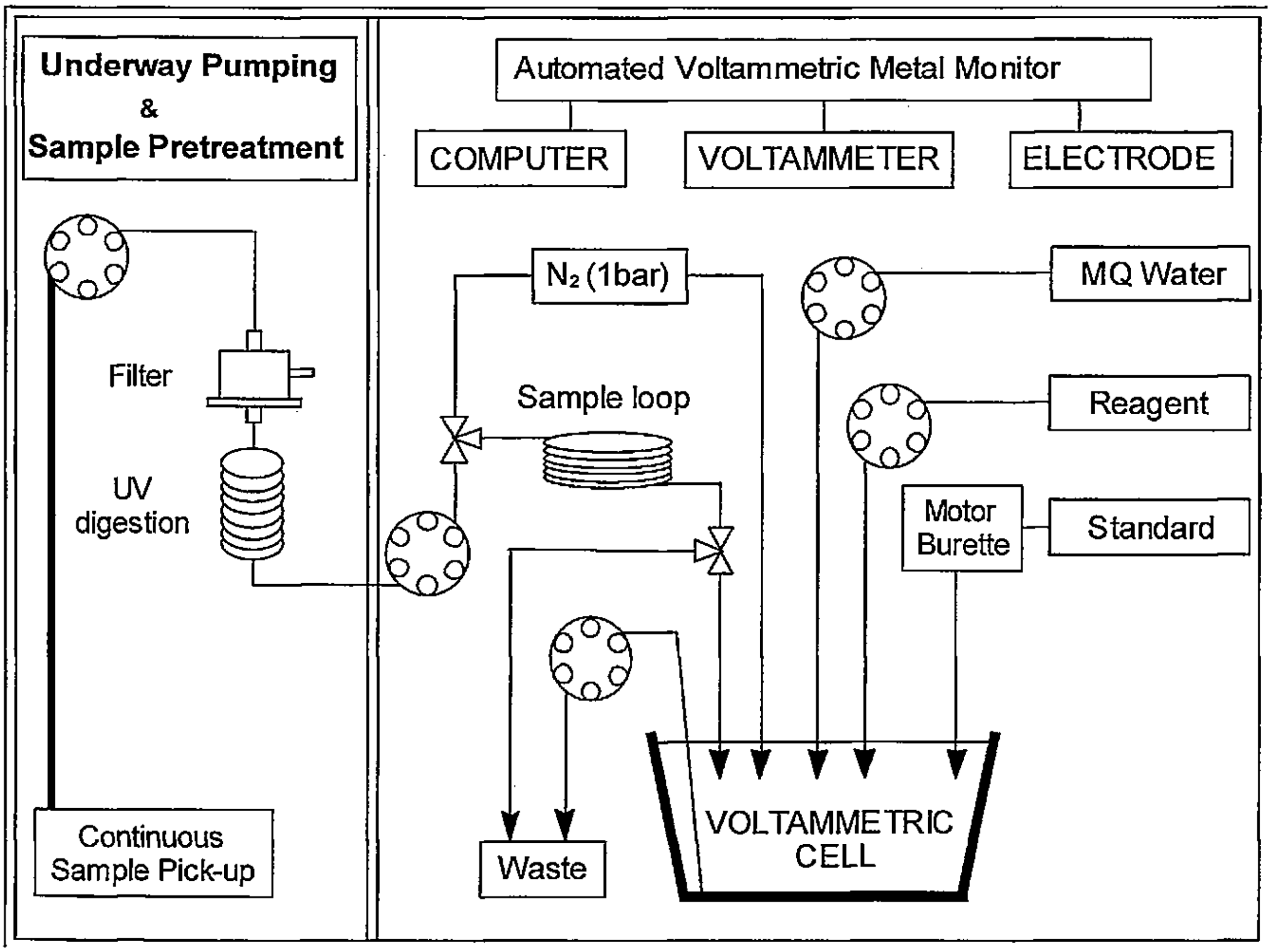


Figure 3. 1 Diagram of the continuous underway pumping system with sample pre-treatment (left box), linked to the computer controlled, automated voltammetric metal monitor operating in batch mode (right box).

On-line filtration (using 0.4 μm pore size membrane filters) followed by on-line organic matter removal by UV digestion are used to obtain total filterable trace metal concentrations in marine waters. The HMDE has been used as the working electrode in automated batch (Whitworth *et al.*, 1998; Braungardt *et al.*, 1998; Achterberg *et al.*, 1999) and flow cell (Achterberg *et al.*, 2002) approaches. The closed seawater and reagent flow approach has greatly reduced the risk of sample contamination, and the automation of the method has resulted in an enhanced sample throughput yielding high resolution environmental data.

This sampling and analytical approach is valuable in the monitoring of dynamic estuarine and coastal waters, where important temporal and spatial changes in metal concentrations occur. Interactive surveys, through near real-time measurements, can be used to ascertain point and diffuse metal pollution sources. For example, Figure 3.2 shows the distribution of total filterable Cu in surface waters of the Gulf of Cadiz, obtained using ship-board voltammetric measurement with continuous underway sampling. This coastal system receives large trace metal inputs from the river-estuarine systems of the Rio Tinto and Odiel and Guadalquivir which are subjected to acid mine drainage (AMD; Elbaz-Poulichet *et al.*, 1999). Cathodic stripping voltammetry (CSV) with automated batch analyses using an HMDE was used during this survey, with 8-hydroxyquinoline as the added CSV ligand, and HEPES as pH buffer (final concentrations 15 μM and 10 mM, respectively; pH 7.8). Figure 3.2 indicates that the highest Cu concentrations were observed near the outflows of the estuarine systems. The high frequency of measurements (4 samples h^{-1}) obtained with the ship-board monitoring approach has resulted in a high spatial resolution of the data, allowing a thorough interpretation of the input and removal mechanisms affecting trace metals in the Gulf of Cadiz.

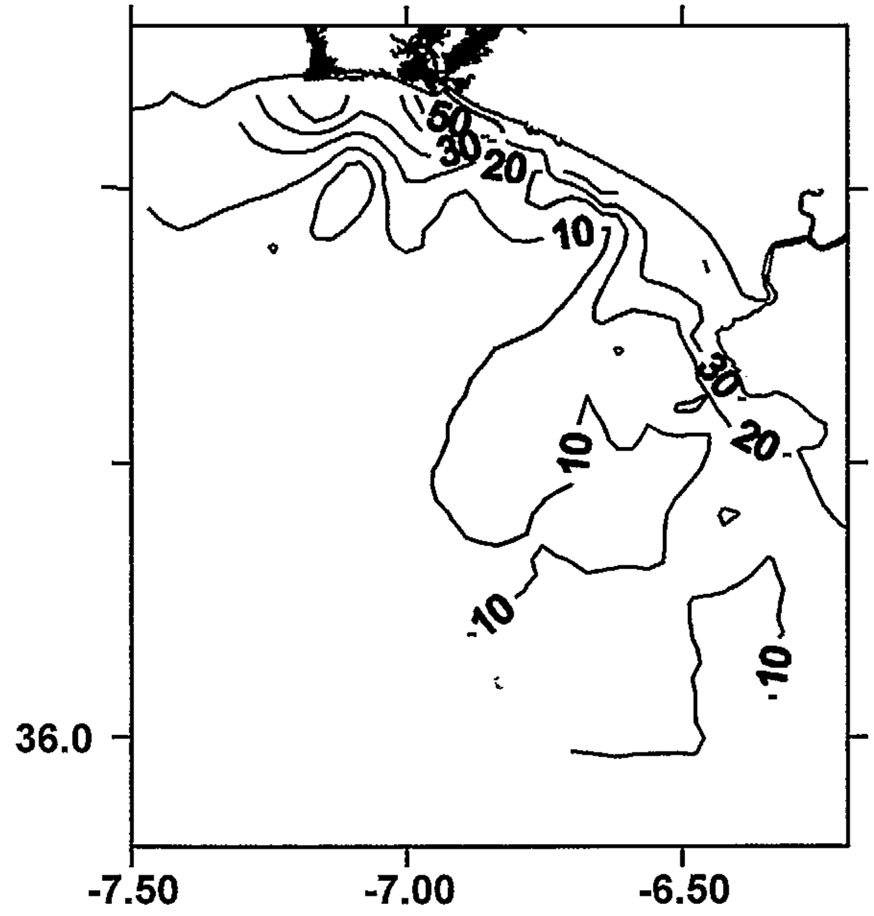


Figure 3. 2 Total filterable Cu (nM) distribution in the Gulf of Cadiz, October 1998. The contour plot was created from ca. 280 on-line measurements, performed during three days of steaming with the vessel *Garcia del Cid*. Axes on the graph depict latitude (y-axis; degrees N) and longitude (x-axis; degrees W). Braungardt *et al.*, 1998.

3.5 In situ Electrochemical Instrumentation: Component Considerations

Advances in flow cell and electrode design, and in the manufacturing of novel working electrodes and miniaturisation of instrumentation, have resulted in the development of submersible voltammetric probes during the last decade. These *in situ* voltammetric probes allow unattended deployment in coastal waters, result in a further minimisation of sample handling, allow depth profiles to be undertaken in

coastal waters, yield spatial and temporal data sets that allow a more thorough interpretation of biogeochemical cycles, and facilitate rapid detection of pollution incidents when combined with satellite telemetry. A further advantage of the submersible probes is that the *in situ* approach allows unperturbed trace metal speciation measurements to be undertaken.

A range of *in situ* voltammetric probes have been described in the literature, (Tercier *et al.,* 1990; Wang *et al.,* 1995; Wang *et al.,* 1997; Herdan *et al.,* 1998; Tercier *et al.,* 1998; Tercier-Waeber *et al.,* 1999; Wang *et al.,* 1999B; Daniele *et al.,* 2000) with the majority of the systems using ASV as the preferred electrochemical method. The choice of working electrode has been crucial for the successful deployment of these systems. Probes using MFEs have been superior, and the application of both Hg-film macro ($r > 100$ μm) and micro ($r < 10$ μm) electrodes has been reported. The current trend is towards the use of micro-electrodes as they have a number of advantages over macro-electrodes, including spherical diffusion that permits trace metal measurements in quiescent solutions (*i.e.* not sensitive to convection in the sample medium), a low ohmic drop, rapid mass transport rates, low capacitive currents and a low signal-to-noise ratio resulting in excellent sensitivity (Buffle *et al.,* 2000). Substrates used for macro- and micro-electrodes include C, Ag, Au, Pt and Ir. Carbon and Au based Hg-film macro-electrodes have been shown to be stable for at least one day, whereas Ir based Hg-film micro-electrodes are stable for more than ten days (Tercier *et al.*, 1995). The advantages of Ir based Hg-film micro-electrodes originate from the superior stability of the Hg-film on the Ir surface due to the good wetability of Ir, and the low solubility in Hg combined with high resistance to oxidation (Kounaves and Deng,, 1991). Ir based Hg-film micro-electrodes are also stable in open circuit and long-term measurements (3 to 4 weeks) can be undertaken in natural waters using the same Hg-film (Buffle *et al.*, 2000).

Recent advances in both thin film technology on chips and photolithographic techniques have allowed the mass manufacturing of Ir based micro-electrode arrays, featuring interconnected Ir micro-disc electrodes (Kounaves and Deng, 1991). A Hg-film is electrochemically coated on the Ir micro-discs (5-10 µm diameter), resulting in Hg hemispheres. The low cost and disposable nature of the Ir micro-electrode array provides advantages over the single Ir micro-electrode, which requires careful mechanical polishing on a regular basis, unlike the array electrodes. However, current micro-electrode array designs have shown no gain in sensitivity compared with the single Ir micro-electrode.

3.6 *In Situ* Electrochemical Instrumentation: Design and Applications

The first fully submersible *in situ* voltammetric monitor was deployed for measurements of trace metals in the marine waters of San Diego Bay (Tercier *et al.*, 1990). The probe consisted of a flow-through Plexiglas voltammetric cell, a Plexiglas submersible housing and a control box with its communication cord. The use of a glassy carbon based Hg-film macro-electrode in the probe allowed for continuous measurements using ASV of Cu, Pb, Cd and Zn, with limits of detection (LODs) of 3-4 pM for each metal.

Wang and co-workers have reported the deployment of *in situ* probes for trace metal (Cr, Cu, Hg, Ni, U) measurements in natural waters (river, sea and ground water) utilising both stripping potentiometry (Wang *et al.*, 1995; Wang *et al.*, 1997; Daniele *et al.,* 2000) and stripping voltammetry (Wang *et al.,* 1999A; Wang *et al.,* 1999B, Daniele *et al.,* 2000). Hg-film coated macro-electrodes (Wang *et al.,* 1997; Wang *et al.,* 1999A) and bare gold micro-electrodes (Wang *et al.,* 1995; Daniele *et al.,* 2000) have been used as working electrodes in these electrochemical probes. Although the probes represent a major advance in in *situ* trace metal measurements in natural waters, most of the systems lacked the sensitivity required for many coastal waters (nanomolar to picomolar concentrations).

An early environmental application of a voltammetric probe with an Ir based micro-electrode array was reported by Herdan *et al.* (1998). An electrode consisting of 20 mercury plated interconnected Ir micro-discs (10 µm diameter; Kounaves *et al.,* 1994) was used for the determination of Cu and Pb in groundwater. The micro-electrode and electrochemical hard and firmware were housed in a PVC tube. *In situ* Cu and Pb measurements showed an excellent correlation with laboratory analysis of filtered groundwater samples. However, interferences from dissolved organic matter were reported during *in situ* analyses, leading to a reduced sensitivity.

The voltammetric *in situ* probe used in the laboratory of the authors is the VIP (voltammetric *in-situ* profiling) system. This probe is the first commercially available system for trace metal measurements in natural waters and was developed by Buffle and co-workers (Tercier *et al.,* 1998; Tercier-Waeber *et al.,* 1999). The submersible probe consists of an upper housing (manufactured from Delrin) containing electronic hardware and firmware, a flow-through voltammetric cell (Plexiglas) and a lower housing (Delrin) containing a preamplifier and peristaltic pump (Figure. 3.3). The voltammetric cell holds a three electrode system which is contained in an internal flow-through cell (working micro-electrode and built-in Pt ring) and an outer cell (reference electrode), both are held together by a cell cover (Figure. 3.4).

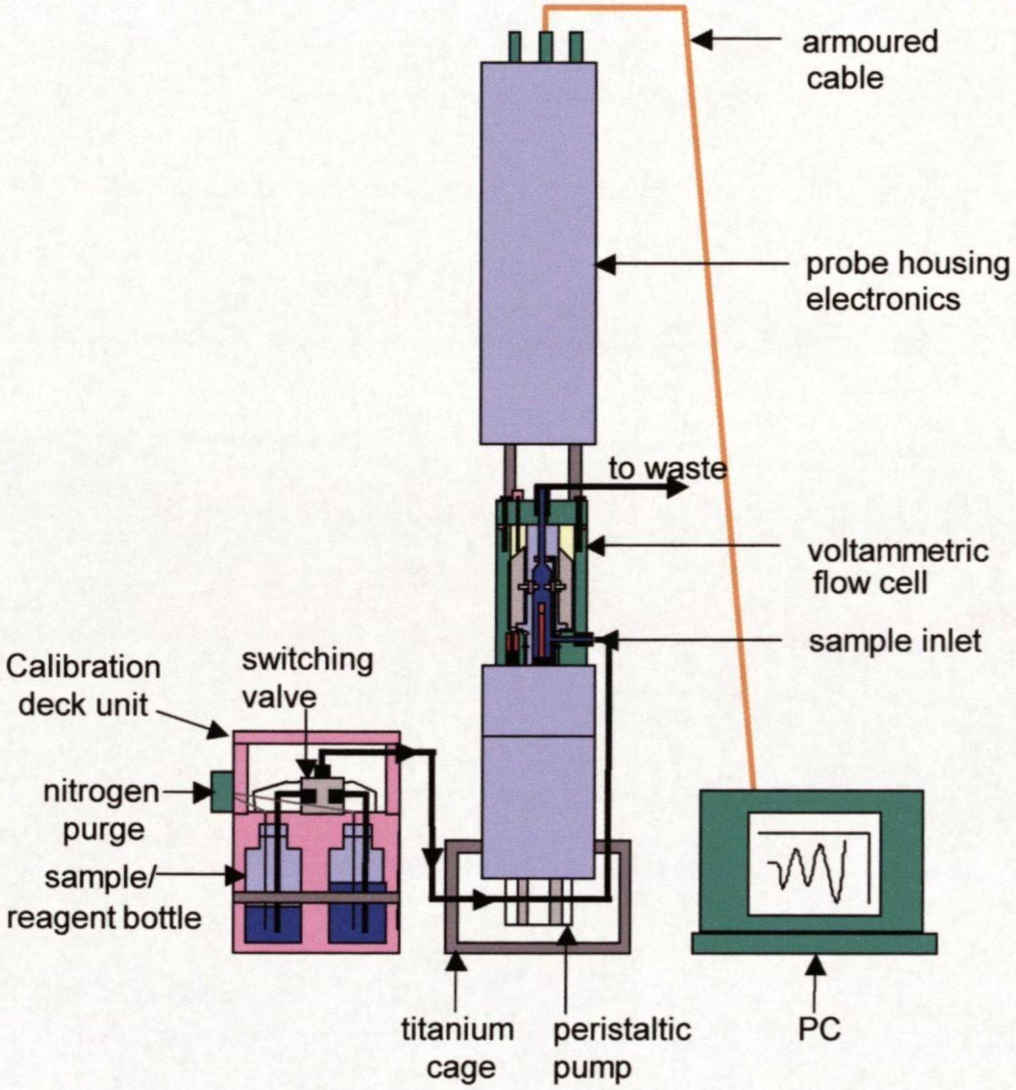


Figure 3. 3 Schematic diagram of the VIP system

The compartment between the inner and outer cells is filled with agarose (1.5 % v/v) / $NaNO_3$ (1 M) gel and acts as a pressure equaliser through the pressure compensator, as a double bridge between the sample and the agarose gel reference

electrode (through two ceramic junctions) and as a shield for the micro- and counter electrodes (Tercier *et al.,* 1998).

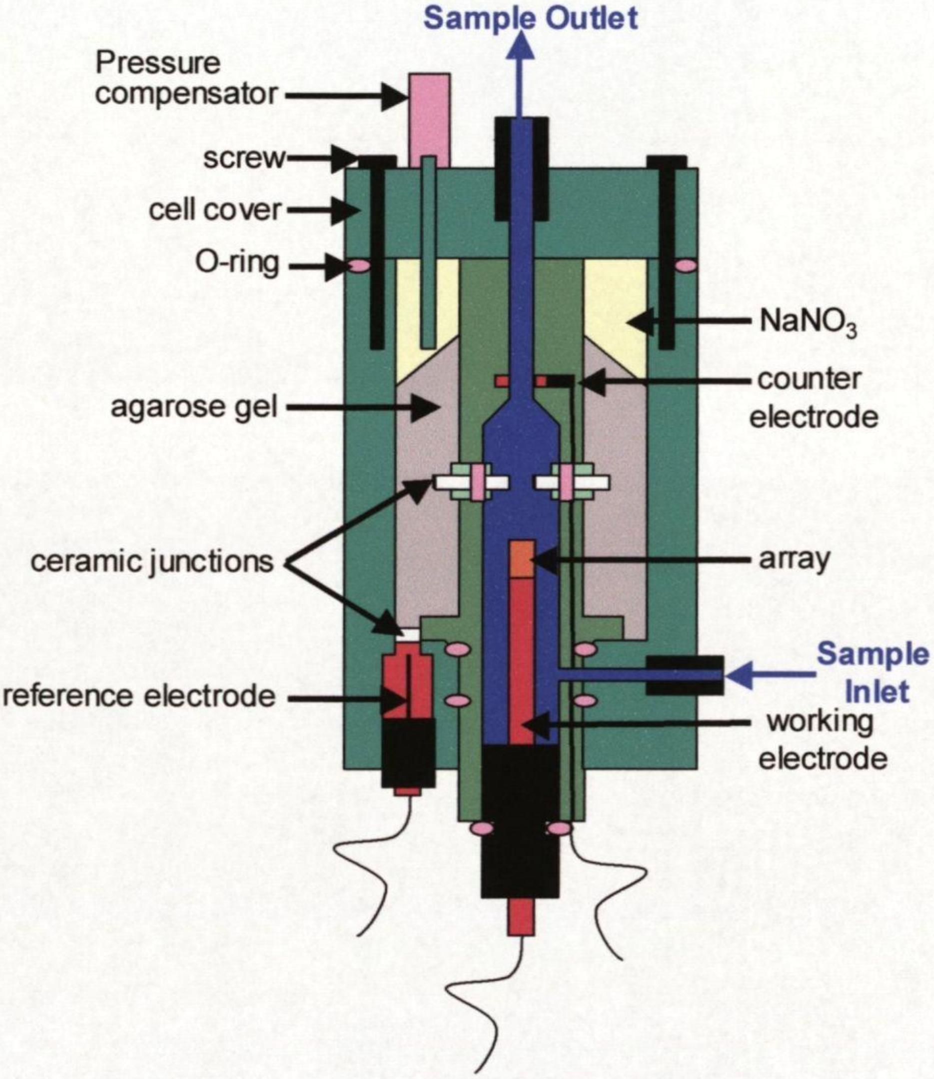


Figure 3. 4 Schematic diagram of voltammetric flow cell used in the VIP system.

The micro-electrode is the heart of the VIP, and the use of both an Ir disc micro-electrode of a few µm diameter (µ-AMMIE; Tercier *et al.,* 1998; Tercier *et al.,* 1995) and an Ir micro-electrode array (µ-AMMIA; Belmont *et al.,* 1996) have been reported. The micro-electrode array consists of 100 interconnected Ir micro-discs with a diameter of 5 µm and a centre to centre spacing of 150 µm. Both types of microelectrodes are coated with a layer of agarose gel (LGL, 1.5 %) with a pore size

of a few nanometres (Tercier and Buffle, 1996; Belmont-Hébert *et al.,* 1998). The gel thickness is between 400 and 600 µm for the µ-AMMIE. The presence of a containment ring on the µ-AMMIA enables reproducible gel thickness of 300 µm. The gel acts as a semi-permeable membrane, permitting diffusion of dissolved metal ions and small complexes whilst hindering the diffusion of colloids and macromolecules to the micro-electrode surface. The trace metal fraction determined by the VIP is termed 'dynamic', and includes these free ions and small complexes with size smaller than the agarose gel pores (Tercier-Waeber *et al.*, 1999). This dynamic fraction is considered bioavailable (Tercier-Waeber *et al.*, 1999), and therefore of importance for ecosystem functioning. The concentration of metals on/in colloids and small (< few nm) non-labile metal-organic complexes (Buffle *et al,* 2000) can be obtained as the difference between measurements of VIP dynamic metal and total filterable metal from discretely collected samples which have been filtered (0.4 µm pore size membrane filter), acidified (pH 2) and UV-digested prior to analysis (Achterberg and van den Berg, 1994). The gel by providing protection against organic and surfactant fouling, thereby allows long-term VIP deployment, but importantly allows trace metal speciation measurements.

To make the Hg film, Hg semi-drops are electrochemically plated through the Agarose gel layer onto the Ir sensor surface and the Hg-coating is stable for up to three weeks (Belmont-Hébert *et al.,* 1998). The life-time of the gel and µ-AMMIA can be up to 1 month.

The VIP system can be deployed at depths of up to 500 m. An armoured coaxial cable is used for communication between the probe and a laptop computer. Connection of the VIP system to a 12 V marine battery enables continuous deployment for 4 days. A calibration deck unit (see Figure 3.3) is used in the laboratory and on-board ship for renewal of the Hg-layer, calibration of the VIP system, and analysis of discrete samples and standard solutions. Trace metal measurements with the VIP system involve pumping sample through the voltammetric cell followed by an equilibration period (6.5 min) and a deposition stage (between 5 and 30 min) during which preconcentration of the metals in the Hg takes place, with a subsequent square wave anodic voltammetric stripping step. Simultaneous determinations of Cd, Cu, Pb, and Zn can be undertaken, with LODs of 50 pM, 200 pM, 50 pM (Tercier-Waeber *et al.,* 1999) and 400 pM (unpublished), respectively, with a 15 min preconcentration time. As the transport of the analyte to the Hg-film and the reaction kinetics are influenced by temperature and hence an Arrhenius temperature correction must be made for *in situ* measurements (Belmont-Hébert *et al.,* 1998; Chapter 4, section 4.3.10).

3.7 Conclusions and Future Trends

Large progress has been made with the development of electrochemical probes for *in situ* trace metal measurements in natural waters. Use of new materials and designs for flow cells and micro-electrode components has greatly improved the reliability, sensitivity and stability of the analytical systems. The automation and miniaturisation of the probes has facilitated their use in fieldwork programmes, providing high quality and high temporal resolution trace metal data. In *situ* probes can now make an impact on the monitoring programs required for the WFD and the OSPAR Convention.

Further miniaturisation of electrochemical *in situ* probes through 'Lab-on-a-Chip' technology is being undertaken. Recent advances in micro-machining technology have enabled complete laboratory analysis systems to be built on a chip. The group of Wang at New Mexico University are testing these miniaturised systems for fully submersed and remote deployment with continuous trace metal analysis (Wang *et al.,* 1999B).

Extending the range of metals that can be determined in seawater using ASV (Cd, Cu, Pb and Zn), will improve the monitoring capabilities of the electrochemical *in situ* probes. Recent work has described *in situ* probes for measurement of Cr using CSV (Wang *et al.,* 1999A) and Ni and U using stripping potentiometry (Wang *et al.,* 1997) with the use of a dialysis membrane to prevent electrode fouling. Further improvements to these systems, resulting in lower LODs will allow their application in dynamic coastal waters experiencing a range of metal concentrations. In the near future we can expect sophisticated *in situ* voltammetric probes which will undertake simultaneous, unperturbed speciation measurements of separate trace metal fractions and hence allow an improved interpretation of geochemical cycling and biological effects of these elements in coastal waters.

Reference List

Achterberg, E. P., Colombo, C., and van den Berg, C. M. G. (2002) Continental Shelf Research, *in press*

Achterberg, E. P., Colombo, C. and van den Berg, C. M. G. (1999) *The Distribution of Dissolved Cu, Zn, Ni, Co and Cr in English Coastal Surface Waters.* Cont. Shelf Res., **19**, 537-558

Achterberg, E. P. and van den Berg, C. M. G. (1994) *Automated Voltammetric System for Shipboard Determination of Metal Speciation in Sea-Water.* Anal.Chim. Acta, **284**, 463-471

Achterberg, E. P. and van den Berg C. M. G (1994) *In-Line Ultraviolet-Digestion of Natural-Water Samples for Trace-Metal Determination Using an Automated Voltammetric System,* Anal. Chim. Acta, **291**, 213-232

Belmont, C., Tercier, M.-L., Buffle, J., Fiaccabrino, G. C., and Koudelka-Hep, M. (1996) *Mercury-Plated Iridium-Based Microelectrode Arrays for Trace Metals Detection by Voltammetry: Optimum Conditions and Reliability.* Anal. Chim. Acta, **329,** 203-214

Belmont -Hébert, C., Tercier, M.-L., Buffle, J., Fiaccabrino, G. C., de Rooij, N. F., and Koudelka-Hep, M. (1998) *Gel-Integrated Microelectrode Arrays for Direct Voltammetric Measurements of Heavy Metals in Natural Waters and Other Complex Media.* Anal. Chem., **70,** 2949-2956

Bloxham, M. J., Hill, S. J., and Worsfold P. J. (1994) *Determination Of Trace-Metals in Sea-Water and the Online Removal of Matrix Interferences by Flow-Injection with Inductively-Coupled Plasma-Mass Spectrometric Detection.* J.Anal. Atom. Spec., **9**, 935-938

Brand, L. E., Sunda, W. G., and Guillard, R. R. L. (1986) *Reduction of Marine-Phytoplankton Reproduction Rates by Copper and Cadmium.* J. Exper. Mar. Biol. Ecol., **96**, 225-250

Braungardt, C., Achterberg, E. P., and Nimmo, M. (1998) *On-line Voltammetric Monitoring of Dissolved Cu and Ni in The Gulf of Cadiz, South-West Spain.* Anal. Chim. Acta, **377**, 205-215

Buffle, J. and Tercier-Waeber, M.-L. (2000), *In situ monitoring of aquatic systems.* In: Chemical Analysis and Speciation, Eds. J. Buffle and G.Horvai, John Wiley & Sons, Chichester, pp. 279-405

Butler, A. (1998) *Acquisition and Utilization of Transition Metal Ions by Marine Organisms.* Science, **281**, 207-120

Campbell, P. G. C. (1995), *Interactions Between Trace Metals and Aquatic Organisms: A Critique of The Free Ion Activity Model.* In: Metal Speciation and Bioavavailibility in Aquatic Systems Eds. A. Tessier and D. R. Turner, John Wiley & Sons, Chichester, pp. 45-103

Colombo, C., vandenBerg, C.M.G. and Daniel A. (1997) *A Flow Cell for On-Line Monitoring of Metals in Natural Waters by Voltammetry with a Mercury Drop Electrode.* Anal. Chim. Acta, **346**, 101-111

Daih, B. J. and Huang H., J. (1992) *Determination of Trace-Elements in Sea-Water by Flow-Injection Anodic-Stripping Voltammetry Preceded by Immobilized Quinolin-8-Ol Silica-Gel Preconcentration.* Anal. Chim. Acta, **258**, 245-252

Daniel, A., Baker, A. R., van den Berg, C. M. G. (1997) *Sequential Flow Analysis Coupled with ACSV for On-Line Monitoring of Cobalt in the Marine Environment.* Fres. J. Anal. Chem., **6**, 703-710

Daniele, S., Bragato, C., Baldo, M. A., Wang, J., and Lu, J. (2000) *The Use of a Remote Stripping Sensor for the Determination of Copper and Mercury in the Lagoon of Venice.* Analyst, **125**, 731-735

Danielsson, L. G., Magnusson, B. and Westerlund, S. (1978) *An Improved Metal Extraction Procedure for the Determination of Trace Metals in Sea Water by Atomic Absorption Spectrometry with Electrothermal Atomization.* Anal. Chim. Acta, **98**, 47-57

Dobney, A. M. and Greenway, G. M. (1994) *Online Determination of Chromium by Adsorptive Cathodic Stripping Voltammetry.* Analyst, **119**, 293-297

Economou, A., Fielden, P. R., Packham, A. J. (1994) *Batch and Flow Determination of Uranium(VI) by Adsorptive Stripping Voltammetry on Mercury-Film Electrodes.* Analyst, **119**, 279-285

Elbaz-Poulichet, F., Morley, N. H., Cruzado, A., Velasquez, Z., Green, D., Achterberg, E. P. and Braungardt C. B. (1999) *Trace Metal and Nutrient Distribution in an Extremely Low pH (2.5) River-Estuarine System, The Rio of Huelva (South-West Spain).* Sci. Total. Environ., **227**, 73-83

European Commission, (1995) Official Journal of the European Commission, L327.

Gilmartin, M. A. T and Hart J. P. *Sensing with Chemically and Biologically Modified Carbon Electrodes*, Analyst, **120**, 1029-1045

Herdan, J., Feeney, R., Kounaves, S.P., Flannery, A.F., Storment, C.W., Kovacs, G.T.A. and Darling, R.B. (1998) *Field Evaluation of an Electrochemical Probe for In Situ Screening of Heavy Metals in Groundwater.* Environ. Sci. Technol., **32**, 131-136

Kissinger, P. T. and Heineman, W. R. (1995) *Laboratory Techniques in Electroanalytical Chemistry*, 2 nd Ed. Marcel Dekker, New York

Kounaves, S. P., Deng, W., Hallock, P. R., Kovacs, G. T. A. and Storment, C. W. (1994) *Iridium-Based Ultramicroelectrode Array Fabricated by Microlithography.* Anal. Chem., **66**, 418-423

Kounaves, S. P.and Deng, W. (1991) *An Iridium Based Mercury Ultramicroelectrode - Fabrication and Characterization.* J. Electroanal. Chem., **301**, 77-85.

OSPAR Commission, (2000) *Quality Status Report 2000.* OSPAR Commission. Assessment and Monitoring. London, 111, pp 1-108

Tercier-Waeber, M.-L., Buffle, J., Confalonieri, F., Riccardi, G., Sina, A., Graziottin, F., Fiaccabrino, G. C., and Koudelka-Hep, M. (1999) *Submersible Voltammetric Probes for in Situ Real-Time Trace Element Measurements in Surface Water, Groundwater and Sediment-Water Interface.* Meas. Sci. Technol., **10**, 1202-1213

Tercier, M- L., Buffle, J. and Graziottin, F. (1998) *Novel Voltammetric In-Situ Profiling System for Continuous Real-Time Monitoring of Trace Elements in Natural Waters.* Electroanalysis, **10**, 355-363

Tercier, M.-L. and Buffle, J. (1996) *Antifouling Membrane-Covered Voltammetric Microsensor for in Situ Measurements in Natural Waters.* Anal. Chem., **68**, 3670-3678.

Tercier, M.-L., Parthasarathy, N. and Buffle, J. (1995) *Reproducible, Reliable and Rugged Hg-Plated Ir-Based Microelectrode for In-Situ Measurements in Natural-Waters.* Electroanalysis, **7**, 55-63.

Tercier, M.-L., Buffle, J., Zirino, A., and de Vitre, R. R. (1990) *In situ Voltammetric Measurement of Trace-Elements in Lakes and Oceans.* Anal. Chim. Acta, **237**, 429-437

Wang, J., Wang, J., Lu, J., Tian, B., MacDonald, D. and Olsen, K. (1999A) *Flow Probe for In Situ Electrochemical Monitoring of Trace Chromium.* Analyst, **124**, 349-352

Wang, J., Tian, B., Wang, J., Lu, J., Olsen, C., Yarnitzky, C., Olsen, K., Hammerstrom, D., and Bennett W. (1999B) *Stripping Analysis into the 21st Century: Faster, Smaller, Cheaper, Simpler and Better.* Anal. Chim. Acta, **385**, 429-435

Wang, J. Lu, J., Luo, D., Wang, J., Jiang, M., and Tian, B. (1997) *Renewable-Reagent Electrochemical Sensor for Monitoring Trace Metal Contaminants.* Anal. Chem., **69**, 2640-2645

Wang, J., Foster, N., Armalis, S., Larson, D., Zirino, A. and Olsen, K. (1995) *Remote Stripping Electrode for In-Situ Monitoring of Labile Copper in the Marine-Environment.* Anal. Chim. Acta, **310**, 223-231

Wang, J., Greene, B. (1983) *Stripping Analysis of Zinc in Natural-Waters Utilizing the Medium Exchange Method.* Watar Res., **11**, 1635-1638

Whitworth, D. J., Achterberg, E. P. Nimmo, M. and Worsfold, P. J. (1998) *Validation And In Situ Application of an Automated Dissolved Nickel Monitor for Estuarine Studies.* Anal. Chim. Acta, **371,** 235-246

Zirino, A., Lieberman, S. H., and Clavell, C. (1978) *Measurement of Cu and Zn in San Diego Bay by Automated Anodic Stripping Voltammetry.* Environ. Sci. Technol., **12** 73-79

CHAPTER 4

Optimisation of the Voltammetric *In situ* Profiling system for Use in Estuarine and Coastal Systems

Abstract

This chapter presents the optimisation and validation of a Voltammetric *In situ* Profiling (VIP) system for the simultaneous determinations of dynamic Cd(II), Cu(II) and Pb(II) in estuarine and coastal waters, where master variables can exhibit marked spatial and temporal variability. Systematic studies in $NaNO_3$ (as a supporting electrolyte) and seawater, were conducted and indicated that variations in ionic strength, pH and dissolved oxygen did not affect the response of the instrument, whereas an Arrhenius type temperature response was observed. During developmental work, physical and voltammetric settings for each separate operation of the VIP system analysis were considered and tested in detail in order to achieve the analytical requirements to perform measurements in the laboratory and during in *situ* deployments. The VIP instrument has a detection limit (defined as 3σ) in seawater for Cd(II) of 15 pM, Cu(II) 0.25 nM, and Pb(II) 15 pM. The accuracy of the VIP system was determined by the measurement of the total dissolved concentrations of Cd(II), Cu(II) and Pb(II) in two certified reference materials, a river water (SLRS-3) and an estuarine water (SLEW-2).

4.1 Introduction

Voltammetry, and in particular the VIP system, was selected as the method for laboratory and in *situ* determination of trace metals in estuarine and coastal waters because it presented many advantages over other methods including:

- Simultaneous determination of several metals
- Speciation capabilities
- Sensitivity and selectivity
- Cheap, robust and portable instrumentation
- Minimal sample handling

The types of micro-electrodes that can be used in the VIP system have been described in detail in Chapter 3, Section 3.6. The µ-AMMIA micro-electrode (Belmont *et al*, 1996; Belmont-Hebert *et al*, 1998) was chosen for the determination of Cd(II), Cu(II) and Pb(II) because it offered important advantages over the µ-AMMIE (Tercier *et al*, 1995; Tercier *et al*, 1996; Tercier *et al*, 1998), namely, thinner, reproducible agarose gel layers, produced by the use of a containment ring to give uniform thickness gels of 300 µm (Belmont-Hebert *et al*, 1998). Also, the preparation of the µ-AMMIA for use is less time consuming than for the µ-AMMIE, and finally, specialist equipment is not needed for polishing of the electrode or cutting of the agarose gel.

The VIP system has been successfully applied in lake, (Tercier-Waeber *et al.*, 1999; Tercier-Waeber and Buffle, 2000) river (Tercier *et al.*, 1995) and fjord waters (Tercier, *et al.,* 1998; Tercier-Waeber and Buffle, 2000) to measure the dynamic fractions (free metal, electrochemically labile and other small, mobile species) of Cd(II), Cu(II), Pb(II), Zn(II) and Mn(II) (Tercier-Waeber, 1998), but to date no application in seawater and estuarine water has been published. The aim of this chapter is to evaluate the analytical aspects of the dynamic trace metal measurements using the VIP system in marine waters and in particular estuarine and coastal waters, where master variables (salinity, temperature, pH, dissolved oxygen) can exhibit marked spatial and temporal variability. We have therefore conducted systematic studies, using seawater as supporting electrolyte, to examine the effect of each master variable on instrumental response. In addition experiments in sodium nitrate ($NaNO_3$), a commonly used supporting electrolyte for electrochemical studies, were conducted for comparison. $NaNO_3$ is the recommended electrolyte for VIP system use (Idronaut, s.r.l., 1998) and is used for calibration.

4.2 Experimental

All reagents were purchased from VWR (Poole, UK) and were of AnalaR grade unless otherwise stated. Handling of reagents, solutions and samples was undertaken in a Class 100 laminar flow cabinet, using clean techniques for trace metals (Howard and Statham, 1993). All aqueous reagents were prepared using water purified by reverse osmosis (Milli-RO, Millipore, Bedford, USA) followed by ion exchange (MQ water; Millipore, 18.2 MΩ cm^{-1}).

4.2.1 Supporting Electrolyte

The supporting electrolyte for laboratory VIP measurements was 0.1 M $NaNO_3$ (Trace select, Sigma Aldrich, Dorset, UK) unless otherwise stated. This commercial $NaNO_3$ is low in trace metals, and was used in the range 0.05–0.6 M to approximate the ionic strength of seawater (in the salinity range 2.5 to 35). Figure 4.1 illustrates the relationship between conductivity, salinity and $NaNO_3$ concentration for the salinity range 2.5–4 (Figure 4.1A; slope of 0.578 mS salinity unit^{-1}; $R^2 = 0.999$) and 4–35 (Figure 4.1B; slope of 0.731 mS; salinity unit^{-1}, $R^2 = 0.997$).

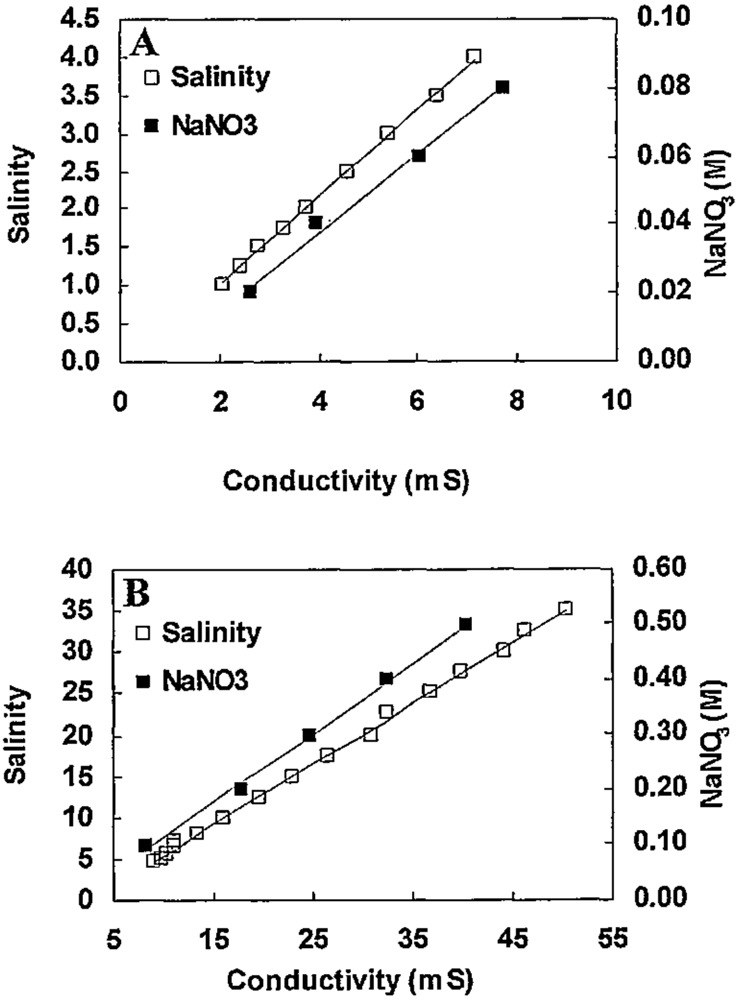


Figure 4.1 Relationships between conductivity, concentration of $NaNO_3$ and salinity at **(A)** low (0.02- 0.08 M; salinity range 2.5–4), and **(B)** high concentrations of $NaNO_3$ and salinities (0.1- 0.5 M; salinity range 4–35).

4.2.2 Standards and Buffer Preparation

Standard solutions of 1×10^{-4} M Cd(II), Cu(II) and Pb(II) were prepared from atomic absorption spectrometry standard solutions (SpectrosoL; 1000 mg L^{-1}), acidified to pH 2 using sub-HNO_3. (Chapter 2, section 2.2.2). Acetate buffers in the pH range 2-6 were prepared from sub-CH_3COOH and iso-NH_4OH (Chapter 2, section 2.2.2)., to give a final concentration of *ca* 1 M. An aqueous solution of 1 M *n*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES, pH buffer) was prepared by dissolution in MQ water and the pH adjusted to 7.8 using iso-NH_4OH.

4.2.3 Trace Metal Free Seawater

Coastal seawater (salinity 35) was sampled off Plymouth, filtered through acid washed polycarbonate membrane filters (0.4 μm, Cyclopore, Whatman, Kent, UK) and subsequently UV-digested using the method of Achterberg *et al.*, (1994; 400 W medium pressure UV lamp, Photochemical Reactors, Slough, UK) in 30 mL quartz tubes for 5 h, after the addition of H_2O_2 (8.8 mM, final concentration). Low trace metal seawater was prepared by passage through Chelex-100 resin (Bio-Rad, Hercules, CA, USA) packed into a column comprising low density polyethylene tubing (Nalgene) fitted with a quartz wool plug. Prior to use, the column was conditioned with three 150 mL aliquots of sub-HNO_3 (0.5 M) followed by six 150 mL aliquots of MQ water. The cleaned seawater was UV-digested for a second time for 5 h.

4.2.4 Micro-electrode Preparation

LDL agarose gel (Biofinex, Neuchâtel, Switzerland) was prepared by agitation of agarose (1.5 % v/v) in MQ water at 80 °C for 10 minutes until a transparent gel was formed. The gel was cooled to 60 °C before coating the micro-electrode array. A Hg film was electrochemically deposited (-400 mV for 6 min) onto the μ-AMMIA using a 5.5 mM $Hg(CH_3COO)_2$ / 0.1 M $HClO_4$ solution, resulting in Hg hemisphere radii in the range of 5.1-5.8 μm. The size of the Hg hemispheres was calculated from the current obtained during the electro-deposition of Hg on the μ-AMMIA (Belmont, 1996).

Following each series of experiments, the Hg was electrochemically removed in a solution of 1 M KSCN by a scan from –300 to +300 mV (scan rate of 5 mV s^{-1}).

4.2.5 VIP system Measurements

In the laboratory, trace metal measurements using the VIP instrument were made in acidified (pH 2) deoxygenated (using humidified N_2 gas; BOC, Surrey, UK) 0.1 M $NaNO_3$, or acidified (pH 2) deoxygenated seawater unless otherwise stated. As shown in Figure 4.2, sample was pumped through the VIP flow cell for 3 min (7 mL min^{-1}), equilibrated with the agarose gel on the micro-electrode (6.5 min), followed by a second pumping stage of sample (3 min). Subsequently, a deposition stage was conducted at –1100 mV (unless otherwise stated) for a period between 5 to 40 min during which metals were pre-concentrated in the Hg. After a 10 s equilibration period, a square wave (SW) ASV scan was carried out using a frequency of 200 Hz (pulse amplitude 25 mV and step amplitude 8 mV, unless otherwise stated).

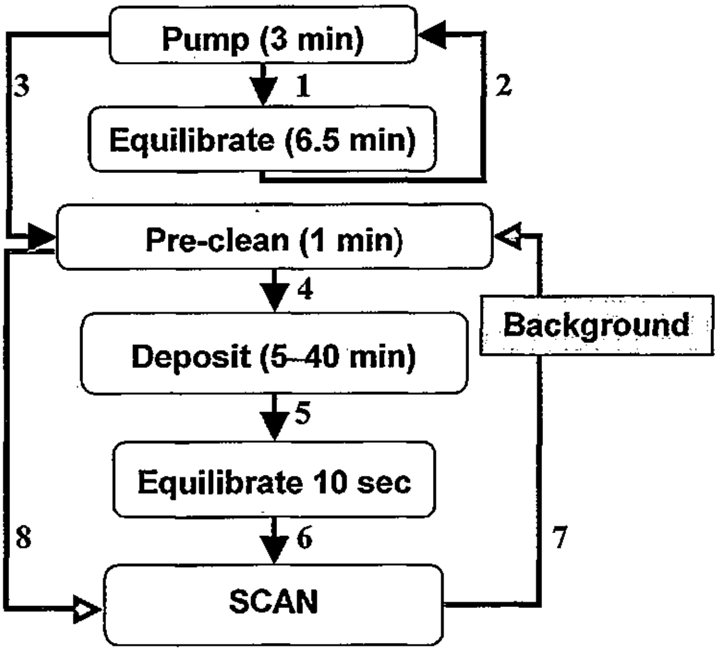


Figure 4.2 Flow chart of the analytical procedure used for the determination of trace metals using VIP system. The number sequence represents the order of events during analysis. Black lines represent sample stripping analysis and red lines represent background analysis.

The background scan was recorded subsequently, without prior deposition, and the background current was numerically subtracted from the sample stripping current using the VIP firmware (Tercier *et al*, 1993). An example of a voltammetric scan in $NaNO_3$, showing the stripping current, the background current and the background corrected current is given in figure 4.3. All quoted potentials are *versus* the Ag/AgCl/3M KCl/1M $NaNO_3$ reference electrode.

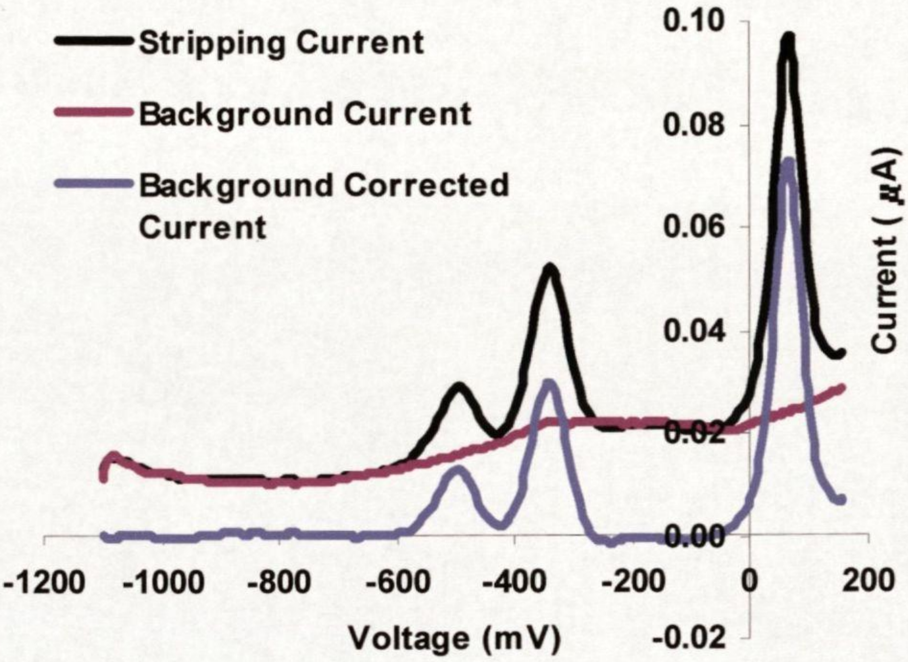


Figure 4.3 Anodic stripping voltammetric (ASV) scan of N_2 degassed, pH 2, 0.1M $NaNO_3$, in the presence of 2 nM of Cd and Pb and 10 nM of Cu and with voltammetric conditions: deposition at –1100 mV for 10 minutes; pre-cleaning potential at +150 mV.

4.3 Results and Discussion

4.3.1 Deoxygenation of Reagents, Samples and Standards

Dissolved oxygen (DO) can interfere with the voltammetric signal in unacidified $NaNO_3$ samples and standards, through reduction to hydrogen peroxide (~ -100 mV) (which can be further reduced to water (~ -900 mV; Tercier-Waeber and Buffle,

2000). In reagents, the presence of oxygen can lead to the formation of insoluble mercury oxides, reducing sensitivity and performance of the array. It was therefore important to determine the time needed to reach complete deoxygenation of the sample or standard.

A nitrogen (N_2) purge was used to remove DO from the solutions. A DO meter (model 55, Yellow Springs Industries (Y.S.I.) U.K. Ltd, Lynford House, Farnborough, Hampshire) was used to monitor the change in DO concentration of a fixed volume solution (125 to 500 mL) of $NaNO_3$ (0.1 M) with time (0 to 40 min) at a fixed gas flow rate of 1.97 L min^{-1}. Subsequent to a reaching a minimum, stable, DO concentration, the solution continued to be purged for a further 10 minutes and after which the purge was removed. The DO concentration continued to be monitored for a further period of 20 minutes. Figure 4.4 shows the effect of N_2 purging on a 125 mL sample of $NaNO_3$ and the effect on DO concentrations after removal of the purge.

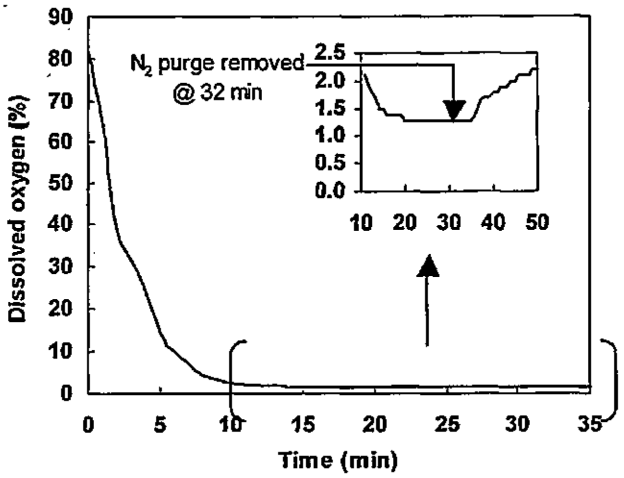


Figure 4.4 The effect of a nitrogen purge on a fully oxygenated 0.1M solution of $NaNO_3$ (125 mL with time at gas flow rate of 1.97 L min^{-1})

Upon removal of the purge, a slight increase in DO concentration was observed after 5 minutes, from 1.25 % to 2.25 % DO over the 20 minute period. This small amount of DO was found not to have an effect on measurements that took place within the VIP system during this time period. However, the purge was kept on when using $Hg(CH_3COOH)_2$ and KSCN solutions in order to prevent the formation of insoluble mercury oxide on the electrode surface (Idronaut, s.r.l, 1988).

A graph of the minimum time solutions of different volumes needed to be purged to ensure absence of DO prior to use is presented in Figure 4.5. This figure shows linearity over the range 150 mL to 500 mL (the volumes of solutions most often used with the VIP system).

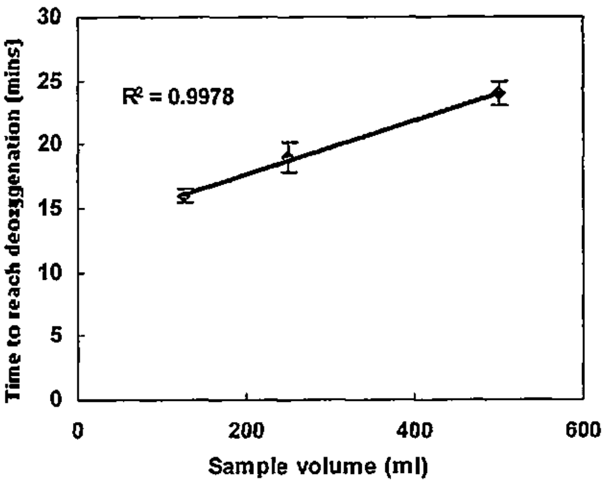


Figure 4.5 Time required to fully deoxygenated 0.1 M $NaNO_3$ as a function of sample volume (n=3 for each volume)

4.3.2 Calibration for Trace Metal Determination

Calibration of the VIP system was achieved by way of measurement of peak height produced by analysis of known concentration standards at a fixed deposition time. Figure 4.6 shows an example of a typical calibration in pH 2, $NaNO_3$ (0.1 M) for Cd(II), Pb(II) and Cu(II).

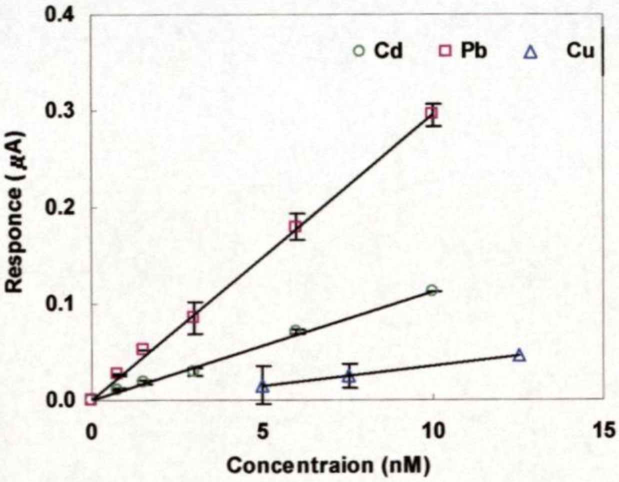


Figure 4.6 Effect of concentration of Cd(II), Pb(II) and Cu(II) on peak current response in deoxygenated, pH 2, 0.1 M $NaNO_3$ at 20 minutes deposition. (n=3 for each value)

Good linearity [$R^2 > 0.99$ for Cd(II), Cu(II) and Pb(II)] for the relationship between peak current response and concentration was observed in $NaNO_3$ (Figure 4.6) and seawater. Concentrations typically in the range 5-20 nM were used in the studies reported in this chapter, yielding easily measurable peak current responses. Voltammetric signals are, however, proportional to both the concentration of analyte and to the preconcentration or 'deposition' time. For calibration purposes, both deposition time and analyte concentrations were recorded in order to calculate the sensitivity of the instrument determined by Equation 1.

$$\text{Sensitivity (nA nM}^{-1}\text{ min}^{-1}) = \frac{response(nA)}{concentration(nM) \times deposition \quad time(\min)} \qquad \text{Eq. (1)}$$

Good linearity ($R^2 > 0.98$ for Cd(II), Cu(II) and Pb(II)) for the relationship between peak current response and deposition time was observed in seawater (Figure 4.7)

and 0.1 M $NaNO_3$. A deposition time typically in the range 5-15 minutes was used in the studies reported in this chapter, giving rise to easily measurable peak currents.

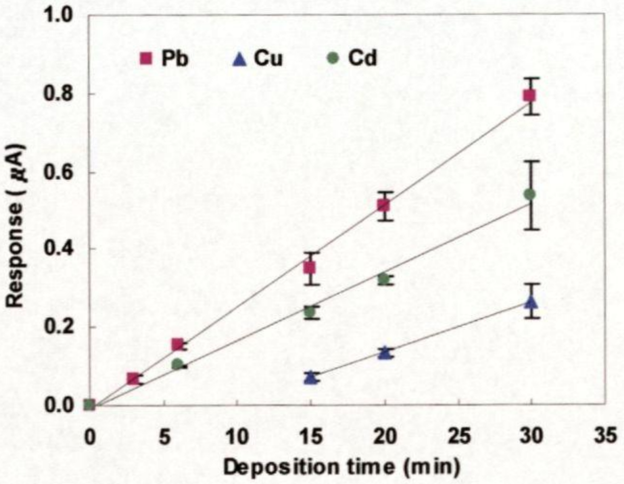


Figure 4.7 Effect of deposition time on peak current response in deoxygenated, pH 2, 0.1 M seawater containing 5 nM Cd(II), 5 nM Cu(II) and 5 nM Pb(II). (n=3)

This proportional relationship holds true until, a well-defined, upper point is reached, whereby the plot becomes non-linear due to the saturation of the mercury film with trace metal, at the end of the linear dynamic range. The extent of the linear dynamic range was investigated and the results reported in section 4.3.3 of this Chapter.

4.3.3 Linear Dynamic Range

Metal concentrations in estuarine and coastal waters vary a great deal, both spatially and temporally, mainly due to influence of tide, riverine input and industrial effluents. Over the course of a tidal cycle, the concentration of trace metal may vary over one or two orders of magnitude. In some contaminated estuaries, during times of high metal concentration from riverine inputs, the instrument sensitivity must be decreased by changing the deposition time, to ensure that the linear dynamic range is not exceeded. Determination of the of the highest concentration in the linear dynamic range was achieved by analysing Cd(II), Pb(II) and Cu(II) at fixed concentrations (of

10 to 20 nM), using preconcentration times increasing from 10 to 60 minutes, until the linear range was exceeded. The results are presented in Figure 4.8.

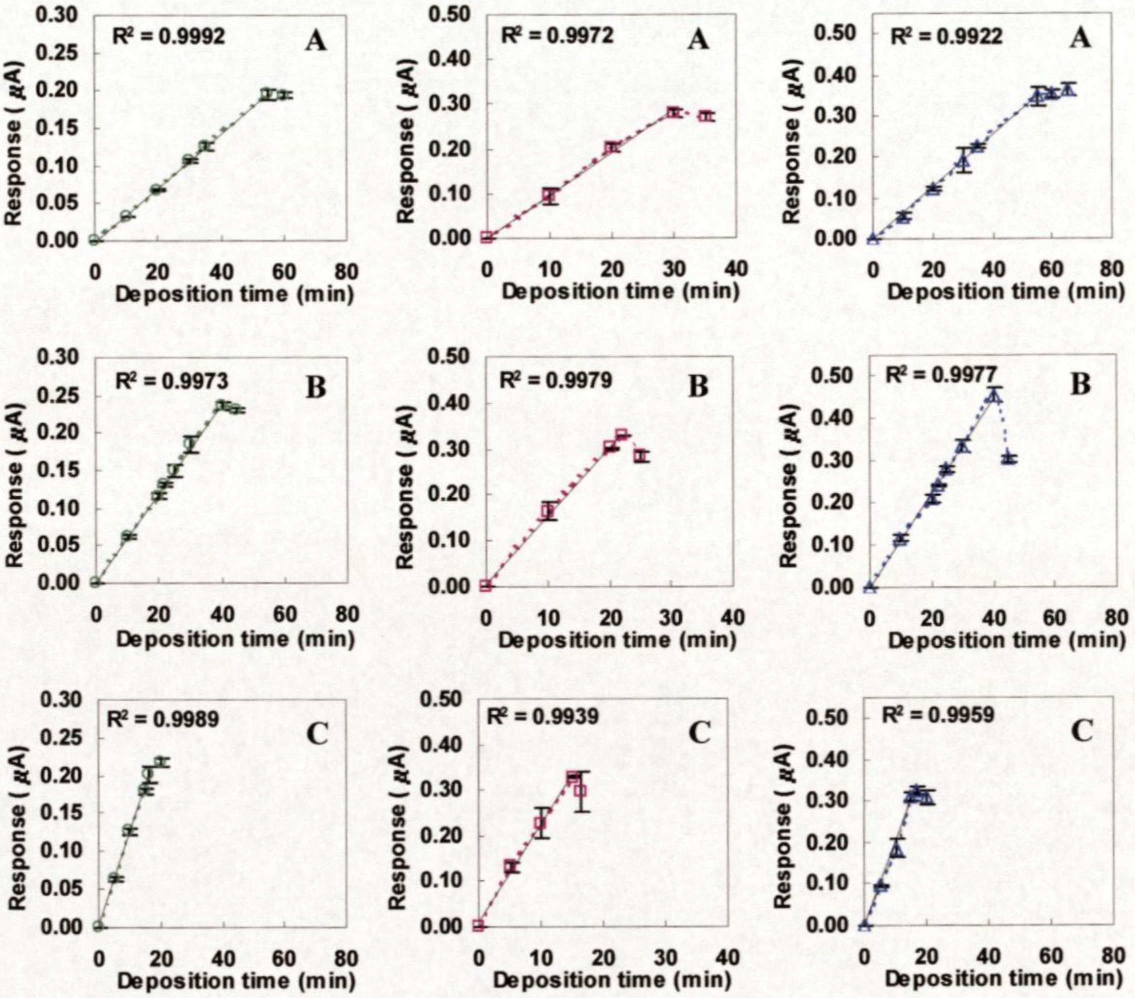


Figure 4.8 Effect of deposition time on the peak current response for deoxygenated, pH 2, 0.1 M $NaNO_3$ containing 10 nM, Cd(II), Pb(II) and Cu(II) **(A)** 15 nM Cd(II), Pb(II), and Cu(II) **(B)** and 20 nM Cd(II), Pb(II) and Cu(II) **(C)**. The correlation coefficient (R^2) relates to deposition times up to the point that the calibration deviates from a straight line *i.e.* up to the end the linear dynamic range. The green circles, pink squares and blue triangles represent Cd(II), Pb(II) and Cu(II) respectively. (n=3 for each deposition time)

All three metals have unique sensitivity values, derived from their solubility in Hg and hence the linear range was exceeded at different deposition times for each metal. The pre-concentration time at which the linear dynamic range was exceeded, at specified concentration, was recorded and a graph of maximum deposition time [illegible] at 2.0 ms, using preconcentration times varied from 10 to 60 minutes. A [illegible] linear curve was recorded. The results are presented in Figure 4.8.

Figure 4.8. Effect of [illegible] time on the peak current response of [illegible] [illegible] [illegible] [illegible]

[illegible]

against concentration was plotted (Figure 4.9). This was found to be linear for Cd (II) (R^2=0.997), Pb(II) (R^2=0.997) and Cu(II) (R^2=0.998) over the range of concentrations and deposition times tested. The lines of best fit can be extrapolated to take into account higher and lower trace metal concentrations. The system can therefore be deployed in contaminated coastal waters by changing the deposition time to ensure the linear dynamic range is not exceeded.

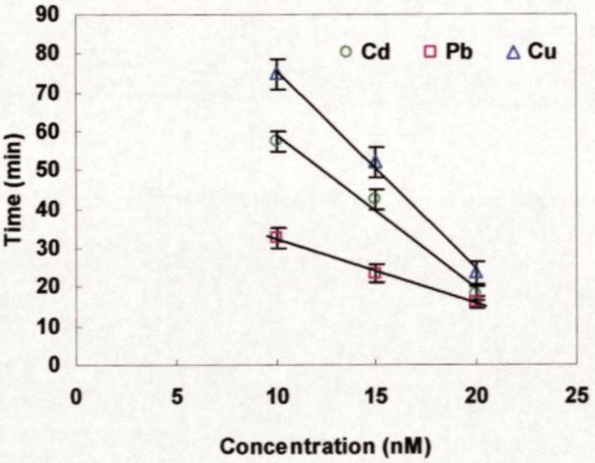


Figure 4.9 Maximum concentrations that can be analysed on VIP at different deposition times without exceeding the linear dynamic range. (n=3 for each concentration)

4.3.4 Influence of Deposition Potential, and Pulse and Step Amplitudes

The potential applied during the deposition step in voltammetric measurements may affect analytical sensitivity. The effect of deposition potential on the peak current response over the range -800 mV to -1200 mV in $NaNO_3$ and seawater was examined to optimise voltammetric conditions (results are presented in Figure 4.10A and B). Relative standard deviations (RSD) < 4 % (n=15) were obtained for repeated measurements of the peak currents over the whole deposition potential range for Cu(II) and Pb(II) in both matrices. Cd(II) showed a marked decrease in sensitivity for both matrices at potentials more positive than -1100 mV. These observations for

Cd(II), Cu(II) and Pb(II) are in agreement with previously reported results from similar investigations using 0.1 M $NaNO_3$ (pH 2) (Belmont-Hebert *et al.*,1998; Tercier *et al.*, 1996).

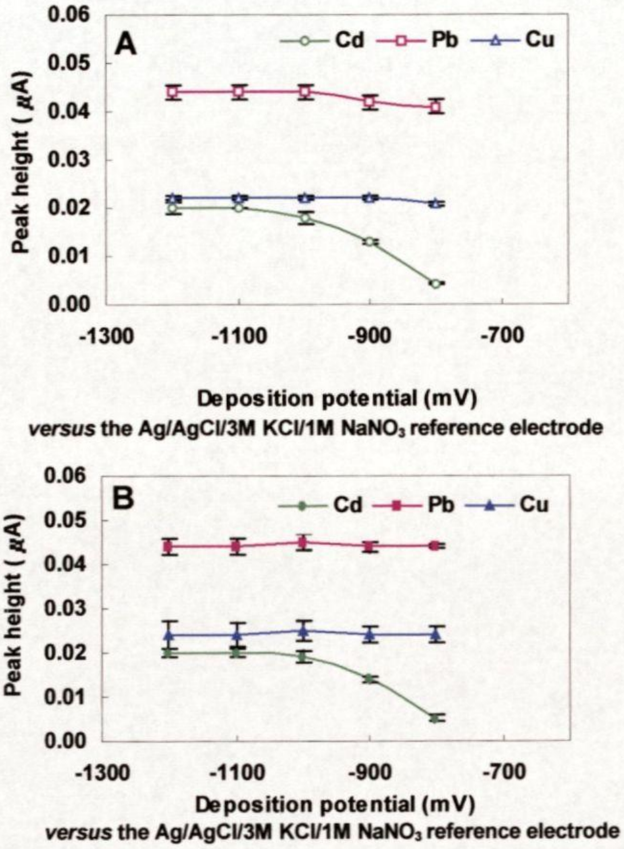


Figure 4.10 Effect of deposition potential on the peak current response for **(A)** deoxygenated, pH 2, 0.1 M $NaNO_3$, and **(B)** deoxygenated, pH 2 seawater. The solutions contained 5 nM Cd(II), 10 nM Cu(II) and 5 nM Pb(II), and a deposition time of 10 min was used. (n=3 for each depostition potential)

At potentials more negative than –1200 mV, the irreversibility of the reduction of hydrogen ions resulted in a large current response in the acidified solutions (pH 2) (van den Berg, 1991). A deposition potential of –1100 mV was considered optimal for both matrices and used during further laboratory and field studies.

The pulse and step amplitudes influence analytical sensitivity and peak shape and were optimised by systematic adjustment in the range 12.5-50 mV and 1-16 mV, respectively. A pulse amplitude of 25 mV and step amplitude of 8 mV resulted in

good sensitivity and peak shapes for all metals, and an excellent resolution of the Cd(II) and Pb(II) peaks, in both $NaNO_3$ and seawater. These conditions were used during further measurements and are consistent with previously reported optimum conditions for 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffered (pH 6.7), deoxygenated 0.1 M $NaNO_3$ (Tercier *et al.*, 1998).

4.3.5 Optimisation of Sample Transport and Gel Equilibration Times

Sharp changes in trace metal concentrations may influence dynamic trace metal determinations using the VIP system, which is because of memory effects between samples when the voltammetric flow cell is insufficiently flushed, or when insufficient time is allowed for metals in the sample to equilibrate with the agarose gel on the micro-electrode prior to the deposition step. The effect on the peak current response of measurements involving solutions with significantly different metal concentrations was examined by alternating analyses between $NaNO_3$ solutions spiked with Cd(II), Cu(II) and Pb(II) in the range 10–20 nM and a $NaNO_3$ blank (*i.e.* negligible trace metal content).

Allowing a period of 5 minutes to achieve equilibration between the sample solution and the agarose gel, and using a sample volume of 12.6 mL pumped through the voltammetric cell between measurements, a memory effect was observed (Figure 4.11A), resulting in a RSD for the different solutions in the range 1-7 % (n=3). An increase in flushing volume between measurements, from 12.6 mL to 21 mL, alleviated the memory effect for the $NaNO_3$ blank by sufficiently cleaning the voltammetric cell to give RSDs < 1 % (n=3) for all metals (see Figure 4.11B).

An increase in gel equilibration time, from 5 to 6.5 min, for the metal spiked $NaNO_3$ solutions improved the RSD < 2 % (n=3) for all metals (see Figure 4.11B), this increase allowed sufficient time for the metals to diffuse through the agarose gel of the µ-AMMIA. These conditions were subsequently used for all further measurements.

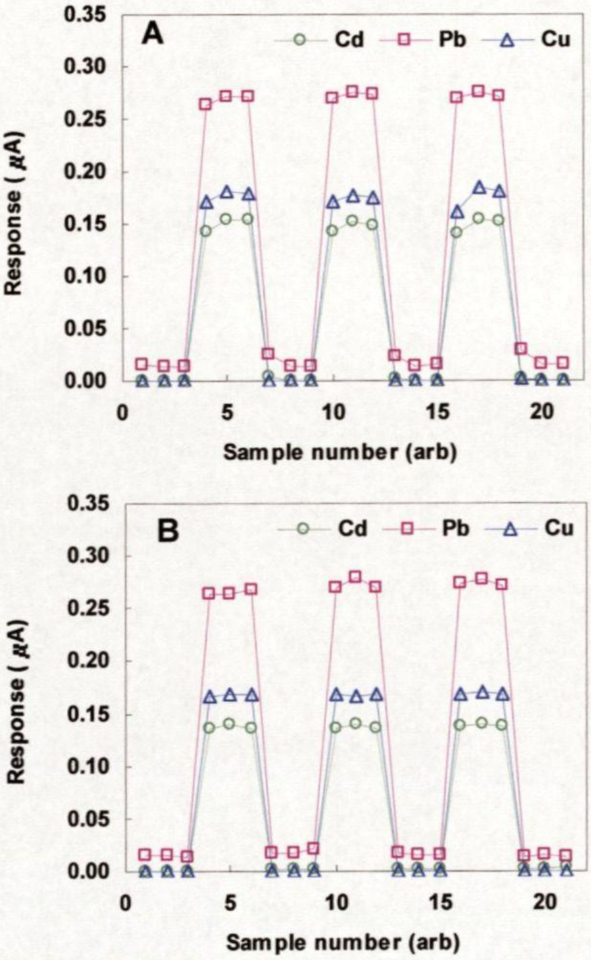


Figure 4.11 Effect of repeat changes in sample metal concentrations on peak current response for **(A)** 5 min gel equilibration time and 12.6 mL flow cell flushing volume between measurements, with deoxygenated, pH 2, 0.1 M $NaNO_3$ containing 10 nM Cd(II), 20 nM Cu(II) and 10 nM Pb(II) (denoted spiked) and $NaNO_3$ blank (*i.e.* negligible trace metal content) (denoted blank), at a deposition time of 15 min, and **(B)** optimised conditions at 6.5 min gel equilibration time and 21 mL of flow cell flushing volume.

4.3.6 Influence of pH on Dynamic Metal Determinations

Estuaries, particularly within the low salinity regions, may be subject to marked variations in pH (typically 6.5-8.1) as a result of changes in tidal state, biological

respiration, primary productivity, riverine and waste water discharges. The effect of pH on the peak current response of dynamic trace metal measurements with the VIP system was investigated in metal spiked $NaNO_3$ and seawater. The pH may affect the peak potential during the electrochemical analysis as a result of the Nernstian pH behaviour (*e.g.* 60 mV pH^{-1}) of the type of Ag/AgCl reference electrode used in the VIP system, and the pH has an influence on the negative limit of the polarisation range of Hg (Buffle and Tercier-Waeber 2000). The UV-digested seawater was subjected to an additional filtration step using a 0.1 μm pore size polycarbonate membrane filter (Whatman, Cyclopore); the reasons for which are outlined below.

Figures 4.12A and B show the peak current as a function of pH in $NaNO_3$ and seawater with added HNO_3 (pH 2-3.2), NH_4Ac buffer (0.01 M; pH range 4-7.1), HEPES buffer ($NaNO_3$ only; 0.01 M; pH 7.8) or no addition (seawater only; pH 8.3). Figure 4.12A shows that the pH did not significantly affect the peak current response in $NaNO_3$ over a pH range between 2 and 7.8, and in seawater between pH 2.6 and 4.6 (Figure 4.12B). However, a marked drop in peak current response was observed in seawater at pH ≥5.4 for Cd(II), Cu(II) and Pb(II).

The stable peak current response with changing pH for $NaNO_3$ is because the only speciation change in the matrices is hydrolysis. For example Cu at higher pH values, three hydrated species will be present ($Cu(H_2O)_n^{2+}$, $Cu(H_2O)_{n-1}^{+}$, $Cu(H_2O)_{n-2}$), with n=6 (Stumm and Morgan, 1996). We can expect that these species have very similar diffusion coefficients and therefore similar peak current responses. Trace metal determinations using the VIP in seawater at pH values below 5.4 yielded similar sensitivity as in $NaNO_3$ because of similar diffusion coefficients of the hydrated metal species (also see Table 4.1). The pH therefore does not appear to affect the electrochemical measurement of trace metals using the VIP. The decrease in the peak current response at pH ≥5.4 for Cd(II), Cu(II) and Pb(II) in seawater could most likely be explained by metal speciation changes in the seawater solution. The additional filtration (0.1 μm pore size filter) of the seawater was undertaken in an attempt to remove metal scavenging colloids from the 0.4 μm filtered UV-digested (at pH 8.1) solution. However, at higher pH values, a fraction of the metals was not detected by the VIP system, probably because they were unable to pass through the agarose gel covering the micro-electrode.

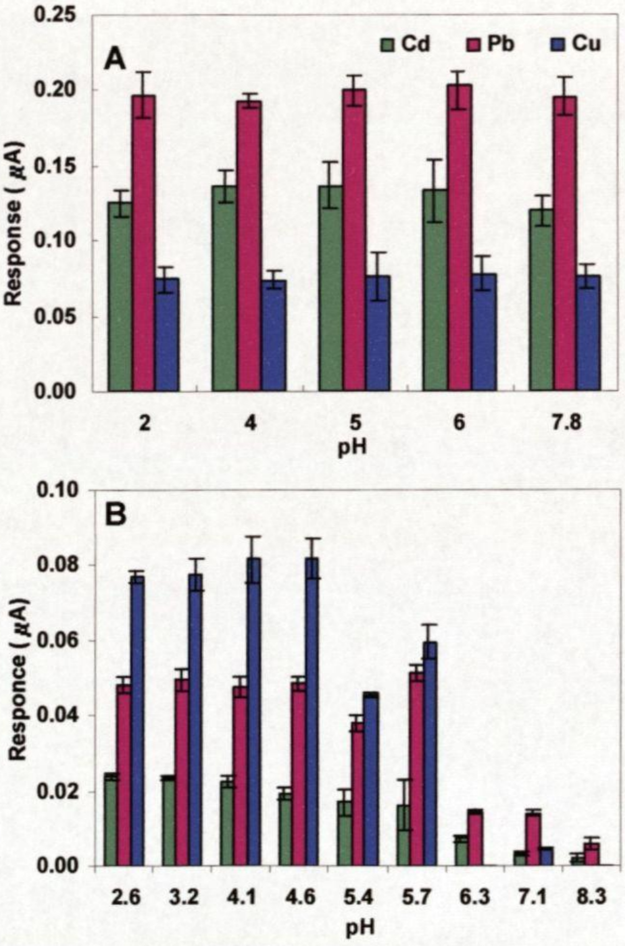


Figure 4.12 Effect of pH on peak current response in **(A)** deoxygenated, 0.1 M $NaNO_3$ containing 10 nM Cd(II), 20 nM Cu(II) and 10 nM Pb(II), and using a 15 min deposition time, and **(B)** deoxygenated seawater with addition of 4 nM Cd(II), 40 nM Cu(II) and 4 nM Pb(II), and using a 5 min deposition time. Adjustment of pH in $NaNO_3$ and seawater with added HNO_3 (pH 2-3.2), NH_4Ac buffer (0.01 M; pH range 4-7.1), HEPES buffer ($NaNO_3$ only; 0.01 M; pH 7.8) or no addition (seawater only; pH 8.3). (n≥3 for each pH value)

If metal colloids were not completely removed during the ultrafiltration step, then the observation of rapid removal at pH ≥5.4 is consistent with sorption of ionic metal onto metal colloids (*e.g.* Mn oxides) in seawater which is free of metal buffering organic ligands (Stumm and Morgan 1996).

The pH experiments indicate that Cd(II), Cu(II) and Pb(II) determinations using the VIP system can be undertaken over the pH range that is typically encountered in the estuarine and coastal waters and will yield high quality dynamic metal concentrations.

4.3.7 Influence of Ionic Strength and Salinity on Dynamic Metal Determinations

In estuarine systems, salinity can range from negligible salinity to fully marine salinity (35-36), and this determines the ionic strength of a sample to which the VIP system is exposed. Variations in ionic strength and salinity may influence trace metal measurements using the VIP as these parameters can affect the conductivity of a sample and therefore possibly affect the analytical peak current response for a metal. In addition, it is possible that enhanced ionic interactions with an increase in ionic strength influence the electrochemical response in trace metal measurements.

The effect of ionic strength and salinity changes on the peak current response was investigated using solutions of $NaNO_3$ (0.05-0.60 M) and seawater (salinity 2.5-35; dilutions with MQ water) containing Cd(II), Cu(II) and Pb(II) (in the range 5-10 nM). Figure 4.13 shows that for $NaNO_3$ (Figure 4.13A) and seawater (Figure 4.13B) the response was stable over the ionic strengths and salinities examined (RSD < 3%, n=18 for all metals in $NaNO_3$; RSD < 6 %, n=15, for all metals in seawater). In addition, a *t*-test on the results showed that there was no significant difference between the peak current responses for the different ionic strength or salinities (99 %

confidence interval). The absence of an influence of ionic strength on the peak current response can be explained by the low ohmic drop of the µ-AMMIA, which is typical for micro-electrodes because of their very small surface area (Buffle and Tercier-Waeber, 2000). The results demonstrate that dynamic trace metal measurements using the VIP system are unaffected by variations in ionic strength /salinity and that the probe can therefore be deployed *in situ* throughout the full range of salinities encountered in estuarine and coastal waters.

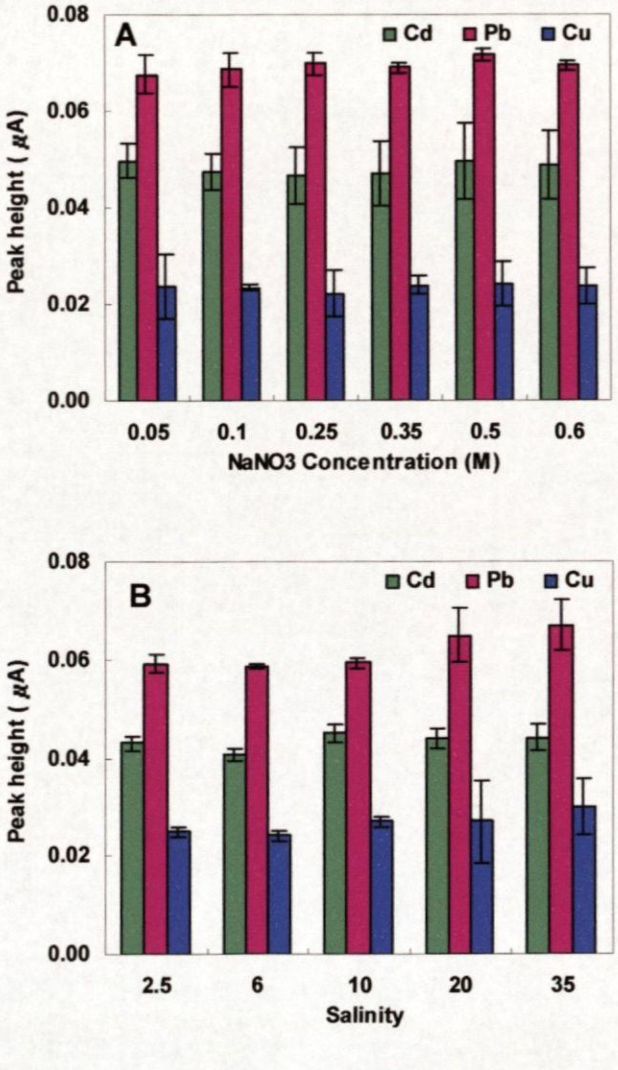


Figure 4.13 Effect of ionic strength on peak current response for **(A)** deoxygenated, pH 2, 0.1 M $NaNO_3$, and **(B)** deoxygenated, pH 2, seawater. The solutions contained 5 nM Cd(II), 10 nM Cu(II) and 5 nM Pb(II), and a deposition time of 15 min was used. (n≥3 for each value)

4.3.8 Influence of Dissolved Oxygen Concentration on Dynamic Metal Determinations

The DO concentration in estuarine waters can change as a result of variations in temperature, salinity, re-aeration, photosynthesis, biological respiration and mixing of different water masses. DO interferes with the voltammetric measurements due to the reduction of oxygen to hydrogen peroxide, resulting in a reduction peak at *ca* -100 mV.

The DO concentrations in saturated waters are much higher ($\sim 3 \times 10^{-4}$ M) than trace metal concentrations and consequently the oxygen reduction current is 4-6 orders of magnitude higher than that produced by trace metals of interest (Buffle and Tercier-Waeber, 2000). In addition, a pH increase (to ~11) may occur at the micro-electrode surface during the reduction of oxygen, resulting in the possible formation of insoluble metal oxy-hydroxides (Buffle, 1981) and a decrease in sensitivity and reproducibility. In the laboratory the oxygen interference is removed by deoxygenation of the sample using N_2 (g), but this degassing procedure is not applicable to *in situ* measurements. The VIP instrument minimises oxygen interference by a combination of background subtraction and the use of SWASV at a fast frequency (200 Hz that is the optimal scanning rate; Tercier, *et al.,* 1998). In the first approach the background current is subtracted from the stripping current, and the second approach makes use of the strongly reduced sensitivity of fast SWASV to irreversible processes (*e.g.* oxygen reduction; Buffle and Tercier-Weaber, 2000). However, the combined approach does not eliminate the reduction of oxygen during the voltammetric scan, with the consequent pH increase at the electrode surface. Therefore, it is applicable to well-buffered aquatic media, such as seawater (Buffle and Tercier-Waeber, 2000) but in the case of natural (unbuffered) freshwater an additional *in situ* deoxygenation step needs to be used (Tercier-Waeber, 2000). To examine the influence of DO on trace metal determinations on the VIP system, solutions of 0.1 M $NaNO_3$ and seawater, both oxygen saturated and deoxygenated (N_2 degassed), containing Cd(II), Cu(II) and Pb(II) in the range 5-15 nM were analysed. The results obtained in 0.1 M $NaNO_3$ are presented in Figure 4.14

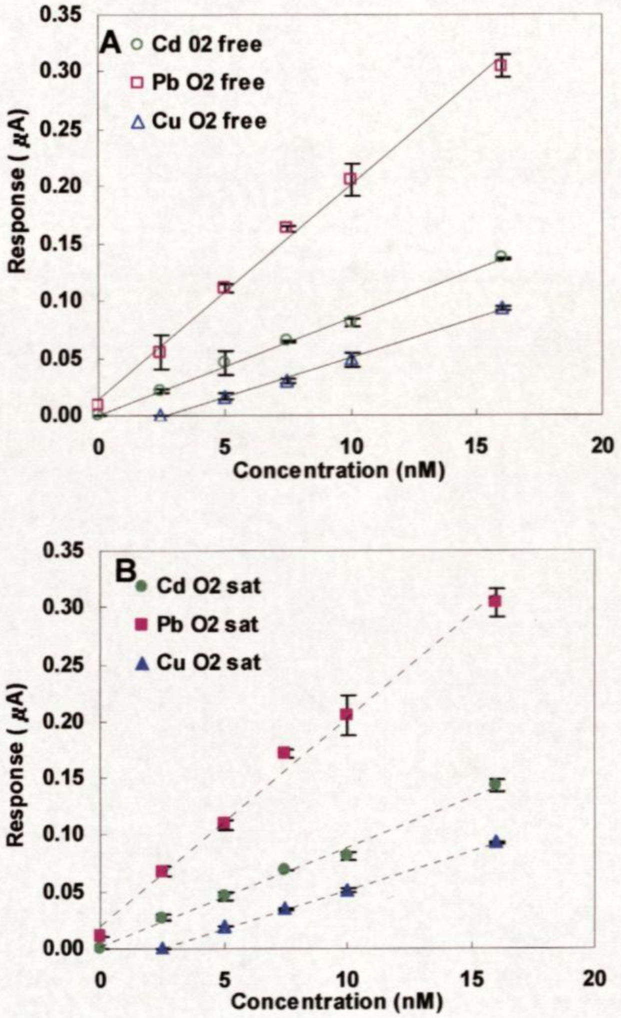


Figure 4. 14 The effect of **(A)** deoxygenation and **(B)** oxygenation calibration graphs for current response against concentration, in pH 2, 0.1 M $NaNO_3$ containing Cd(II), Cu(II) and Pb(II) in the range 1-10 nM, at a deposition time of 10 min. (n=3 for each value)

The peak current response was similar for oxygen saturated and deoxygenated solutions in both matrices, and no differences in sensitivity or slope of calibration graphs were observed for the metals (Table 4.1). These results show that the

sensitivity of the VIP is not dependent on the DO concentration in $NaNO_3$ and seawater. This is critical for VIP deployments in estuarine and coastal waters where significant gradients in DO concentrations may be encountered. Table 4.1 summarises the sensitivity of each metal in each matrix.

Table 4. 1 The effect of oxygenation and deoxygenation on the sensitivity [nA)/concentration (nM)×deposition time (min)] of calibration graphs for current response against concentration in pH 2, 0.1 M $NaNO_3$ and seawater containing Cd(II), Cu(II) and Pb(II) in the range 1-10 nM, at a deposition time of 10 min.

Matix	*Oxygenation*	*Sensitivity (nA/nM.min)*	*Correlation coefficient*
$NaNO_3$	Oxygenated	Cd(II):0.58 Pb(II):1.22 Cu(II):0.45	Cd(II):0.993 Pb(II):0.993 Cu(II):0.999
	Deoxygenated	Cd(II):0.58 Pb(II):1.25 Cu(II):0.47	Cd(II):0.993 Pb(II):0.996 Cu(II):0.997
Seawater	Oxygenated	Cd(II):0.51 Pb(II):1.12 Cu(II):0.43	Cd(II):0.992 Pb(II):0.999 Cu(II):0.993
	Deoxygenated	Cd(II):0.50 Pb(II):1.10 Cu(II):0.43	Cd(II):0.993 Pb(II):0.991 Cu(II):0.995

4.3.9 Sensitivity in Differing Matrices

An important, additional feature represented by Table 4.1 is that sensitivity, calculated from the slope of the graphs, is independent of matrix. The sensitivities between the two matrices have RSD< 8 % for Cd (II) and Pb (II) and <5 % for Cu (II). The implication is that calibration can be completed in readily available, trace metal free, $NaNO_3$ and can be applied to seawater matrix with no change in sensitivity.

4.3.10 Influence of Temperature on Dynamic Metal Determinations

The temperature in the water column of estuarine and coastal systems in mid-latitudinal regions typically varies between 4 and 25 °C. In order to obtain reliable trace metal determinations from *in situ* VIP measurements, the influence of the temperature on the peak current response needs to be established. Changes in the Hg drop geometry can be neglected in the temperature range between 4 and 25 °C, but the diffusion of trace metals through the agarose gel to the micro-electrode is temperature dependent (Tercier-Waeber *et al.*, 1999; Belmont-Hebert *et al.*, 1998) as shown by the Stokes-Einstein relationship:

$$D \propto \frac{RT}{6N_A r\pi} \exp\left(\frac{-Ea}{RT}\right) \qquad \text{Eq. 2}$$

Where D is the diffusion coefficient (m sec^{-1}), r is the radius of the species of interest (m), R is the gas constant, T is temperature (K), E_a is the activation energy (J mol^{-1}) and N_A is Avogadro's number. For the micro-electrode used in this study, the current response [$\imath$ (A)] should be proportional to D, and consequently for reversible, diffusion-controlled systems, a linear relationship is expected (Tercier-Waeber *et al*, 1999) between ln($\imath$) and $1/T$. The quantitative relationship between the peak current response, $\imath$, and temperature therefore should follow the Arrhenius equation:

$$i = i_0 \exp\left(\frac{-\Delta G^*}{RT}\right) \qquad \text{Eq. 3}$$

In order to verify this behaviour, the VIP system was placed in a temperature controlled room and the temperature was varied in the range 4-23 °C in oxygen saturated $NaNO_3$ and seawater, containing Cd(II), Cu(II) and Pb(II) in the range 5-10 nM (for n=3). Indeed, linear responses ($R^2 > 0.98$) were observed for the trace metals by plotting ln(*i*) against 1/*T* in both of the matrices tested. Figure 4.15A illustrates an example results obtained using $NaNO_3$ and Figure 4.15B , the results in seawater.

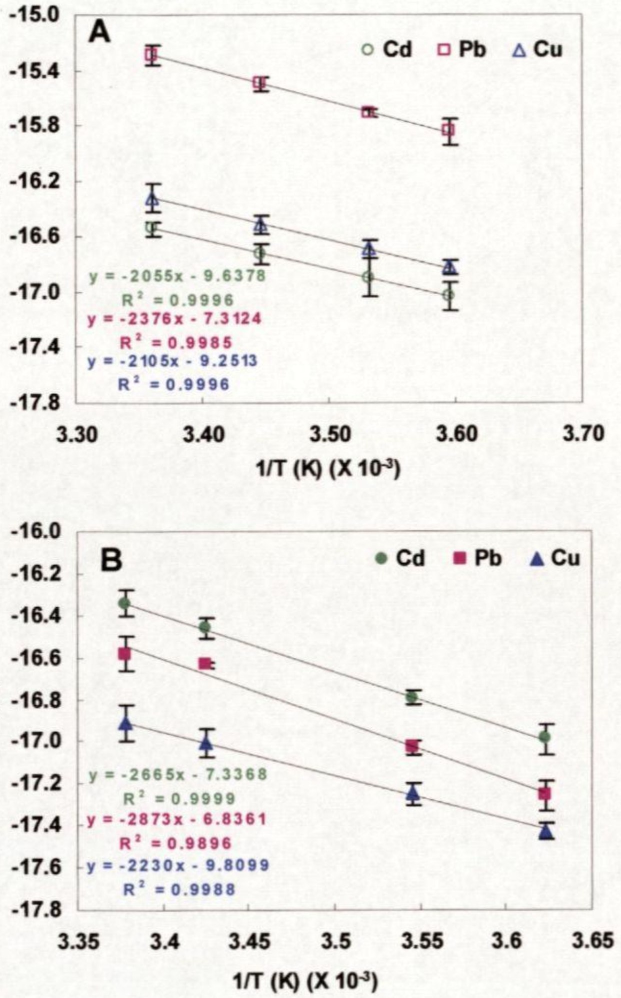


Figure 4. 15 The natural logarithm of the peak current response as a function of the reciprocal of the temperature. The gradient yields a value of $\Delta G/R$ for **(A)** deoxygenated, pH 2, 0.1M $NaNO_3$ and **(B)** deoxygenated, pH 2, 0.1M seawater containing 5 nM Cd(II) and Pb(II) and 10 nM Cu(II) at a deposition time of 5 min. ($n \geq 3$ for each value)

Experimentally determined slopes were compared with theoretical slopes (Tercier-Waeber *et al.*, 1999) for reversible systems, as shown in Table 4.2.

Table 4.2 Theoretical and experimental slopes (ln K) of Arrhenius plots for the temperature dependence of the diffusion coefficients calculated by Equation 1, and experimental SWASV current responses (Equation 2) in oxygen saturated, pH 2, 0.1 M $NaNO_3$ and seawater containing 5 nM Cd(II) and Pb(II) and 10 nM Cu(II) at a deposition time of 5 min. Confidence interval represents 1σ at n=3 for each metal ion in each matrix.

	Cd(II)	*Pb(II)*	*Cu(II)*
Theoretical slopes for $\ln(D)$ versus $1/T$	-2555	-2555	-2555
Experimental slopes for $\ln(i)$ versus $1/T$ in $NaNO_3$	-2245 ± 327	-2324 ± 325	-2240 ± 349
Experimental slope for $\ln(i)$ versus $1/T$ in seawater	-2581 ± 351	-2873 ± 423	-2400 ± 336

For each metal in both matrices, the values for the slope were in good agreement with the theoretical values, thereby verifying that the metal ion oxidation-reduction step is completely reversible in both matrices over the range of temperatures tested. The obtained slopes are used for temperature corrections of dynamic trace metal data obtained during *in situ* deployment of the VIP probe in marine waters.

4.3.11 Stability Test for Long-Term Dynamic Metal Determinations

The long-term *in situ* deployment of the VIP system is important for biogeochemical studies in dynamic and complex estuarine and coastal systems which require high temporal and/or spatial data resolution, and for use of the probe as an early warning system against accidental waste water discharges in these waters. Probe stability may be influenced by deterioration with time of the Hg film and/or the micro-electrode array surface. To examine the stability of measurements using the VIP probe with time, long term determinations of Cd(II), Cu(II) and Pb(II) in the range 5-10 nM in $NaNO_3$ and seawater were undertaken. These laboratory measurements were made hourly for the first 12 h (Figures 4.16A for $NaNO_3$; 4.17A for seawater) with subsequent measurements every 12 h over a period of 5 days (Figures 4.16B for $NaNO_3$; 4.17B, for seawater), using a deposition time of 10 min.

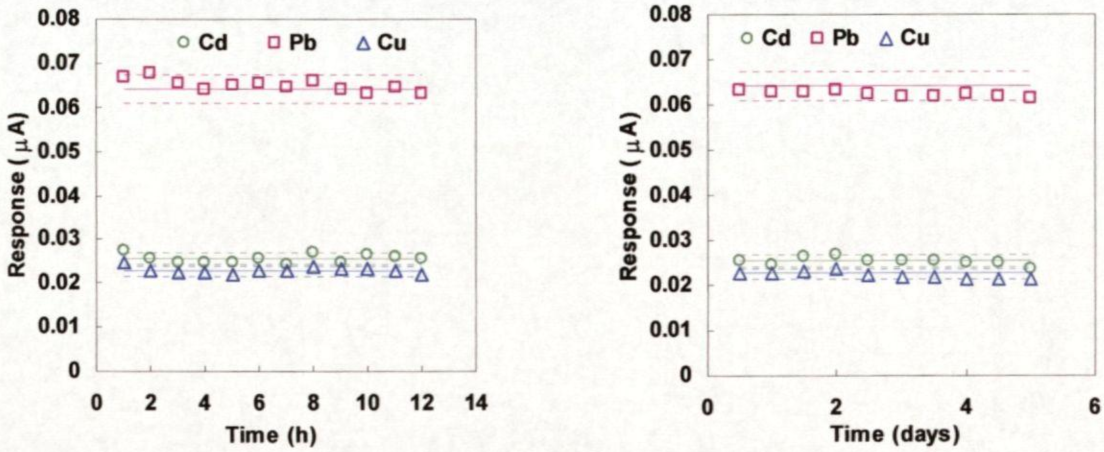


Figure 4. 16 The effect of time over **(A)** 12 hours and **(B)** 5 days on the current response in pH 2, deoxygenated 0.1 M $NaNO_3$ containing 5 nM Pb(II) and Cd(II), and 10 nM Cu(II) at a deposition time of 10 min. The solid lines represent the mean concentration over both time periods and the dotted lines represent ± 5 % of the mean.

Precision remained good over this period to yield an RSD (n=20 for all three metals in both matrices) of 4.1 % and 10.0 % for Cd(II), 4.4 % and 9.9 % for Cu(II) and 2.1 % and 6.6 % for Pb(II) in $NaNO_3$ and seawater, respectively.

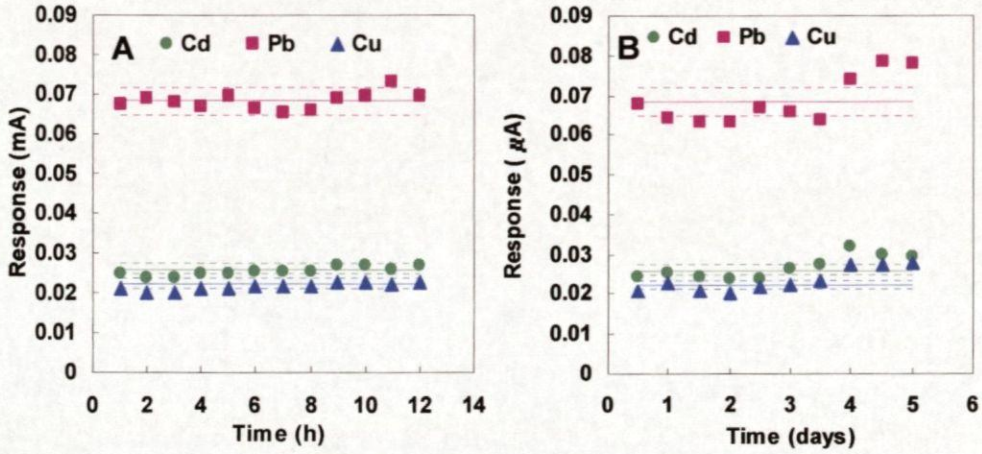


Figure 4. 17 The effect of time on the current response over **(A)** 12 hours and **(B)** 5 days in pH 2, deoxygenated seawater containing 5 nM Pb(II) and Cd(II), and 10 nM Cu(II) at a deposition time of 10 min. The solid lines represent the mean concentration over both time periods and the broken lines represent ± 5 % of the mean.

[illegible] over a period of 6 days [illegible]

[illegible]

Figure 4.[illegible] The effect of [illegible]

[illegible]

[illegible]

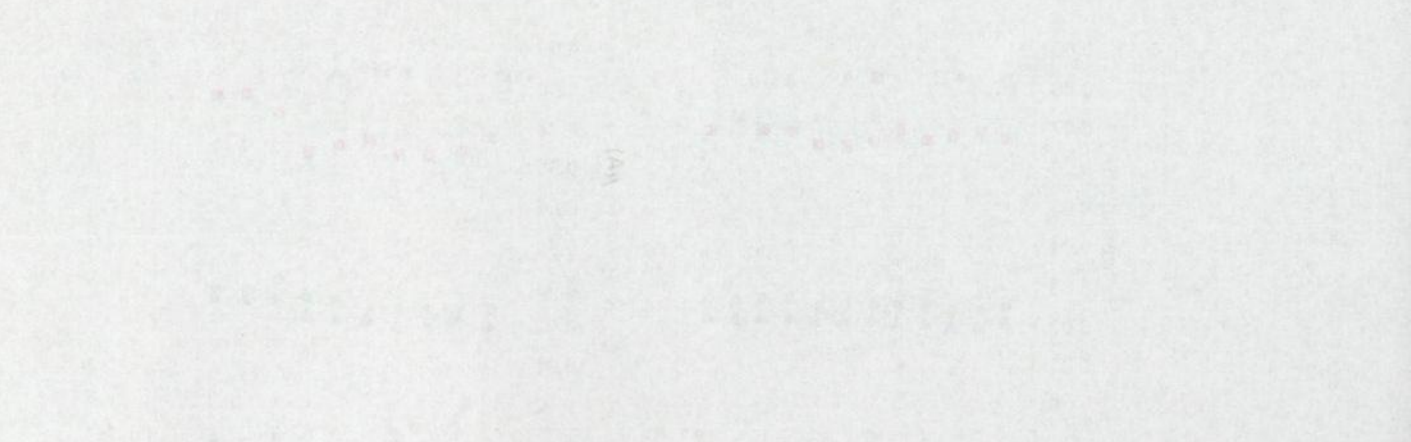


Figure 4.[illegible] The effect of [illegible]

The good stability of peak current response for trace metal determinations using the VIP probe is an important quality with respect to the applicability of the system for *in situ* measurements over periods that can vary from hours up to days.

4.3.12 Analytical Figures of Merit

The limits of detection (LOD) for Cd(II), Cu(II) and Pb(II) were determined using low trace metal $NaNO_3$ and seawater. The LOD (n=3), defined as 3σ of the blank, together with the precision at the LOD, and linear range and correlation coefficient for the conditions used for the LOD determination, are shown in Table 4.3. These LOD values are in reasonable agreement with values reported by Tercier-Waeber *et al* (Tercier-Waeber *et al*, 1999) for 0.1 M $NaNO_3$, 15 min deposition time: Cd(II) 50 pM, Cu(II) 200 pM, and Pb (50 pM) (LOD based on a signal to noise ratio of 2). The LOD results for seawater indicate that the VIP system is ideally suited for dynamic trace metal measurements in estuarine and coastal waters, where trace metal concentrations are somewhat elevated compared to open ocean environments. Furthermore, if required for VIP deployments in trace metal contaminated waters, the linear range of the measurements can be extended by decreasing deposition time.

Table 4.3 Analytical figures of merit for analysis (n=3) in deoxygenated, pH 2, 0.1 M $NaNO_3$ and in pH 2 seawater, using a 20 min deposition time. LOD is defined as 3σ.

Matrix	*Metal ion*	*LOD (pM/ nM)*	*Precision (at LOD, %)*	*Linear Range (nM)*	*Correlation Coefficient (R^2)*
$NaNO_3$	Cd(II)	58 pM	5.1	0.06 to 20.0	0.998
$NaNO_3$	Pb(II)	26 pM	8.4	0.03 to 17.6	0.999
$NaNO_3$	Cu(II)	0.75 nM	5.2	0.75 to 23.0	0.996
Seawater	Cd(II)	23 pM	4.8	0.02 to 21.3	0.995
Seawater	Pb(II)	23 pM	7.1	0.02 to 18.8	0.994
Seawater	Cu(II)	1.13 nM	7.6	1.13 to 24.0	0.989

The accuracy of the trace metal analyses using the VIP system was assessed using CRMs (National Research Council, Ottawa, Canada) for dissolved metals. For this purpose, analyses were undertaken in UV-digested CRMs of a riverine water (SLRS-3) and an estuarine water (SLEW-2). The results shown in Table 4.4 indicate a good agreement between the observed and certified values, with confidence intervals for measurements quoted as ± 2σ.

Table 4.4 Results of analysis of CRMs using the VIP system, with confidence intervals for measurements quoted as ± 2σ. (n≥3)

Metal ion	*CRM*	*Certified Value (nM)*	*VIP Determined Value (nM)*
Cd(II)	SLRS-3	0.12 ± 0.02	0.13± 0.01
	SLEW-2	0.17 ± 0.02	0.17 ± 0.02
Pb(II)	SLRS-3	0.42 ± 0.03	0.43 ± 0.01
	SLEW-2	0.13 ± 0.02	0.12 ± 0.01
Cu(II)	SLRS-3	21 ± 1	22 ± 1
	SLEW-2	26 ± 2	25 ± 1

4.4 Conclusions

The VIP system can be used for the determination of Cd(II), Cu(II) and Pb(II) in estuarine and coastal waters. The trace metal measurements using the VIP were not affected by changes in pH (in the range 2-7.6 and 2-4.8 in $NaNO_3$ and seawater respectively), salinity (in the range 2.5- 35) or oxygen (in the range 1.25-100 %), whereas an Arrhenius type temperature response was observed which is used for temperature correction of *in situ* dynamic trace metal data. In addition, determinations of trace metals using the VIP system are not affected by pressure in the range 1-600 bar (Belmont-Hebert *et al*,1998). The limits of detection (3σ) in $NaNO_3$ were 58 pM for Cd(II), 26 pM for Pb(II) and 0.75 nM for Cu(II) and in seawater were 23 pM for

Cd(II), for 23 pM Pb(II) and 1.13 nM for Cu(II). The analytical procedures were shown to be accurate, owing to the achievement of good agreement between measured values and the certificated values for riverine and estuarine CRMs.

Reference List

Achterberg, E. P. and van den Berg C. M. G (1994) *In-Line Ultraviolet-Digestion of Natural-Water Samples for Trace-Metal Determination using an Automated Voltammetric System.* Anal. Chim.Acta, **291**, 213-232

Belmont C., Tercier M.-L., Buffle J., Fiaccabrino G. C. and Koudelka-Hep, M. (1996) *Mercury-Plated Iridium-Based Microelectrode Arrays for Trace Metals Detection by Voltammetry: Optimum Conditions and Reliability.* Anal. Chim. Acta, **329,** 203-214.

Belmont-Hebert, C., Tercier, M.-L. Buffle, J., Fiaccabrino, G. C., de Rooij, N. F., and M. Koudelka-Hep (1998) *Gel-Integrated Microelectrode Arrays for Direct Voltammetric Measurements of Heavy Metals in Natural Waters and other Complex Media.* Anal. Chem., **70**, 2949-2956.

Buffle, J. and Tercier-Waeber, M.-L. (2000) In: *In Situ Monitoring of Aquatic Systems*, Eds Buffle, J. and Horvai. G, J. Wiley and Sons, Chichester, pp.279-406.

Buffle, J. (1981) *Calculation of the Surface Concentration of the Oxidized Metal During the Stripping Step in the Anodic-Stripping Techniques and its Influence on Speciation Measurements in Natural-Waters.* J. Electroanal. Chem., **125**, 273-278.

Howard, A. G. and Statham, P. J. (1993) In: *Inorganic Trace Analysis: Philosophy and Practice*, Eds. A. G. Howard and P. J. Statham, J. Wiley & Sons, Chichester, pp. 182.

Idronaut srl (1998) *VIP Reference Manual.* Milan, Italy.

Stumm, W. and Morgan, J. J. (1996) in *Aquatic Chemistry; Chemical Equilibria and Rates in Natural Waters*, ed W. Stumm and J. J. Morgan, J. Wiley & Sons, Chichester, pp. 516-608.

Tercier-Waeber, M.-L. and Buffle, J. (2000) *Submersible Online Oxygen Removal System Coupled to an In Situ Voltammetric Probe for Trace Element Monitoring in Freshwater.* Environ. Sci. Technol., **34**, 4018-4024.

Tercier-Waeber, M.-L., Buffle, J., Confalonieri, F., Riccardi, G., Sina, A., Graziottin, F., Fiaccabrino, G. C. and Koudelka-Hep, M. (1999) *Submersible Voltammetric Probes for In Situ Real-Time Trace Element Measurements in Surface Water, Groundwater and Sediment-Water Interface.* Meas. Sci. Technol., **10**, 1202-1213.

Tercier, M.-L., Buffle, J. and Graziottin, F. (1998) *Novel Voltammetric In-Situ Profiling System for Continuous Real-Time Monitoring of Trace Elements in Natural Waters.* Electroanalysis, **10**, 355-363.

Tercier-Waeber, M.-L., Belmont-Hebert, C. and Buffle, J. (1998) *Real-Time Continuous Mn(II) Monitoring in Lakes Using a Novel Voltammetric In Situ Profiling System.* Environ. Sci. Technol. 32, 1515-1521

Tercier, M.-L. and Buffle, J. (1996) *Antifouling Membrane-Covered Voltammetric Microsensor for In Situ Measurements in Natural Waters.* Anal. Chem., **68,** 3670-3678.

Tercier, M.-L. Parthasarathy, N. and Buffle, J. (1995) *Reproducible, Reliable and Rugged Hg-Plated Ir-Based Microelectrode for In-Situ Measurements in Natural-Waters.* Electroanalysis, **7,** 55-63.

Tercier, M.-L. and Buffle, J. (1993) *In situ Voltammetric Measurements in Natural Waters – Future Prospects and Challenges.* Electroanalysis, **5**, 187-200.

van den Berg, C. M. G.(1991) *Potentials and Potentialities of Cathodic Stripping Voltammetry of Trace-Elements in Natural-Waters.* Anal. Chim. Acta, **250**, 265-276.

CHAPTER 5

Inter-comparison of VIP Systems and Reference Methods for the Determination of Trace Metals in Estuarine and Fjord Waters

Abstract

A laboratory and shipboard based analytical inter-comparison by 4 EU laboratories was carried out on discrete estuarine samples (<0.2 μm; analysed in laboratory) and fjord water (VIP dynamic fraction; analysed *in situ*) for the determination of Cd(II), Pb(II) and Cu(II).

Estuarine samples (6 samples) were collected and analysed for total filterable (0.2 μm pore filter) Cd(II), Pb(II) and Cu(II) concentrations using VIP systems and reference methods. Statistical analyses of the results (*t*-test) showed that results of the analysis were significantly different at the 95 % confidence interval in the majority of cases (84 %). Poor correlation may be due to (1) incomplete digestion of samples during UV irradiation and/or (2) sensitivity changes from oxidation of the mercury film caused by pumping air through the system.

Statistical analyses (*t*-test) of samples measured *in situ* in the fjord showed good correlation and the null hypothesis was accepted (at the 95 % confidence interval) in 66 % of cases. Significantly different results (34 %) could possibly be due to the inhomogeneity of samples due to different water masses in the fjord.

5.1 Introduction

Trace metals are an integral part of ecosystem functioning (Campbell, 1995) and reliable measurements in natural water matrices are required to ensure consistent research and proper enforcement of environmental legislation. Ideally, instrumentation should be accurate, sensitive (measuring in pM range) and capable of high-resolution laboratory and shipboard monitoring (2 to 4 samples h^{-1}; Howell *et al.*, 2003). To this end, precautions must be taken to ensure methods available for the determination of trace metals in natural water are comparable.

Few publications on inter-laboratory analytical comparison for trace metals in seawater exist to date (*e.g* Brugmann, *et al.,* 1983). However, constraints on ship time, space and costs often make shipboard analytical inter-comparison exercises difficult and hence there have been a small amount of publications with shipboard data (Bewers *et al.,* 1981; Landing *et al.,* 1995; de Jong, *et al.,* 2000). The International Council for the Exploration of the Sea (ICES; Bewers *et al.*, 1981) fourth round inter-comparison exercise provided a comprehensive temporal data set attained on board ship. However, results for Pb were of limited use due to high contamination that led to concentrations greater (1.4 nM) than those expected in open ocean surface waters (typically 0.14 nM; Kremling and Streu, 2001). Similar contamination was reported for Zn.

The first Intergovernmental Oceanographic Commission (IOC) baseline survey for inter-comparison of trace metals in the Atlantic Ocean took place in April 1990 (Landing *et al.,* 1995). Although Cu and Cd showed no significant differences (95 % level) between analyses by 9 different laboratories, Pb, investigated by 4 laboratories, proved problematic and gave significantly different, anomalous data, attributed to inadequate cleaning of sampling equipment.

In situ approaches minimise the risk of sample storage effects and sample contamination and also give rise to high-resolution data which can be used for both current and predictive modelling purposes. However, discrete sampling procedures are often incorporated into monitoring protocols for speciation studies, as highlighted by the work using the VIP system (Chapter 3, section 3.6; Chapter 6;), but can lead to contamination and errors during sampling and analysis.

Analytical comparison of VIP systems used by different laboratories was necessary, in order to evaluate performance between system users and in a variety of matrices (Buesseler *et al.*, 1996). For this reason, a laboratory inter-comparison exercise was carried out on total filterable (0.2 μm pore filter; $TF_{0.2}$; defined Chapter 2, section 2.4) river, estuarine and seawaters and *in situ* tests performed in a fjord.

The objectives of the inter-comparison were to provide a means that enabled participating laboratories to validate VIP protocols and performance; to compare and assess measurements with participant laboratories; to evaluate data quality from measurements of selected trace metals in certified reference materials (CRMs) and natural water samples (namely estuarine and fjord samples) and, finally, to establish if differences due to deployment artefacts could lead to systematic errors in the measurements. The overall aim of the exercise was to improve the quality of measurements of trace metals by participants, University of Plymouth, UK [LAB 1, LAB 2 (LAB 2=author)]; University of Ancona, Italy (LAB 4) and University of Geneva, Switzerland (LAB 3, LAB 5, LAB 6, LAB 7).

The first part of the inter-calibration exercise involved the collection and distribution of 6 homogeneous natural water samples taken from the Tamar Estuary (three samples collected and distributed in November, 2001 and three samples in May 2002). Samples were then analysed for $TF_{0.2}$ concentrations of Cd(II), Pb(II) and Cu(II) by the participating laboratories, by using the VIP system and by other methods specific to each laboratory. The main purpose of this part of the exercise was to confirm that despite differences in the analytical methodologies used, data obtained using the VIP systems and other methods were comparable between all participating laboratories.

The second part of the exercise involved inter-comparison of *in situ* determination of dynamic Cd(II), Pb(II) and Cu(II) species in the Gullmar Fjord, Sweden (August 2002). This study involved the use of VIP systems by each participant, as well as a Multi Physical-Chemical Profiler (MPCP) probe, which is currently undergoing systematic tests and is illustrated in Figure 5.1. The MPCP employed three separate peristaltic pumps and electrochemical VIP cells, and consequently the instrument effectively worked as three independent VIP systems (labelled LAB 5, LAB 6 and LAB 7).

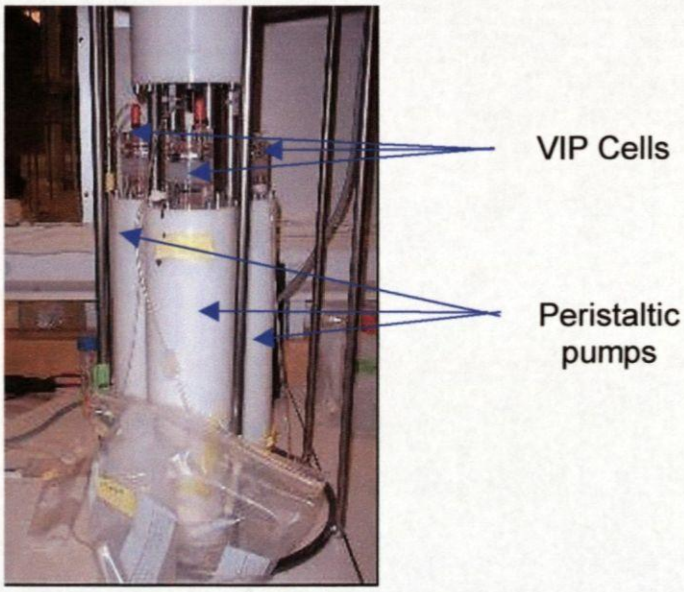


Figure 5. 1 The MPCP with three independent VIP cells and peristaltic pumps.

There is a pressing need to address standardisation of sampling procedures and analytical methods to ensure the reliability, consistency and comparability of reported trace metal data. This inter-comparison was viewed as an interactive trial procedure, and unacceptably poor bias and precision (> 5 %) would lead to further inter-comparisons employing more prescribed approaches.

5.2 Experimental

5.2.1 Description of Sample Sites

Tamar Estaury

The Tamar Estuary has been previously described in Chapter 2, section 2.1 and the same sample sites as illustrated in Chapter 2, Figure 2.1 were used for this study.

The Gullmar Fjord, Kristinaberg, Sweden

The Gullmar Fjord (Figure 5.2) is situated on the Swedish west coast (58°15'8"N; 11°28'4"E). It is 28 km long and between 1-2 km wide, with a maximum depth of 116 m and a sill depth of 43 m (Arneborg and Liljebladh, 2001). The fresh water input from the Orekil River is low, only maintaining a fresh surface layer of 1 m deep in the inner parts of the fjord. Stratification above the sill level in the fjord is dominated by the coastal stratification, which varies strongly due to the fresh water outflows from the Baltic sea and wind driven circulation within the Skaggerak (Arneborg and Liljebladh, 2001). Water below the sill is stagnant for most of the year, being renewed in late winter in most years. The stratification of the fjord is often characterised by a relatively strong pycnocline at 5 to 20 m depth dividing the relatively fresh Kattegat water from the more saline Skagerrak water. A second, less pronounced pycnocline is often situated just below sill level, separating Skaggerak water from the denser, stagnant basin water (Arneborg and Liljebladh, 2001).

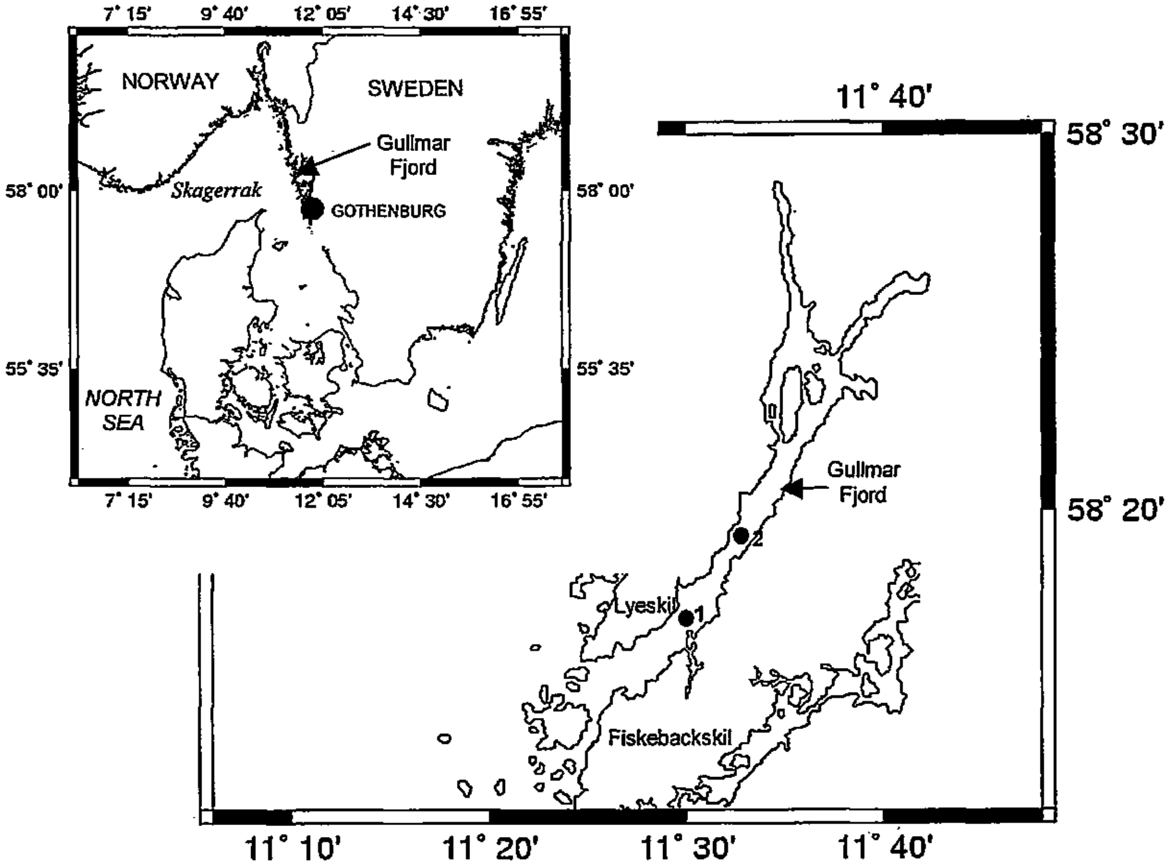


Figure 5. 2 Coastline map of the Gullmar Fjord and locations of stations. Map produced in http://rimmer.ngdc.noaa.gov/coast

5.2.2 Sampling Procedure, Tamar

Bulk samples from the Tamar River and estuary were collected in acid cleaned 10 L carboys (according to procedure in Chapter 2; section 2.2.1) during November 2001 and again during May 2002 from three locations selected for their ease of access, along the salinity gradient. Table 5.1 indicates the sample dates, locations, codes and salinity.

Table 5. 1 The dates, locations, codes and salinity of samples.

Date	Location	Code	Salinity
November 2001	Gunnislake	R1	~0
	Calstock	E1	9.91
	Saltash	E2	33.96
May 2002	Gunnislake	R2	~0
	Calstock	E3	8.58
	Saltash	CW1	31.85

Water samples were taken from the same locations as in Chapter 2, section 2.2.1. The raw samples were returned to the laboratory, and kept at 4°C in the dark prior to filtration and filtered in-line through Sartorius filter capsules [Sartobran 300; cellulose acetate; sterile; nominal 0.45 μm pre-filter, 0.2 μm pore size] within a Class 100 clean bench into a acid cleaned 10 L carboy (High density polyethylene; HDPE). The carboy was rinsed twice with filtrate prior to final filtrate collection. Sub-samples were decanted from the carboy into 1 L bottles (HDPE; Nalgene) and the bottles rinsed twice with filtrate prior to final sample filling. Samples were acidified immediately (pH 2; sub-HNO_3) after filling. The bottles were given codes (Table 5.1) and distributed to the respective laboratories. Trace metal measurements were undertaken on these samples using the VIP system, while measurements of total filterable ($TF_{0.2}$) trace metals were performed using laboratory based analytical techniques (anodic and cathodic stripping voltammetry and ICP-MS).

5.2.3 Deployment Mechanism of VIP Systems in Gullmar Fjord

The VIP systems were deployed in the Gullmar Fjord (Figure 5.2) from onboard *RV Arne Tiselius* (Figure 5.3).

Figure 5. 3 The *RV Arne Tiselius*, based at Kristineberg marine station

In order to maximise the data collection capacity, multiple VIP systems housed in titanium frames (Idronaut s.r.l, Milan, Italy), were deployed at different depths in the water column, illustrated in Figure 5.4.

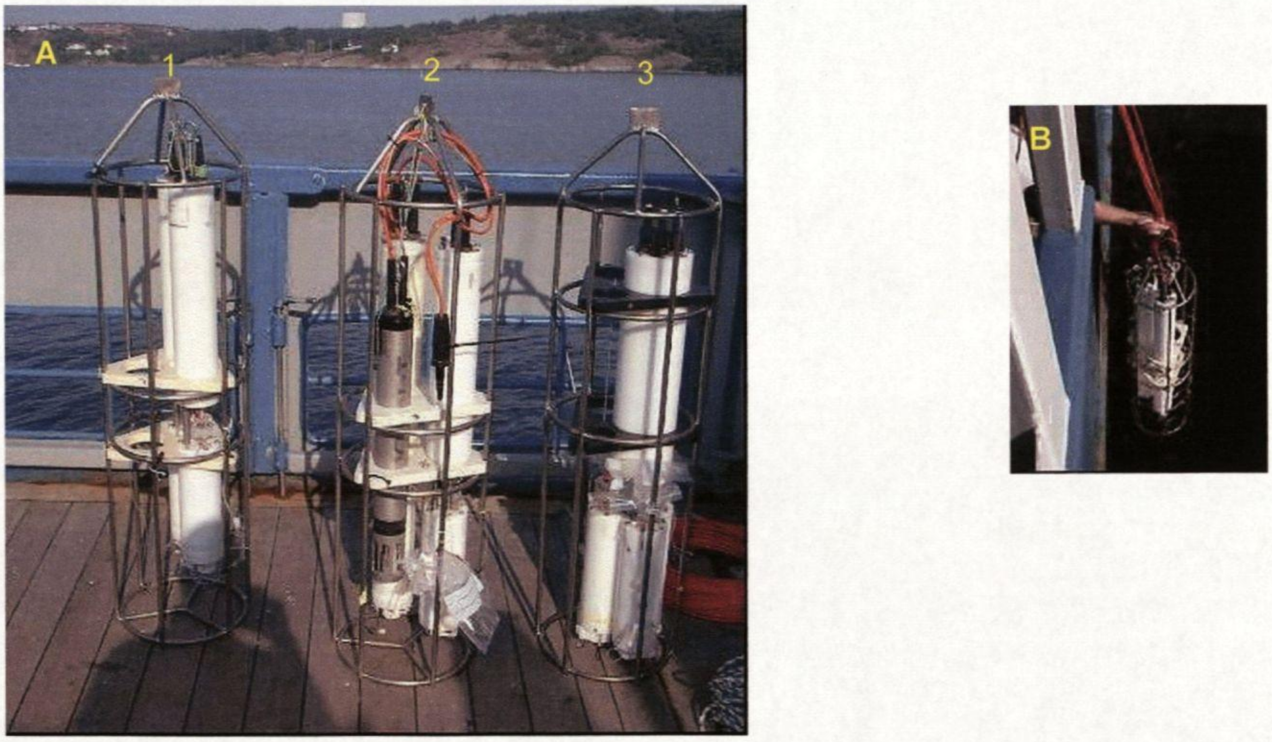


Figure 5. 4 VIP systems in titanium frames were deployed simultaneously (B) with 2 VIPs in one frame (A1), 2 VIPs and an ocean 7 CTD probe (A2) and MPCP (A3).

To facilitate safe deployment at multiple depths, the titanium frames and the VIP telemetry cables were attached to a Kevlar rope using plastic cable ties, which were applied during each deployment and removed during each recovery. The Kevlar rope was knotted through the top of each titanium frame in order to secure the frame to a fixed position on the rope. The deployment arrangement is shown schematically in Figure 5.5. Deployment depths were calculated from the length of rope deployed, and by conductivity-temperature-depth (CTD) probe measurements.

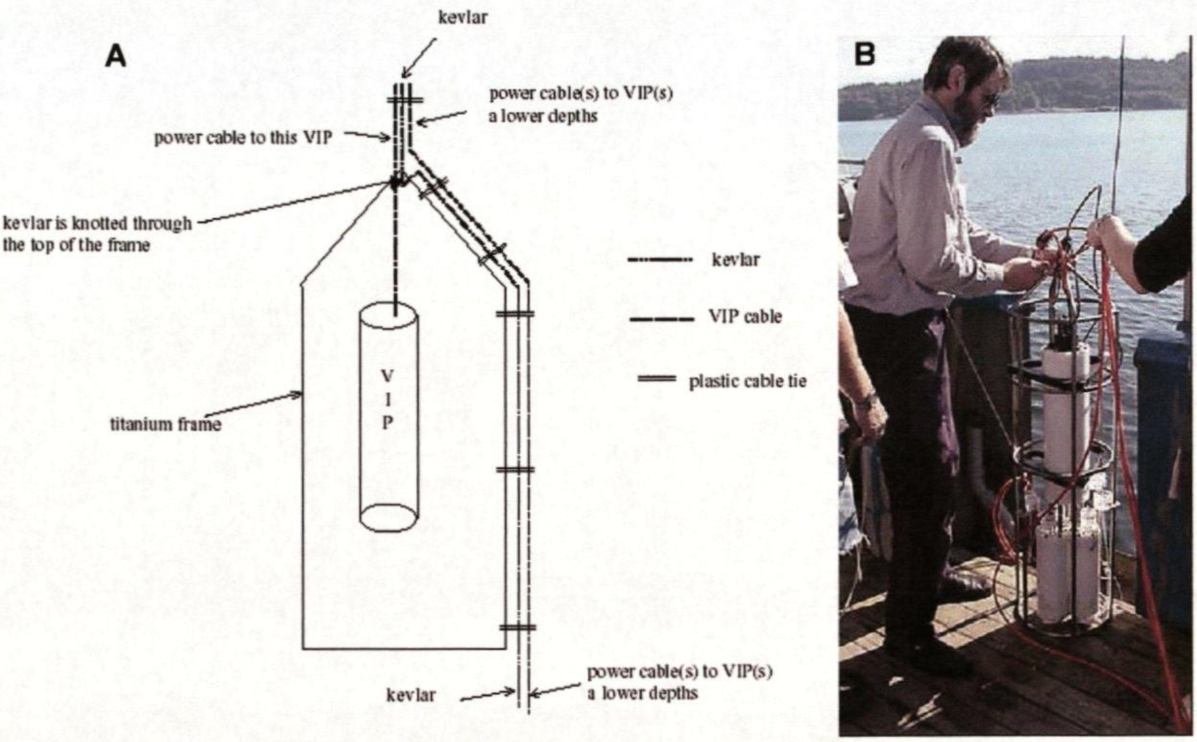


Figure 5. 5 Schematic (A) and photographic (B) representation of the procedure used to secure VIP systems during deployment in the Gullmar Fjord.

5.2.4 Discrete Sampling Procedure

All discrete water sampling was carried out using 8 L Go Flo bottles on a rosette, (Figure 5.6A) deployed on a Kevlar rope (Figure 5.6B).

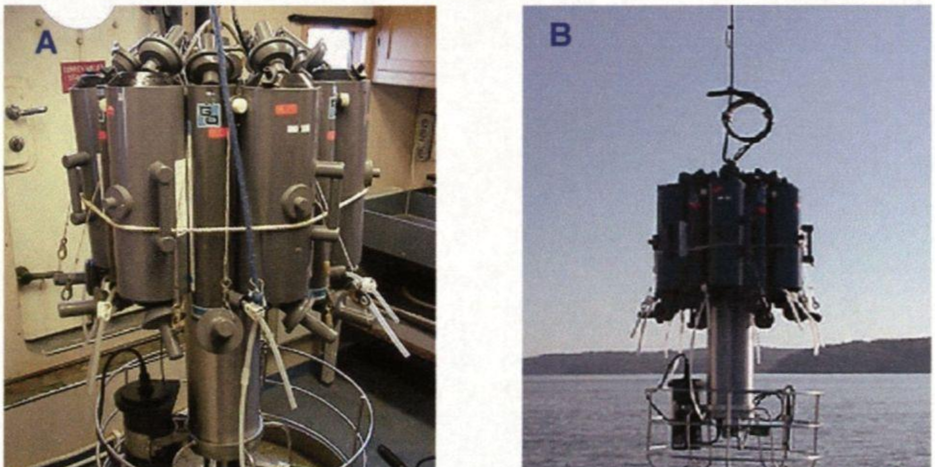


Figure 5. 6 Rosette of Goflo bottles (12 X 8L) and CTD probe in the lab (A) and during deployment on kevlar rope (B).

Filtration for $TF_{0.2}$ sample fraction for the metal analysis was carried out immediately after collection, on board ship using online filter units (with 0.2 μm pore size) coupled directly to the GoFlo bottles, which were pressurised with nitrogen gas (Figure 5.7). Sampling and sample handling were carried out using trace metal clean conditions, using a laminar flow hood for filter manipulations and filling up of sample bottles.

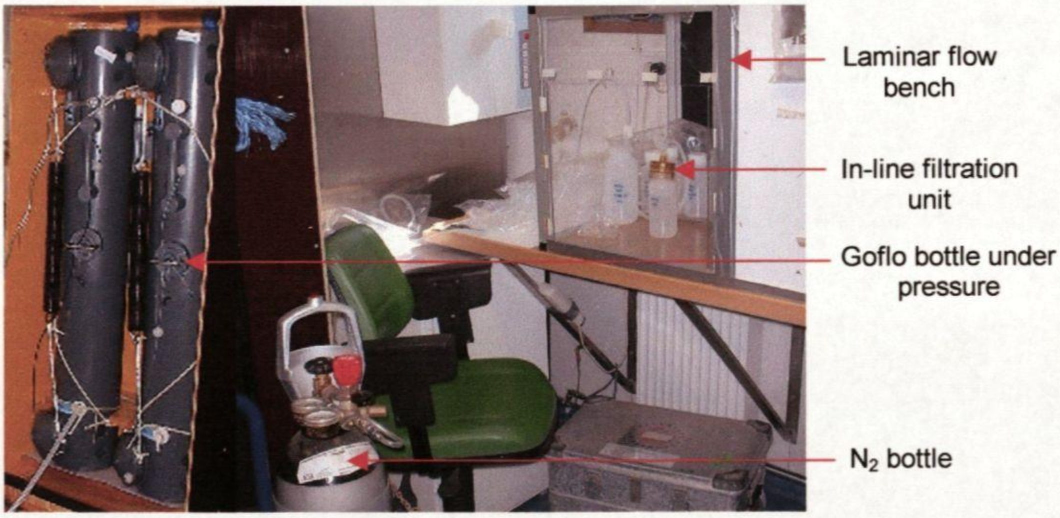


Figure 5. 7 The filtration set-up. Go-Flos were pressurised and connected to an inline filtration unit that was housed in a laminar flow cabinet.

5.2.5 Gullmar Fjord Sampling Sites

Station 1

Deployments were made on *22/08/02* from the *Arne Tiselius* anchored at Station 1 with 60 m water depth and within a short distance from Kristineberg (Figure 5.2, Station 1). The depth profiles presented in Figure 5.8 showed a clear stratification of the water column, with a halocline and thermocline observed at ~18 m separating the surface layer (influenced by fresh water inputs from the Orekil river and less saline Baltic sea) and deep layer comprising denser water (from the Skaggerak). Chlorophyll-a concentrations were highest in upper waters, where light penetration was at its maximum. According to the different water masses found in this site, VIPs were deployed subsequently for 3 h on the Kevlar rope at depths of 5 m (LAB 2, LAB

4) and 30 m (LAB 1, LAB 3). The MPCP (LAB 5, 6 and 7) was deployed at 5 m depth over the stern A-frame After 3 h the VIPs at 5 m were redeployed at 5 m on a separate line, while the VIPs at 30 m were lowered to 47 m for further measurements. *In situ* measurements were completed over a 2 h period. The VIP systems were deployed on the Kevlar rope as follows: LAB 2 and LAB 3 at 5 m, MPCP at 28 m, and LAB 1 and LAB 4 at 33 m.

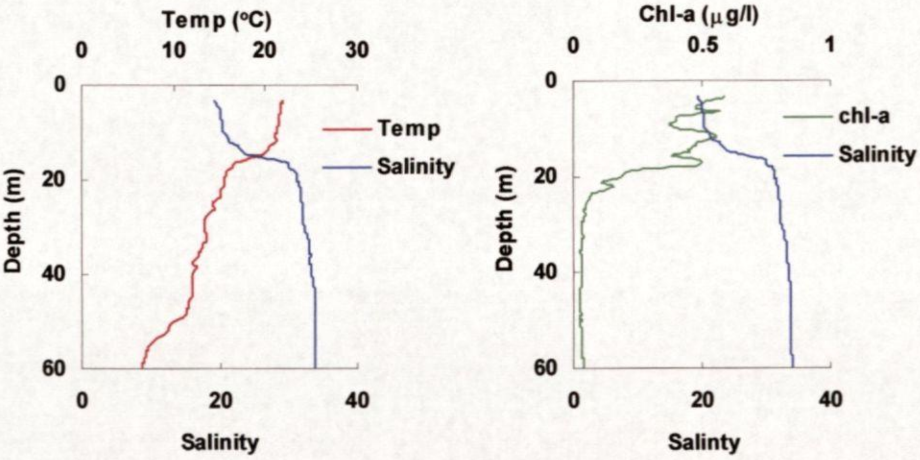


Figure 5. 8 Temperature and Salinity **(A)** and Chlorophyll-a **(B)** concentrations measured as a function of depth at Station 1 in the Gullmar Fjord on 22/08/02.

Station 1 was re-sampled on 26/08/02. The CTD profile showed a similar water column structure (Figure 5.9A,B) compared with 22/08/02 but with a somewhat shallower main thermo and haloclines to ~ 15 m).

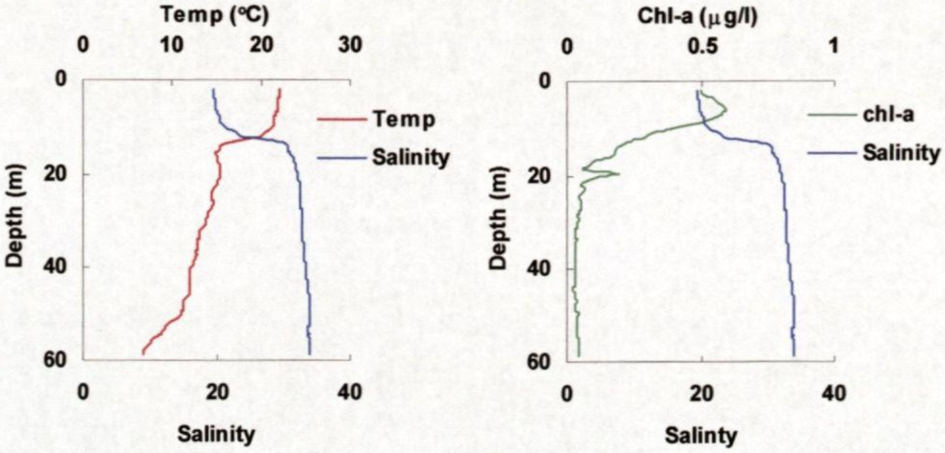


Figure 5. 9 Temperature and Salinity (**A**) and Chlorophyll-a (**B**) concentrations measured as a function of depth at Station 1 in the Gullmar Fjord on 26/08/02

The initial CTD profile (Figure 5.9) indicated suitable sampling depths of 5, 30 and 48 m as they represented the major water masses at this station. However, sampling was restricted to two depths, 5 m and 30 m, in order to obtain *in situ* measurements for as long a period as possible.

Station 2

Deployments were made on 23/08/02 in the deepest part of the fjord in order to sample the water column at a different location. The CTD depth profiles presented in Figure 5.10 show stratification of the water column, similar to that of station 1 in the first 50 m with a halocline and thermocline observed at ~15 m. However a second thermocline was observed situated just below sill level, separating Skaggerak water from the denser, stagnant basin water. Chlorophyll-a concentrations once again were highest in upper waters (<20 m depth).

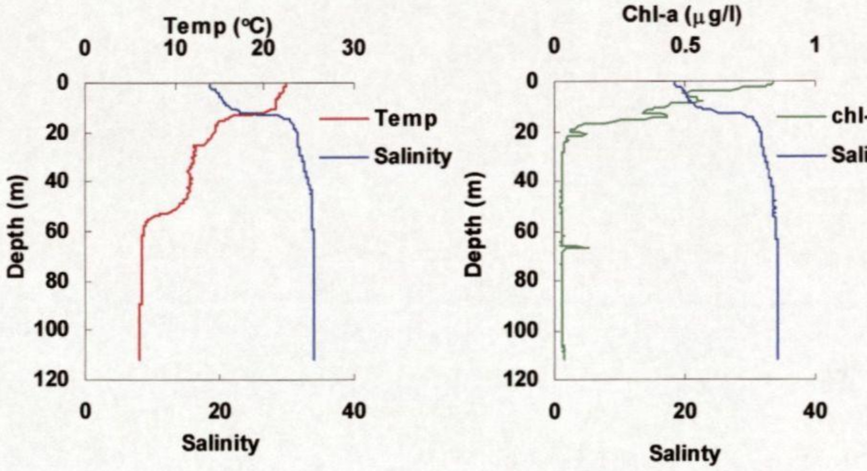


Figure 5. 10 Temperature and Salinity (**A**) and Chlorophyll-a (**B**) concentrations measured as a function of depth at Station 2 in the Gullmar Fjord on 23/08/02.

5 m and 32 and 35 m were selected as the working depths. VIP and MPCP systems were set out attached to the Kevlar line: LAB 2 and LAB 3 at 37 m, MPCP at 32 m, LAB 1 and LAB 4 at 5 m

5.2.6 Analysis

Sample pre-treatment

As an agreed procedure all participants undertook UV digestion of samples immediately prior to analysis (Achterberg and van den Berg, 1994), with the exception of Lab 4. All samples and certified reference materials (CRMs) were UV-digested (60 or 400 W medium pressure UV lamp; Photochemical Reactors, Slough, UK), after the addition of H_2O_2 (8.8 mM, final concentration).

Each group employed recommended procedures and methodologies of the VIP reference manual, (Idronaut srl, 1998) for VIP system operations and used established reference methods for comparison. LABS 5, 6 and 7 did not participate in the Tamar discrete sample inter-comparison as the MPCP was under construction at the time of the exercise. The analytical instruments used by each laboratory are shown in Table 5.2.

Table 5. 2 Laboratory code, and reference method for Tamar samples. VIP=Voltammetric *In situ* Profiling System, HMDE=Hanging Mercury Drop Electrode, RDE=Rotating Disc Electrode and ICP-MS=Inductively Coupled Plasma Mass Spectrometry

	Analysis Type					
Code	R1	E1	E2	R2	E3	CW1
LAB 1	VIP HMDE RDE	VIP HMDE RDE	VIP HMDE RDE	VIP HMDE RDE ICP-MS	VIP HMDE RDE	VIP HMDE RDE
LAB 2	VIP HMDE RDE	VIP HMDE RDE	VIP HMDE RDE	VIP HMDE	NA	VIP
LAB 3	VIP ICP-MS	VIP	VIP	VIP ICP-MS	VIP	VIP
LAB 4	NA	NA	NA	VIP	VIP	VIP

Sample analysis for $TF_{0.2}$ Cd(II), Cu(II), and Pb(II) were undertaken by SWASV using the VIP system by the method described in Chapter 4. Additional $TF_{0.2}$ Cu (II) measurements were made by HMDE, AdCSV with complexing ligand 8-hydroxyquinoline (25 μM final concentration; van den Berg; 1986) in the presence of 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES; pH 7.7; 0.01 M final concentration) as a pH buffer. Additional $TF_{0.2}$ Cd(II) measurements were made with a RDE in the presence of mercury nitrate (30 μM, final concentration) and potassium thiocyanate (5 mM, final concentration) with ammonium acetate (pH 5.5; 0.1 M final concentration) as a pH buffer (Fischer and van den Berg, 1999). ICP-MS measurements were also made for samples R1 and R2 using a VG elemental PQ2 turbo (Winsford, Cheshire) ICP-MS fitted with an Ebdon high solids 'V' groove nebuliser with a fixed plasma gas (Ar) flow (1 l min^{-1}). Replicates of measurements were normally made with $n \geq 5$. The accuracy of the trace metal analyses using the VIP system was assessed using CRMs (National Research Council (NRC), Ottawa, Canada) for dissolved metals.

5.3 Results and Discussion

To avoid bias, an independent collator and processor of the data, Professor Paul Worsfold, University of Plymouth, completed the reporting and processing of data anonymously. An identification code was assigned to the participant laboratories upon submission of their results, continuing to be unidentified until after collation, analysis and presentation of results.

5.3.1 Accuracy and Precision Tests

CRMs (SLRS-4; SLRS-3; SLEW-2; SLEW-3 and CASS-4, NRC) for dissolved metals were used to assess and gauge laboratory performance for accuracy and to provide a way of assessing the comparability of data produced by participating laboratories, for Cd(II), Pb(II) and Cu(II) determinations. For each metal, the performance of the laboratory was considered accurate if the laboratory result for analyses of CRM (±2 σ) was within the confidence interval (±2 σ) quoted for the reference material. Measurements (n=5) were reported for at least two CRMs and the results of the analysis with the VIP systems of participating laboratories are given in Table 5.3, with the exclusion of the Lab 4 that did not provide CRM data.

The results shown in Table 5.3 (with confidence intervals for measurements quoted as ± 2σ) indicate mostly good agreement between the observed and certified values for all measurements undertaken by laboratories.

Table 5. 3 Measured and certified results of analysis of CRMs using the VIP system, with confidence intervals for measurements quoted as ± 2σ. (n=5 for all samples) and precision in %. The authors results are underlined.

	Metal ion	CRM	Measured Value	Certified Value	Accurate	Precision (%)
LAB 1	Cd(II)	SLRS-4	0.11 ± 0.01	0.11 ± 0.02	Yes	9.1
	Pb(II)	SLRS-4	0.45 ± 0.03	0.41 ± 0.03	Yes	6.7
	Cu(II)	SLRS-4	26.9 ± 2.6	28.49 ± 1.2	Yes	9.7
LAB 2	Cd(II)	SLRS-3	0.12 ± 0.02	0.13 ± 0.01	Yes	16.7
		SLEW-2	0.17 ± 0.02	0.17± 0.02	Yes	11.8
	Pb(II)	SLRS-3	0.42 ± 0.03	0.43 ± 0.01	Yes	7.1
		SLEW-2	0.13 ± 0.02	0.12 ± 0.1	Yes	15.4
	Cu(II)	SLRS-3	21.3 ± 1.1	22.0 ± 1.1	Yes	5.2
		SLEW-2	25.5 ± 1.7	25.1 ± 1.2	Yes	6.7
LAB3	Cd(II)	SLRS-4	0.09 ± 0.02	0.11 ± 0.02	Yes	22.2
		SLEW-3	0.29 ± 0.08	0.42 ± 0.03	No	27.6
		CASS-4	0.22 ± 0.08	0.23 ± 0.03	Yes	36.4
	Pb(II)	SLRS-4	0.52 ± 0.09	0.41 ± 0.03	Yes	17.3
		SLEW-3	0.93 ± 0.11	0.04 ± 0.01	No	11.8
		CASS-4	1.07± 0.18	0.05 ± 0.02	No	16.8
	Cu(II)	SLRS-4	25.0 ± 3.25	28.5 ± 1.2	Yes	13.0
		SLEW-3	23.2 ± 3.3	24.4 ± 1.9	Yes	14.2

	CASS-4	7.58 ± 0.8	9.31 ± 0.87	No	10.6

Under estimation of Cu(II) and Cd(II) concentrations was probably due to incomplete destruction of organic complexing ligands during UV irradiation. The over estimation of Pb(II) concentrations is most likely due to typically low concentration of Pb(II) in the environment (*e.g.* 0.13 to 0.32 nM in the Narragansett estuary; Kozelka and Bruland, 1998) that is close to the limit of detection (LOD) of the VIP system (0.015 nM, Chapter 4, Section 4.3.12) and the ubiquitous nature of Pb that connotes easy contamination of samples. Precision was poor for most CRMs and varied in the range 5.2 to 36.4 % at CRM concentrations and again this is probably due to the inherently low concentrations (pM) of these trace metals in the CRMs, that are close to the LODs of the instrument (Chapter 4, section 4.3.12). Table 5.4 shows the results from analyses with reference methods used by each particular laboratory for the same samples.

Table 5. 4 Measured and certified results of analysis of CRMs using reference methods, with confidence intervals for measurements quoted as ± 2σ. The authors results are underlined.

	Metal ion	CRM	Method	Measured Value (nM)	Certified Value (nM)	Accurate	Precision (%; n=3)
LAB 1	Cd(II)	SLRS-4	ICP-MS	0.10± 0.01	0.11 ± 0.02	Yes	10
		SLRS-3	RD	0.12 ± 0.01	0.13 ± 0.01	Yes	8.3
	Pb(II)	SLRS-4	ICP-MS	0.44 ± 0.06	0.41 ± 0.03	Yes	13.6
	Cu(II)	SLRS-4	ICP-MS	21.4 ± 1.1	28.5 ± 1.2	No	5.1
		SLRS-3	HMDE	21.2 ± 0.6	22.0 ± 1.1	Yes	2.8
LAB 2	Cd(II)	SLRS-3	RD	0.12 ± 0.01	0.13 ± 0.01	Yes	8.3
		SLEW-2	RD	0.18 ± 0.01	0.17± 0.02	Yes	5.6
	Cu(II)	SLRS-3	HMDE	21.4 ± 0.7	22.0 ± 1.1	Yes	3.3
		SLEW-2	HMDE	25.8 ± 1.0	25.1 ± 1.2	Yes	3.9
LAB 3	Cd(II)	SLRS-4	ICP-MS	0.07 ± 0.01	0.11 ± 0.02	No	14.3
	Pb(II)	SLRS-4	ICP-MS	0.65 ± 0.01	0.41 ± 0.03	No	1.5
	Cu(II)	SLRS-4	ICP-MS	27.8 ± 0.46	28.5 ± 1.2	Yes	1.7

Excellent agreement between certified and measured concentrations for the three metals in the CRMs was observed for most laboratories. Under estimation of the Cu(II) and Pb(II) was most likely due to a high blank. Overestimation of Pb(II) may be due to sample contamination or inaccuracy due to measuring close to the LOD of the ICP method (typically 10 pM; Ebdon, *et al* 1998). Precision was acceptable (<15 %) at the concentrations reported for the CRMs.

5.3.2 Tamar Samples

General Observations

Regression graphs for comparison between measured concentrations in each pairing were constructed to assess systematic errors during analysis. LAB 4 was excluded from testing due to insufficient data. Data from LAB 1, LAB 2 and LAB 3 were considered for Cd(II) and Pb(II) and are represented in Figure 5.11 (A-C). Cu(II) concentrations were 1-3 orders of magnitude higher and in order to prevent bias are plotted in Figure 5.11 (D, E, F).

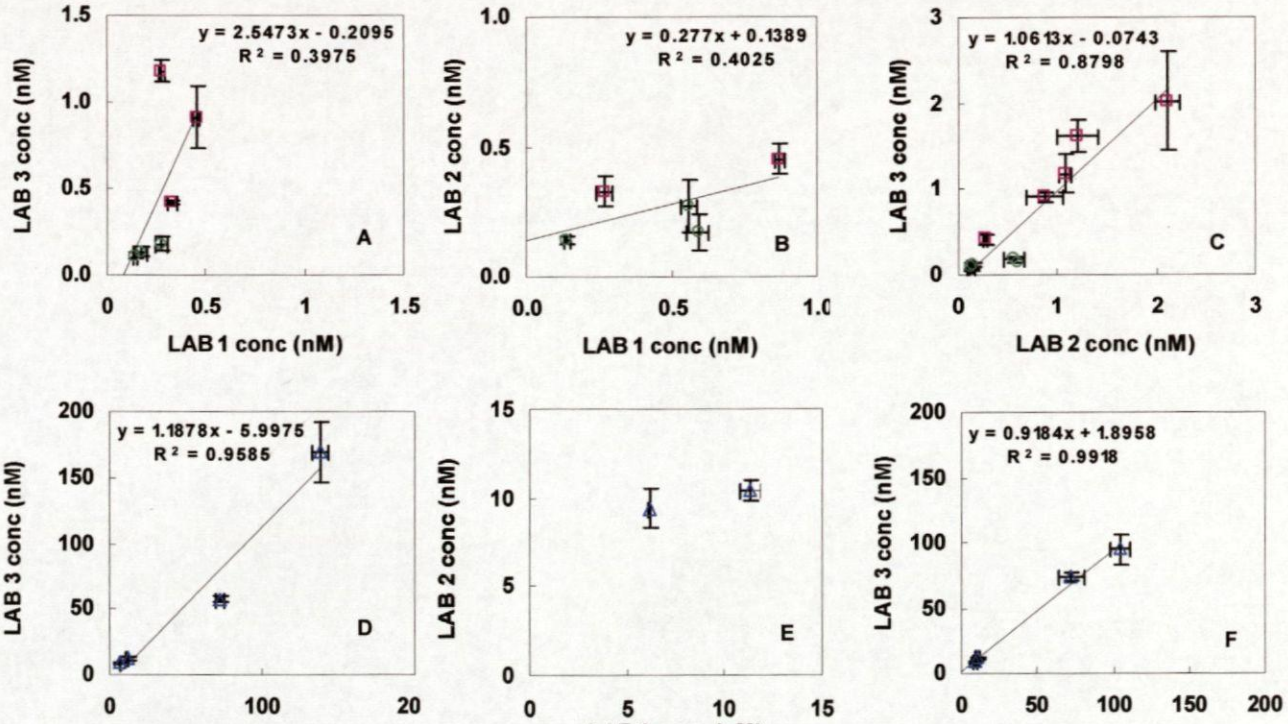


Figure 5. 11 Regressions graphs comparing paired laboratories for Cd(II) (green circles) and Pb(II) (pink squares) (A-C) and Cu(II) (blue triangles)(D-F).

Figure 5.11 shows product-the moment correlation coefficient r^2 in the range 0.3975 to 0.9918. Deviation from the ideal (r^2=1) was caused by both random and systematic errors.

Statistical Analysis

In order to determine whether variation of results obtained by the participating laboratories were significant, *t*-tests (at 95 % confidence level) comparing two experimental means were performed by examining the difference between each pair of laboratories (if data was available) for each metal in each sample. In addition, *t*-tests were preformed for each laboratory between the results obtained with reference analytical methods employed by that laboratory. In applying a significance test to the data, the null hypothesis that the means of the results given by two methods should not be significantly different was tested according to the method of Miller and Miller, (2000) and the results shown in Table 5.5.

Examination of Laboratory based inter-comparison

The poor correlation shown in Table 5.5 indicates that systematic errors are a source of significant differences between the analyses of samples, with no specific analyte or specific water mass giving rise to an appreciably greater number of significantly different results. The results demonstrate that within a small population (6 samples) all groups exhibited a systematic bias at the 95 % confidence interval. The most likely cause of systematic errors was from the UV digestion that was necessary to release trace metals from metal-organic complexes prior to analysis (Achterberg and van den Berg, 1994; Gueguen *et al.*, 1999). Incomplete UV digestion probably led to the underestimation of concentrations. Even though users followed standard procedures (Achterberg and van den Berg, 1994), the variation in design of UV digestion equipment is large, due to the in-house manufacture of such equipment, the power of the UV lamp and the age of the lamp.

Table 5. 5 Results from *t*-tests on each set of paired data at the 95 % confidence interval (*P*=0.05). Critical value of t in each case was 2.31 at 95 % confidence level. No result (nr) indicates samples that were not analysed by that laboratory. Green colour indicates pairings that were not significantly different and red indicates the pairings that were significantly different. The author's results are underlined.

		Lab								
		LAB 1			LAB 2			LAB 3		
		Metal ion			Metal ion			Metal ion		
Sample	Lab	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)
R1	LAB 2	nr	nr	nr						
	LAB 3	nr	nr	nr	1.88	5.38	0.55			
E1	LAB 2	nr	nr	nr						
	LAB 3	nr	nr	nr	2.82	0.55	2.35			
E2	LAB 2	0.49	12.3	10.5						
	LAB 3	4.95	55.1	6.13	4.24	1.47	1.91			
R2	LAB 2	9.81	3.13	nr						
	LAB 3	6.62	9.85	4.69	12.5	8.57	nr			
	LAB 4	nr	11.8	57.1	nr	12.2	nr	nr	3.57	18.1
E3	LAB 2	nr	nr	nr						
	LAB 3	60.3	14.3	15.1	nr	nr	nr			
	LAB 4	20.5	14.0	62.8	nr	nr	nr	2.83	50.8	51.9
CW1	LAB 2	18.8	23.5	3.97						
	LAB 3	3.10	8.93	0.78	21.4	0.75	2.51			
	LAB 4	nr	8.62	29.9	nr	5.45	20.1	nr	6.97	10.7

Digestion efficiency is directly proportional to the intensity of the source *i.e.* the energy efficiency (number of UV photons emitted per watt (W) of input power), demonstrated by Achterberg and van den Berg (1994) and Achterberg *et al.* (2001). Both papers indicate that 100 W and 125 W lamps were not as efficient per unit time as higher wattage 1000 W and 400 W lamps respectively. In addition, the lifetime of the lamp can have a significant effect on output as UV radiation drops significantly with age. Consequently, length of time of radiation required varies with the age of the lamp (medium pressure lamps typically last for 1000 h and low pressure for 9000 h). In addition the digestion is inversely proportional to the distance between the UV source and samples. Hence incomplete digestion could occur as a result of using aged lamps. Variability between samples could arise from proximity to the lamp if samples were not equidistant from it.

Other sources of errors could be from degradation of the mercury film (by oxidation) due to trapped air in the system leading to a loss of sensitivity. Sensitivity could also be affected by small changes in temperature (Chapter 4, section 4.3.10; affecting mass transport and electron transfer) and pH effects (Chapter 4, section 4.3.6). Care should be taken to avoid such systematic errors or, if unavoidable (such as temperature changes), should be accounted for and corrected.

Accuracy of the methods was possibly affected through contamination of samples. As the samples were collected, processed, homogenised, acidified (to preserve samples) and packaged by one group disagreements of trace metal concentrations reported by each laboratory were possibly introduced through subsequent sample handling procedures (*e.g.* sample-bottle protection and unwrapping procedures).

Comparison Between Reference Method and VIP Measured Samples

In order to determine whether variation of results obtained by using VIP system and reference methods in participating laboratories were significant, *t*-tests were carried out by comparing pairs of experimental means. As demonstrated in Section 5.3.1, of this chapter, ICP-MS was not sufficiently accurate and was therefore excluded from the *t*-tests, allowing statistical analyses on RD and HMDE (Table 5.6). Results indicate that VIP analyses gave significantly different results from reference methods and the poor correlation indicates that systematic errors are the source of significant differences between the analyses of samples.

Table 5. 6 Results from t-tests on each set of paired data at the 95 % confidence interval (*P*=0.05). Critical value of t in each case was 2.31 at 95% confidence level. No result (nr) indicates samples that were not analysed by that laboratory. Green colour indicates pairings that were not significantly different and red indicates the pairings that were significantly different. The author's results are underlined.

		Lab			
		LAB 1		**LAB 2**	
		VIP measured		VIP measured	
		Metal ion		Metal ion	
Sample	Reference method	Cd(II)	Cu(II)	Cd(II)	Cu(II)
R1	RD	21.96	nr	nr	
	HMDE		3.16		nr
E1	RD	3.16		nr	
	HMDE		1.95		nr
E2	RD	3.83		4.73	
	HMDE		2.78		11.76
R2	RD	nr		1.08	
	HMDE		nr		0.01
E3	RD	nr		16.7	
	HMDE		nr		9.3
CW1	RD	nr		3.51	
	HMDE		nr		0.58

Examination of Laboratory based inter-comparison of VIP and Reference Methods.

The systematic errors most probably arose from inadequate UV digestion of samples. Quantification of peaks obtained using the HMDE and RD was by standard addition and therefore the techniques should not be affected by changes in sensitivity of the instrument.

5.3.3 In situ Analysis in the Gullmar Fjord

General Observations

As concentrations varied considerably (Appendix A) statistical tests were carried out to determine which group, water mass and metal (if any) gave rise to inaccurate results. Regression graphs for comparison of concentrations measured by each pair of laboratories were constructed (Figure 12).

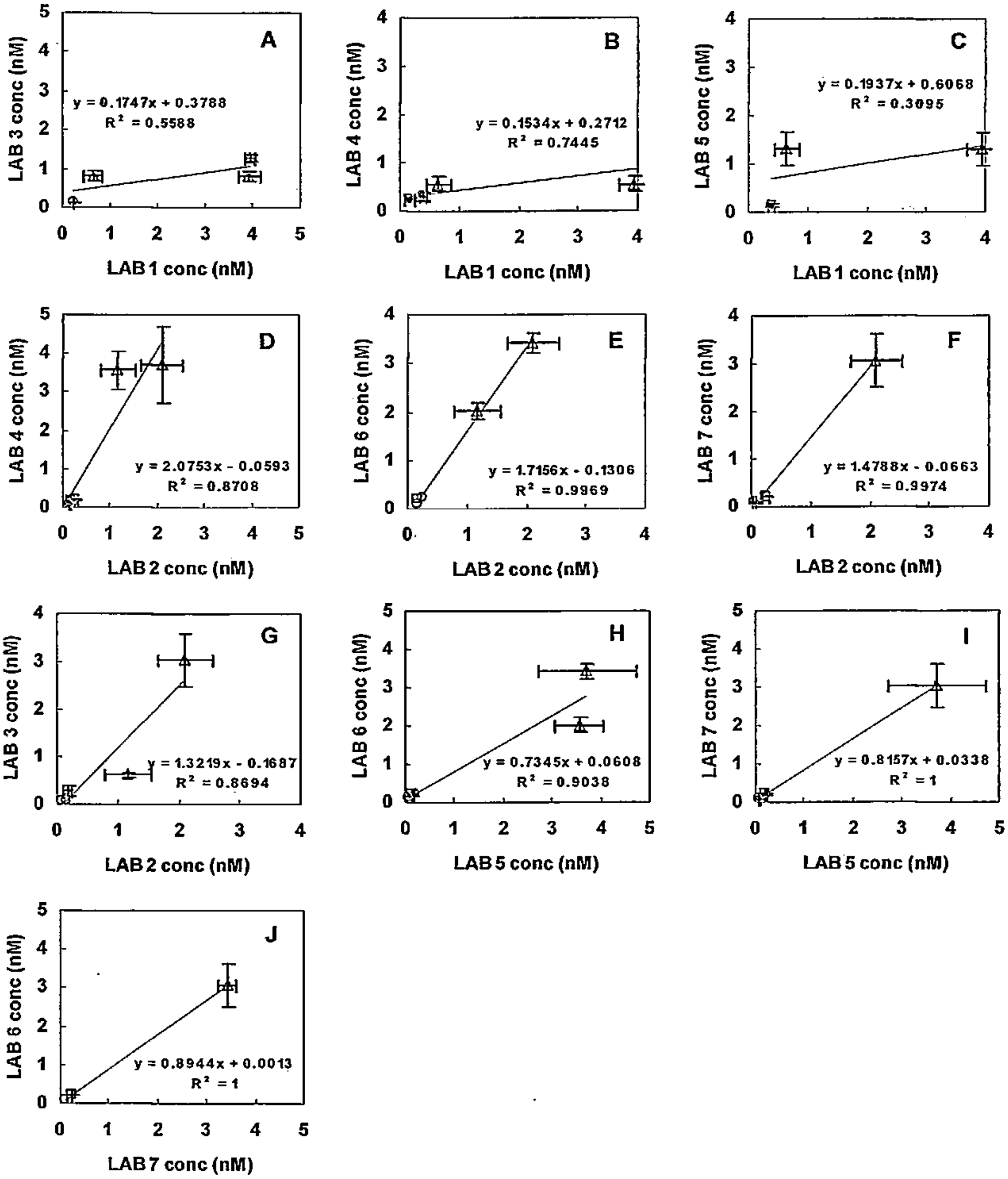


Figure 5.12 Regressions graphs comparing paired laboratories for *in situ* measurements for Cd(II) (green circles) and Pb(II) (pink squares) and Cu(II) (blue triangles).

Figure 5.12 shows r^2 values in the range 0.31 to 1.00, slopes in the range 0.15 to 2.08 and intercepts in the range 0.63 to 0.61. Figure 5.12 graphs D-J are close to theoretical value for r^2, slope and intercept. Deviation from theoretical values is greatest with LAB 1, paired with LAB 3, LAB 4 and LAB 5 (Figure 5.12 graphs A-C). Deviation from the ideal (r^2=1) was caused by both random and systematic errors.

Statistical Analysis of the Gullmar Fjord Samples

Station 1

Variation of results obtained by participating laboratories using VIP systems *in situ* was assessed to determine if differences were significant by performing *t*-tests between each pair of laboratories (provided data was available) for each metal. Results for Station 1 are given in Table 5.7

Table 5.7 Results of *t*-test with VIP systems at 5 m (A) and 35 m (B). Green indicates accepted null hypothesis at 95 % confidence interval, and red indicates rejected null hypothesis, indicating the measurements were significantly different. /t/ for critical value is indicated in brackets.

A

	LAB 1		
	Cd(II)	Pb(II)	Cu(II)
LAB 4	0.65 (4)		16.18 (8)

B

	LAB 2			LAB 3			LAB 5		
	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)
LAB 3	0.11 (4)	6.24 (8)	10.2 (8)						
LAB 5		1.14 (7)	12.1 (7)		2.07 (6)	17.7 (6)			
LAB 6		0.79 (6)	5.27 (3)				3.47 (7)	3.71 (6)	6.27 (5)

At 5 m (Table 5.7A) the pairing LAB 1/LAB 4 gave significantly different results (at the 95 % significance level). As LAB 1 gave poor correlation in the regression analyses this result is expected. At 35 m (Table 5.7B) for Cd(II) the null hypothesis was accepted for pairing LAB 2/LAB 3 (95 % confidence interval) but rejected for LAB 6/LAB 5 (95% confidence interval). For Pb(II) LAB 3/LAB 2 gave significantly different results, however LAB 2/LAB 5 and LAB 2/LAB 6 did not give significantly different results, indicating results for LAB 3 probably led to the significantly different results. For Cu(II) LAB 3/LAB 2 gave no significantly different result (at 95 %), however pairings LAB 2/LAB 3, LAB 2/LAB 5 and LAB 5/LAB 6 all gave significantly different results and so indicating results for LAB 5 and LAB 6 most likely led to the significantly different results.

Station 2

Variation of results for *t*-tests between each pair of laboratories for each metal at Station 2 are given in Table 5.8

Table 5. 8 Results of *t*-test with VIP systems at 5 m (A), 30 m (B), and 47 m (C). Green indicates accepted null hypotheses at 95 % confidence interval, and red indicates rejected null hypotheses, indicating the measurements were significantly different. /t/ for critical value is indicated in brackets.

A

	LAB 2			LAB 4			LAB 5			LAB 6		
	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)
LAB 4	1.66 (2)											
LAB 3	12.5 (9)		4.96 (9)									
LAB 5	1.28 (5)			1.13 (3)								
LAB 6	0.44 (3)			1.18 (3)			1.39 (3)		2.00 (7)			
LAB 7	1.13 (3)			1.30 (3)			1.19 (4)		1.77 (7)	0.09 (1)		1.70 (6)

B

	LAB 1			LAB 4		
	Cd(II)	Pb(II)	Cu(II)	Cd(II)	Pb(II)	Cu(II)
LAB 4	1.39 (14)		16.7 (9)			
LAB 3			1.66 (12)			
LAB 5	18.27 (12)			7.25 (12)	15.8 (11)	7.28 (9)

C

	LAB 1		
	Cd(II)	Pb(II)	Cu(II)
LAB 3	2.99 (2)		17.4 (1)

At 5 m (Table 5.8A) all pairings were not significantly different (at 95 % confidence) with exception of LAB 3/LAB 2 pairing. As LAB 2 paired with LAB 4, LAB 5, LAB 6 and LAB 7 gave no significantly different results (at the 95 % significance level), results obtained with LAB 3 probably in error. At 30 m (Table 5.8B) for Cd(II) null hypothesis was correct for LAB 1/LAB 4 pairing. However the LAB 4/LAB 5 pairing gave significantly different results. For Pb(II) all pairings gave significantly different results. For Cu(II) LAB 1/LAB 3 gave no significantly different result (at 95%).

However parings LAB 2/LAB 3, LAB 2/LAB 5 and LAB 5/LAB 6 all gave significantly different results and so only the results for LAB 3 and LAB 2 were deemed to be accurate. For VIP systems at 47 m (Table 5.8C) results for LAB 1/LAB 3 pairing were not significantly different.

5.3.4 Assessment of the In situ Analyses

For the *in situ t*-tests, the null hypothesis was accepted in 66 % of cases compared to just 14 % for laboratory based, discrete sample measurements. This indicates that there is a considerable systematic bias for laboratory-based analyses at the 95 % confidence level (discussed in section 5.3.2) and is most likely due to variable UV digestion of samples. For *in situ* analyses systematic bias is much less by comparison and differences are almost certainly due to environmental changes in the dynamic nature of matrix and/or lack of homogeneity of samples. Systematic errors for *in situ* analysis may also originate from degradation of agarose gel coating and fouling of electrode surface with organic matter, reducing sensitivity (discussed in Chapter 3, section 3.6).

Overall Cd(II), Pb(II) and Cu(II) produced 71 %, 50 % and 36 % accepted null hypotheses for the *in situ* measurements. The low Cu(II) value maybe due to incorrect measurement of the Cu(II) during *in situ* scans. The Cu(II) peak appears close to 0 mV (versus Ag/AgCl/3M KCl/1M $NaNO_3$ electrode) but can vary slightly (-50 to +20 mV), due to the nature of the KCl gel in the reference electrode. In this case it is viable that Cu(II), (reduced in the mercury film during the deposition stage) was not completely removed during the voltammetric scan, but remained within the mercury film where it became pre-concentrated over the course of several scans. This would lead to a systematic error causing underestimation of Cu(II).

Different water masses in the fjord affect the outcome of the *t*-tests with correct null hypotheses of 53 % at 30 m (n=19) 88 % at 5 m (n=17) and 100 % at 47 m (n=2). It may be that the water masses are more stable at 5 and 47 m. At *Station 2,* a very small thermocline were observed at about 30 m (Figure 5.10), indicting that different water masses were present at this depth. It is possible that the significant differences at this depth could be due to measuring inhomogeneous samples.

5.4 Conclusions

Regardless of the generic nature of the VIP instrumentation, the majority of laboratories gave significantly different $TF_{0.2}$ concentrations for Tamar samples at the 95 % confidence interval (using a paired *t*-test). Systematic discrepancies between reporting laboratories were due to either: (1) incomplete digestion of samples during UV irradiation or (2) sensitivity changes from oxidation of the mercury film caused by pumping air through the system. Accuracy errors most likely originated from contamination during sub-sample processing and sample handling at each individual laboratory.

Each group calibrated their own instrument by obtaining a standard calibration curve for a batch of samples. It could be more advantageous to use standard additions to quantify concentrations (but this may be more time consuming than calibration although it may be necessary) in order to achieve the desired accuracy.

For *in situ* measurements, only 34 % of all tested pairings were significantly different (at 95 % confidence level) results compared to 86 % for laboratory based, discrete sample measurements. This indicates that there was a considerably less systematic bias for *in situ* measurements compared to laboratory based determinations. This is most likely attributed to (1) the variable nature of UV digestion equipment between laboratories that led to incomplete digestion of discrete samples and (2) the absence of contamination artefacts during deployment. No single laboratory indicated a very low percentage of accepted null hypotheses, (all groups obtained 45-66 % null hypotheses). Significant differences for *in situ* determinations were attributed to (1) incomplete measurement of Cu(II) during the stripping stage and (2) variations in water column structure at 30 m.

The inter-comparison exercise highlighted several areas of VIP use that were unfavourable. Enhancing of VIP methodologies and processes will only become apparent through further inter-comparison exercises held both at sea and in land-based laboratories to ensure fieldwork and analytical techniques are harmonised to lead to improved comparability between EU laboratory data. Future inter-comparisons will require pre-exercise discussions to ensure that all partners are following prescribed procedures.

Standard Protocol

From the overall conclusions given above, a set of guidelines for further inter-comparison exercises is suggested:

1) Ensure all laboratories can measure CRMs within the errors quoted, prior to commencing analyses of inter-comparison samples
2) Reduce contamination by ensuring all phases of the pre-sampling operation, from the initial cleaning of bottles; sampling; processing; storage to handling and analyses be carried out under the direct control of skilled persons applying clean and protective measures outlined by Howard and Statham (1993).

3) The manufacturer of agarose gel used should be standardised.
4) For laboratory based inter-comparison, although protocols suggested by Achterberg *et al.*, (1994) for UV digestion were followed, laboratories should ensure that UV digestion is complete by performing a UV digestion efficiency experiment, as described by Achterberg *et al.*, (2001), to ensure efficient digestion of samples. This should be completed in a variety of matrices as this can affect the UV digestion period (*e.g.* Achterberg and van den Berg (1994) demonstrated that humic acids are broken down more efficiently in Milli-Q water than in seawater).
5) For laboratory based methods, if standard addition is not possible due to time constraints, a maximum prescribed number of samples should be run before re-calibration (that should be completed as frequently as realistically possible to ensure unchanging sensitivity)
6) For *in situ* inter-comparison in stratified water columns, care should be taken to avoid sampling the halocline/ thermocline as this could lead to greater inhomogeneity of samples than samples taken in the more stable water masses.
7) For Cu(II) *in situ* measurements, the scan should always finish at +20 mV to ensure complete oxidation of the Cu(II) during the stripping stage.

Reference List

Achterberg, E. P. and van den Berg, C. M. G. (1996) *Automated Monitoring of Ni, Cu and Zn in the Irish Sea.* Mar. Pollut. Bull., **32**, 471-479.

Achterberg. E. P. and van den Berg C. M. G (1994) *In-Line Ultraviolet-Digestion of Natural-Water Samples for Trace-Metal Determination using an Automated Voltammetric System.* Anal. Chim.Acta, **291**, 213-232

Ackroyd, D. R., Bale, A. J., Howland, R. J. M., Knox, S., Millward, G. E. and Morris, A. W. (1986) *Distributions and Behavior of Dissolved Cu, Zn And Mn in the Tamar Estuary,* Estuar. Coast. Shelf Sci., **23**, 621-640.

Arneborg, L. and Liljebladh, B. (2001) *The Internal Seiches in Gullmar Fjord. Part I: Dynamics.* J. Phys. Oceanogr., 31, 2549-2566.

Bewers, J. M., Dalziel, J. A., Yeats, P.A. and Barron, J.L. (1981). *An Intercalibration for Trace Metals in Seawater.* Mar. Chem., **10**, 173-193.

Brugmann, L., Danielsson, L. G., Magnusson, B., and Westerlund, S. (1983) *Intercomparison of Different Methods for the Determination of Trace Metals in Seawater.* Mar. Chem., **13**, 327-339.

Buesseler ,K. O., Bauer, J. E., Chen, R. F., Eglinton, T. I., Gustafsson, O., Landing, W., Mopper, K., Moran, S. B., Santschi, P. H., Vernon-Clark, R. and Wells, M. L. (1996) *An Intercomparison of Cross-Flow Filtration Techniques used for Sampling Marine Colloids: Overview and Organic Carbon Results.* Mar. Chem. **55**, 1-31.

Campbell, 1995: Campbell, P.G.C. (1995), *Interactions Between Trace Metals and Aquatic Organisms: A Critique of the Free Ion Activity Model.* In: Metal speciation and bioavavailibility in aquatic systems. Eds. Tessier, A. and Turner D. R., John Wiley & Sons, Chichester, pp. 45-103.

Coale, K. H., Johnson, K. S., Stout, P. M. and Sakamoto, C. M. (1992) *Determination of Copper in Seawater Using a Flow-injection Method with Chemiluminescence Detection.* Anal. Chim. Acta, **266**, 345-351.

Daih, B.-J. and Huang, H.-J. (1992) *Determination of Trace Elements in Seawater By Flow-Injection Anodic Stripping Voltammetry Preceded by Immobilized Quinolin-8-Ol Silica Gel Preconcentration.* Anal. Chim. Acta, **258**, 245-252.

de Jong, J. T. M., Boye, M., Schoemann, V. F., Nolting, R. F., and de Baar, H. J. W., (2000) *Shipboard Techniques Based on Flow Injection Analysis for Measuring Dissolved Fe, Mn and Al in Seawater.* J. Environ. Monit., **2**, 496-502.

Ebdon, L., Evans, E. H., Fisher, A. and Hill, S. J. (1998) *An Introduction to Analytical Atomic Spectrometry,* 1st Ed.; Wiley: Chichester.

Fischer E. and van den Berg C. M. G (1999) *Anodic Stripping Voltammetry of Lead and Cadmium Using a Mercury Film Electrode and Thiocyanate.* Anal. Chim. Acta, **385**, 273-280.

Howard, A. G. and Statham P. J. (1993) in *Inorganic Trace Analysis: Philosophy and Practice*, Eds. A. G. Howard and P. J. Statham, J. Wiley & Sons, Chichester, pp. 182.

Idronaut srl (1998), *VIP Reference Manual.* Milan, Italy.

Kozelka, P. B. and Bruland, K. W. (1998) *Chemical Speciation of Dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island.* Mar. Chem. **60**, 267-282

Kremling, K. and Streu, P. (2001) *The Behaviour of Dissolved Cd, Co, Zn, and Pb in North Atlantic Near-Surface Waters (30 Degrees N/60 Degrees W-60 Degrees N/2 Degrees W)*, Deep Sea Res.Pt I, **48**, 2541-2567

Landing, W. M., Cutter, G. A., Dalziel, J. A., Flegal, A. R., Powell, R. T., Schmidt, D., Shiller, A., Statham, P., Westerlund, S. and Resing, J. (1995) *Analytical Intercomparison Results from the 1990 Intergovernmental-Oceanographic-Commission Open-Ocean Base-Line Survey for Trace-Metals – Atlantic Ocean.* Mar. Chem. **49**, 253-265.

Miller J. C. and Miller J. N. (2000) *Statistics and Chemometrics for Analytical Chemistry.* 4th. Ed., Prentice-Hall, Harlow, England, pp 42-50.

Pei, J. H., Tercier-Waeber, M. L. and Buffle, J. (2000) *Simultaneous Determination and Speciation of Zinc, Cadmium, Lead, and Copper in Natural Water with Minimum Handling and Artifacts, by Voltammetry on a Gel-Integrated Microelectrode Array,* Anal. Chem., **72**, 161-171.

Tercier, M. L. and Buffle, J. (1996) *Antifouling Membrane-Covered Voltammetric Microsensor for In Situ Measurements in Natural Waters.* Anal. Chem., **68**, 3670-3678.

Tercier-Waeber, M-L., Buffle, J., Confalonieri, F., Riccardi G., Sina, A., Graziottin, F., Fiaccabrino G. C. and Koudelka-Hep, M. (1999) *Submersible Voltammetric Probes for In Situ Real-Time Trace Element Measurements in Surface Water, Groundwater and Sediment-Water Interface.* Meas. Sci. Technol., **10**, 1202-1213.

van den Berg C. M. G. (1991) *Monitoring of Labile Copper and Zinc in Estuarine Waters using Cathodic Stripping Chronopotentiometry.* Mar. Chem., **34,** 211-223.

van den Berg C. M. G. (1986) *Determination of Copper, Cadmium and Lead in Seawater by Cathodic Stripping Voltammetry of Complexes with 8-Hydroxyquinoline.* J. Electroanal. Chem., **215**, 111-121.

CHAPTER 6

Application of the Voltammetric Profiling System to Estuaries of the South West of England, U.K

Abstract

This chapter presents the field deployment of the automated Voltammetric *In situ* Profiling (VIP) system for the simultaneous determinations of dynamic Cd(II), Cu(II) and Pb(II) in three estuaries located in the Southwest of England, UK, namely, the Tamar, Plym, and Restronguet Creek. The VIP instrument allowed the *in situ* determination of 2-4 samples h^{-1}. In addition, the VIP system accurately measured the total filterable (through 0.4 μm pore size filter) concentrations of Cd(II), Pb(II) and Cu(II) in discrete estuarine water samples. Through a series of 7 tidal cycle studies lasting 7-12 h each high resolution data sets, including speciation information was built up that enabled an interpretation of geochemical processes in the studied estuaries.

6.1 Introduction

A complex interplay of physical, (Turner and Millward, 2002) chemical, (Morris *et al.,* 1982) and biological (Butler, 1998) mechanisms are involved in controlling the biogeochemical behaviour of trace metals in estuarine and coastal systems (as described in Chapter 1). The macrotidal estuaries of southwest England are characterised by strong variations in water flow and turbidity and are pronouncedly influenced by tides, fresh water run off and biological activity (Morris *et al.,* 1982). These factors considerably determine the biogeochemical behaviour of trace metals in estuarine systems and a quantitative understanding of trace metal biogeochemistry in these systems is important to ensure public and aquatic ecosystem health.

Rivers usually provide the main supply of trace metals to estuaries (Chapter 1, section 1.5), but interpretation of data for the estuarine environment based solely on riverine inputs can give an incomplete, ambiguous biogeochemical picture. Significant point source inputs of trace metals along an estuary can result in enhanced concentraions. In the southwest of England, estuaries have been subject to enhanced trace metal inputs through both solid and liquid mining wastes discharged into these estuaries for many years and metal concentrations can be very high as a result (Boult *et al.*, 1994).

Mining activity in the region has left a legacy of contaminated sites with a variety of histories yielding variable water column conditions. For example, in the lower salinity stretches of the Fal Estuary, pH values fall as low as 3.8 owing to inflows of acidic mine drainage (AMD) waters (Bryan *et al.*, 1987). Such pH conditions can cause marked changes in metal speciation, bioavailability and toxicity, and as a consequence can weaken and damage fishing industries and limit or alter faunal variation (Warwick, 2001). Fishing and tourism form an integral part the economy of the southwest of England and there is an urgent need to understand the behaviour of trace metals at these sites, in order to enable safe rehabilitation and regeneration these of delicate ecosystems.

This chapter presents and discusses results from field monitoring campaigns in the Tamar, Plym and Carnon Estuaries undertaken to examine VIP dynamic and total filterable (0.4 µm pore size; $TF_{0.4}$) concentrations of Cd(II), Pb(II) and Cu(II), together with master variables (suspended particulate matter [SPM], temperature pH, dissolved oxygen [DO] and salinity). A total of seven tidal cycles were performed in contrasting estuaries at different times of year and under various tidal states at one fixed station per estuary. A high resolution data set including metal speciation information was built up enabling an interpretation of geochemical processes in these estuaries.

6.2 Experimental

6.2.1 Field Campaigns

Conductivity, pH, DO, and temperature were measured *in situ*. Conductivity was measured using a conductivity meter (model HI9635, Hanna Instruments Ltd, Happy Valley Industrial Park, Herts, UK). Measurements of pH were performed using a pH meter (model HI9025, Hanna Instruments Ltd) fitted with a GELPLAS probe (BDH). The pH meter was calibrated before each field campaign using buffer solutions of pH 7.0 and 4.0 (NIST), taking temperature into account. SPM was determined gravimetrically after filtration of a set volume of sample through a 0.4 µm filter. Temperature and DO concentrations were determined using a combined meter (model 55, Yellow Springs Industries (Y.S.I.) U.K. Ltd, Lynford House, Farnborough). During the Restronguet Creek surveys, samples were taken at regular intervals and chlorinity measured in the laboratory from which the salinity was calculated according to Equation 1 (Eq 1; Head, 1985). For the Plym and Tamar surveys, *in situ* conductivity measurements were converted to salinity using equations derived from Chapter 4, Figure 4.1.

$$\text{Salinity} = \text{Chlorinity } (\text{g kg}^{-1}) \times 1.80655 \qquad \text{Eq 1}$$

6.2.2 *Description of Sample Sites*

The Tamar River and Estuary, England, UK

The Tamar system has been previously described in Chapter 2, section 2.2.1 Sampling site location for these surveys are shown in Figure 6.1.

Plym Estuary

The River Plym (Figure 6.1) is located on the eastern side of Plymouth Sound. The Plym Estuary is macrotidal, with a length of 21.3 km and a mean river flow of 2.25 m^3 s^{-1} (C.E.H., 2003). It rises on west Dartmoor granite and passes southwest onto upper Devonian slates before draining into Plymouth Sound where it joins the Tamar Estuary, discharging into the sea. The catchment area is 79.2 km^2 and land is used mainly for grazing, forestry and moorland (C.E.H., 2003). Several anthropogenic activities (including metalliferous mining, china clay workings, sewage treatment) and natural geology of the catchment influence the chemistry of the estuarine waters. The lower part of the estuary is of importance to commercial shipping and fishing industries; hence channels are surveyed every year and are dredged if necessary, and rich mudflats are located above Laira Bridge (Hiscock and Moore, 1986).

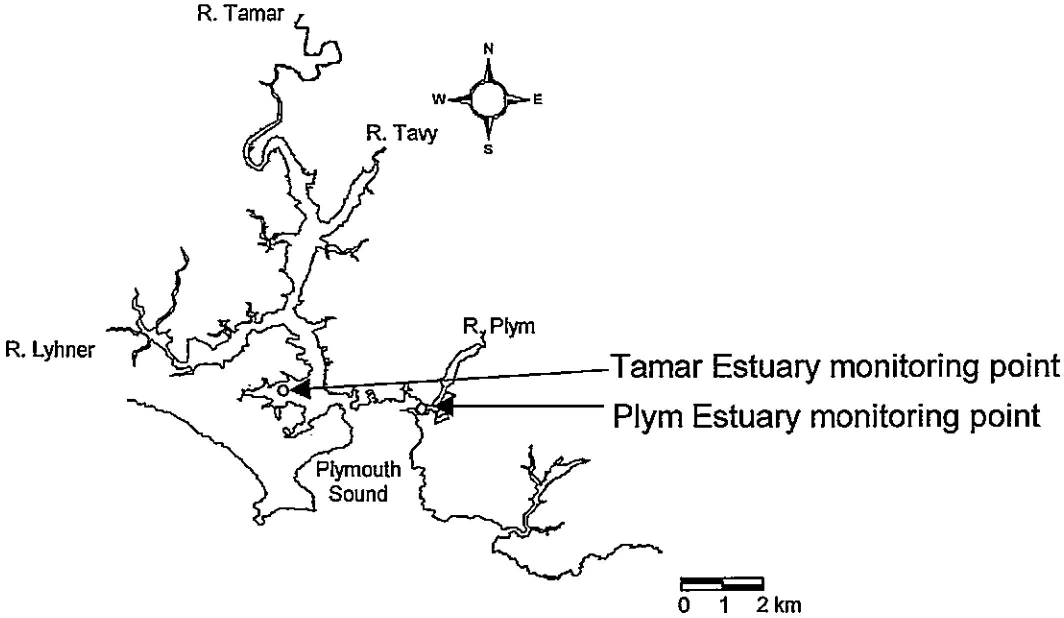


Figure 6. 1 Tamar and Plym Estuaries with the sampling location marked in red.

Restronguet Creek

The river Carnon (Figure 6.2) is located in Cornwall, S. W England and flows through an area with an ancient Sn and Cu mining history (Pirrie *et al.,* 1997). It discharges into Restronguet Creek which flows into the Fal Estuary, an area popular for sailing and fishing activities that comprises a complex series of creeks and tidal rivers extending 17 km inland. The mean annual flow rate of the Restronguet Creek into the Fal is 2.10 m^3 s^{-1}, with a catchment area of 87.0 km^2 and land used mainly for low grade agriculture and grazing (C.E.H., 2003A). Restronguet Creek is subjected to acid mine drainage (AMD) that forms when metal sulphide minerals, *e.g.* pyrite (FeS_2), that are commonly present in rock layers overlying coal seams are oxidised through weathering or industrial processes (Banks *et al.*, 1997), resulting in the formation of metal hydroxide and sulphuric acid. An example is the oxidation of pyrites:

The pyrite oxidises upon contact with air and water [and can be catalysed by *Thiobacillus ferrooxidans* (Forstner and Salomons, 1988)]:

Chemical oxidation of pyrite described in the following reaction:

$$4\,FeS_{2\,(s)} + 14\,O_{2(g)} + 4\,H_2O_{(l)} \rightarrow 4\,Fe^{2+}_{(aq)} + 8\,SO_4^{2-}{}_{(aq)} + 8\,H^+_{(aq)}$$

Fe(II) is oxidised to form Fe(III):

$$4\,Fe^{2+}_{(aq)} + O_{2(g)} + 4\,H^+_{(aq)} \rightarrow 4\,Fe^{3+}_{(aq)} + 2\,H_2O_{(l)}$$

Fe(III) hydrolysed in water to form Fe(III) hydroxide:

$$4\,Fe^{3+}_{(aq)} + 12\,H_2O_{(l)} \rightarrow 4\,Fe(OH)_{3(s)} + 12\,H^+_{(aq)}$$

Stoichiometric equation:

$$4\,FeS_{2(s)} + 15\,O_{2(g)} + 14\,H_2O_{(l)} \rightarrow 4\,Fe(OH)_{3(s)} + 8\,SO_4^{2-}{}_{(aq)} + 16\,H^+_{(aq)}$$

As can be seen from the stoichiometric equation, the products of AMD formation are acidity and metal hydroxides (pH dependant). The acid can leach heavy metals such as Pb, Zn, Cd, and Cu resulting in high (mM) concentrations of these metals *e.g.* Huelva Estuary in SW Spain (Nelson and Lamothe, 1993; van Geen *et al.*, 1997) and coating streambeds with iron hydroxide that forms an orange colour. Sampling location for the Restronguet Creek surveys is shown in Figure 6.2

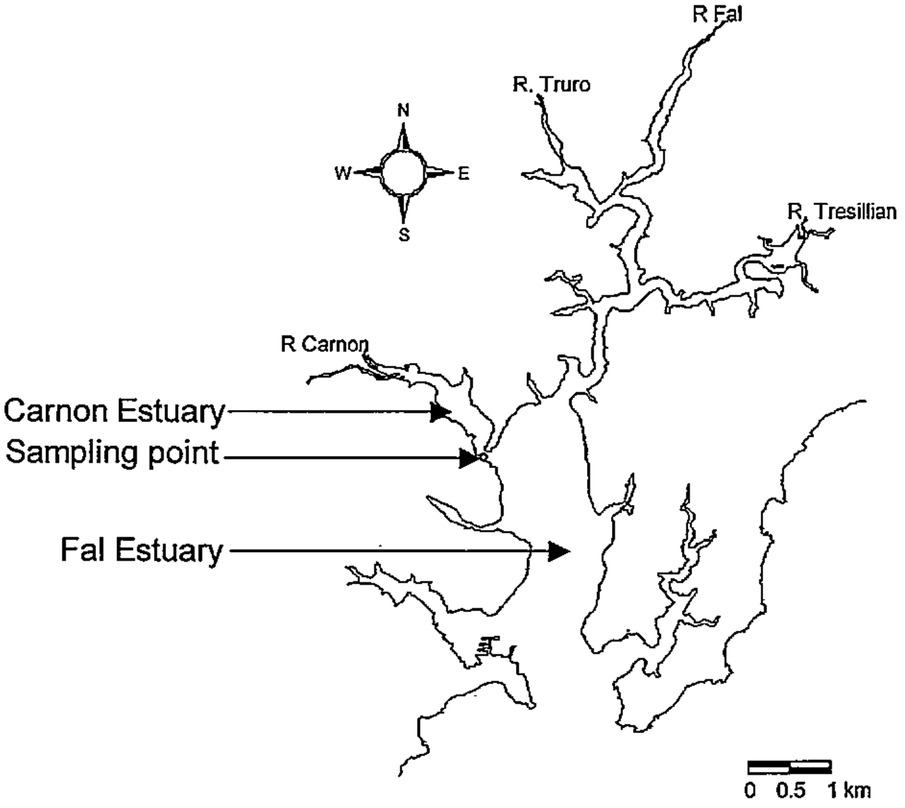


Figure 6. 2 Restronguet Creek with the sampling location marked in red.

6.2.3 Sampling Procedure

The microelectrode for the VIP system was prepared in the manner described in Chapter 4, section 4.2.4 and the system calibrated in $NaNO_3$ (0.1 M, pH 2) prior to and following each deployment. *In situ* measurements were made with the VIP system using the optimised conditions for seawater presented in Chapter 4 and adapting the deposition times accordingly for each estuary in order to remain within the linear range of the instrument. Discrete water samples were taken as described in Chapter 2, section 2.2.1 and filtered on site through acid cleaned filters (0.40 μm; polycarbonate; Cyclopore, Whatman, UK), using a filtration unit (Nalgene) fitted with a hand pump (Nalgene). Samples were acidified (pH 2; sub-HNO_3) upon return to the laboratory.

The discrete samples were analysed for total filterable (0.4 μm pore filter; $TF_{0.4}$) concentrations in the laboratory. The samples were UV digested immediately prior to analysis (Achterberg and van den Berg, 1994; 400 W medium pressure UV lamp; Photochemical Reactors, Slough, UK), after the addition of H_2O_2 (8.8 mM, final concentration). $TF_{0.4}$ Cd(II) and Pb(II) concentrations were determined using the VIP system, while for speed, $TF_{0.4}$ Cu(II) measurements were performed using AdCSV with complexing ligand 8-hydroxyquinoline (25 μM final concentration) in the presence of 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES; pH 7.7; 0.01 M final concentration) as a pH buffer (van den Berg, 1986).

6.3 Results and Discussion

Tidal cycles were carried out in the Tamar (1 cycle) and Plym (2 cycles) estuaries and Restronguet Creek (4 cycles) during the period November 2001 to December 2002. Monitoring locations on the estuaries have been indicated for the Tamar (Figure 6.1), Plym (Figure 6.1) and Fal (Figure 6.2).

6.3.1 Tamar Estuary

Tidal Cycle (15/11/01)

This survey was performed over a period of 7 h on 15/11/01 in St Johns Lake (Figure 6.3A) commencing at low water (11:41 h GMT; Stevens, 2001) on a spring tide. *In situ* measurements were undertaken at *ca* 2 m depth at a fixed station from an anchored vessel (RV *Catfish;* Figure 6.3B). The results of the campaign are shown in Figure 6.4.

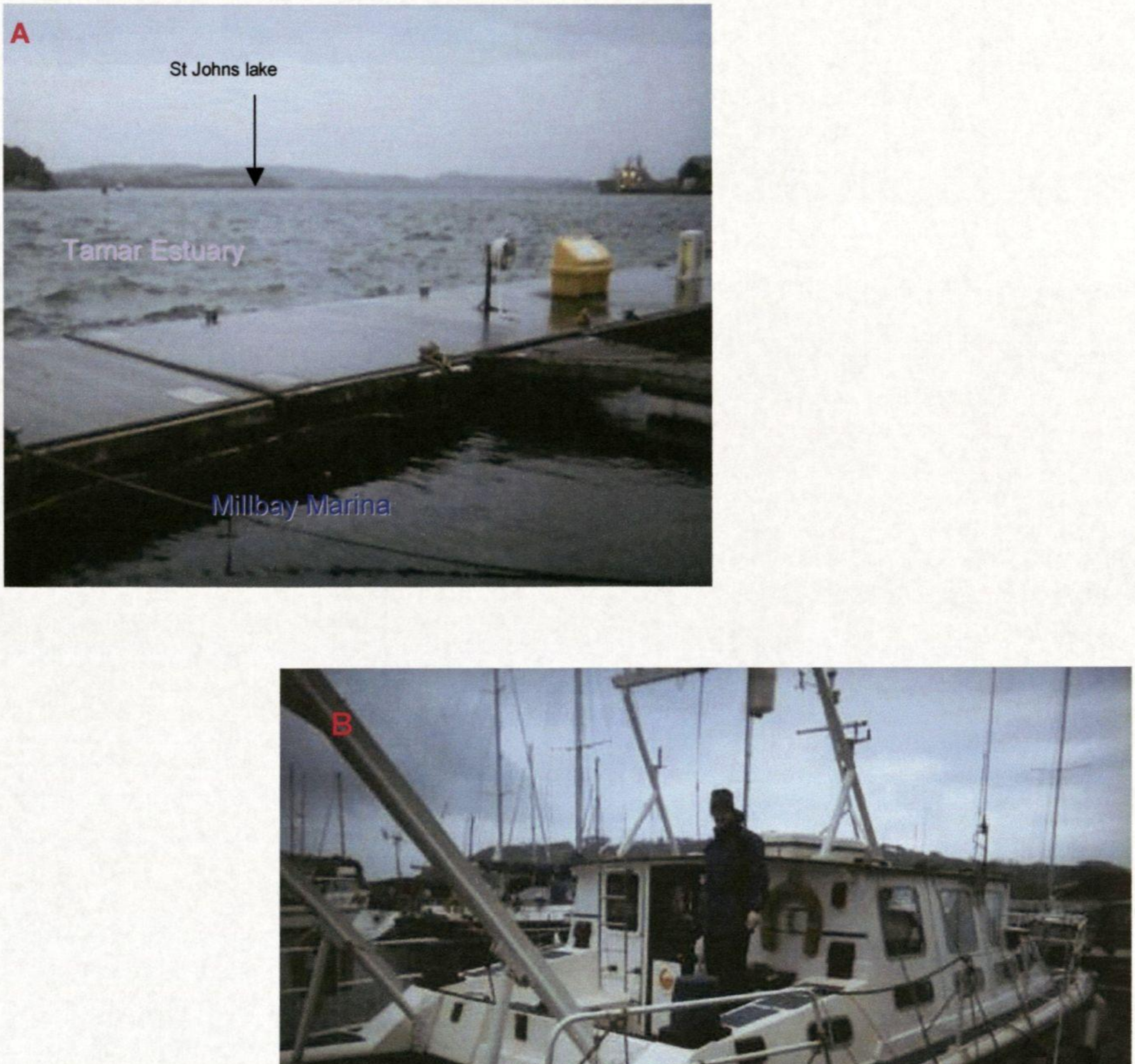


Figure 6.3 St Johns lake on the Tamar Estuary (**A**) and the *RV* Catfish (**B**), used for the Tamar tidal cycle campaign.

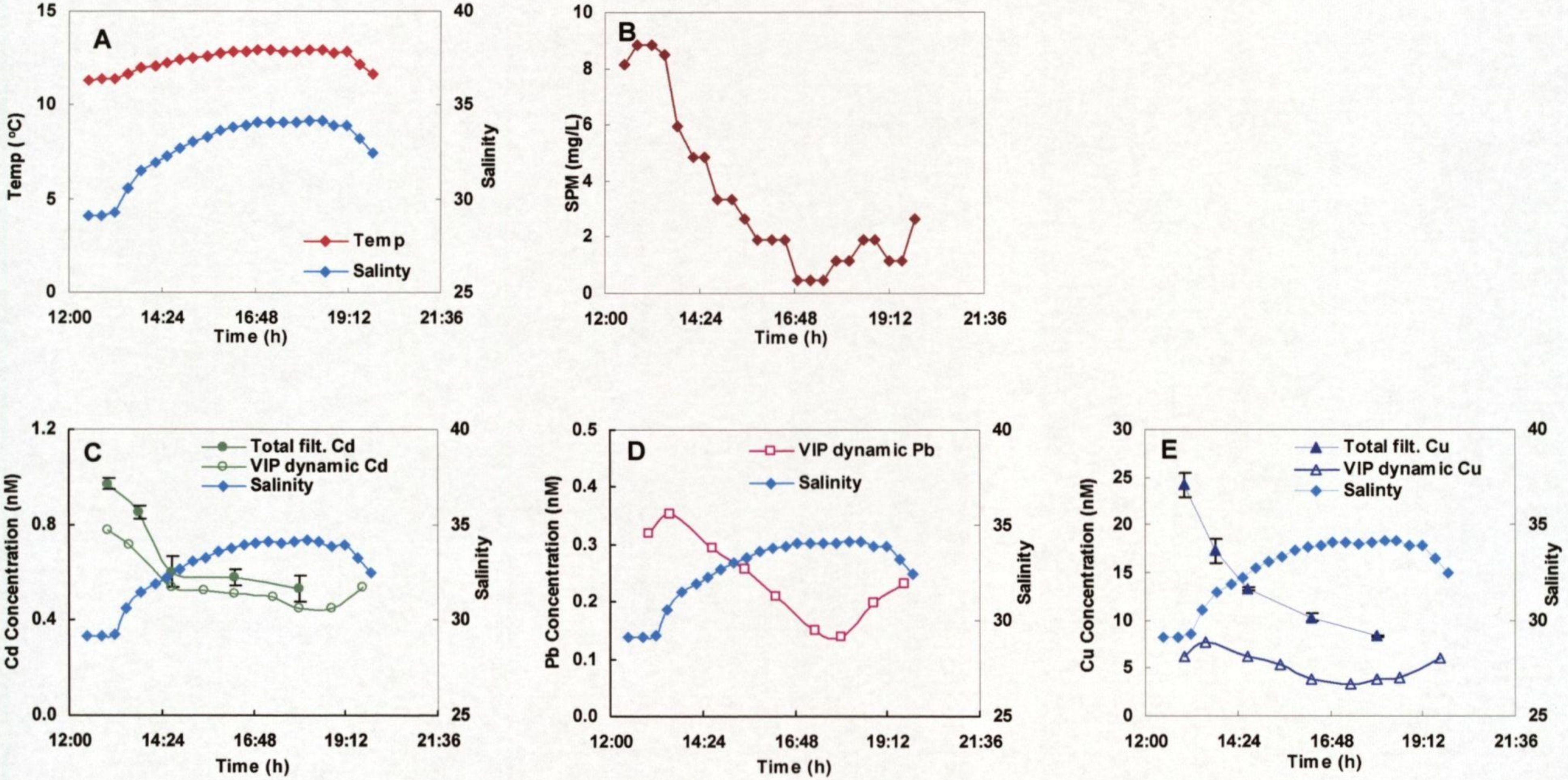


Figure 6.4 Time series measurements over a 7 h period on 05/11/01 in the Tamar Estuary of salinity and temperature **(A),** SPM concentrations **(B)** and *in situ* VIP measured dynamic concentrations of Cd(II) **(C)**, Pb(II) **(D)** and Cu(II) **(E)**. In addition graphs **(B)** and **(E)** also show $TF_{0.4}$ (measured in laboratory in discrete samples), concentrations of Cd(II) and Cu(II) respectively. Low and high water occurred at *ca* 11:40 h and 17:00 h, respectively. Colloidal fractions of Cd(II) and Cu(II) are plotted against salinity (**F** and **G** and SPM (**H** and **I**) respectively.

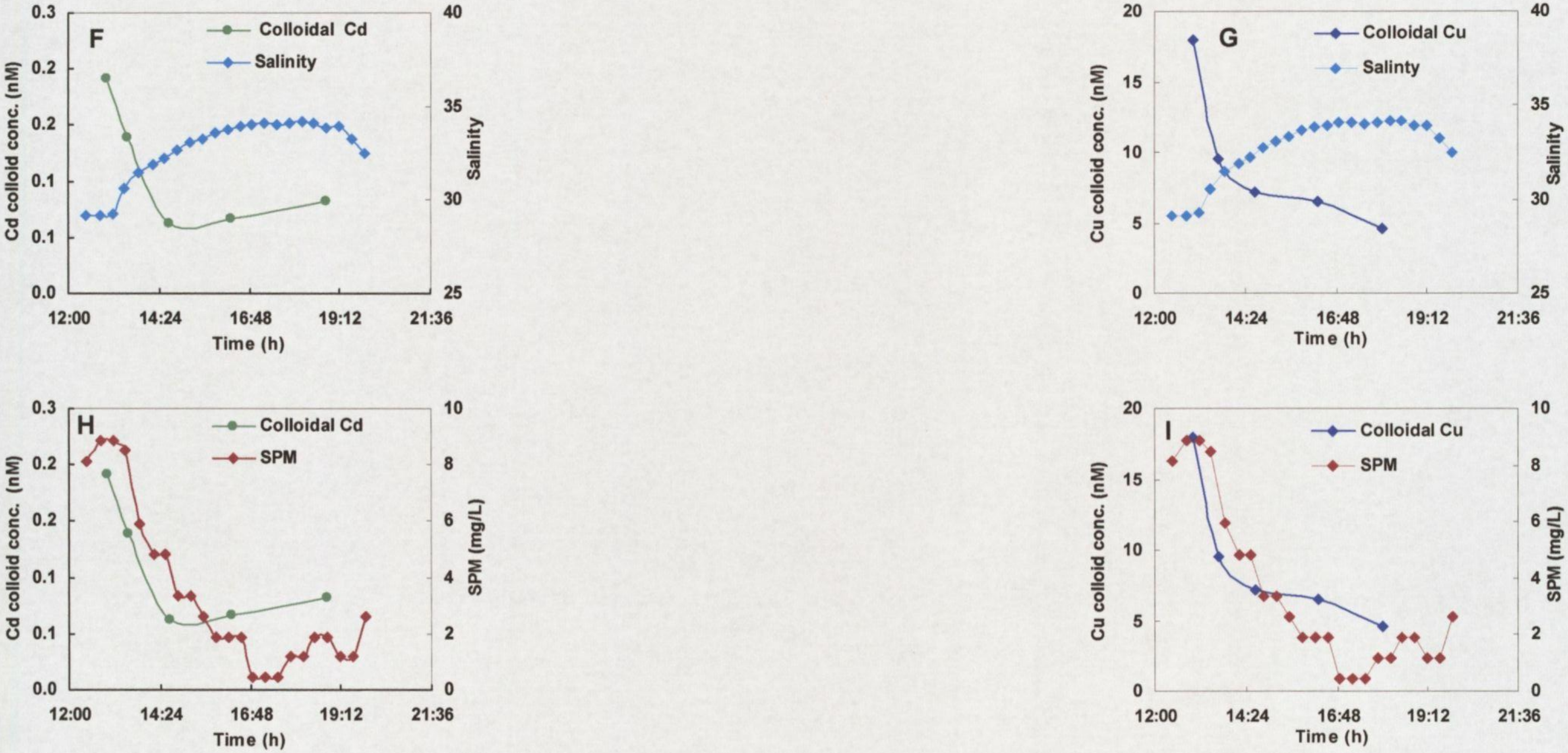


Figure 6.4 Continued...

Figure 6.4A indicates that as the tide ebbed, colder, fresher water from the River Tamar was transported to the station, and both salinity and temperature were lowest between 12:00 h to 13:10 h. After this time, an increase in salinity and temperature was observed as the tide rose bringing warmer, Atlantic water to the station. Figures 6.4C-E illustrate the results of *in situ* measurements of trace metals Cd(II), Pb(II) and Cu(II), respectively. The river Tamar is subject to run-off from disused metalliferous mines and consequently has enhanced trace metal concentrations (Ackroyd *et al.*, 1986). Figures 6.4C-E show that significant concentration changes in the VIP dynamic fraction were noted for all three metals over the monitoring period. Concentrations of Cd(II), Cu(II) and Pb(II) were highest (*ca* 13:00 h) close to low water as a result of the influence of freshwater river runoff with enhanced trace metal concentrations. The lowest concentrations for all metals were observed at high water (*ca* 17:00 h), resulting from the inflow of cleaner Atlantic seawater into the Tamar Estuary.

The trace metal fraction determined by the VIP is termed 'dynamic', and includes free ions and small complexes with a size smaller than a few nm that pass through the agarose gel (as described in Chapter 3, Section 3.6) and this dynamic fraction is considered bioavailable (Tercier-Waeber *et al.*, 1999). The concentration of metals associated with colloids and small non-labile metal-organic complexes (< few nm) can be obtained as the difference between measurements of VIP dynamic metal and $TF_{0.4}$ metal from discretely collected samples which have been filtered; (Buffle *et al.*, 2000; Chapter 3, section 3.6), acidified (pH 2) and UV-digested (Achterberg and van den Berg, 1994) prior to analysis and is termed the 'colloidal' fraction. Figure 6.4F-I shows Cd(II) and Cu(II) colloidal fraction as a function of salinity and SPM concentration (mg/ L).

The largest colloidal fractions were observed close to low water (13:00 h) where dynamic Cd(II) constituted 78 % of $TF_{0.4}$ concentration and dynamic Cu(II) only 27 % of $TF_{0.4}$ indicating the importance of freshwater (rivers and mine run-off) as a source of metal colloids and small non-labile metal-organic complexes to the estuarine system. This can be compared to high water with less riverine input where dynamic Cd(II) constituted 85 % of $TF_{0.4}$ and dynamic Cu(II) constituted 45 % of $TF_{0.4}$.

6.3.2 Plym Estuary

Plym Tidal Cycle 1 (05/09/02)

This survey was performed over a period of 12 h on 05/09/02 at Oreston, Plym Estuary and commencing 2 h before low tide (high water 05:07 h BST, 4.8 m and 17:24 h BST, 5.2 m; low water 11:14 h BST, 1.3 m; Jones, 2003) four days before spring tides. *In situ* measurements were undertaken at *ca.* 2 m depth at a fixed station from an anchored vessel (*Harlequin;* Figure 6.5). Results of the tidal cycle are represented in Figure 6.5

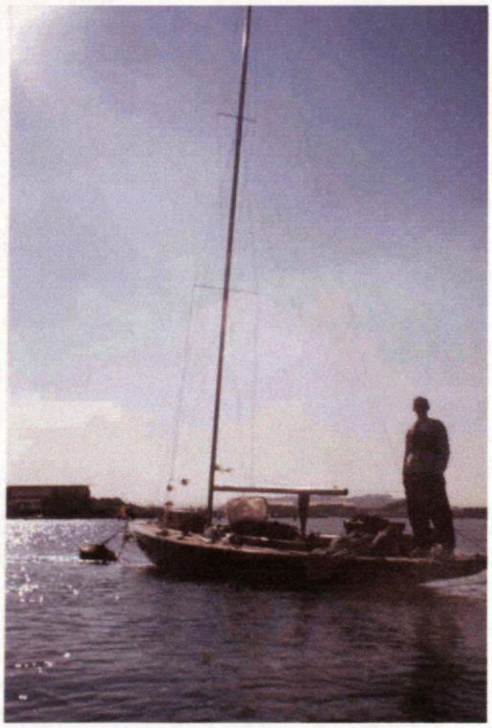


Figure 6.5 *Harlequin,* a fixed location boat, used for the Plym tidal cycles.

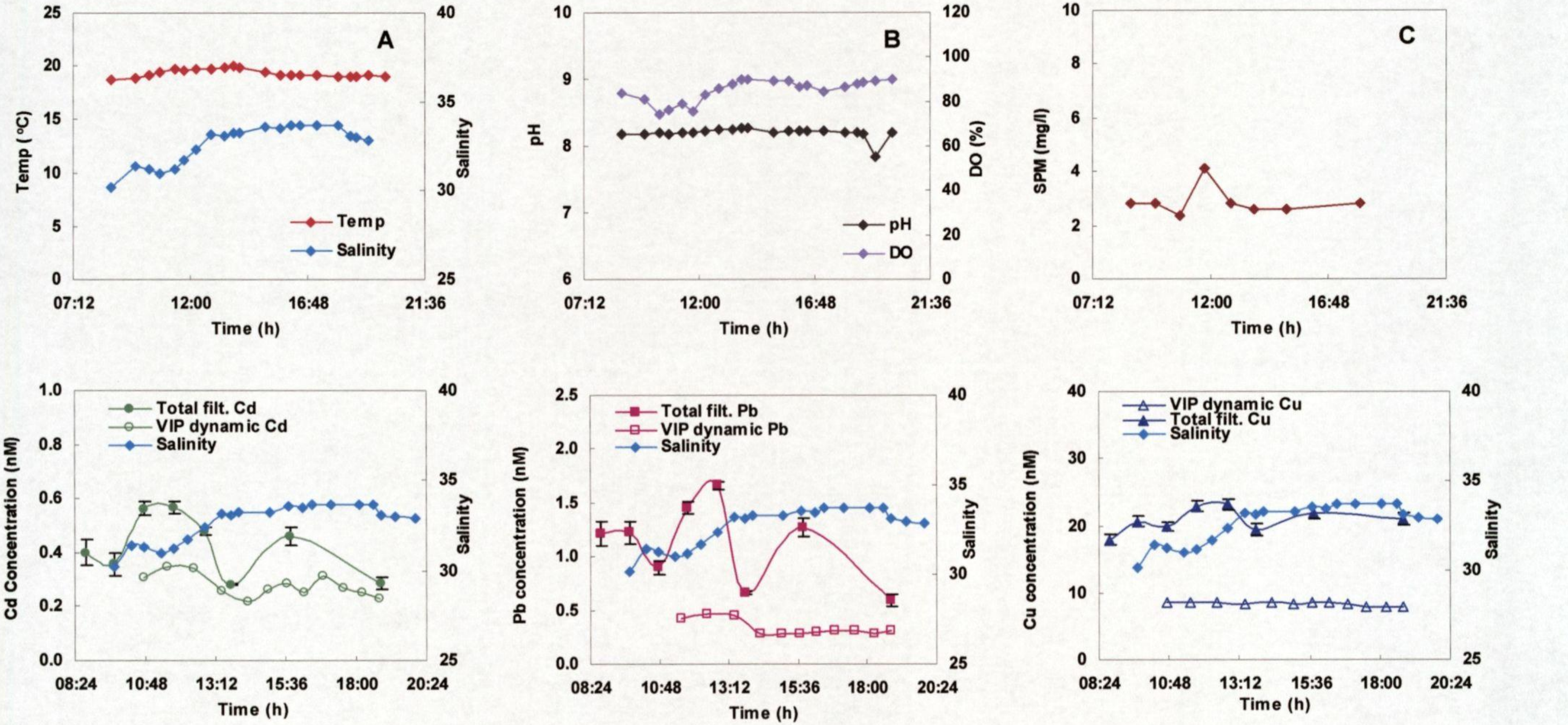


Figure 6.6 Time series measurements over an 11 h period on 05/09/02 in the Plym Estuary of salinity and temperature **(A)**, pH and DO **(B)**, SPM **(C)** and *in situ* VIP measured dynamic concentrations and laboratory measured $TF_{0.4}$ concentrations of Cd(II) **(D)**, Pb(II) **(E)** and Cu(II) **(F)**. Low and high water occurred at *ca* 11:14 h and 17:24 h, respectively. Colloidal fractions of Cd(II), Pb(II) and Cu(II) are plotted against salinity (**G**, **H**, **I**) and SPM (**J**, **K**, **L**) respectively.

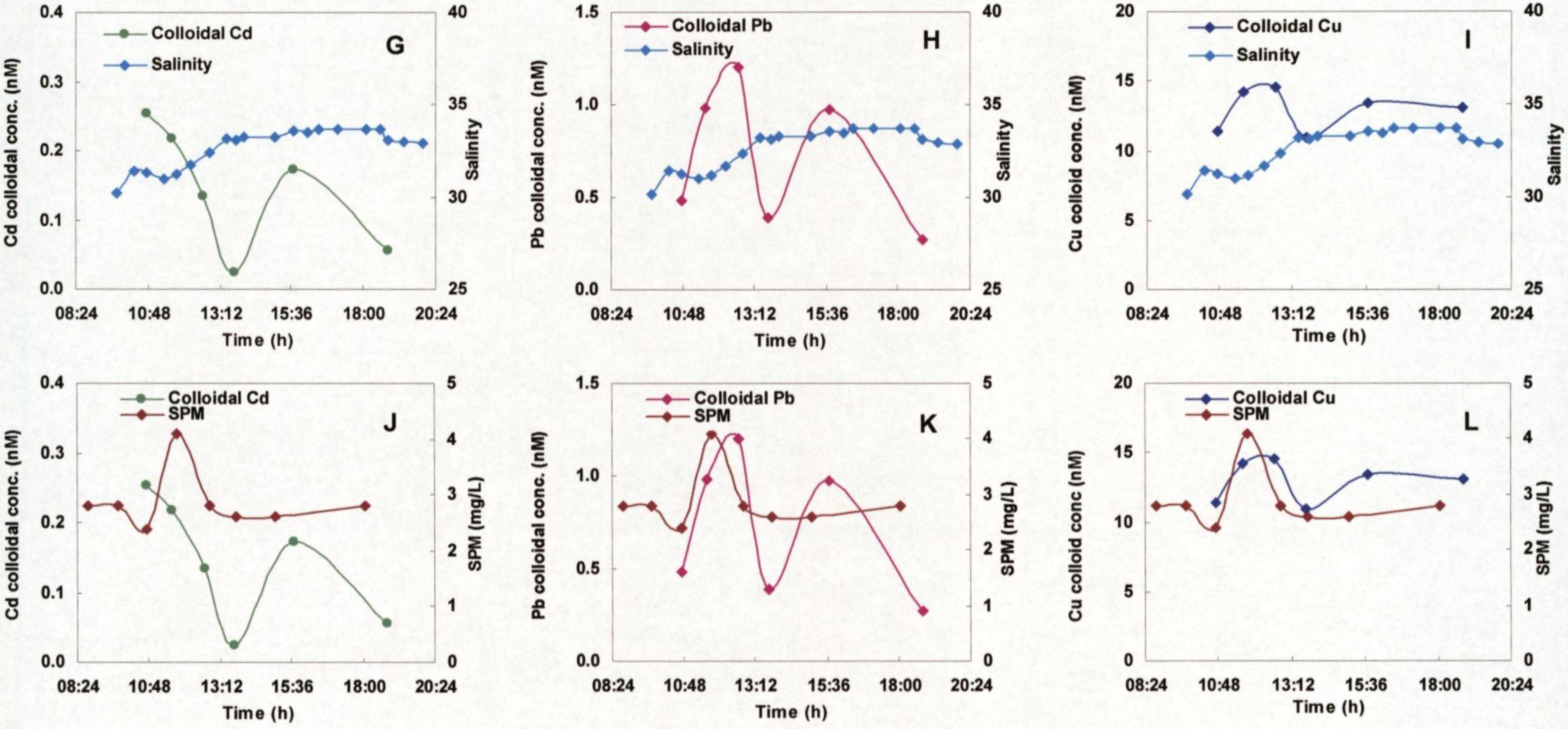


Figure 6.6 Continued…

Figure 6.6A shows relatively little change in the salinity over the time period but a small decline in salinity was observed from 08:45 h to high tide at the station (*ca* 11:20 h), after which, recorded values increased and reached a plateau (at 12:50 h). A similar trend was noted for pH and DO (Figure 6.6B). DO concentrations were below saturation. VIP dynamic and $TF_{0.4}$ trace metal concentrations showed an inverse relationship with salinity, reaching a maximum concentration at low tide for all three metals. Cd(II) (Figure 6.6G,J) showed a maximum concentration at low tide and showed a large VIP dynamic fraction of 85 % and only a small fraction in the colloidal fraction (15 %) at high water. The colloidal fraction increased to 40 % at low water. Pb(II) (Figure 6.6H,K) demonstrated a colloidal fraction of 60 % at high tide increasing to 80 % at low tide, probably due to influence of trace metal complexing colloids in the fresh waters. The Cu(II) (Figure 6.6F) dynamic fraction was not affected by riverine inputs (possibly due to similar localised concentration and a less vigorous mixing process). Trace metal concentrations were similar to those recorded in the Tamar tidal cycle, probably due to the similar geology and land use in the catchment area.

Plym Tidal Cycle 2 (14/09/02)

This survey was performed over a period of 11 h on 05/09/02 at Oreston, Plym Estuary, commencing 2 h before high tide (High water 06:44 h BST, 5.4 m and 18:28 h BST, 5.5 m; low water 12:21 h BST, 0.7 m; Jones, 2003) two days before spring tides. *In situ* measurements were undertaken at *ca* 2 m depth at a fixed station from *Harlequin*. The results of the tidal cycle are presented in Figure 6.7. An inverse relationship between salinity and temperature was noted with minimum salinity values at low water (18:15 h). Dissolved oxygen data (Figure 6.7B) exhibited an opposite trend to salinity, increasing during ebb flow, with variations probably due to

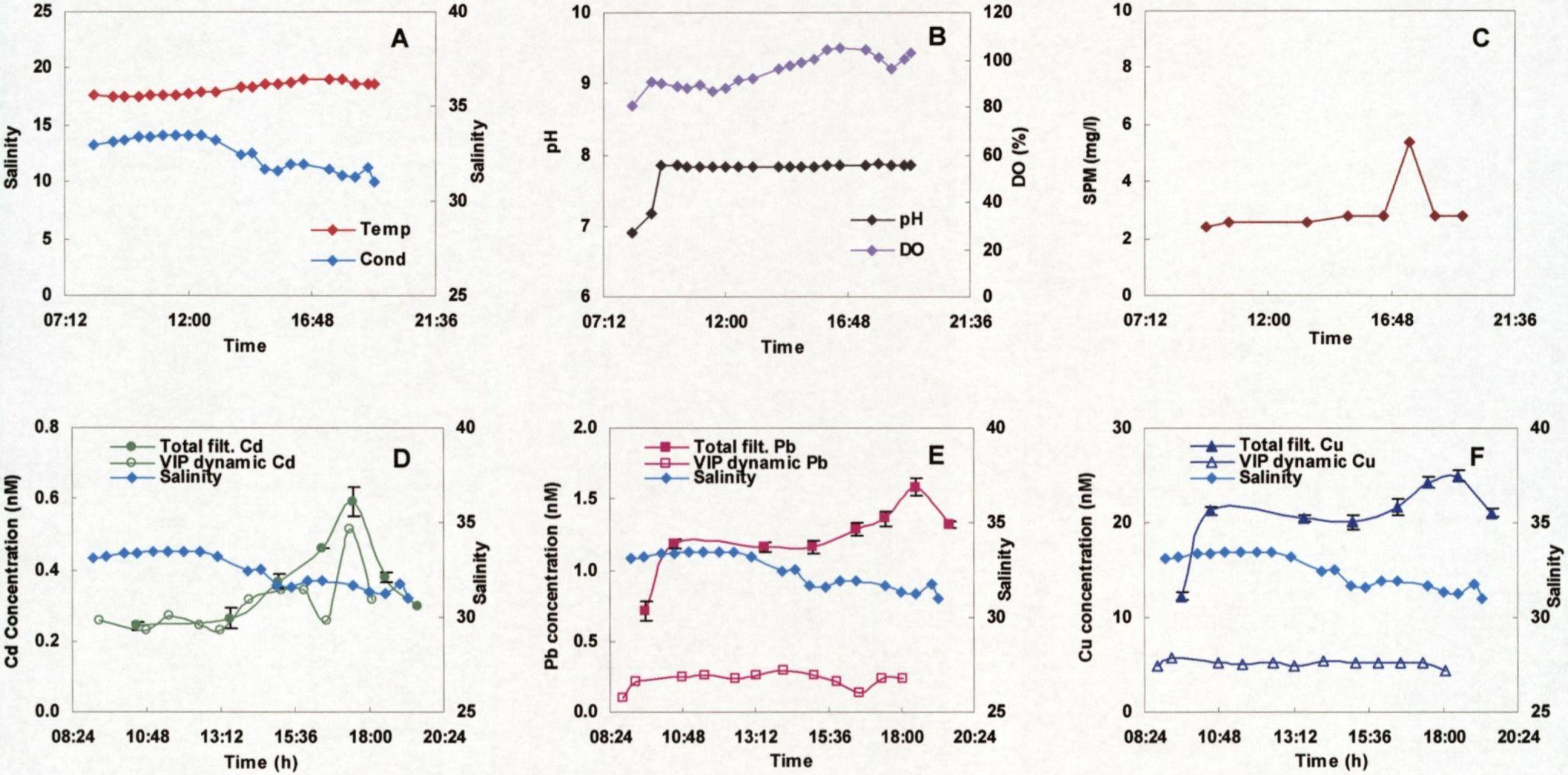


Figure 6.7 Time series measurements over an 11 h on 14/09/02 period in the Plym Estuary of salinity and temperature **(A),** pH and DO **(B)**, SPM **(C)** and *in situ* VIP measured dynamic concentrations and lab measured $TF_{0.4}$ concentrations of Cd(II) **(D),** Pb(II) **(E)** and Cu(II) **(F)**. Low and high water occurred at *ca* 12:21 h and 18:28 h, respectively. Colloidal fractions of Cd(II), Pb(II) and Cu(II) are plotted against salinity (**G, H, I**) and SPM (**J, K, L**) respectively.

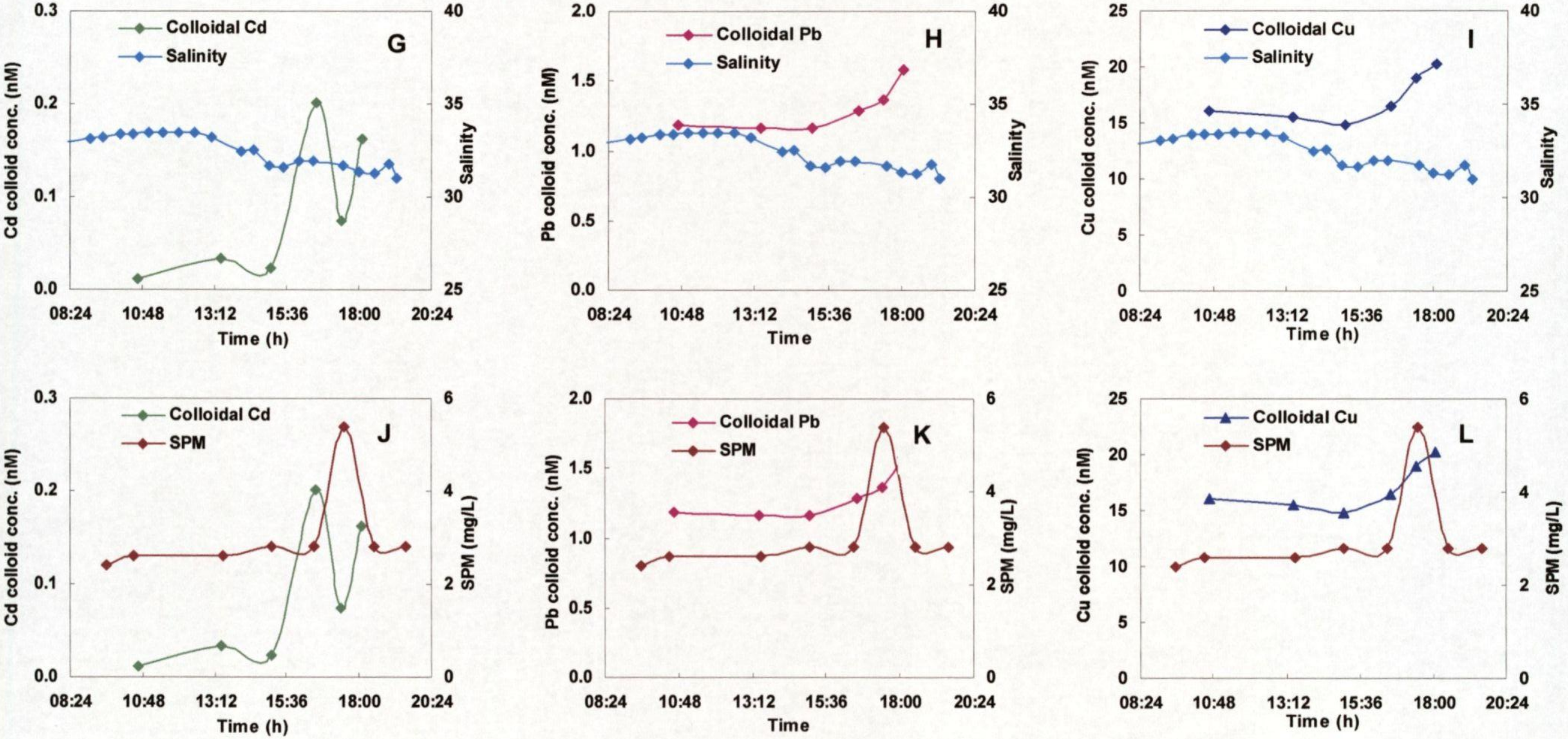


Figure 6.7 Continued…

mixing of different water masses and some primary productivity in the estuary. The pH remained stable over the course of the tidal cycle measurements. Figures 6.7D-F indicate similar behaviour for all metals, *i.e.* inversely correlated with salinity, with minimum concentrations observed close to high tide at 08:40 h and maximum concentrations observed at low tide at 12:20 h. Trace metal concentrations were very similar to those observed during Plym tidal cycle 1. Cd(II) showed a large VIP dynamic fraction at low water, increasing to nearly all VIP dynamic Cd(II) at higher salinities. Once again Pb(II) (Figure 6.7H,K) and Cu(II) (Figure 6.7I,L) showed colloidal fractions that were about 75 % of the $TF_{0.4}$ over the course of the tidal cycle. Concentrations of the metals were very similar to the two tidal cycles, probably due to the similar tidal states and lack of rain affecting the fresh water inputs to the estuary at this time of the year, reflected in the flow data that was 0.32 m^3sec^{-1} for this day (Lewis, 2003) compared to an annual average of 2.25 m^3sec^{-1} (C.E.H., 2003).

6.3.3 Restronguet Creek

Restronguet Creek Tidal Cycle 1 (27/06/02)

This survey was performed over a period of 12 h on 27/06/02 at Restronguet Point, Restronguet Creek, commencing near to high water in the morning (High water 07:43 h BST, 4.9 m and 18:52 h, 5.2 m; low water at 14:20 h, 1.1 m; Jones, 2003) one day after spring tides. *In situ* measurements were undertaken at *ca* 2 m depth at a fixed station from an anchored vessel. Results are presented in Figure 6.8. Salinity (Figure 6.8A) was relatively stable up to 11:35 h, and showed a slight decrease in values, until low water at *ca.* 12:50 h. The pH (range 8.13 to 8.26) showed the opposite trend to salinity. The DO values increased as sunlight levels increased throughout the day presumably as a result of increased photosynthesis, producing dissolved oxygen levels over saturation (100 %) at 09:00 h, reaching a maximum of 114 % at 16:30 h

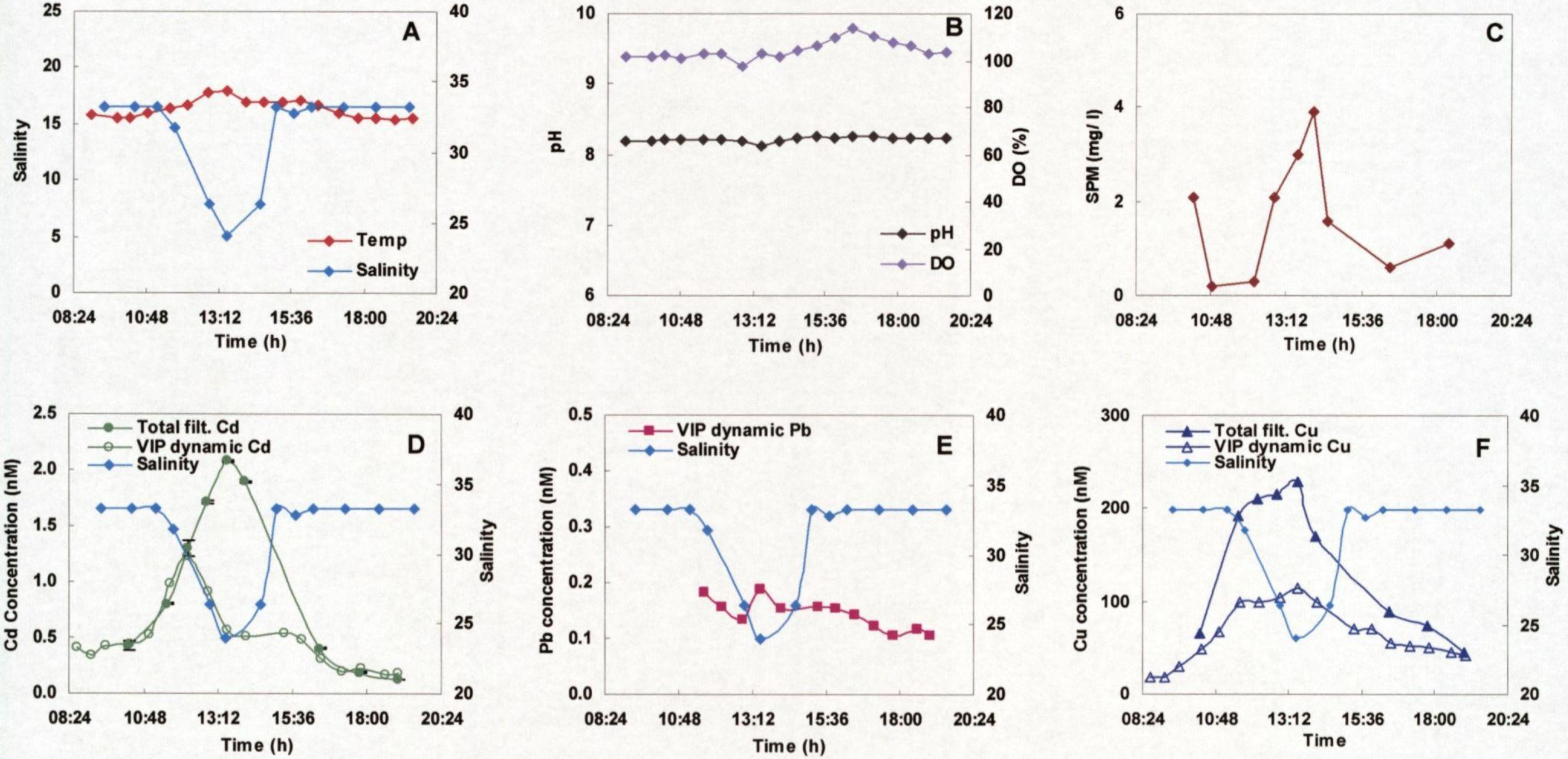


Figure 6.8 Time series measurements over an 11 h period on 27/06/02 in Restronguet Creek of salinity and temperature **(A)**, pH and DO **(B)**, SPM **(C)** and *in situ* VIP measured dynamic concentrations of Cd(II) **(D)**, Pb(II) **(E)** and Cu(II) **(F)**. In addition graphs **(D)** and **(F)** also show $TF_{0.4}$ (measured in lab in discrete samples), concentrations of Cd(II) and Cu(II) respectively. Low and high water occurred at *ca* 14:20 h and 18:52 h, respectively. Colloidal fractions of Cd(II) and Cu(II) are plotted against salinity (**G, H**) and SPM (**I, J**) respectively.

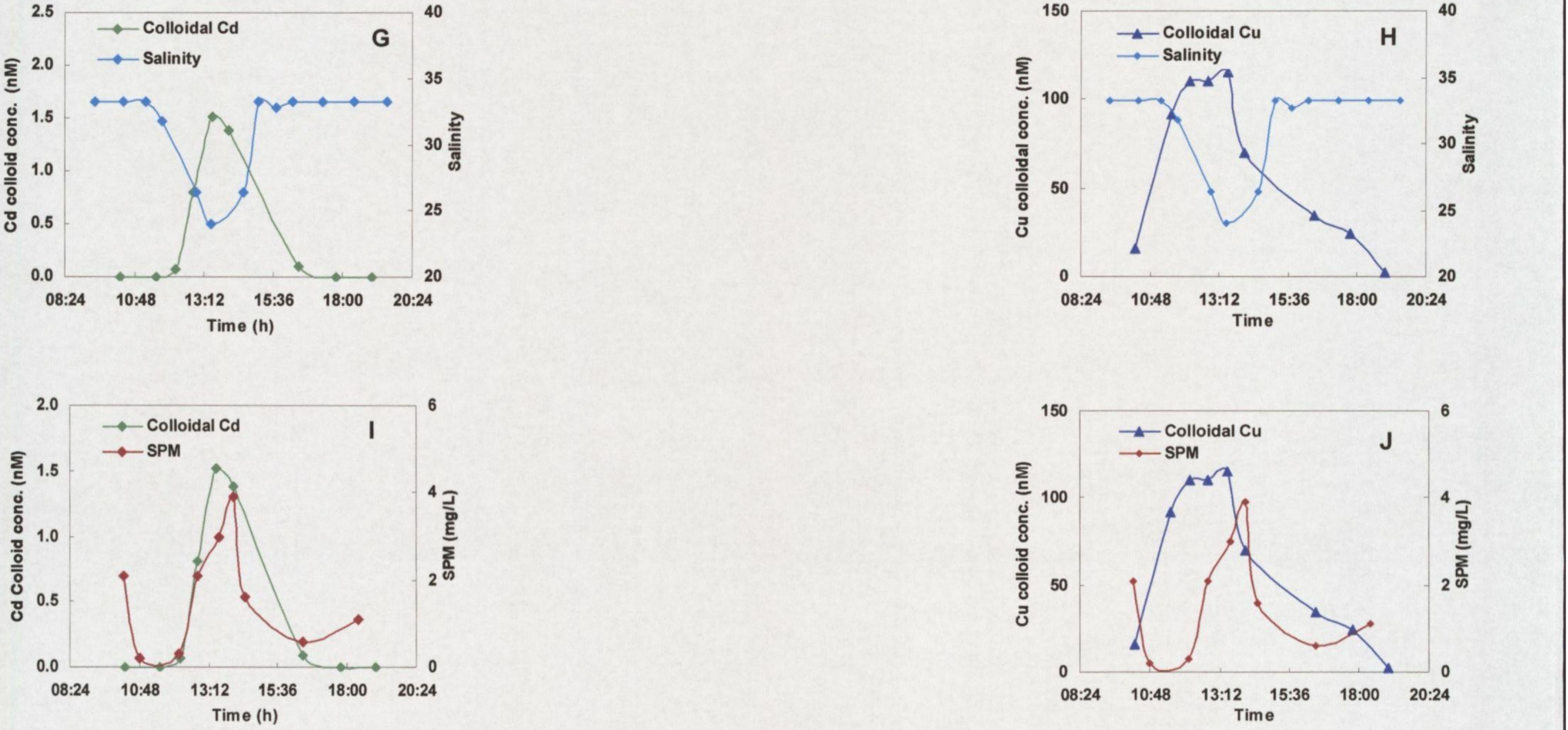


Figure 6.8 Continued...

The contribution of freshwater from the upper part of the estuary increased the temperature, relative to that of the seawater and it remained higher during low tide (Figure 6.8B). Fluctuations of trace metal concentrations over the tidal cycle showed a strong inverse relationship with salinity thereby presenting the same trend as temperature values. Figure 6.8 illustrates results of *in situ* measurements of Cd(II), (Figure 6.8D) Pb(II) (Figure 6.8E) and Cu(II) (Figure 6.8F). Cd(II) was mostly in the VIP dynamic fraction (90-100%), indicating little or no complexation with colloids (Figure 6.8G,I). This is probably a direct result of competition from the high concentration of chloride ions that have a high complexation affinity for the Cd(II) and kept the metal ions in solution. Both VIP dynamic and $TF_{0.4}$ (Figure 6.8D) metal profiles follow an initial increase as the salinity began to decrease up to 12:00 h. Between the periods 12:00 h to 13:30 h the Cd(II) colloidal fraction also increased. It is feasible that adsorption of the Cd(II) onto colloids was effectively able to out compete the chloride ions for metal complexation as the salinity decreased. As the salinity increased and returned to baseline, the colloidal fraction decreased from 75 % at 13:30 h until baseline values of ~0 % colloidal fraction were reached at 17:50 h. The colloidal fraction at low tide was higher than in the Tamar and Plym Estuaries and may be due to a higher concentration of colloids in the water column of Restronguet Creek, originating from the high Fe inputs from the AMD, that provide Fe oxyhydroxides (see section 6.2.2). This inference appears to be reiterated by the consistently high colloidal Pb(II) fraction observed in this estuary. As seen for Chapter 2, Pb(II) is often found associated with colloidal material and over 95 % of the TF_{04} concentrations of Pb(II) in Restronguet Creek was colloidally associated, compared the Plym (60 % at low tide and 80 % at high tide) over all tidal cycles. Concentrations of VIP dynamic Pb(II) were consistently low over the duration of the tidal cycle compared to Cd(II) and Cu(II). Pb(II) is a particle reactive metal (Elbaz-

Poulichet *et al.*, 1984) and hence much of the unbound Pb(II), that is available to the VIP dynamic fraction, is rapidly adsorbed onto colloids and suspended particulate matter giving rise to the low observed concentrations (0.12 to 0.35 nM) compared to Cd(II) (0.25 to 1.75 nM) and Cu(II) (3.5 to 85 nM). Highest concentrations of both VIP dynamic and $TF_{0.4}$ Cd(II), Pb(II) and Cu(II) largely occurred at lower salinities, due to riverine inputs. The Cu(II) profiles showed an increase in concentrations with decreasing salinity followed by a decrease in concentration after low tide. This is probably due to the input of AMD into the estuary from the River Carnon. At lower salinities the estuary becomes increasingly turbid (Figure 6.8F) and partitioning between VIP dynamic and $TF_{0.4}$ fraction is larger. At the higher salinities (≥ 30) less than 50 % of the $TF_{0.4}$ Cu(II) was in the colloidal fraction (Figure 6.8H,J) whereas this percentage increased to < 80 % as salinity decreased (less than 30). In seawater systems a greater proportion of Cu(II) metal is bound to organic complexes as compared to inorganic complexes (Bruland *et al.*, 1991) and although the degree of colloidal complexation for Cu(II) was less than that reported in other estuarine and marine systems (*e.g.* > 99 % reported by Kozelka and Bruland (1998) in Narragansett Bay) it was similar to those reported for the Firth of Clyde (typically 76-96 %; Muller, 1998) and this is characteristic of the effect of increasing particulate load on the partitioning of metals (Balls *et al.*, 1997).

Restronguet Creek Tidal Cycle 2 (01/08/02)

This survey was performed over a period of 12 h on 01/08/02 at Restronguet Point, Restronguet Creek, commencing on the tidal push (High water 10:59 h BST, 4.3 m; low water 17:13 h, 1.9 m; Jones, 2003) six days after spring tides. *In situ* measurements were undertaken at *ca.* 2 m depth at a fixed station from an anchored vessel. Results are presented in Figure 6.9.

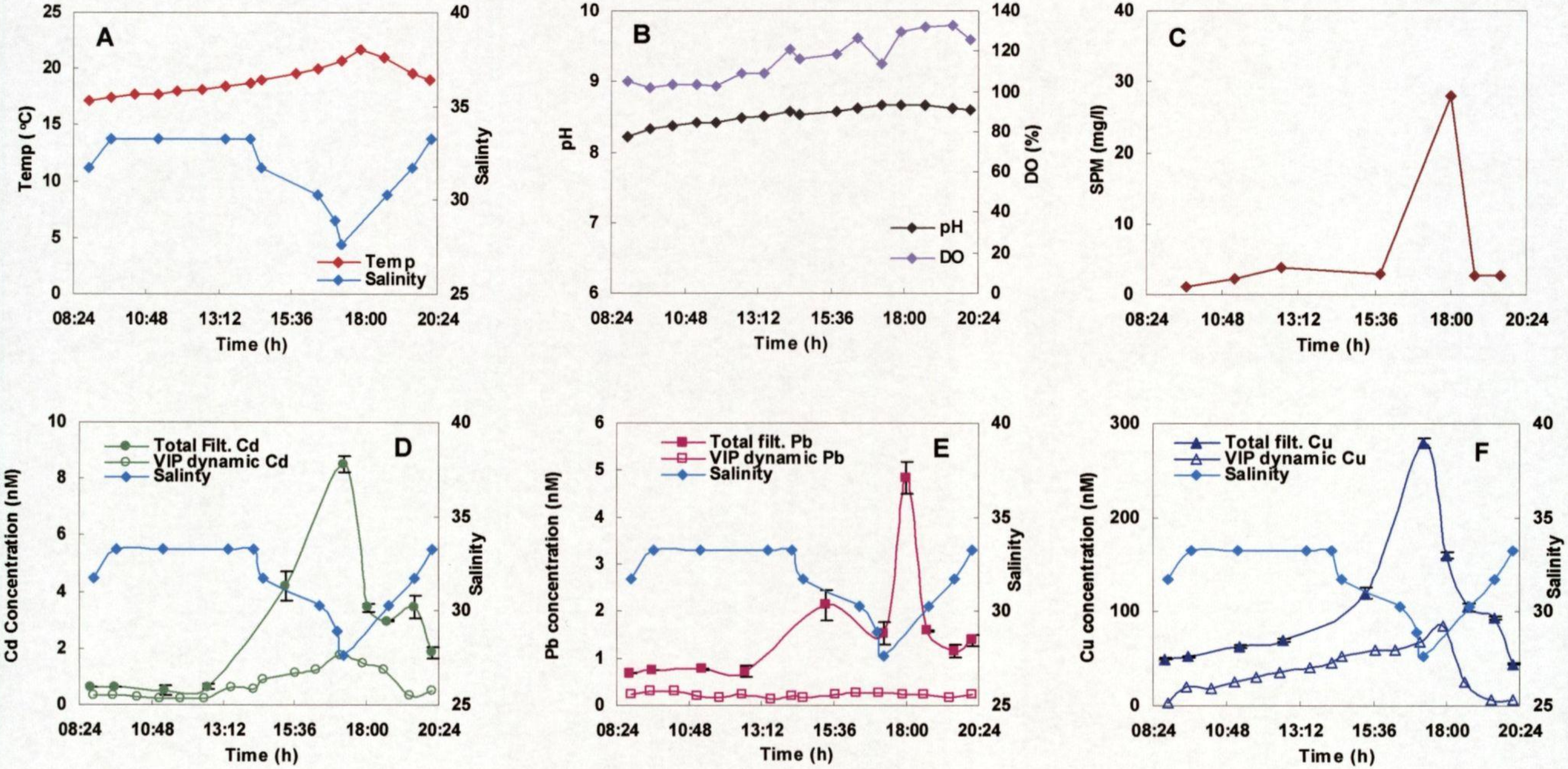


Figure 6. 9 Time series measurements over an 11 h period on 01/08/02 in Restronguet Creek of salinity and temperature (**A**), pH and DO (**B**), SPM (**C**) and *in situ* VIP measured dynamic concentrations and lab measured $TF_{0.4}$ concentrations of Cd(II) (**D**), Pb(II) (E) and Cu(II) (**F**). Low and high water occurred at *ca* 10:59 h and 17:13 h, respectively. Colloidal fractions of Cd(II) and Cu(II) are plotted against salinity (**G, H, I**) and SPM (**J, K, L**) respectively.

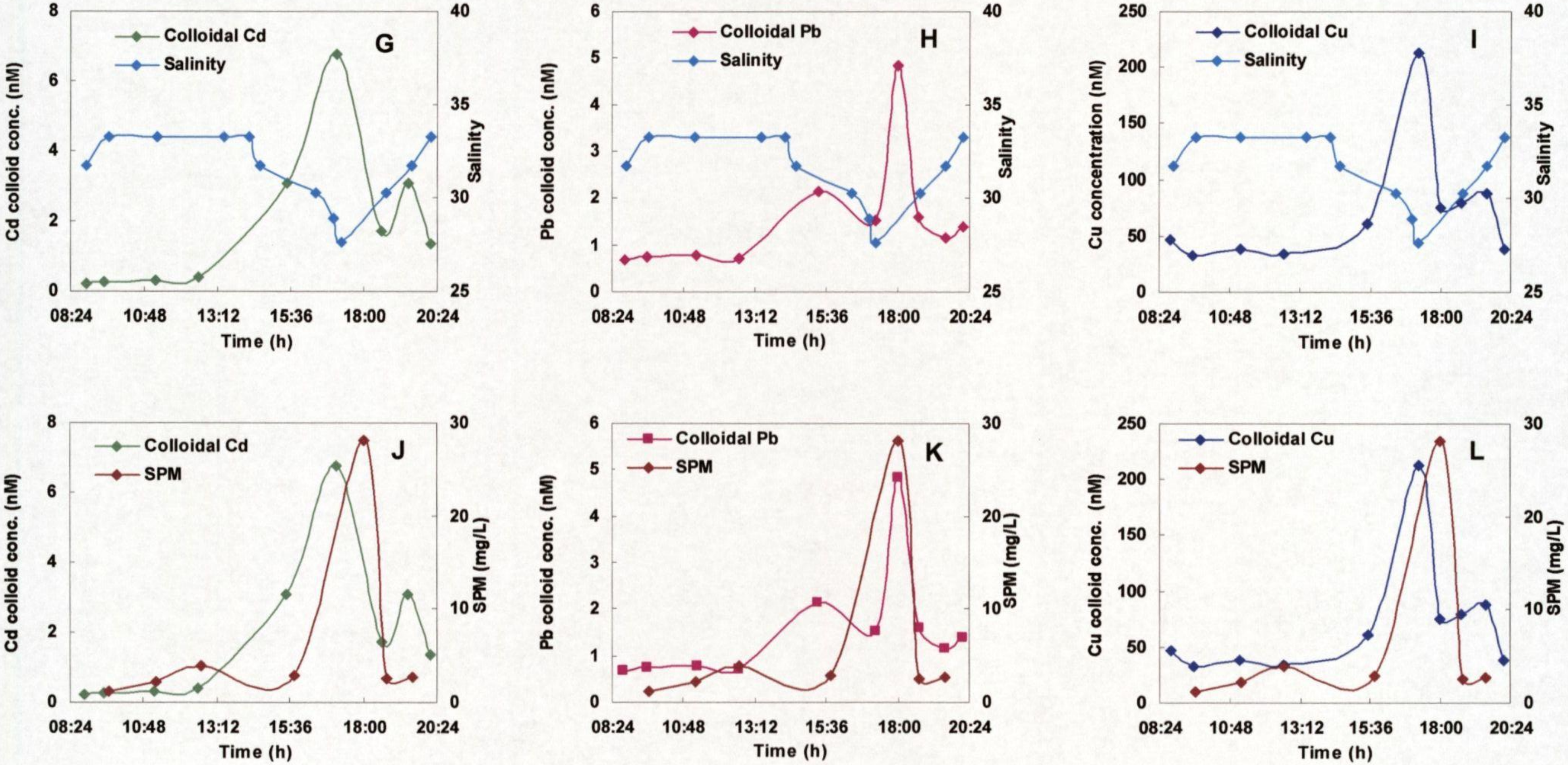


Figure 6.9 Continued...

Figure 6.9A and B show that after the sample at 12:40 h marked changes were noted for most of the variables. Temperature and DO exhibited slight increases with the highest values at low tide (Figure 6.9A,B), with the inverse true for salinity. Trace metal concentrations started a pronounced and rapid increase during low water (Figure 6.9C-E). Trace metal concentration profiles were similar to those shown by Tidal cycle 1, with an increase in concentrations for all three metals as the salinity decreased. Kozelka and Bruland (1998) estimated the colloidal Cd(II) fraction in $TF_{0.4}$ to be 70 to 80 % in Narragansett Bay and similar results have been shown in this tidal cycle where at low tide the colloidal fraction (Figure 6.9G,J) constituted 80 % of the $TF_{0.4}$ (Figure 6.9D) compared to 60 % at high tide. According to Muller (1996) and Kozelka and Bruland (1998) within estuarine systems $TF_{0.4}$ or $TF_{0.2}$ filtrates contain 65 to 99 % colloidally complexed Pb(II), consequently free Pb(II) concentrations are expected to be much lower than $TF_{0.4}$ Pb(II) concentrations. This was the case for Restronguet Creek samples taken during this tidal cycle that showed 80-99 % colloidal Pb(II) fraction over the course of the tidal cycle. There was however no increase in VIP dynamic Pb(II) concentration with input of fresh water, probably as most was bound to colloidal material (Figure 6.9H,K) and hence unavailable for measurement in the VIP dynamic fraction. Again at the higher salinities, observed between 09:30 h and 14:15 h, more than 50 % of the $TF_{0.4}$ Cu(II) was found in the colloidal fraction and this proportion increased to 75 % as salinity decreased to a minimum value (at 17:15 h) showing results very similar to Restronguet Creek tidal cycle 1.

In general both the VIP dynamic fraction and $TF_{0.4}$ concentrations were higher compared with tidal cycle 1, even though the tidal state was closer to spring at this time. Flow rates in the Carnon on 27/06/02 were measured at 0.475 m^3sec^{-1} and

0.544 m^3sec^{-1} on 01/08/02 (Lewis, 2003) and hence differences were likely to be due attributed to the amount of rain preceding the sampling campaigns.

Restronguet Creek Tidal Cycle 3 (02/08/02)

This survey was performed over a period of 12 h on 02/08/02 at Restronguet Point, Restronguet Creek, commenced on the flood tide at 08:00 h (low water 05:47 h, 1.9m and 17:13 h BST, 1.9 m; High water 11:51 h BST, 4.2 m; Jones, 2003) seven days after spring tides. *In situ* measurements were undertaken at *ca* 2 m depth at a fixed station from an anchored vessel.

Figures 6.10A,B show similar behaviour of temperature, DO and pH. Changes in salinity were much less pronounced than in Tidal cycle 2 (as a result of neap tide conditions). During ebb flow of the tide, concentration values of $TF_{0.4}$ Cd(II), Pb(II) and Cu(II) and VIP dynamic fraction Cd(II) and Cu(II) increased significantly (and also the colloidal fraction) to reach approximately 4 fold the concentration at high tide. VIP dynamic concentrations of Pb(II) did not show correlation with salinity probably because most of Pb(II) remained bound to colloidal material (Figure 6.9H,K). Concentrations of both $TF_{0.4}$ and VIP dynamic fractions and hence colloidal fractions for all three metals were of a similar to those in previous tidal cycles.

Concentrations of $TF_{0.4}$ Cd(II) were higher in June than August, which may be explained by the rainfall preceding the June study, (flows were lower in August than June (Restronguet Creek tidal cycle 2)] and leaching of Cd(II) from the metalliferous catchment. Nevertheless, concentrations of dynamic Cd(II) were similar for the two periods.

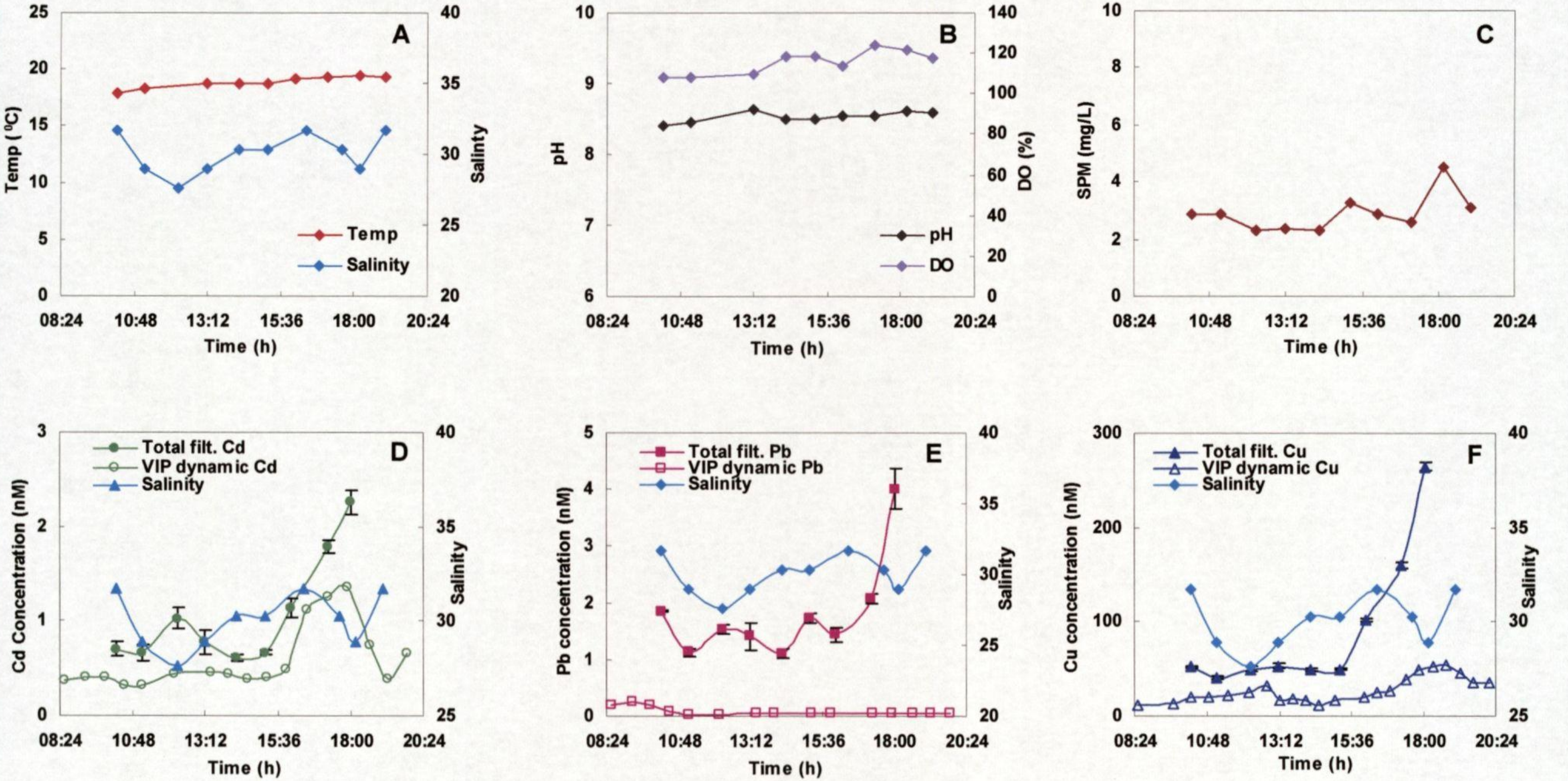


Figure 6. 10 Time series measurements over an 11 h period on 02/08/02 in Restronguet Creek of salinity and temperature **(A)**, pH and DO **(B)**, SPM **(C)** and *in situ* VIP measured dynamic concentrations and lab measured $TF_{0.4}$ concentrations of Cd(II) **(D)**, Pb(II) **(E)** and Cu(II) **(F)**. Low and high water occurred at *ca* 11:51 h and 17:13h, respectively. Colloidal fractions of Cd(II) and Cu(II) are plotted against salinity (**G, H, I**) and SPM (**J, K, L**) respectively.

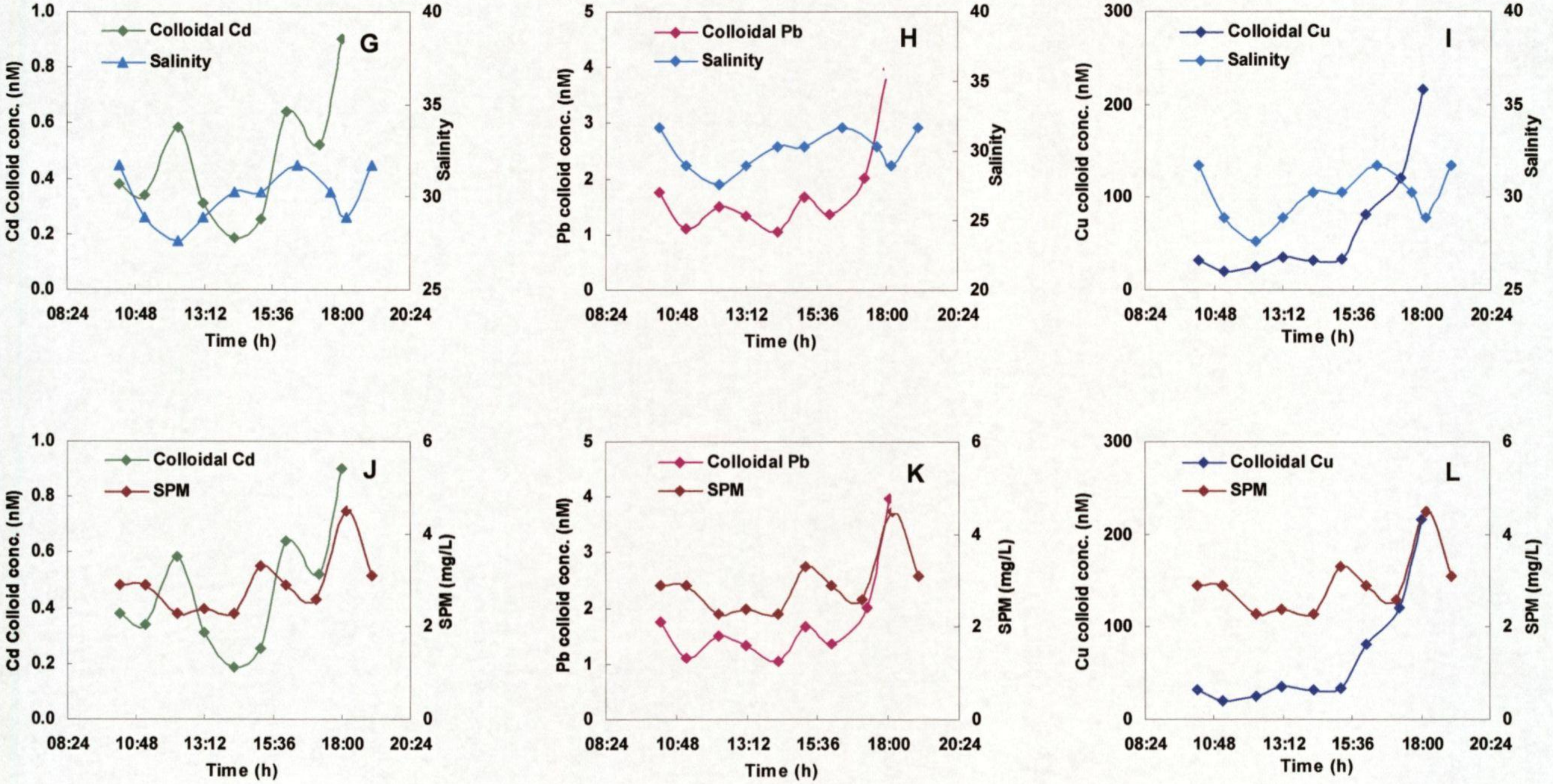


Figure 6.10 Continued…

Dynamic Cd(II) constituted 27-99 % of the total Cd(II) in June, and 57-100 % in August. In both cases, concentrations of Cd(II) were highest at lowest salinities, indicating the importance of AMD as a source of this metal in this system.

Restronguet Creek Tidal Cycle 4 (05/12/02)

This survey, performed over a period of 12 h on 05/12/02 at Restronguet Point commenced at the morning high water (High water 05:30 h GMT, 5.6 m and 17:54 h, 5.4 m; low water 12:11 h GMT, 0.7 m; Jones, 2003) one day before spring tides and undertaken at *ca* 2 m depth at a fixed station from an anchored vessel. Figure 6.11A shows a steady decrease in salinity during the flood tide at the station (*ca.* 11:45 h), after which, recorded values increased quickly. A similar trend was noted for pH and DO. Anomalies in DO and pH measurements were probably due to the effect of wind producing swells. The combination of the spring tide and the contribution of more freshwater from the upper estuary were evident with lower salinity values during low tide compared to previous tidal cycle. Trace metal concentrations were however very similar to those recorded previously (in tidal cycles 1-3).

Trace metal behaviour over time (Figure 6.11C-E) once again demonstrated a strong inverse relation with salinity. Cd(II) profiles showed that at higher salinities that most of the $TF_{0.4}$ Cd(II) was in the VIP dynamic fraction and not bound to colloidal material. However at high tide with the greater influence of river water the colloidal fraction increased and continued to be evident until flood tide (at *ca.* 14:45 h), when it reached <20 % once again. Pb(II) and Cu(II) profiles showed similar trends to previous tidal cycles.

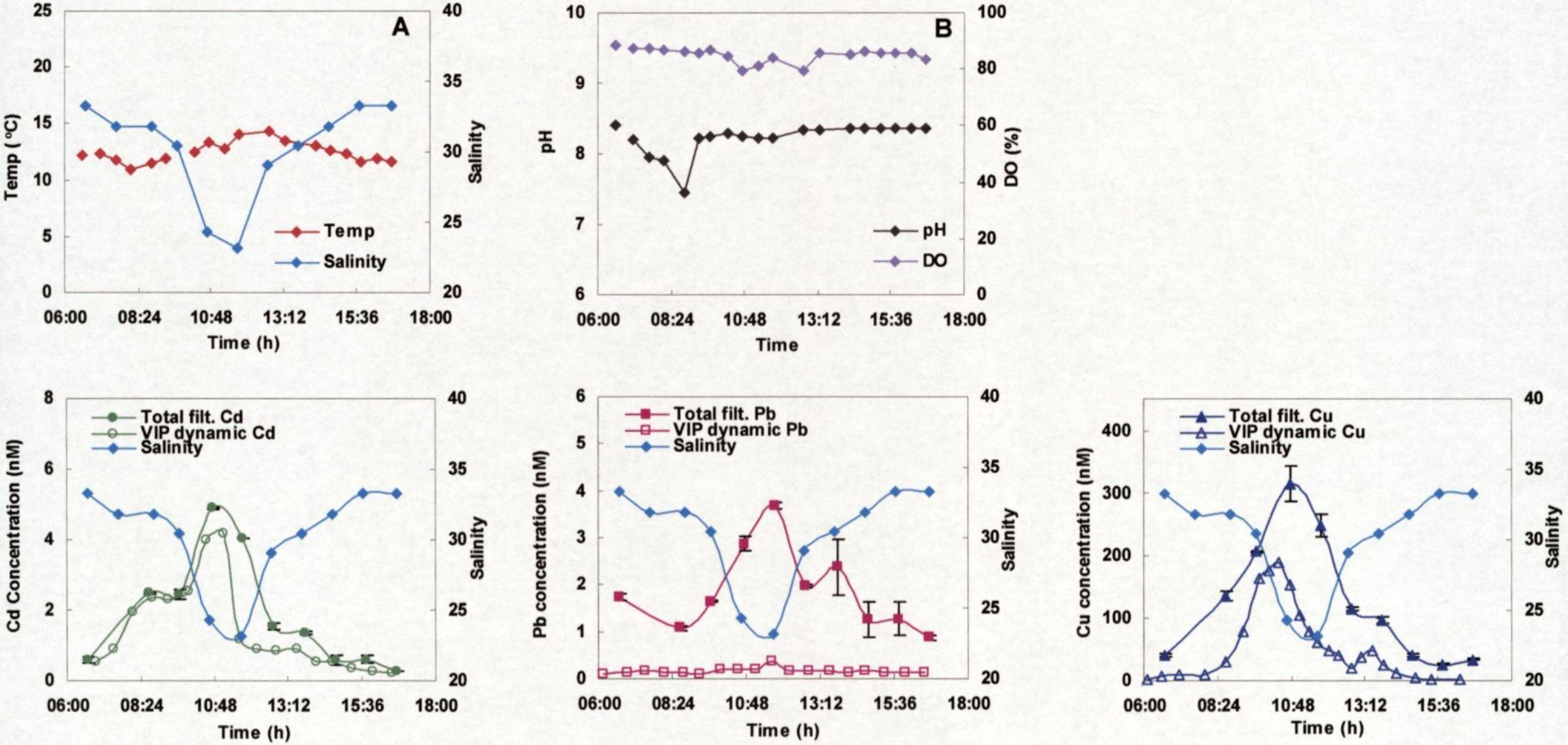
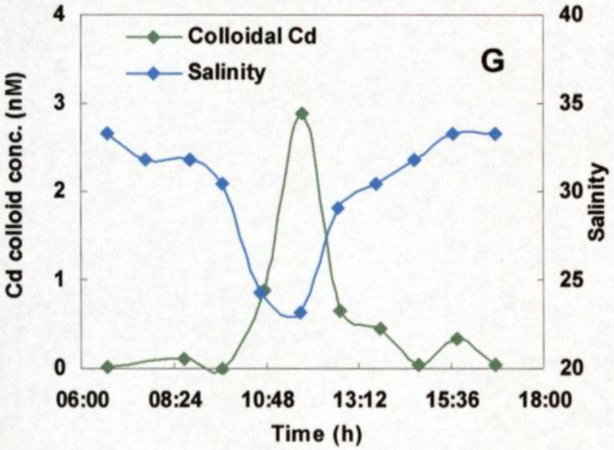
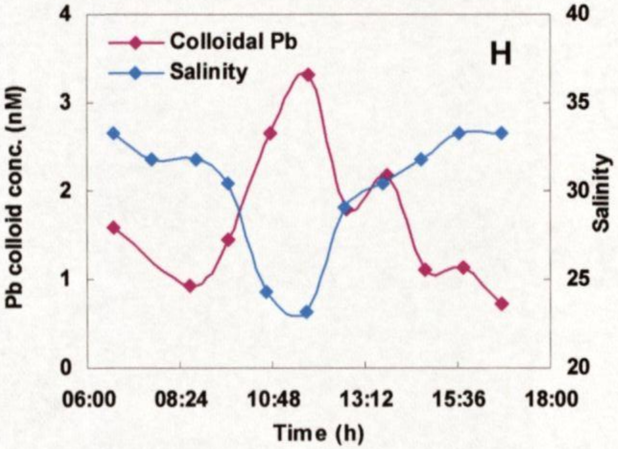
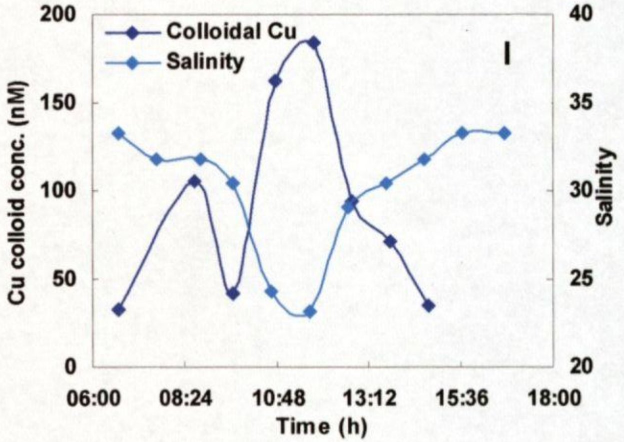


Figure 6. 11 Time series measurements over an 11 h period on 05/12/02 in Restronguet Creek of salinity and temperature (A), pH and DO (B) and *in situ* VIP measured dynamic concentrations and lab measured $TF_{0.4}$ concentrations of Cd(II) (C), Pb(II) (D) and Cu(II) (E). Low and high water occurred at *ca* 11:51 h and 17:13 h, respectively. Colloidal fractions of Cd(II) and Cu(II) are plotted against salinity (**G, H, I**).


G
Colloidal Cd
Salinity
Cd colloid conc. (nM)
0
1
2
3
4
Salinity
20
25
30
35
40
Time (h)
06:00
08:24
10:48
13:12
15:36
18:00


H
Colloidal Pb
Salinity
Pb colloid conc. (nM)
0
1
2
3
4
Salinity
20
25
30
35
40
Time (h)
06:00
08:24
10:48
13:12
15:36
18:00


I
Colloidal Cu
Salinity
Cu colloid conc. (nM)
0
50
100
150
200
Salinity
20
25
30
35
40
Time (h)
06:00
08:24
10:48
13:12
15:36
18:00

6.3.4 Comparison of Trace Metal Concentrations with Environmental Quality Standards (EQS)

The EC Dangerous Substances Directive (76/464/EEC) requires routine monitoring of environmental concentrations of all substances in List I and List II by the statutory regulatory authority wherever there is a consented discharge of a listed substance. In addition, Trace metals are included in List I and II and as a consequence the Environment Agency (EA) regularly monitors the Plym, Tamar and Carnon estuaries. Based on toxicity levels, 'Environmental Quality Standards' (EQS) for $TF_{0.4}$ trace metals in the water column have been set (EA, 2003). The EA is required to ensure that the EQS are met at the monitoring locations.

The standard practice of the EA is to sample on a quarterly basis in order to obtain average annual $TF_{0.4}$ concentrations of trace metals. Even though our sampling campaigns were temporally less frequent than the EA's, the number of samples taken from each estuary during each tidal cycle allowed a maximum, minimum, and median concentration to be determined from all discrete samples taken from the estuaries on the monitoring days. Cd(II), Pb(II) and Cu(II) in waters of the three estuaries were compared with the statutory EQS for coastal and estuarine waters (EA, 2003) and are results shown in Table 6.1.

Table 6.1 The table gives the EQS levels for Cd(II), Pb(II) and Cu(II) and median concentrations for samples collected from the estuaries examined in this work. n=number of samples.

	Cd(II) (nM)	Pb(II) (nM)	Cu(II) (nM)
EQS	22.3	126.0	78.7
Tamar	0.6 (n=5)	1.0 (n=5)	13.4 (n=5)
Plym	0.4 (n=15)	1.2 (n=16)	21.0 (n=16)
Restronguet Creek	1.1 (n=38)	1.5 (n=27)	92.0 (n=38)

The table indicates that the EQS limits for Cd(II) and Pb(II) were not exceeded for median results from the water column for all of the sites. For Cu(II), the Tamar and Plym values fall below the EQS with concentrations, whilst the Restronguet Creek showed values exceeding the EQS, indicating that the site has levels of considerable toxicity.

Environmental data is often not ‘normally distributed,’ demonstrating large natural variations in analyte levels, therefore common statistical tools are not usually used for the interpretation of such data and non-parametric representations and statistical tests are often applied (Miller and Miller, 2000). In accord with this, a box and whisker plot of the data from $TF_{0.4}$ is given in Figure 6.12. The plot shows the interquartile range, median and spread and range of data. It is clearly observed that Restronguet Creek gives the highest concentrations and largest spread of data. This is probably due to the nature of the heavily impacted head of estuarine water mixing with the comparatively cleaner Fal estuarine water and water sampled over contrasting tidal cycle states.

Comparison of Concentrations to Published Values

$TF_{0.4}$ metal concentrations reported above are in good agreement with previously reported data for the Tamar. Cu concentrations (8.36 to 24.8 nM) were similar to those reported by and van den Berg (1991) and Ackroyd *et al.*, (1986) of 15 to 150 nM (at salinities of 28 to 32). $TF_{0.4}$ Cd concentrations were in the range 0.6 to 1.7 nM and were similar to those reported by Morris *et al.*, (1986) of 0.3 to 1.1 nM (at salinities ranging 30 to 35). In Restronguet Creek, Rijstenbil, (1991) reported $TF_{0.4}$ concentrations of between 98 to 395 nM for Cu (at salinities ranging 29.6 to 33.9). This is similar, to the $TF_{0.4}$ concentrations found in our surveys (26 to 280 nM).

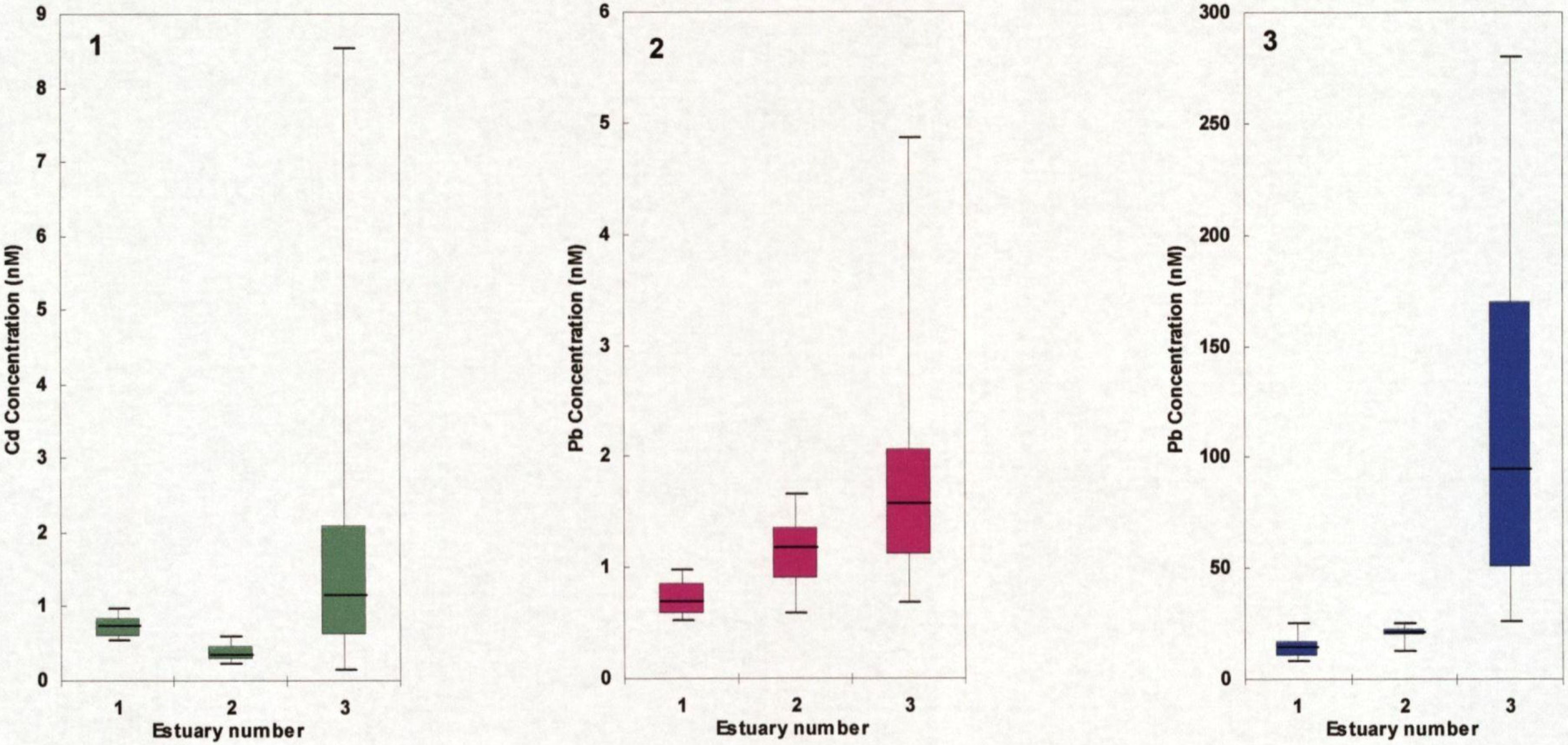


Figure 6.11 Box and whisker plots of the spread of data for **(A)** Cd(II), **(B)** Pb(II) and **(C)** Cu(II) for the Tamar (Estuary number 1), Plym (Estuary number 2) and Restronguet Creek (Estuary number 3). The box indicates the interquartile range, and the line crossing the box, the median. The whiskers (lines above and below the box) indicate the range in concentrations.

No published trace metal data on the Plym exists to date, but concentrations our observed concentrations are similar to those reported in the Tamar.

6.4 Conclusions

The results from all tidal cycle surveys undertaken in the three estuaries reaffirm the importance of reliable measurements of trace metals and master variables (salinity, conductivity, pH, temperature, dissolved oxygen and suspended solids) to aid understanding of trace metal estuarine biogeochemical processes. The employment of the VIP system allowed high quality speciation measurements and the importance of colloids to these systems was observed. The application of the VIP during the surveys resulted in high-resolution trace metal data (2-4 measurements h^{-1}) showing dynamic changes in concentrations as a result of tidal variations. The VIP allowed unattended, multi-elemental measurements for periods up to 12 h and the deployment of this submersible *in situ* probe provided significant advantages in terms of metal speciation measurements and temporal resolution.

The overall behaviour of both $TF_{0.4}$ and VIP dynamic trace metals during these tidal cycles showed an inverse relationship with salinity. Maximum trace metal concentrations were observed during periods of low water. In the Tamar, VIP dynamic Cd(II), Pb(II) and Cu(II) concentrations varied from 0.45 to 0.77 nM, 0.14 to 0.35 nM and 3.44 to 7.77 nM, respectively. Concentrations of metals in the $TF_{0.4}$ fraction varied from 0.53 to 0.97 nM, 0.59 to 1.66 nM and 8.36 to 24.8 nM respectively. In the Plym VIP dynamic Cd(II), Pb(II) and Cu(II) concentrations varied, from 0.21 to 0.51 nM, 0.25 to 0.47 nM and 3.28 to 8.50 nM respectively. Concentrations of metals in the $TF_{0.4}$ fraction varied from 0.24 to 0.59 nM, 0.59 to 1.66 nM and 12.38 nM to 25.0 nM respectively. Restronguet Creek VIP dynamic

Cd(II), Pb(II) and Cu(II) concentrations varied, from 0.17 to 4.2 nM, 0.06 to 0.37 nM and 17.5 to 190 nM respectively. Concentrations of metals in the $TF_{0.4}$ fraction varied from 0.13 to 8.53 nM, 0.68 to 4.85 nM and 26 to 280 nM respectively.

Concentrations of trace metals in the Tamar and Plym estuaries were similar owing to the geology of the catchment and anthropogenic inputs. The high concentrations in the Restronguet Creek were the result of the long historical mining background of the area, with numerous inputs from mining adits.

It was noticeable that for all metals at higher turbidity conditions and higher riverine contributions *i.e.* lower salinity, the concentration of the colloidal fraction, defined as the difference between the $TF_{0.4}$ and the VIP dynamic fraction increased significantly compared to that of seawater. Also, there appeared to be a significantly larger contribution of colloidal material to Restronguet Creek from riverine inputs, scavenging the trace metals and making them less bioavailable. In the case of Pb(II) and Cu(II) for all conditions in all estuaries the VIP defined colloidal fraction constituted over 40 % of the $TF_{0.4}$ concentrations, indicating the importance of these colloids for acting as sorbants for trace metals. Cd(II), however, demonstrated more complex behaviour in all three estuaries. It appears at salinities < 30, the colloids competed with the chloride ions for Cd(II) complexation.

Rivers form an integral source of trace metals to estuaries, as demonstrated by our work. It is essential to highlight the importance of a heavily impacted river on the inputs of trace metals to these systems from activities such as mining. $TF_{0.4}$ trace metal concentrations in the Tamar and Plym estuaries are comparatively low and all metals fall comfortably within the EQS. The low concentrations are most likely due (to some extent) to the turbid nature of these estuaries that results in removal of surface reactive metals from solution onto particles. According to our findings, this is Restronguet Creek does not meet the EQS level of Cu(II) in the $TF_{0.4}$. A large amount of the metals discharged into the estuary are probably adsorbed onto sediments and hence the sediment now remains an important source of metals to the water column. Areas where the potential to damage or threaten the ecosystem from such high trace metal concentrations should be primary targets for concentrated remediation efforts to reduce inputs and to achieve background levels.

Reference List

Achterberg, E. P. and van den Berg C. M. G (1994) *In-Line Ultraviolet-Digestion of Natural-Water Samples for Trace-Metal Determination Using an Automated Voltammetric System,* Anal. Chim. Acta, **291**, 213-232

Ackroyd, D. R., Bale, A. J., Howland, R. J. M., Knox, S., Millward, G. E., and Morris, A. W. (1986) *Distributions and Behavior of Dissolved Cu, Zn and Mn in the Tamar Estuary.* Estuar. Coast. Shelf. Sci., **23**, 621-640.

Balls, P.W., Hull, S., Miller, B. S., Pirie, J. M. and Proctor, W. (1997) *Trace Metals in Scottish Estuarine and Coastal Sediments.* Mar. Pollut. Bull. **34**, 42-50.

Banks, D., Younger, P, L. Iversen E. R., Banks, S. B. and Arnesen, R. T. (1997) *Mine-Water Chemistry: The Good, the Bad and the Ugly,* Environ. Geol., **32**, 157-174

Bryan, G. W., Gibbs, P. E., Hummerstone, L. G. and Burt, G. R. (1987) *Copper, Zinc, and Organotin as Long-Term Factors Governing the Distribution of Organisms in the Fal Estuary in Southwest England.* Estuaries, **10**, 208-219.

Bruland, K. W., Donat, J. R. and Hutchins, D. A. (1991) *Interactive Influences of Bioactive Trace Metals on Biological Production in Oceanic Waters.* Limnol. Oceanogr., **36**, 1555-1577.

Boult, S., Collins, D. N., White, K. N., Curtis, C. D. (1994) *Metal Transport In A Stream Polluted By Acid-Mine Drainage - The Afon Goch, Anglesey, UK.* Environ. Pollut., **84**, 279-284

Butler, A. (1998) *Acquisition and Utilization of Transition Metal Ions by Marine Organisms.* Science, **281**, 207-210

C.E.H. (2003) *Plym at Carn Wood,* Centre for Ecology and Hydrology, URL., http://www.nwl.ac.uk/ih/nrfa/station_summaries/047/011.htm. Viewed 29th May 2003

C.E.H. (2003A), *Fal at Tregony,* Centre for Ecology and Hydrology, URL., http://www.nwl.ac.uk/ih/nrfa/station_summaries/048/003.htm. Viewed 29th May 2003

EA (2003) *Dangerous Substances Directive, Environmental Quality Standards (EQS),* Environment Agency, http://www.environment-agency.tv.ye/qa-ae-docs/s-enviro/viewpoints/3compliance/2fwater-equal/3-3-4tab3, Viewed 12th June 2003

Elbaz-Poulichet, F., Holliger, P., Huang, W. W., and Martin, J.-M. *(1984) Lead Cycling in Estuaries, Illustrated by the Gironde Estuary, France,* Nature, **308,** 409-414

Forstner, U and Salomons, W., (1988) *Environmental Management of Solid Waste.* Eds Forstner, U and Salomons, W., 1st Ed., Springer-Verlag, Berlin, Heidelberg.

Head, P. C. (1985) *Practical Estuarine Chemistry: A Handbook.* Ed., Head, P. C. Cambridge Univ. Press, pp 337.

Hiscock, K., and Moore, J. (1986) *Surveys of Harbours, Rias and Estuaries in Southern Britain: Plymouth Area including the Yealm.* Nature Conservancy Council, CSD Report, No. 752.

Kozelka, P. B. and Bruland, K. W. (1998) *Chemical Speciation of Dissolved Cu, Zn, Cd and Pb in Narragansett Bay, Rhode Island.* Mar. Chem., **60**, 267-282

Lewis, A. (2003) Environment Agency, Exeter, UK, personal communication, 20th May, 2003

Miller J. C. and Miller J. N. (2000) *Non –parametric Robust Methods.* In: Statistics and Chemometrics for Analytical Chemistry. 4th Ed., Prentice-Hall, Harlow, England, pp 151-181.

Morris, A. W., Bale A. J., Howland, R. J. M, Millward, G. E., Ackroyd, D. R., Loring, D. H. and Rantala, R. T. T. (1986) *Sediment Mobility and its Contribution to Trace-Metal Cycling and Retention in a Macrotidal Estuary.* Wat. Sci. Technol., **18**, 111-119.

Morris, A. W., Bale, A. J., Howland, R. J. M. (1982) *Chemical Variability In The Tamar Estuary, Southwest England,* Estuar. Coast. Shelf Sci., **14**, 649-661

Muller, F. L. L. (1998) *Colloid/Solution Partitioning of Metal-Selective Organic Ligands, and its Relevance to Cu, Pb And Cd Cycling in the Firth of Clyde.* Estuar. Coast. Shelf Sci., **46**, 419-437.

Muller, F. L. L. (1996) *Interactions of Copper, Lead and Cadmium with the Dissolved, Colloidal and Particulate Components of Estuarine and Coastal Waters.* Mar. Chem., **52,** 245-68.

Nelson, C .H. and Lamothe, P. J., (1993) *Heavy Metal Anomalies in the Tinto and Odiel River and Estuary System, Spain.* Estuaries, **16**, 496–511.

Pirrie, D., Camm, G. S., Sear, L. G., Hughes, S. H. (1997) *Mineralogical and Geochemical Signature of Mine Waste Contamination, Tresillian River, Fal Estuary, Cornwall, UK.* Environ. Geol., **29**, 58-65

Rijestenbil, J. W., Merks, A. G. A., Peene, J, Poortviliet, T. C. W. and Wijnholds, J. A. (1991) *Phytoplankton Composition and Spatial Distribution of Cupper and Zinc in the Fal Estuary (Cornwall, U. K.)* Hydrobiol. Bull., **25**, 37-44

Stevens, (2001) Stevens tide tables, Able Printers, Saltash, UK

Tercier-Waeber, M. L., Buffle, J., Confalonieri, F., Riccardi, G., Sina, A., Graziottin, F, Fiaccabrino, G. C. and Koudelka-Hep, M. (1999) *Submersible Voltammetric Probes for In Situ Real-Time Trace Element Measurements in Surface Water, Groundwater and Sediment-Water Interface.* Meas. Sci. Technol. **10** 1202-1213

Turner, A. and Millward, G. E. (2002) *Suspended Particles: Their Role in Estuarine Biogeochemical Cycles.* Estuar. Coast. Shelf Sci., **55**,857-883

van den Berg C. M. G. (1991) *Monitoring of Labile Copper and Zinc in Estuarine Waters using Cathodic Stripping Chronopotentiometry.* Mar. Chem., **34**, 211-223.

Warwick, R. M. (2001) *Evidence for the Effects of Metal Contamination on the Intertidal Macrobenthic Assemblages of the Fal Estuary.* Mar. Pollut. Bull., **42,** 145-148

van Geen, A., Adkins, J. F., Boyle, E. A., Nelson, C. H. and Palanques, A. (1997). *A 120 Yr Record of Widespread Contamination from Mining of the Iberian Pyrite Belt.* Geology, **25**, 291–294.

CHAPTER 7

Determination of VIP Dynamic Concentrations of Cd (II), Pb(II) and Cu(II) in the Po Estuary and Adriatic Sea

Abstract

In situ determination of VIP dynamic metal concentrations in the Po plume and Adriatic Sea showed values of 0.13 to 0.29 nM for Cd(II), 0.12 to 0.30 nM for Pb(II) and 1.4 to 4.3 nM for Cu(II). These concentrations were similar to total filterable (0.4 µm pore size) concentrations previously reported for this coastal sea system. Pb(II) and Cu(II) were negatively correlated with salinity, whilst Cd(II) was not correlated with salinity indicating that its distribution was not controlled by simple mixing and dilution and highlighting the importance of local riverine inputs close to the river mouth for this element. The data demonstrated that the Adriatic was not heavily contaminated with trace metals, and elevated concentrations were only found close to significant boundary inputs, that included the river Po.

7.1 Introduction

The River Po, Italy, is one of the most heavily polluted rivers in Europe (Justic *et al.*, 1994) and the Po Estuary receives large quantities of industrial and domestic pollutants from the River Po (Renoldi, 1997; Barghigiani *et al.*, 2001) as a consequence. The Po flows into the Northern Adriatic Sea and influences trace metal concentrations in the Adriatic as a result (Tankere and Statham, 1996). An important feature of the coastal waters surrounding the Po is the often highly visible chlorophyll plume (Figure 7.1; Grancini and Cescon, 1973; Crema *et al.*, 1991; Revelante and Gilmartin, 1992) present as a result of the enhanced nutrient loads (McIntyre, 1992, Justic *et al.*, 1994). Due to the prevailing marine currents, northward along the Croatian coast and southward along the Italian coast (Malacic *et al.*, 2001; Kourafalou, 2001) polluting substances discharged by the river Po, mainly affect the Italian coast from the mouth of the river Po to Ancona (de Wit and Bendoricchio, 2001). The presence of the Po plume signifies the high input of anthropogenic chemicals into this area (that include trace metals; Renoldi *et al.*, 1997).

Figure 7.1 The highly visible 'Po plume' that extends from the Po Estuary into the Nothern Adriatic Sea. Satellite image provided by the SeaWiFS Project, NASA/Goddard Space Flight Center, and ORBIMAGE.

7.1 Introduction

The Foyle River is one of the most [illegible] rivers in Europe [illegible] [illegible] [illegible] [illegible] from the River [illegible] 1997; [illegible] et al., 2003; [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] et al., [illegible] [illegible] [illegible] 2007) [illegible] [illegible] 2001). The presence of the [illegible] [illegible] of [illegible] [illegible]

Figure 7.1 [illegible]

The aim of this study was to:

- ensure that the VIP system performed accurately during extended deployments in both polluted estuarine and more pristine coastal waters.
- determine VIP dynamic concentrations of Pb(II), Cd(II) and Cu(II) in Po plume and the Adriatic Sea.

7.2 Experimental

7.2.1 Description of the Po

The river Po is the longest river in Italy, crossing the industrialised northern part of the country and stretching 650 km from source before discharging into the Adriatic Sea, through a delta of 400 km^2 (Pettine, 1997). The river Po with a flow of 2,900 m^3 sec^{-1} (Tankere and Statham, 1996) contributes about 50 % of the total freshwater discharge into the shallow Northern Adriatic basin. This basin has an average depth of 30 m; (Degobbis, *et al.*, 1986; Degobbis and Gilmartin, 1990). The Adriatic Sea is characterised by a low tidal range (0.3 to 1.0 m; Baric, 2000).

Cruise stations

The VIP system was deployed from *RV G Dallaporta* (Figure 7.2) in the Adriatic coastal waters during the period 27/10/02 to 01/11/02.

Figure 7.2 The RV *G Dallaporta anchored in the Adriatic Sea, Italy*

the end of the study was 5C.

However, these two systems performed [illegible] requirements [illegible] (Fig. [illegible]) [illegible] and the [illegible]

7.2 [illegible]

7.2.1 Description of the site

[illegible] the country and [illegible] from source before [illegible] (Figure [illegible]) [illegible] 2003).

[illegible]

[illegible] period [illegible]

[illegible]

The location and numbering of the sampling stations are illustrated in Figure 7.3.

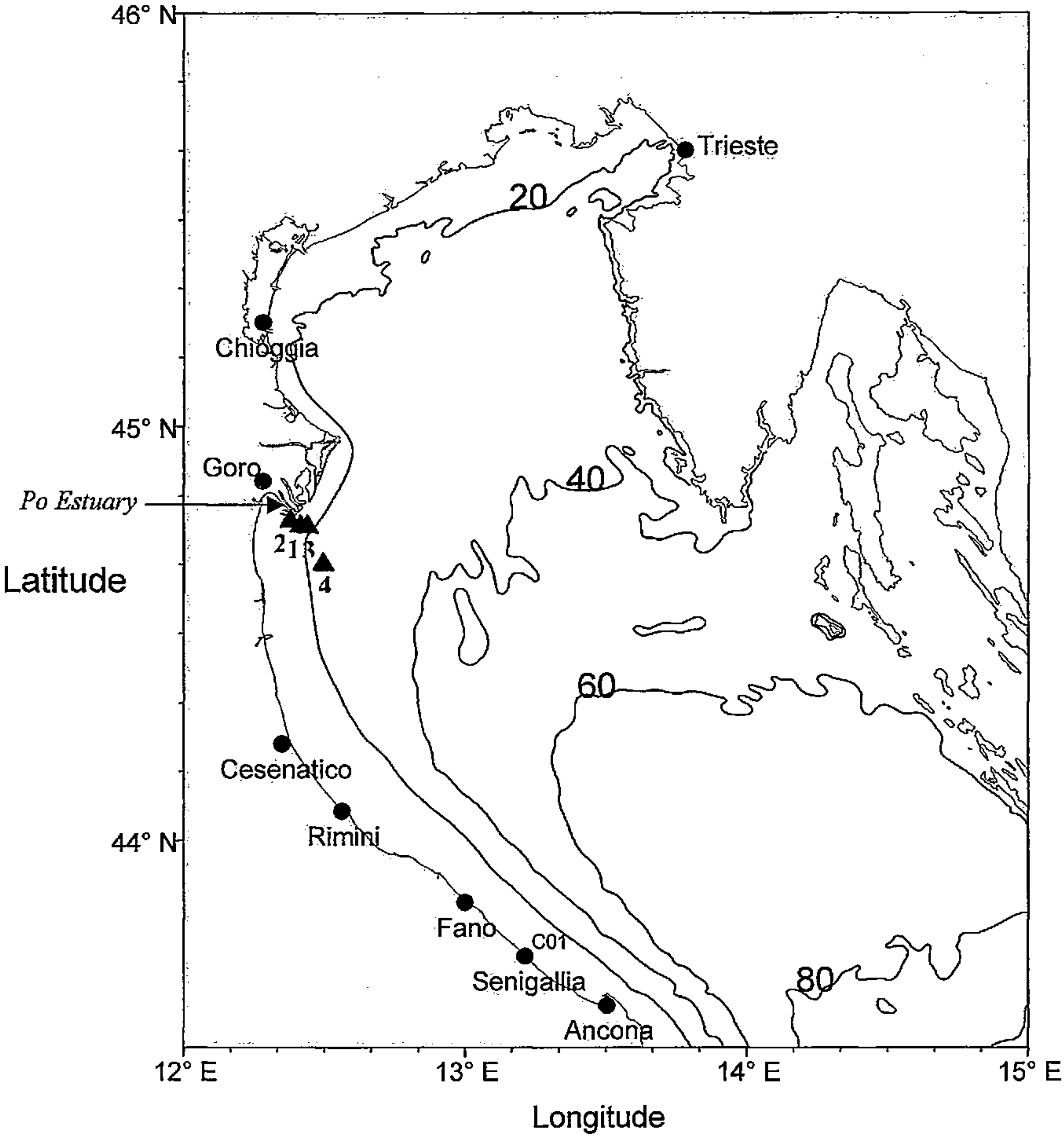


Figure 7.3 Location and numbering of sampling stations in the Adriatic Sea for the IMTEC Cruise 2, 2002.

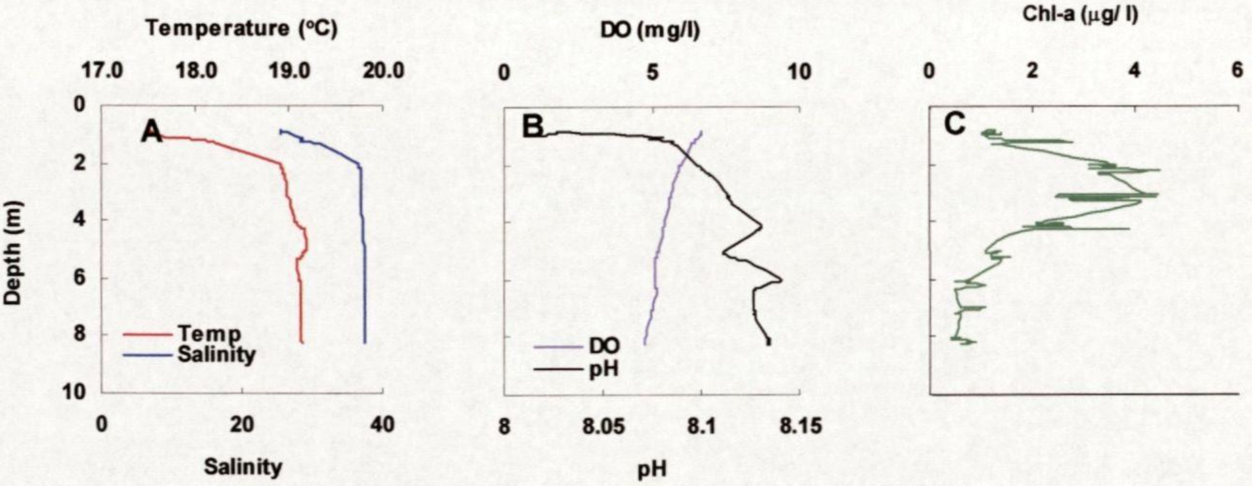
7.2.2 Deployment of the VIP System and Analysis

The vessel was stopped at each station and a conductivity-temperature-depth (CTD) profile taken. The calibrated CTD recorded salinity, temperature, depth, dissolved

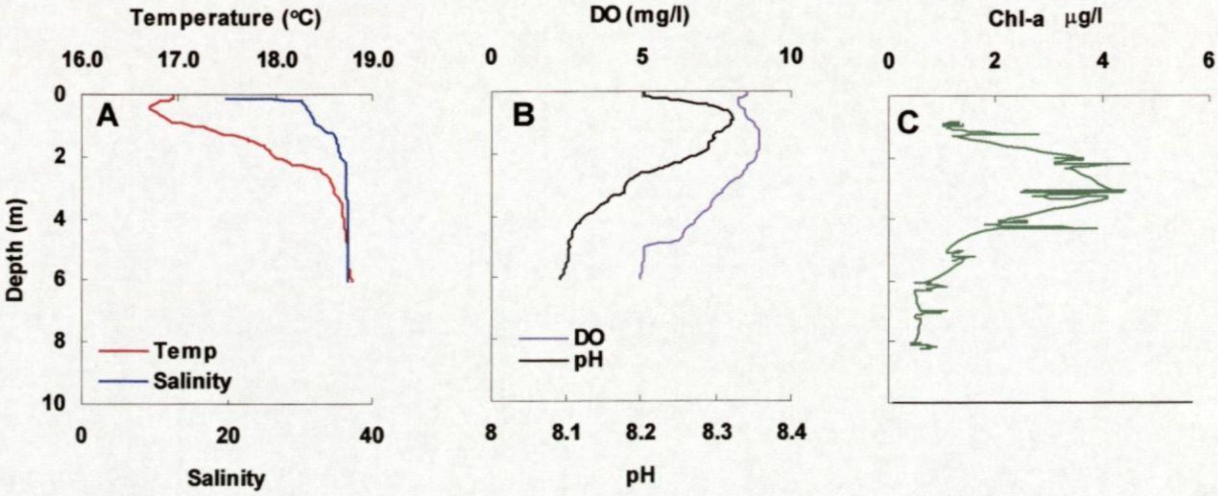
oxygen and fluorescence. The VIP system was deployed (by the method described in Chapter 5, section 5.2.3), at 1 to 2 depths at each station to determine trace metals in different water masses for ~4 h at each depth. The procedures and methodologies of *in situ* VIP operation (described in Chapter 4, section 4.2) were employed for *in situ* analysis, and laboratory calibration and analysis.

7.2.3 *CTD Profiles*

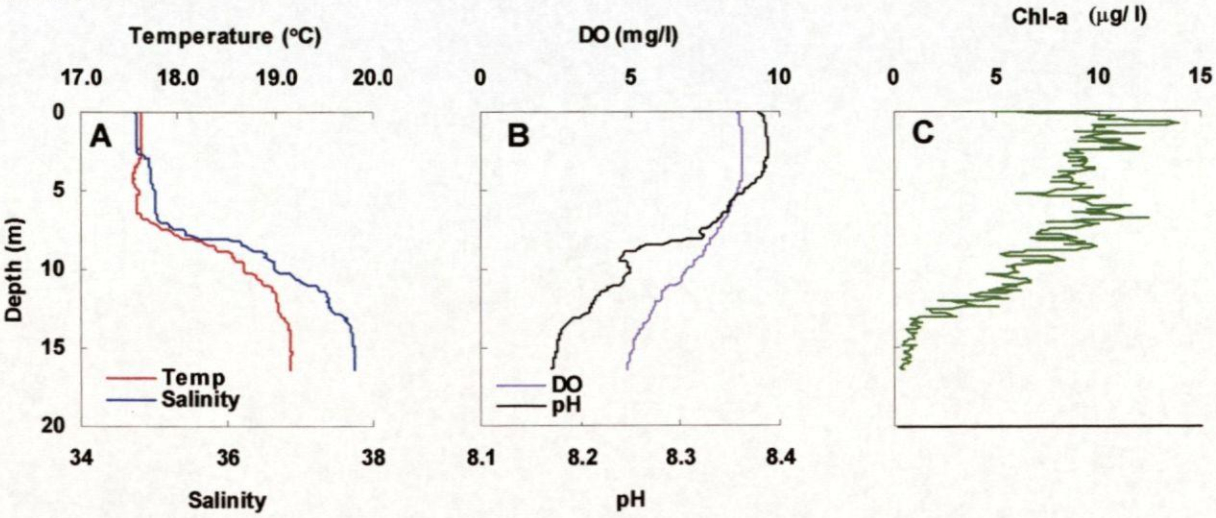
Station 1



Station 2



Station 3



Station 4

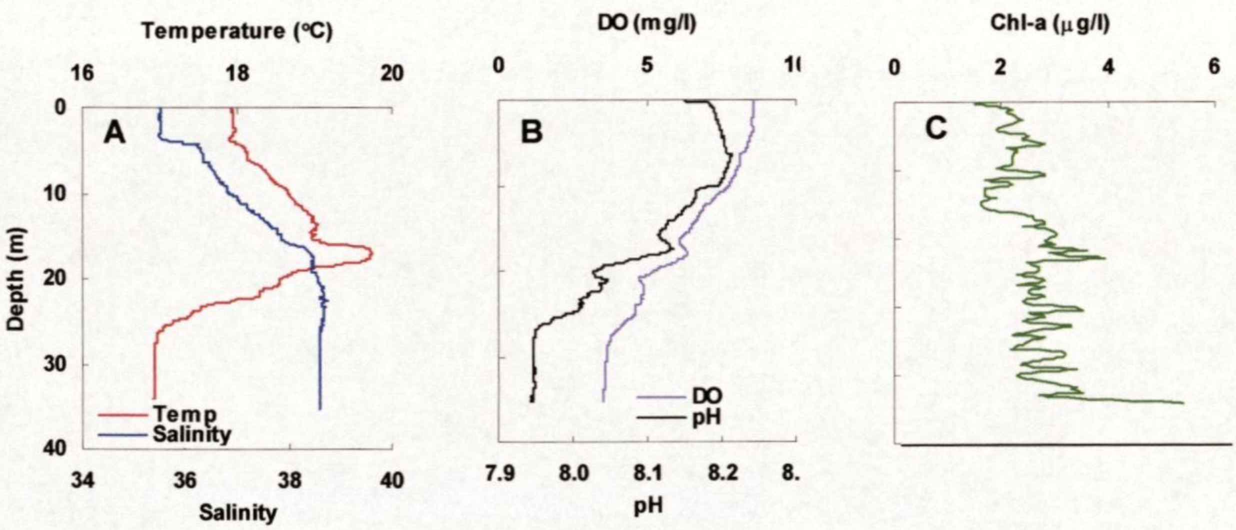


Figure 7.4 Temperature and Salinity (A), DO and pH (B) and Chlorophyll-a (C) concentrations measured as a function of depth at Stations 1, 2, 3 and 4.

The depth profiles presented in Figure 7.4 showed water column stratification. At all stations the halocline and thermocline separated the surface layer (influenced by fresh water inputs from the River Po) from the deeper, more saline (Adriatic) water and were observed at ~2 m depth at Station 1; ~1.5 m depth at Station 2; ~8 m depth at Station 3 and 4 to 20 m depth at Station 4. The dissolved oxygen (DO) was highest in the surface waters (as were Chlorophyll-a concentrations) at all stations where light penetration was maximum. The pH increased with depth, probably due to

the influence of the more alkaline Adriatic water. Deployment and sampling depths, representing major water masses were chosen with depths indicated in Table 7.1. *In situ* monitoring, however, was restricted to depths greater than 4 m as contamination from the hull of the vessel could influence results at shallower depths. Discrete samples were taken at 0.2 m depth, 50 m away from the vessel using a *zodiac* and the samples immediately analysed in the shipboard laboratory using the VIP system.

Table 7. 1 Date of sampling, station number, co-ordinates of station and depths of sampling for the IMTEC Cruise 2.

Date	Station #	Co-ordinates	Discrete sample depths	Deployment depths
28/10/02	1	44°46,20'N, 12°24,00'E	0.2 m	5.0 m
28/10/02	2	44°45,60'N, 12°18,00'E	0.2 m	5.0 m
29/10/02	3	44°40,40'N, 12°23,50'E	0.2 m	5.0 m
31/10/02	4	44°25,25'N, 12°44,80'E	0.2 m	5.0 and 18.5 m

7.2.4 Performance of the VIP System During the Cruise

The sensitivity of the VIP system was measured over the period of the cruise to ensure no loss of sensitivity. Two full calibrations were performed on two separate mercury films in a land based laboratory prior to deployment. The results are illustrated in Figure 7.5.

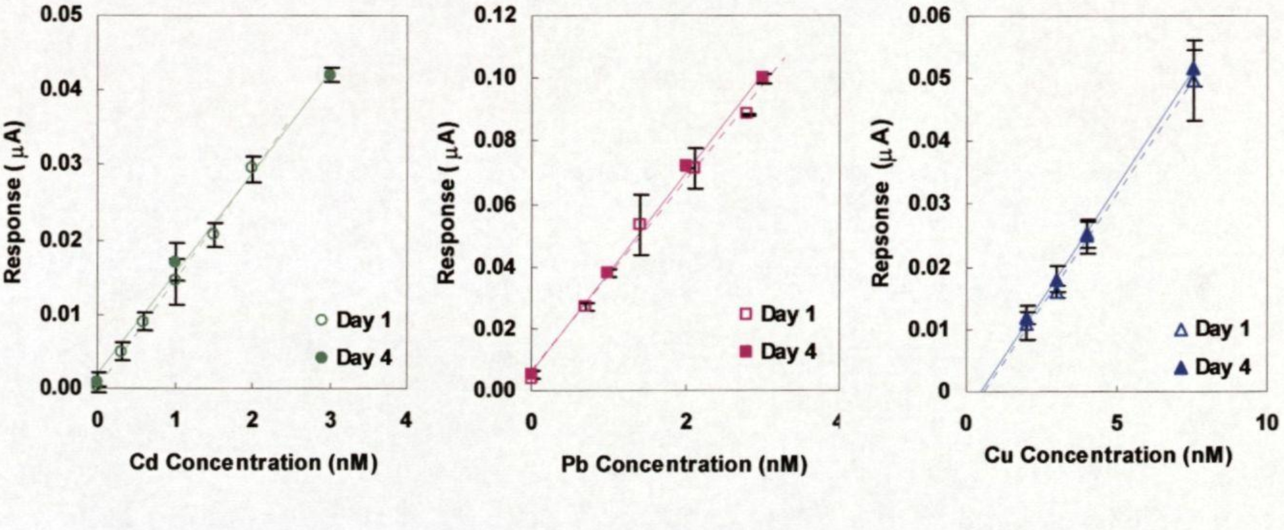


Figure 7.5 Full calibrations of Cd(II) (green circles) Pb(II) (pink squares) and Cu(II) (blue triangles) on day 1 (open) and day 4 (filled).

The results demonstrate unchanging sensitivity between each calibration, indicating the VIP was functioning well. Each day, after deployment, 2 standards that were used in the calibration were analysed for each metal in the shipboard laboratory. The mercury film was renewed on day 3 and 10 and the sensitivities of the system on each day are shown in Table 7.2. The reproducibility of sensitivity was acceptable [RSD of 2.9 % for Cd(II); 7.5 % for Pb(II) and 4.5 % for Cu(II)] over the duration of the cruise and indicated that all mercury was removed during the renewal of the mercury film, that the sensor was free from fouling by organic matter and that no oxidation of the mercury film had taken place over the duration of use.

Table 7. 2 Performance of the VIP system over the course of the IMTEC Cruise 2. Day of sensor's use, location of the calibration and achieved sensitivities of Cd(II) Pb(II) and Cu(II) are indicated.

		Sensitivity (nA min^{-1} nM^{-1})		
Day #	Location	Cd(II)	Pb(II)	Cu(II)
1	Land	0.72	1.30	0.24
2	Land	0.69	0.95	0.21
3	Land	N/A	N/A	N/A
4	Land	0.76	1.10	0.25
5	Land	0.75	1.12	0.23
6	Land	0.74	1.11	0.24
7	Ship	0.74	1.11	0.24
8	Ship	0.74	1.11	0.24
9	Ship	0.71	1.10	0.23
10	Ship	N/A	N/A	N/A
11	Ship	0.74	1.10	0.23
12	Ship	0.71	1.10	0.24

The VIP system was therefore working well for the duration of the cruise.

7.3 Results and Discussion

7.3.1 Biogeochemistry of Cd(II), Pb(II) and Cu(II) During the Cruise

Total filterable concentrations of Cd(II), Pb(II) and Cu(II) discrete samples collected during the cruise are yet be analysed by IMTEC partners. However the *in situ* VIP system data is discussed.

Cadmium

VIP dynamic Cd(II) concentrations measured during the IMTEC cruise 2, ranged from 0.13 to 0.25 nM. Figure 7.6 illustrates the Cd(II) concentrations in the water column at Stations 1, 3 and 4 on the cruise.

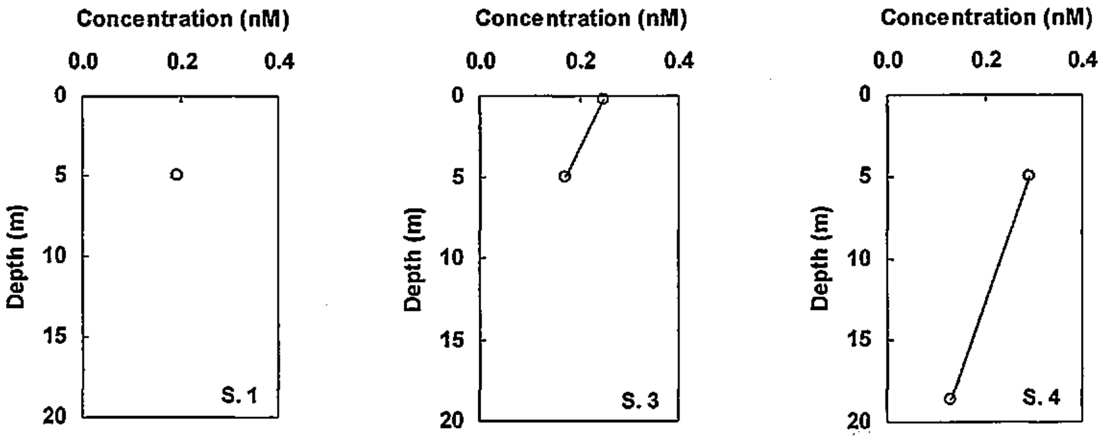


Figure 7.6 VIP dynamic concentrations of Cd(II) measured at Station 1 (S.1), Station 3 (S.3) and Station 4 (S.4) visited between 26/10/02 and 01/11/02.

The highest VIP dynamic Cd(II) concentration occurred at 5 m depth at Station 4. The lowest concentration was recorded at 20 m depth at station 4. Although the most important source of Cd(II) into the Northern Adriatic appeared to be the Po estuary with possible atmospheric inputs (*e.g.* as reported in the Mediterranean; Guerzoni *et al.,* 1999; Herut *et al.,* 2001), it is possible however that localised sources other than the Po are contributing inputs to the region (*e.g.* industrial waste point discharges). This is also demonstrated by the relatively poor salinity-Cd(II) relationship (Figure 7.9). Only a modest number of papers have been published in the literature on trace metal concentrations in the Po plume and Adriatic (*e.g.* Camusso *et al.,* 1997; Pettine *et al.,* 1997). Tankere and Statham, (1996) observed total filterable (0.4 μm pore; $TF_{0.4}$) Cd(II) concentrations in samples collected in this area of 0.05 to 0.15 nM, and VIP dynamic concentrations (varied from 0.13 to 0.25 nM). In estuarine work (Chapter 6) VIP dynamic Cd(II) concentrations were close to total filterable concentrations (*i.e.* low colloidal fraction) and therefore the concentrations reported in this study show good agreement with the literature concentrations.

Lead

VIP dynamic Pb(II), concentrations measured during the IMTEC cruise 2, ranged from 0.12 to 0.30 nM. Figure 7.7 illustrates the Pb(II) concentrations in the water column at the stations on the cruise.

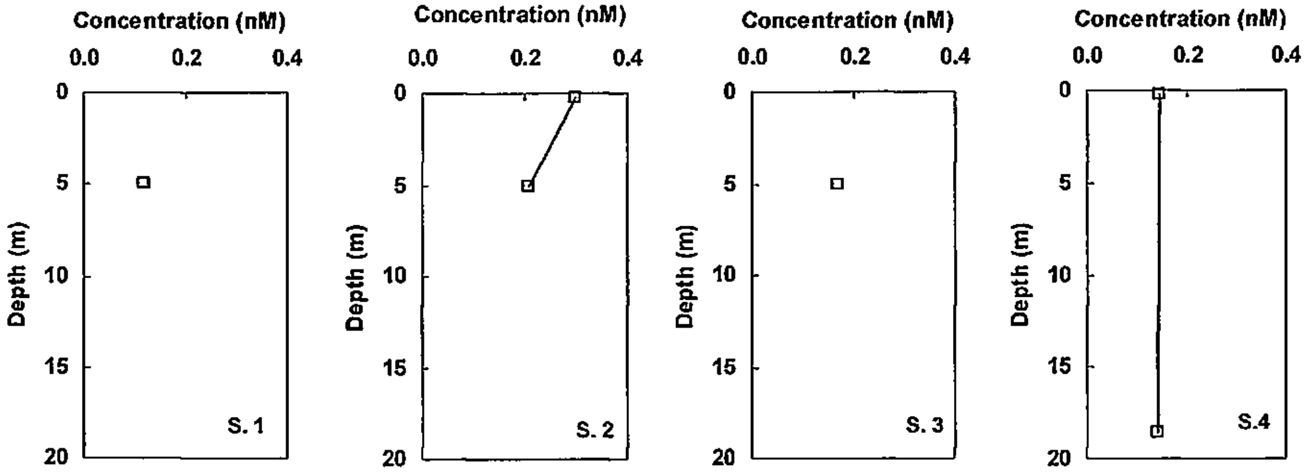


Figure 7.7 VIP dynamic concentrations of Pb(II) measured at Station 1 (S.1), Station 2 (S.2), Station 3 (S.3) and Station 4 (S.4) visited between 26/10/02 and 01/11/02.

The highest concentration was observed at 0.2 m depth at Station 2. This station was located near to the mouth of the Po Estuary, and the high concentration was probably due to the contribution from the river water input of the Po (indicated by salinity of surface waters of ~20; Figure 7.4) with enhanced trace metal concentrations. At this station, lower VIP dynamic metal concentrations were observed with increasing depth as a result of the cleaner Adriatic seawater (salinity ~38), indicating the importance of freshwater (rivers and run-off) as a source metals to the Adriatic. Station 3 showed a lower concentration than Station 2 probably because of the dilution of the plume by the cleaner Adriatic water. Samples at Station 4 were considerably lower in Pb(II) concentrations than those of Station 2, probably due to the lesser influence of the Po river waters on surface water at this Station, which was ~35 km from the mouth of the Po. This was reflected in the salinity that was higher in the surface waters of Station 4 (~35) compared to other stations *e.g.* Station 2 that had a salinity of ~ 20 in surface waters. Dassenakis *et al.*, 1997 observed total filterable Pb(II) concentrations in samples collected in the eastern Mediterranean ranged from 1.61 to 2.14 nM. VIP dynamic concentrations were lower (0.13 to 0.25 nM) than those reported $TF_{0.4}$ as Pb(II) has a large colloidal fraction as observed in our studies of estuaries (Chapter 6).

Copper

VIP dynamic Cu(II) concentrations measured during the IMTEC cruise 2, ranged from 1.2 to 4.7 nM. Figure 7.8 illustrates the Cu(II) concentrations in the water column at stations on the cruise.

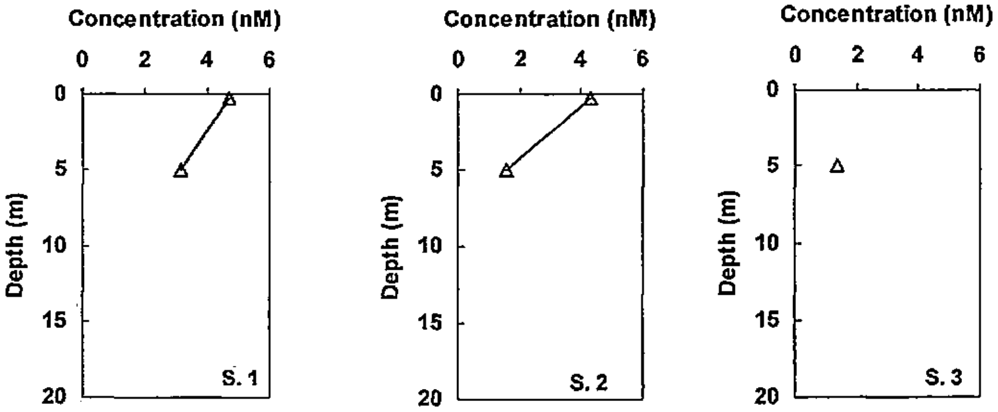


Figure 7.8 VIP dynamic concentrations of Cu(II) measured at Station 1 (S.1), Station 2 (S.2) and Station 3 (S.3) visited between 26/10/02 and 01/11/02.

The highest concentrations were observed at 0.2 m depth at Station 1 and 2. These stations were located near to the mouth of the Po and the high concentration was due to influence from the river Po (indicated by the lower salinities (20 to 25) on the CTD profiles; Figure 7.4). Concentrations decreased with depth as a result of the influence of the cleaner Adriatic seawater (indicated by the higher salinities on the CTD profiles). The lowest concentration was observed at 5 m depth at Station 3. Tankere and Statham, 1996 observed total filterable (0.4 μm pore size filter) concentrations in samples collected in this area ranged from 2 nM to 25 nM for Cu(II). These $TF_{0.4}$ concentrations are slightly higher than VIP dynamic fraction observed in our work (1.4 to 4.7 nM), due to the influence of organic complexation and colloidal association of Cu(II) as observed in our estuarine work (Chapter 6).

7.3.2 *Correlation of Salinity with Metal Concentrations*

Metal- salinity diagrams for each metal are presented in Figure 7.9.

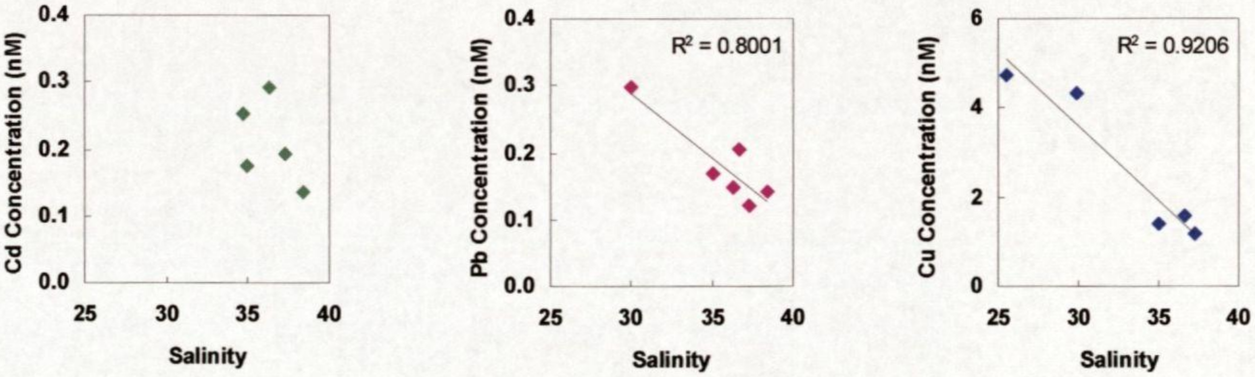


Figure 7.9 VIP dynamic trace metal- salinity diagrams for Cd(II) (**A**), Pb(II) (**B**) and Cu(II) (**C**)

The metal-salinity diagrams for Pb(II) and Cu(II) (Figure 7.9) illustrate a conservative mixing behaviour of the metal rich Po waters with the cleaner Adriatic waters for these elements. This was also reported previously for Cu (Tankere and Statham, 1996). The Cd(II)–salinity relationship is scattered and thus highlights the importance of Cd(II) sources other than the Po to the coastal waters around the Po estuary. This

poor correlation could also indicate the Cd(II) was actively involved in biogeochemical cycles such as particle–solution reactions, biological uptake, benthic inputs and the elements distribution was not simply controlled by mixing and dilution.

7.4 Conclusions

The VIP performed satisfactorily over the duration of the cruise, with no loss of sensitivity as a result of deployments. *In situ* measurements of VIP dynamic Cd(II), Pb(II) and Cu(II) in the Adriatic Sea indicate concentrations were typically of similar values *i.e.* (0.13 to 0.29 nM for Cd(II) and 1.4 to 4.3 nM for Cu(II) to those previously reported in the same coastal sea system by Tankere and Statham, 1996. *In situ* measurements of VIP dynamic Pb(II) in the Adriatic Sea were lower *i.e.* (0.12 to 0.30 nM) than those previously reported in a Mediterranean sea system by Dassenakis *et al.* (1997). Pb(II) and Cu(II) were negatively correlated with salinity, at the stations visited, whilst Cd(II) was not correlated, indicating the importance of localised riverine sourced and atmospheric inputs which can lead to higher concentration of metals close to the river mouth for this element. During this cruise elevated concentrations of trace metals were only found close to the River Po.

Reference list

Barghigiani, C., Ristori, T., Scerbo, R., Cini, C., Nottoli, R., Moschini, L. and Giaconi, V. (2001) *Assessment of Water Pollution and Suitability to Fish Life in Six Italian Rivers.* Environ. Monitor. Assess., **66**, 187-205

Baric, A. (2000) *Implications of Accelerated Sea-Level Rise* for Croatia: *Proceeding of SURVAS Expert Workshop on European Vulnerability and Adaptation to impacts of Accelerated Sea-Level Rise (ASLR),* Hamburg, Germany, June 2000

Camusso, M., Crescenzio, S., Martinotti, W., Pettine, M., and Pagnotta, R. (1997) *Behaviour Of Co, Fe, Mn And Ni In The Po Estuary (Italy).* Water Air Soil Poll., **99**, 297-304

Crema, R., Castelli, A. and Prevedelli, D. (1991) *Long-Term Eutrophication Effects on Macrofaunal Communities in Northern Adriatic Sea.* Mar. Pollut. Bull., **22**, 503-508

Dassenakis, M., Scoullos, M. and Gaitis, A. (1997) *Trace Metals Transport and Behaviour in the Mediterranean Estuary of Acheloos River.* Mar. Pollut. Bull., **34**, 103-111

Degobbis, D. and Gilmartin, M. (1990) *Nitrogen, Phosphorus, And Biogenic Silicon Budgets For The Northern Adriatic Sea*, Oceanol. Acta, **13**, 31-45

Degobbis, D., Gilmartin, M. and Revelante, N. (1986) *An Annotated Nitrogen Budget Calculation For The Northern Adriatic Sea.* Mar. Chem., **20**, 159-177

de Wit M and Bendoricchio G (2001) *Nutrient Fluxes in the Po Basin.* Sci. Tot. Environ., **273**, 147-161

Guerzoni, S., Chester, R., Dulac, F., Herut, B., Loye-Pilot, M. D., Measures, C., Migon, C., Molinaroli, E., Moulin, C., Rossini, P., Saydam, C., Soudine, A. and Ziveri, P. (1999) *The Role of Atmospheric Deposition in the Biogeochemistry of The Mediterranean Sea.* Prog. Oceanogr., **44**,147-190

Herut, B., Nimmo, M., Medway, A., Chester, R., and Krom, M. D. (2001) *Dry Atmospheric Inputs of Trace Metals at the Mediterranean Coast of Israel (SE Mediterranean): Sources and Fluxes.* Atmos. Environ., **35**, 803-813

Justic, D., Rabalais, N. N. and Turner R. E. (1994) *Riverbourne Nutrients, Hypoxia and Coastal Ecosystem Evolution: Biological Responses to Long-Term Changes in Nutrient Loads Carried by the Po and the Missisippi Rivers.* In: Changes in fluxes in Estuaries: Implications from Science to Management (K.R. Dyer and R. J. Orth eds.), Olsen and Olsen, Fredensberg., pp 161-167,

Kourafalou, V. H. (2001) River Plume *Development in Semi-Enclosed Mediterranean Regions: North Adriatic Sea and Northwestern Aegean Sea.* J. Mar. Sys., **30**, 181-205

Malacic, V., Viezzoli, D. and Cushman-Roisin, B. (2000) *Tidal Dynamics in the Northern Adriatic Sea.* J. Geophys. Res., **105**, 26265-26280

McIntyre, A. D. (1992) *The Current State Of The Oceans.* Mar. Pollut. Bull., **25**, 28-31

Pettine, M., Mastroianni, D., Camusso, M., Guzzi, L., and Martinotti, W. (1997) *Distribution of As, Cr and V species in the Po-Adriatic mixing area, (Italy).* Mar. Chem., **58**, 335-349

Revelante, N. and Gilmartin, M. (1992) *The Lateral Advection Of Particulate Organic-Matter From The Po-Delta Region During Summer Stratification, And Its Implications For The Northern Adriatic.* Estuar. Coast. Shelf Sci., **35**, 191-212

Renoldi, M., Camusso, M. and Tartari, G. (1997) *The Highly Polluted Lambro River (N Italy): Dissolved and Solid Transport Of Cu, Cr and Fe.* Wat. Air Soil Pollut., **95**, 99-118

Tankere, S. P. C. and Statham, P. J. (1996) *Distribution of Dissolved Cd, Cu, Ni and Zn in the Adriatic Sea.* Mar. Pollut. Bull., **32**, 623-630

CHAPTER 8

Conclusions and Future work

8.1 Introduction

Conclusions specific to each chapter are presented at the end of that chapter. This chapter presents the overall conclusions from the research and makes suggestions for future work.

8.2 General Conclusions

Water filtration typically forms part of discrete sampling procedures. Optimum protocols for filtration are metal specific, with the most effective filtration protocols for each metal as follows: For Al, Cd and Pb volumes ≤ 100 ml should be filtered on each filter; for Mn, the first 50 ml of filtrate should be discarded as this contains an under representation of subsequent filtrate and as Co, Cr, Cu, U and Zn remain relatively unperturbed by filtration artefacts, filters can be used until exhaustion. Filtration rate can be used as a qualitative parameter to judge the onset of membrane clogging and hence when the filter should be discarded and replaced. Given the differences of concentrations of trace metals found in the filtrate of samples filtered though 0.2 and 0.4 μm pore filters, it is concluded that these pore sizes cannot be used interchangeably.

New technologies have allowed the design of *in situ* probes for trace metal determination in natural waters that circumvent the need for filtration and the associated artefacts, such as membrane clogging. New materials and designs for flow cells and micro-electrode components have greatly improved the reliability, sensitivity and stability of *in situ* electrochemical probes. Automation and miniaturisation of the probes has facilitated their use in fieldwork programmes, providing high quality and high temporal resolution trace metal data. The voltammetric in situ profiling (VIP) system was used in this study. This system has allowed lower limits of detection (LODs) that enabled their application in natural waters improving the quality of data the interpretation of geochemical cycling and biological effects of elements in natural waters.

The VIP was optimised for use in estuarine and coastal waters through systematic laboratory based tests. Trace metal measurements using the VIP system were unaffected by changes in pH (in the range 2.0 to 7.6 and 2.0 to 4.8 in $NaNO_3$ and seawater respectively), salinity (in the range 0.02 to 0.50 M $NaNO_3$ and 2.5 to 35.0) or dissolved oxygen (in the range 1 to 100 % in both $NaNO_3$ and seawater) however a temperature correction is required for *in situ* dynamic trace metal data. The limits of detection (3σ) in seawater were low at 15 pM for Cd(II) and Pb(II) and 750 pM for Cu(II). Analytical procedures were shown to be accurate through good agreement between measured values and certified values for riverine and estuarine CRMs.

Analytical procedures for VIP system application during *in situ* monitoring and laboratory determinations were examined through the inter-comparison of VIP systems and reference methods for the measurement of trace metals in estuarine and fjord waters by three EU laboratories. This was needed in order to harmonise fieldwork and analytical procedures to lead to improved compatibility between European laboratory VIP system data. The results of the inter-comparison showed that the majority of laboratories gave significantly different (paired *t*-test) total filterable (0.2 μm pore size membrane filter) concentrations for laboratory measured estuarine (Tamar, UK) samples at the 95 % confidence interval. Systematic discrepancies between reporting laboratories were due to either: (1) incomplete digestion of samples during UV irradiation or (2) sensitivity changes from oxidation of the mercury film caused by pumping air through the system. There was considerably less systematic bias for *in situ* measurements compared to laboratory based determinations. This was attributed to (1) the variable nature of UV digestion equipment between laboratories that led to incomplete digestion of discrete samples and (2) the absence of contamination artefacts during deployment. Significant differences for *in situ* determinations were attributed to (1) incomplete measurement of Cu(II) during the stripping stage and (2) variations in water column structure at 30 m. The inter-comparison exercise highlighted several areas of VIP use that are unfavourable and further inter-comparison exercises sea and land-based laboratories are required, using more prescribed measures, to ensure fieldwork and analytical techniques are in agreement to lead to improved comparability between EU laboratory data.

The authors laboratory showed good results for CRM analysis during the inter-comparison exercise and was hence considered accurate. The VIP system was deployed in the field for automated, simultaneous determinations of dynamic Cd(II), Cu(II) and Pb(II) in three estuaries (Tamar, Plym, and Restonguet Creek) located in the southwest of England, UK. The application of the VIP during the surveys resulted in high-resolution (at least 2 measurements h^{-1}), unattended, multi-elemental trace metal measurements for periods up to 12 h over 7 tidal surveys. The deployment of this submersible *in situ* probe provided metal high temporal resolution speciation data, enabling an interpretation of geochemical processes in the studied estuaries. The overall behaviour of both total filterable and VIP dynamic trace metal concentrations during tidal cycles showed an inverse relationship with salinity and turbidity. The concentrations of VIP dynamic trace metals in the Tamar and Plym estuaries were found to be similar (in the range 0.21 to 0.77 nM for Cd(II), 0.14 to 0.47 nM for Pb(II) and 3.28 to 8.50 nM for Cu(II)) owing to the geology of the catchment and the comparable anthropogenic inputs. The high Cd (II) and Cu(II) concentrations (typically 5 fold higher than in the Tamar/ Plym Estuaries) in the Restonguet Creek were probably a result of the high metal inputs into the estuary as a result of the long historical acid mine drainage.

The VIP system was exposed to a range of metal concentrations and performed well in both contaminated and more pristine environments during a cruise in the Po Estuary and Adriatic Sea allowing interpretation of geochemical cycling of Cd (II), Pb(II) and Cu(II). *In situ* measurements of VIP dynamic Cd(II), Pb(II) and Cu(II) varied from 0.13 to 0.29 nM for Cd(II); 1.4 to 4.3 nM for Cu(II) and 0.12 to 0.30 nM for Pb(II). Concentrations were typically higher for Cu(II) and Pb(II) in the surface waters and waters near to the Po and these elements were negatively correlated with salinity. Cd(II) was not correlated with salinity, indicating the importance of localised riverine sourced inputs leading to higher concentration of metals close to the river mouth for this element. During this cruise elevated concentrations of trace metals were only found close to the river Po.

8.3 Suggestions for Future Work

Further work arsing from the research reported in this thesis includes:

8.3.1 Filtration Experiment

- Investigate more seasonal and spatial effects by sampling with higher temporal and spatial resolution.
- Investigate a wider range of samples taken from estuaries of different composition *e.g.* organic rich estuaries to determine effects on filtration.

- Apply a tangential flow filtration device to investigate whether the tangential flow lessens filtration artefacts.

8.3.2 *Optimisation of the VIP System*

- Incorporate a switching valve in the VIP system that can be switched between environmental samples and standards that allows *in situ* determination of a trace metal standard. This would ensure quality control during deployments.
- Optimise the use of readily available, cheap electrolyte such as 'Instant Ocean' as an alternative to $NaNO_3$ (that is expensive) and seawater (that requires rigorous cleaning).

8.3.3 *Inter-comparison of VIP systems*

- Repeat and improve inter-comparisons exercise by ensuring all participating laboratories analyse all samples (both discrete and *in situ*) and also analyse riverine, estuarine and coastal certified reference materials, using the same prescribed protocols for handling and period of storage.

8.3.4 *Estuarine Biogeochemistry of Trace Metals*

- Increase deployment frequency and also the duration of the deployments, in order to acquire long term high resolution data, *e.g.* monitor bimonthly variations of Cd(II), Pb(II) and Cu(II) over 12 hour periods for 1 year. Monitoring in different seasons and over the duration of several days would allow greater understanding of the estuarine processes controlling the trace metal distributions.
- Complete transects along the length of the estuaries and to monitor at several points for extended periods along the transect. This would allow the

determination of locations of point sources within the estuary, in addition to improving the information about trace metals in such a dynamic environment.

- Depth profiling to determine effects of variables in the water column on VIP dynamic concentrations (*e.g.* atmospheric and sediment inputs) on the water column.

APPENDIX A

In Situ Data for Inter-comparison Exercise

In situ concentrations of trace metals in the Gullmar Fjord at *Station 1*. Date, laboratory, sample depth (m) and concentrations are indicated.

Day	Depth	Sampler	VIP Cu
26/08/02	5	LAB 2	1.40
26/08/02	5	LAB 2	1.79
26/08/02	5	LAB 2	2.17
26/08/02	5	LAB 2	2.53
26/08/02	5	LAB 2	2.25
26/08/02	5	LAB 2	2.52
26/08/02	5	LAB 3	2.05
26/08/02	5	LAB 3	3.42
26/08/02	5	LAB 3	3.15
26/08/02	5	LAB 3	3.18
26/08/02	5	LAB 3	3.32
22/08/02	5	LAB 5	4.30
22/08/02	5	LAB 5	4.91
22/08/02	5	LAB 5	3.86
22/08/02	5	LAB 5	3.16
22/08/02	5	LAB 5	2.32
22/08/02	5	LAB 6	3.60
22/08/02	5	LAB 6	3.56
22/08/02	5	LAB 6	3.32
22/08/02	5	LAB 6	3.20
22/08/02	5	LAB 7	3.64
22/08/02	5	LAB 7	3.45
22/08/02	5	LAB 7	2.60
22/08/02	5	LAB 7	2.55

Day	5	Sampler	VIP Cd
22/08/02	5	LAB 2	0.28
22/08/02	5	LAB 2	0.21
22/08/02	5	LAB 4	0.22
22/08/02	5	LAB 4	0.55
22/08/02	5	LAB 5	0.08
22/08/02	5	LAB 5	0.06
22/08/02	5	LAB 5	0.10
22/08/02	5	LAB 5	0.10
22/08/02	5	LAB 6	0.11
22/08/02	5	LAB 7	0.11
22/08/02	5	LAB 7	0.09

Day	5	Sampler	VIP Pb
26/08/02	5	LAB 2	0.17
26/08/02	5	LAB 2	0.14
26/08/02	5	LAB 2	0.13
26/08/02	5	LAB 2	0.18
26/08/02	5	LAB 2	0.18
26/08/02	5	LAB 2	0.15
22/08/02	5	LAB 5	0.21
22/08/02	5	LAB 5	0.23
22/08/02	5	LAB 5	0.19
22/08/02	5	LAB 5	0.20
22/08/02	5	LAB 5	0.21
22/08/02	5	LAB 6	0.24
22/08/02	5	LAB 6	0.23
22/08/02	5	LAB 6	0.23
22/08/02	5	LAB 7	0.22
22/08/02	5	LAB 7	0.20
22/08/02	5	LAB 7	0.20

Day	Depth	Sampler	VIP Cu
22/08/02	30	LAB 3	0.74
22/08/02	30	LAB 3	0.91
26/08/02	30	LAB 1	0.26
26/08/02	30	LAB 1	0.49
26/08/02	30	LAB 1	0.70
26/08/02	30	LAB 1	0.73
26/08/02	30	LAB 1	0.78
26/08/02	30	LAB 1	0.82
26/08/02	30	LAB 1	0.78

Day	Depth	Sampler	VIP Cd
22/08/02	30	LAB 1	0.195
22/08/02	30	LAB 1	0.30
26/08/02	30	LAB 1	0.36
26/08/02	30	LAB 1	0.34
26/08/02	30	LAB 1	0.40
26/08/02	30	LAB 1	0.38
26/08/02	30	LAB 1	0.44
26/08/02	30	LAB 1	0.46
26/08/02	30	LAB 1	0.43
22/08/02	30	LAB 4	0.15
26/08/02	30	LAB 4	0.24
26/08/02	30	LAB 4	0.26
26/08/02	30	LAB 4	0.38
22/08/02	30	LAB 4	0.39
26/08/02	30	LAB 4	0.35
26/08/02	30	LAB 4	0.32

Day	Depth	Sampler	VIP Cu
22/08/02	45	LAB 1	3.94
22/08/02	45	LAB 3	1.21
22/08/02	45	LAB 3	1.33

Day	Depth	Sampler	VIP Cd
22/08/02	45	LAB 1	0.232
22/08/02	45	LAB 3	0.13
22/08/02	45	LAB 3	0.12

Day	Depth	Sampler	VIP Pb
22/08/02	45	LAB 3	0.26
22/08/02	45	LAB 3	0.28

In situ concentrations of trace metals in the Gullmar Fjord at *Station 2*. Date, laboratory, sample depth (m) and concentrations are indicated.

Day	Depth	Sampler	VIP Cu(II) (nM)
23/08/02	5	LAB 1	4.14
23/08/02	5	LAB 1	5.03
23/08/02	5	LAB 1	5.46
23/08/02	5	LAB 1	5.91
23/08/02	5	LAB 1	6.03

Day		Sampler	VIP Cd(II) (nM)
23/08/02	5	LAB 1	0.11
23/08/02	5	LAB 1	0.18
23/08/02	5	LAB 1	0.28
23/08/02	5	LAB 1	0.09
23/08/02	5	LAB 4	0.24
23/08/02	5	LAB 4	0.25
23/08/02	5	LAB 4	0.25
23/08/02	5	LAB 4	0.19

Day	Depth	Sampler	VIP Cu(II) (nM)
23/08/02	35	LAB 2	0.72
23/08/02	35	LAB 2	0.88
23/08/02	35	LAB 2	1.27
23/08/02	35	LAB 2	1.68
23/08/02	35	LAB 2	1.34
23/08/02	35	LAB 6	2.18
23/08/02	35	LAB 6	2.06
23/08/02	35	LAB 6	1.82

Day	Depth	Sampler	VIP Cd(II) (nM)
23/08/02	35	LAB 2	0.09
23/08/02	35	LAB 3	0.09
23/08/02	35	LAB 3	0.09
23/08/02	35	LAB 3	0.08
23/08/02	35	LAB 3	0.07
23/08/02	35	LAB 3	0.09

Day	Depth	Sampler	VIP Pb(II) (nM)
23/08/02	35	LAB 2	0.29
23/08/02	35	LAB 2	0.13
23/08/02	35	LAB 2	0.13
23/08/02	35	LAB 2	0.18
23/08/02	35	LAB 2	0.15
23/08/2002	35	LAB 5	0.15
23/08/2002	35	LAB 5	0.19
23/08/2002	35	LAB 5	0.14
23/08/2002	35	LAB 5	0.13
23/08/2002	35	LAB 6	0.20
23/08/2002	35	LAB 6	0.19
23/08/2002	35	LAB 6	0.20

In situ concentrations of trace metals in the Gullmar Fjord at *Station 1*. Date, laboratory, sample depth (m) and concentrations are indicated.

Day	Depth	Sampler	VIP Cu
26/08/02	5	LAB 2	1.40
26/08/02	5	LAB 2	1.79
26/08/02	5	LAB 2	2.17
26/08/02	5	LAB 2	2.53
26/08/02	5	LAB 2	2.25
26/08/02	5	LAB 2	2.52
26/08/02	5	LAB 3	2.05
26/08/02	5	LAB 3	3.42
26/08/02	5	LAB 3	3.15
26/08/02	5	LAB 3	3.18
26/08/02	5	LAB 3	3.32
22/08/02	5	LAB 5	4.30
22/08/02	5	LAB 5	4.91
22/08/02	5	LAB 5	3.86
22/08/02	5	LAB 5	3.16
22/08/02	5	LAB 5	2.32
22/08/02	5	LAB 6	3.60
22/08/02	5	LAB 6	3.56
22/08/02	5	LAB 6	3.32
22/08/02	5	LAB 6	3.20
22/08/02	5	LAB 7	3.64
22/08/02	5	LAB 7	3.45
22/08/02	5	LAB 7	2.60
22/08/02	5	LAB 7	2.55

Day	5	Sampler	VIP Cd
22/08/02	5	LAB 2	0.28
22/08/02	5	LAB 2	0.21
22/08/02	5	LAB 4	0.22
22/08/02	5	LAB 4	0.55
22/08/02	5	LAB 5	0.08
22/08/02	5	LAB 5	0.06
22/08/02	5	LAB 5	0.10
22/08/02	5	LAB 5	0.10
22/08/02	5	LAB 6	0.11
22/08/02	5	LAB 7	0.11
22/08/02	5	LAB 7	0.09

APPENDIX B

Publications

Chapter 5

Field Application of an Automated Voltammetric System for High-Resolution Studies of Trace Metal Distributions in Dynamic Estuarine and Coastal Waters

Eric P. Achterberg, Charlotte B. Braungardt, and Kate A. Howell

Department of Environmental Sciences, Plymouth Environmental Research Centre,
University of Plymouth, Plymouth PL4 8AA, United Kingdom

The increasing environmental pressures on estuarine and coastal waters call for improved monitoring techniques of chemical constituents to aid management decisions. Automated stripping voltammetry is a suitable technique for continuous, near real-time monitoring of trace metals in marine systems. This contribution describes the application of voltammetric monitoring techniques in estuarine and coastal waters of the UK and Spain. The high spatial and temporal resolution of the data obtained, allows a thorough interpretation of the trace metal sources and behaviour. Future trends in this field research include submersible sensors which can be remotely deployed for a period of several weeks.

Introduction

Estuaries and coastal waters are dynamic environments with often important temporal and spatial variability in physical, chemical and biological parameters. Many coastal systems are strongly influenced by anthropogenic activities, with 40 percent of the world's population living within 100 km of a coastline. Population increases, changes in agricultural practices and spread of aquaculture, in addition to further industrialisation and natural resources exploitation are enhancing the environmental pressures on coastal environments. Coastal systems are of ecological, economical and recreational importance. They are biologically productive, and form nursery grounds for a high proportion of commercial fish and shellfish species. The need for sustainable management of coastal zones, has led the European Union to implement integrated coastal zone management practices (*1*) and draft a comprehensive and far reaching Water Framework Directive. In the United States, the Estuaries and Clean Water Act of 2000 (S. 835) and the Oceans Act of 2000 (S. 2327) (http://www.house.gov) have improved legislation concerning the marine environment. In order to achieve the objectives of coastal management plans, it is essential that naturally occurring biogeochemical processes are understood, particularly as processes in coastal waters often have complex cause-effect relationships. For this reason, our ability to monitor coastal waters forms an important management tool.

The traditional monitoring approach for marine waters involves the collection of discrete samples using a survey vessel. Discrete surface water samples can be obtained using a pump with a bottom-weighted hose. At greater depths, sampling is carried out with the use of specialised samplers (e.g. Niskin or Go-Flo bottles) which are attached to a hydrowire or rosette frame and deployed with the use of a winch. The samples are often filtered on-board ship and analysed in a land-based laboratory. Commonly used laboratory techniques for dissolved trace metal analysis in seawater include Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (after liquid-liquid or solid-phase extraction for trace metal preconcentration and matrix removal), chronopotentiometry, colorimetry, chemiluminecence and stripping voltammetry. This approach of laboratory based analyses of discrete samples is time-consuming and therefore expensive. Only a limited number of samples can be collected using discrete sampling techniques and as a result important changes in water quality may not be noticed.

Trace metal concentrations in estuarine and coastal waters are often low. Inadequate sampling and sample treatment techniques have for a long time posed a high risk of contamination during collection and analysis of seawater samples. Sample contamination may arise from components of the sampling gear and from sample handling. Advances in equipment design and sampling protocols have improved contamination-free sample collection (*2*). Furthermore, improved understanding of post-sampling contamination has resulted in the introduction of ultra-trace working practices (*3*). These include the cleaning of sample bottles, filters and filtration equipment with acid prior to use, and the handling of samples in a clean environment (class-100 laminar flow hood in a clean room). These precautions against sample contamination are essential in order to obtain high quality trace metal data, but also importantly reduce the number of samples per day that can be processed by a worker.

In the marine field, the use of continuous near-real-time and in-situ automated monitoring techniques is becoming more widespread (*4,5*). A major advantage of this approach is the reduced contamination risk due to a minimisation of sample handling. In addition, the computer controlled nature of the automated analytical techniques enhances the quality of the data. Furthermore, the approach results in a higher sample throughput and hence an increased amount of environmental data. The application of high resolution monitoring approaches in estuarine and coastal waters is of particular importance due to the dynamic nature of these systems, and will therefore allow small-scale processes to be investigated (*6*).

Electrochemical techniques for marine waters

Electrochemical techniques are commonly used for water quality assessments. The measurements of pH (hydrogen ion selective glass electrode; potentiometry) and oxygen (Clark electrode; amperometry) are often part of monitoring exercises. A sensitive and versatile technique for the determination of trace metals in seawater is stripping voltammetry. Voltammetric techniques are based upon the measurement of a current response as a function of the potential applied to an electrochemical cell. Stripping voltammetry combines a preconcentration step with a stripping step (*7,8*), thereby enhancing the sensitivity and selectivity. With the low concentrations of trace metals in seawater (typically $< 10^{-8}$ M), the preconcentration step is important to reduce the limit of detection of the methods. During the preconcentration step, the trace metal of interest is collected onto or in a working electrode and during the stripping step the collected metal is oxidised or reduced back into solution. Anodic stripping voltammetry (ASV) and adsorptive cathodic stripping voltammetry (AdCSV) are the most suitable stripping voltammetric techniques for the determination of trace metals in seawater. ASV typically involves the addition of a small amount of acid (nitric acid or hydrochloric acid) or pH buffer (e.g. acetate) to the sample prior to analysis, whereas AdCSV requires the addition of an AdCSV ligand in addition to a pH buffer. The AdCSV ligand complexes the trace metal of interest and for most AdCSV methods, this metal-ligand complex has electroactive properties (*8*). In ASV metals are reduced during the preconcentration step, and their oxidation current is determined during the potential scan towards more positive potentials. In the majority of the AdCSV methods, the metal-ligand complex is adsorbed onto the working electrode during the preconcentration step, and the reduction current of the metal is determined during the potential scan towards more negative potentials. Important advantages of the stripping voltammetric techniques include the extremely low detection limits (10^{-10} – 10^{-12} M), their multi-elemental and speciation capabilities, and their suitability for on-line, ship-board and in-situ applications (*7,8,9*). ASV has been successfully applied for trace measurements of Cu, Cd, Pb and Zn in seawater (*10*). ASV is suitable for the determination of other elements (e.g. In, Tl), however their typical seawater concentrations are too low, or the analysis is subject to interferences. AdCSV allows for the determination of more than 20 metals in natural waters (*11,8,12*).

The instrumentation used for stripping voltammetry is relatively low-cost, and typically consists of a voltammetric analyser, a three-electrode-cell (working, reference and counter electrodes), and a computer for automated measurements and data acquisition. For automated on-line measurements, inert valves and peristaltic pumps for sample and reagent transport are required. Hanging mercury drop electrodes (HMDE) and mercury film electrodes (MFE) are the most commonly used working electrodes for trace metal measurements in seawater using stripping voltammetry. The HMDE is a reliable working electrode, both in the laboratory and field. With the HMDE, a new electrode surface is produced with the formation of each new drop, which eliminates analyte memory effects between scans, and also is important for unattended trace metal monitoring activities. The HMDE is the preferred working electrode for trace metal measurements in seawater using AdCSV, but can also be used for ASV. The MFE is the preferred working electrode for dissolved metal analysis using ASV. The MFE may be formed by in-situ plating of Hg on glassy carbon (*13*), or by preliminary deposition. The advantages of the MFE are the robustness and the high sensitivity due to the high surface to volume ratio (*13*). Glassy carbon is the most commonly used material for MFE, but the use of gold (*14*), iridium (*15,16*), graphite pencil (*17*) and carbon fibre (*18*) have also been reported.

The manifold of an automated monitoring system using a HMDE (Metrohm, VA Stand 663) and a voltammetric analyser (μAutolab, Ecochemie) for analysis of trace metals in field situations is presented in Figure 1. Such systems have been deployed on-board ships and from the banks of estuaries (*19,6,20,21*). The analysis is performed in an automated batch-mode, whereby aliquots of ca. 10 ml are analysed at a rate of one complete fully calibrated measurement every ca. 10-20 min (*22*). The collection of water is conducted using a strong polyethylene (PE) or polyvinyl chloride (PVC) hose and a peristaltic pump (Watson Marlow).

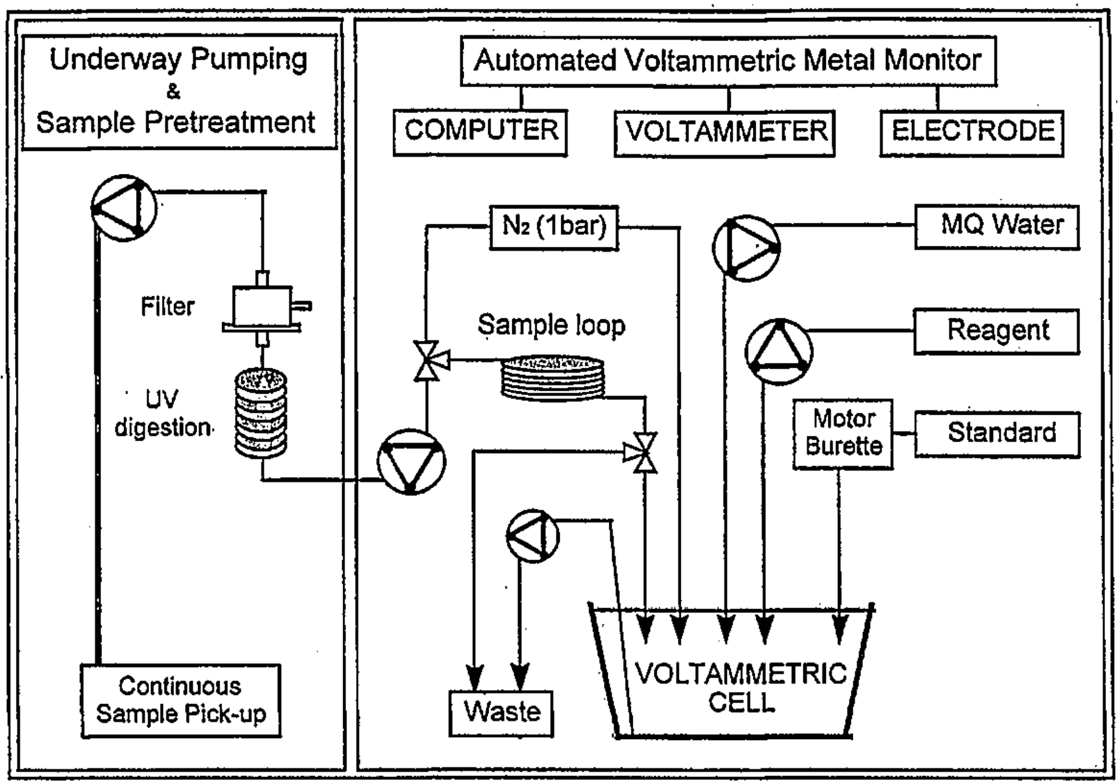


Figure 1. Manifold of an automated voltammetric system for ship-board analysis of trace metals (Reproduced with permission from reference 8. Copyright 1999 Elsevier).

Water is typically pumped at 1-2 l min^{-1}, which results in a minimum residence time of the sample in the hose and a constant conditioning of the hose surface with fresh sample. For the analysis of the dissolved metal fraction, suspended particulate matter is removed from the water using on-line filtration. In turbid estuarine and coastal waters, a tangential filtration device (with membrane filters, 0.4 or 0.2 µm) is most appropriate because of its self-cleansing ability (*6*). However, in clearer marine waters the use of filtration capsules (e.g. Sartobran P, Sartorius; 0.45 µm prefilter and 0.2 µm final filter) is practical, as they can be used for large volumes of water (and hence long periods of time (several days)). On-line UV-digestion is applied in order to destroy surfactants which may interfere with the voltammetric measurement, and to break down metal complexing organic compounds and hence allow the determination of total dissolved trace metals in seawater (*23*). The efficiency of UV-digestion in releasing metals (incl. Ni and Cu) from organic complexes is >99% (*23*). The UV-digestion unit contains a medium pressure mercury vapour lamp (400 W, Photochemical Reactors) surrounded by a quartz glass coil (i.d. 1.0 mm, length ca. 3.5 m) (*24*). The filtered and UV-treated sample is pumped into a sample loop (9.90 ml, Teflon®) using a peristaltic pump, which form part of an automated sample and reagent transport system. This system also rinses the voltammetric cell between samples and delivers reagents to the cell. The sample loop is enclosed by two inert three-way valves (Teflon®, Cole Parmer). The sample loop is emptied into the voltammetric cell using nitrogen gas. Teflon® tubing is used throughout the monitor, with the exception of the peristaltic pump tubing (Santoprene®). A high precision syringe pump (Cavro) is used for metal standard addition to the sample for quantification purposes. Figure 2 shows a flow diagram of a software-controlled analytical cycle during automated analysis. A typical trace metal analysis involves the addition of reagent to the sample by pump, a purging step using oxygen-free nitrogen gas (4 min), a sequence of 3 deposition periods, each followed by voltammetric scans (typically square wave), an internal standard addition with a subsequent sequence of 3 deposition periods followed by voltammetric scans. Dedicated software is used for data acquisition, peak evaluation and data storage. The software is self-decisive, and is able to reject sub-quality scans with a standard deviation above a pre-set value (typically 6%) and will initiate additional measurements. The software is also able to initiate additional standard additions, in case the increase in peak height as a consequence of the first standard addition was insufficient (i.e. less than 100%). The calibration of each sample using standard additions results in high quality data. This approach is important in estuarine and coastal waters, as it takes into account the changes in sample matrix (e.g. as a result of salinity variations), which may result in changes in the sensitivity of the measurement.

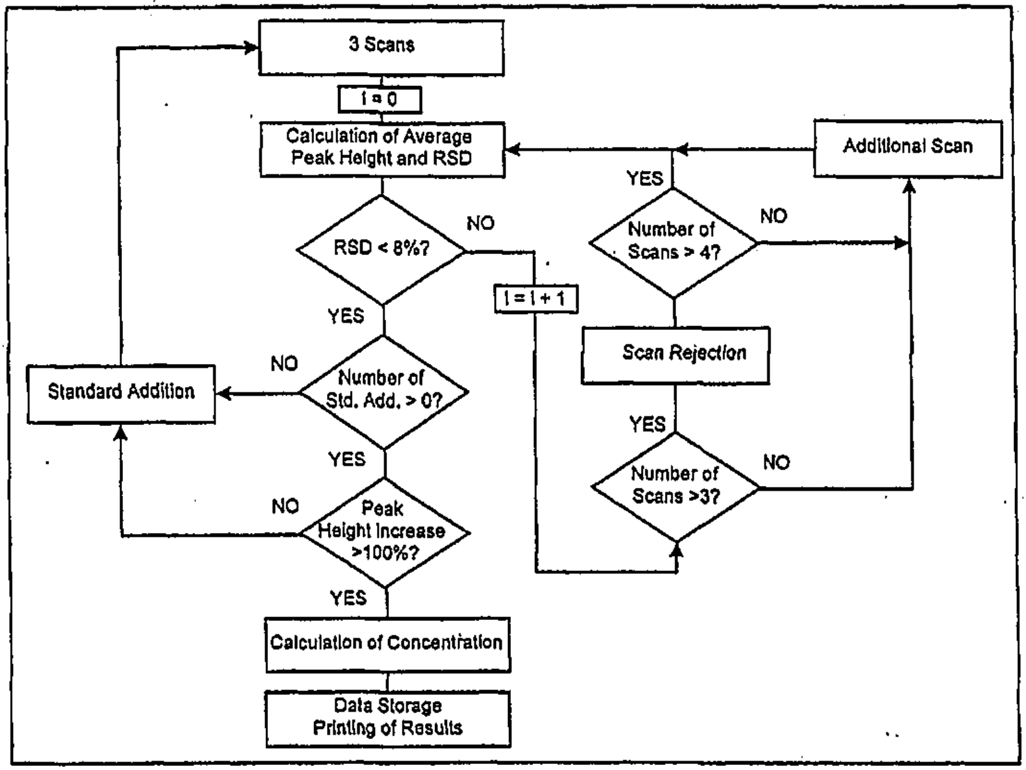


Figure 2. Flow diagram of soft-ware controlled analytical cycle during automated trace metal analysis with voltammetric monitor. The cycle starts with 3 voltammetric scans and ends with data storage and printing of results.

Trace metal monitoring in estuarine environments

Estuaries are highly dynamic and reactive zones, where fluvial discharges mix with seawater and dissolved elements interact with organic material and particles in the water column. Important factors influencing trace metal behaviour in estuarine waters include freshwater inputs, pH, redox conditions, tidal mixing and resuspension of sediments, colloid formation and coagulation, sorption, biological cycling and organic complexation (*25,26*). The dynamic nature of estuarine waters requires trace metal monitoring activities at a resolution similar to the processes affecting trace metal distributions (*20*).

Study at single geographic point

The instrumental set-up employed for the automated determination of dissolved trace metals at a geographical point in an estuary is shown in Figure 3. The instrumentation is operated from a van, and powered by a portable generator. A braided PVC hose is used to collect the water samples from the estuarine channel. The hose is submerged to a depth of ca. 0.5 m using a float and an anchor.

An important feature of a tidal cycle study at a single geographical point in an estuary is that the trace metal concentrations are affected only by water column processes (end-member mixing, adsorption, desorption, precipitation, coagulation etc.) and hence this approach allows a thorough interpretation of biogeochemical processes. In contrast, in an axial transect study, metal concentrations along a transect are affected by processes occurring in the water column in addition to metal inputs (run-off and discharges), and consequently the data is often more difficult to interpret.

Trace metal behaviour in the Tamar estuary

Figure 4 shows the results of automated measurements of dissolved Ni in the Tamar estuary (southwest England) during a tidal cycle (July 1997). The Tamar is situated in a mineral rich region, and has a number of abandoned metal mines in its catchment area. The Tamar receives important trace metal loads (Cu, Zn, Pb, As, U, Sn, Ni), as a consequence of surface run-off (*27,28*). The Ni analyses were performed using AdCSV, with dimethyl glyoxime as the added AdCSV ligand and HEPES as the pH buffer (final concentrations 0.2 mM and 10 mM (pH 7.8), respectively) (*21*). In order to circumvent problems with slow kinetics of Ni-DMG formation following addition of standard for sample calibration, DMG (0.2 mM) (instead of acid) was added to the Ni standard to ensure rapid

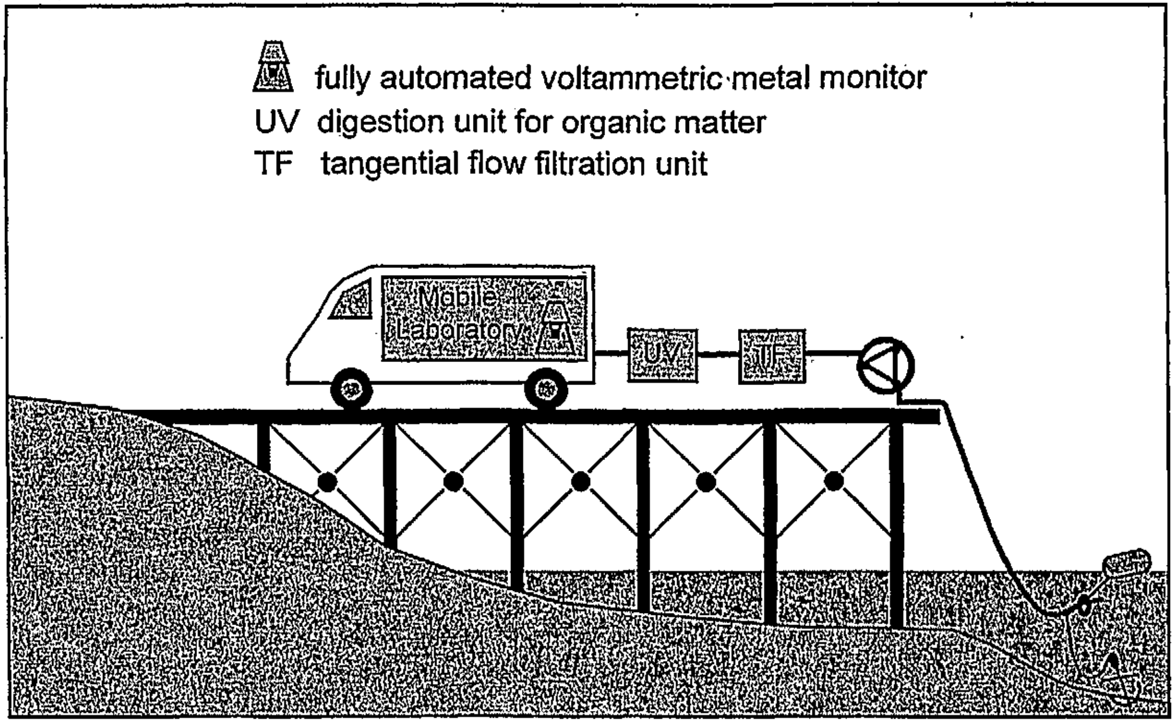


Figure 3. Set-up for estuarine trace metal monitoring with continuous sample collection and automated AdCSV trace metal analysis (Reproduced with permission from reference 8. Copyright 1999 Elsevier).

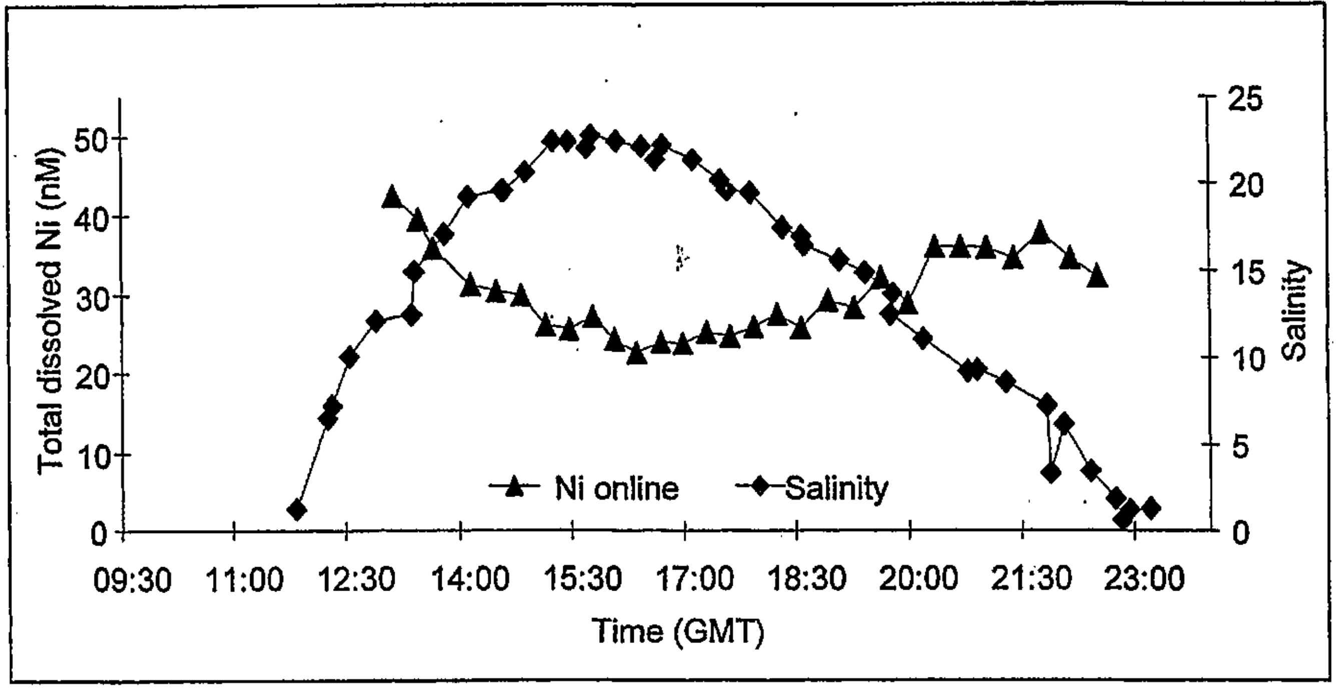


Figure 4. On-line continuous dissolved total Ni measurements in the Tamar estuary during a tidal cycle (July 1997). Discrete salinity data were obtained from conductivity measurements (Reproduced with permission from reference 21. Copyright 1998 Elsevier).

establishment of Ni equilibrium (< 30 s) and the provide stability of the metal standard. The limit of detection and working range for the AdCSV Ni method are presented in Table I, whereas the results of method validation using a certified reference material are presented in Table II.

Table I: Limits of detection and linear ranges of Ni, Cu and Zn analysis using square wave AdCSV for field measurements in the Tamar (Ni), surface waters of the Gulf of Cadiz (Cu, Zn; nM range) and in the mid-Odiel estuary (Cu, Zn; μM range). Values in brackets refer to the simultaneously analysed metal (i.e. Zn).

	Ni	Cu (Zn) nM range	Cu (Zn) μM range
Limit of detection (LOD)	0.1 nM	0.48 (0.81) nM	-
Linear range	100 nM	150 (300) nM	4 (7) μM
R^2 for linear range	0.99	0.99	0.99

Table II: Analysis of UV-irradiated seawater reference material (CASS-3) by AdCSV in batches of 10 ml. Confidence intervals refer to ± 2 SD of the sample mean.

CASS-3	n	ACSV result (nM)	certified (nM)
Ni	6	6.48 ± 0.40	6.58 ± 1.06
Cu	4	8.17 ± 1.05	8.14 ± 0.98
Zn	8	17.7 ± 2.58	19.0 ± 3.82

The advantages of the continuous, near real-time measurements are evident from Figure 4, with ca. 4 fully calibrated dissolved Ni analyses per hour. The dissolved Ni concentrations ranged between 21 and 43 nM. The tidal cycle was undertaken during a period with low river flow (ca. 6 m^3 s^{-1}; long-term mean flow 34 m^3 s^{-1} (*29*)), a tidal range of 3 m (mean tidal range: 3.5 m) and consequently a large variation in salinity was observed (24 salinity units) at our sampling point. The highest Ni concentrations were observed in waters with a low salinity (e.g. [Ni] = 38 nM at salinity 7), and low Ni concentrations were observed during periods with enhanced salinity (e.g. [Ni] = 21 nM at salinity 23). A direct inverse relation between salinity and dissolved Ni was observed (Figure 5), and indicates that dilution of Ni enriched freshwater with cleaner seawater was an important process regulating Ni behaviour in our estuarine

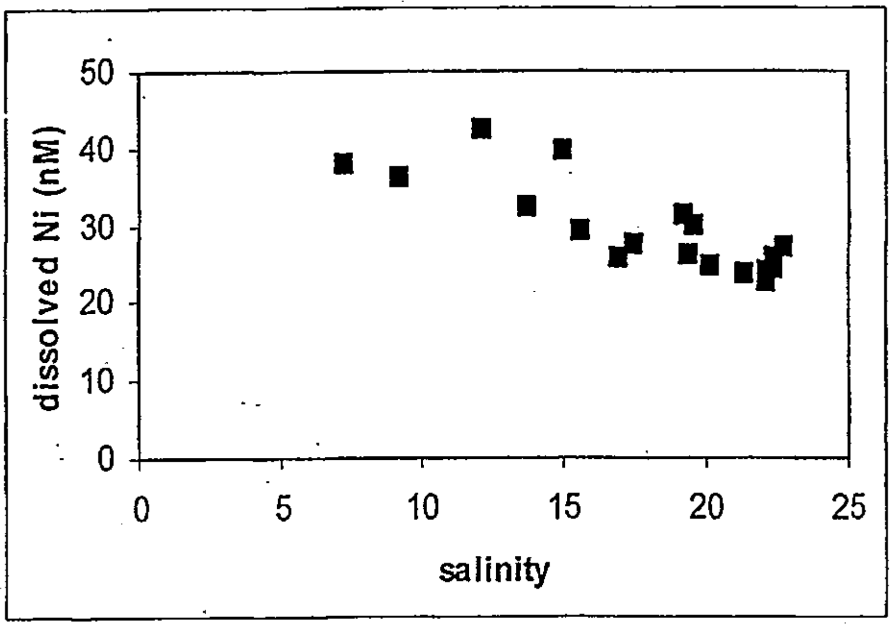


Figure 5. Total dissolved Ni versus salinity for tidal cycle experiment in the Tamar (July 1997).

system. A conservative behaviour for dissolved Ni has previously been observed for the higher salinity regions of the Severn (*30*) and Forth (*31*), in line with our findings. However, important removal of dissolved Ni by suspended particulate matter has been reported for the low salinity region (not covered during our tidal cycle) of the Tamar (*32,33*).

Trace metal behaviour in the Huelva estuary

The Huelva estuary is situated in southwest Spain (Figure 6), and receives metal-rich waters from the rivers Tinto and Odiel, which rise in an important mining district (Iberian Pyrite Belt; Cu, Zn, Fe, Au mines) and flow through heavily industrialised zones. Oxidation of iron pyrite in the orebodies and spoil heaps at the mining sites results in the formation of sulphuric acid and dissolved Fe, with additional release of metals. An automated voltammetric monitor was applied in this system in June 1997. The estimated freshwater discharge for the Tinto and Odiel in June 1997 was 0.08 and 0.3 m^3 s^{-1}, respectively (long-term mean water flows 3 and 15 m^3 s^{-1}). In Figure 7, the variations in dissolved Cu, Zn, salinity and pH are presented over a tidal cycle in the mid-estuary. Dissolved Cu and Zn were determined using AdCSV, with 8-hydroxyquinoline as AdCSV ligand and HEPES as pH buffer (final concentrations 15 μM and 10 mM, respectively), following 100 times dilution of the samples. The dilution was required as a result of the extreme conditions with respect to the metal concentration and sample matrix encountered at this site. The limit of detection and working range of the AdCSV Cu and Zn methods can be found in Table I. The quality of the trace metal data was validated through the analysis of certified reference material (see Table II), and furthermore corroborated by land-based ICP-MS analysis of hourly sampled discrete water samples, which showed close agreement with the continuous, near real-time determined trace metal concentrations (*6*). A strong temporal variability in metal concentrations was observed in this estuarine system as a result of enhanced metal inputs and tidal water movements. The mean tidal range in the Huelva estuary is 2.5 m. The metal concentrations at the single geographic point in the Huelva estuary were high, with Cu (Zn) concentrations ranging between ca. 2 (40) and 120 (200) μM. In line with observations in the Tamar estuary for Ni, the lowest Cu and Zn concentrations occurred during the periods with high tide (high salinity), and the highest concentrations during periods with low tide (low salinity). Figure 7 shows that the variations in pH were the mirror image of the metal variations. A plot of Cu and Zn versus conductivity shows an inverse relationship, indicating a conservative behaviour of these metals in the Huelva estuary (Figure 8). This conservative behaviour was also observed for these elements during axial transects in the Huelva (*34*). Although such a behaviour has been observed in

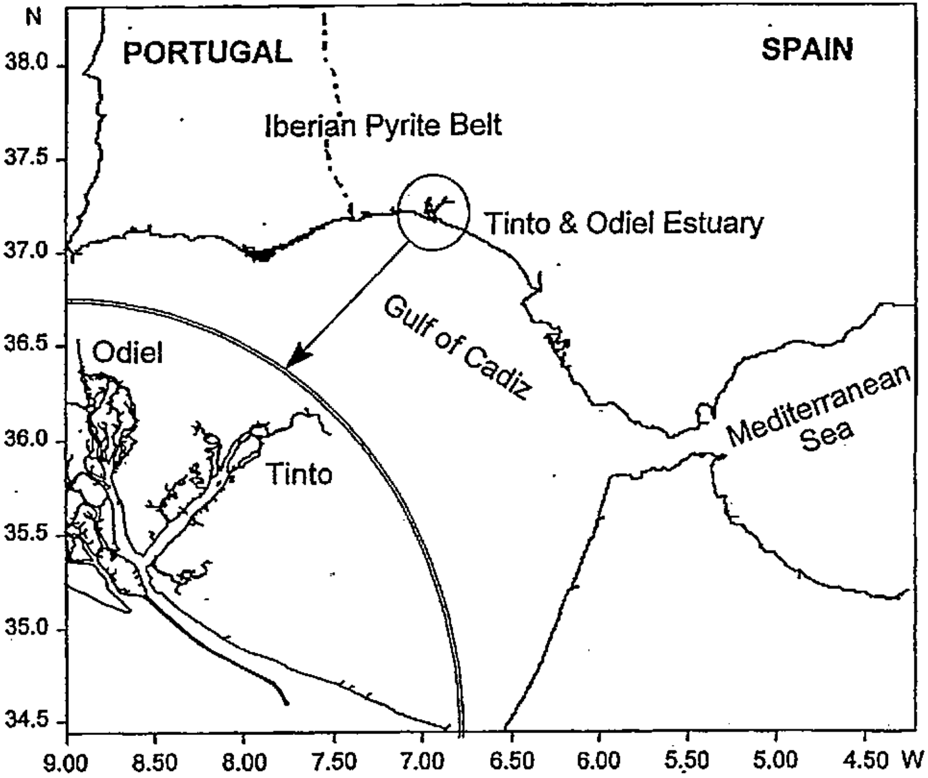


Figure 6. Location of the Gulf of Cadiz and the sulphide bearing Iberian Pyrite Belt in the southwest of the Iberian Peninsular. The inset shows the confluence of the Tinto and Odiel estuaries to form the Huelva estuary.

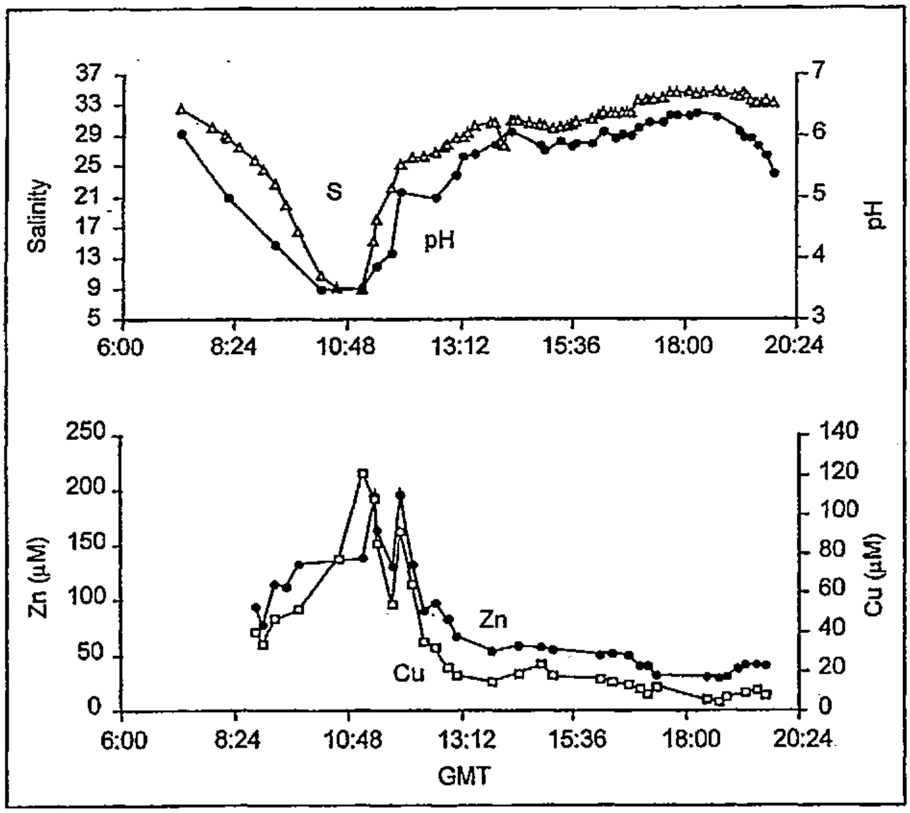


Figure 7. Time series of pH, salinity, total dissolved Cu and Zn over a tidal cycle at a fixed position in the Huelva estuary (June 1997).

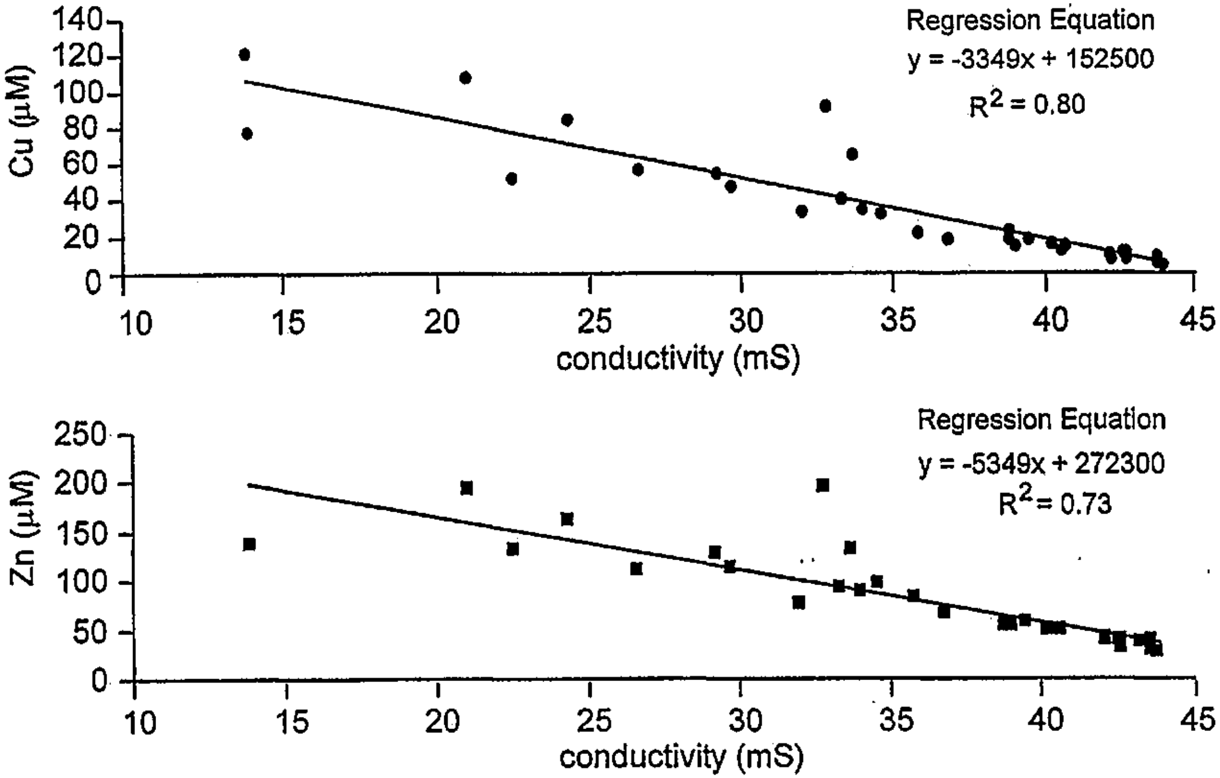


Figure 8. Total dissolved Cu and Zn versus conductivity for tidal cycle experiment in the Huelva estuary (June 1997).

other estuarine systems (e.g. Severn (*30*)), Cu and Zn are often reported to show a non-conservative behaviour in estuaries, with typically removal in the low salinity turbidity maximum zone and a mid-estuarine maximum due to anthropogenic and sedimentary inputs (Cu and Zn in Tamar (*35*) and Scheldt (*36*), and Zn in Forth (*31*)). The main difference which distinguishes the Huelva from other estuarine systems is its acidity, and the conservative Cu and Zn behaviour in the Huelva can be explained by the low pH in this system, which keeps trace metals in solution by proton competition for the available sorption sites on suspended particulate material (*37*).

Trace metal monitoring in coastal waters

The application of automated voltammetric instrumentation on-board ship, in conjunction with a continuous underway sampling approach, has allowed the acquisition of high quality and high resolution coastal trace metal data. Figure 9 shows the set-up for continuous ship-board sampling and analysis. Underway pumping is used as a means of sample collection, and this obviates the need for the vessel to halt for the collection of discrete samples. An effective pumping system involves a peristaltic pump and a braided PVC hose. The hose is hung overboard and attached to a 'fish' (torpedo-like structure, KIPPER-1) which is towed from a strong cable attached to a winch. The fish stays at a constant depth (ca. 2-4 m), even at speeds exceeding 10 knots, due to its design and weight (ca. 40 kg). The fish is made from solid carbon steel with an inlet at the front and a hole through the centre for the sampling hose. The fish is painted with a non-metallic epoxy-based paint.

Distribution and behaviour of trace metals in the Gulf of Cadiz

Figure 10 shows the results of a times series of salinity and dissolved Zn over a full tidal cycle undertaken ca. 3 km off the mouth of the Huelva estuary (see Figure 6), whilst the vessel (*B/O Garcia del Cid*) was anchored (October 1998). The salinity time series shows a limited salinity range (S = 36.33-36.35), indicating that the physical characteristics of the marine waters at the sampling position were not strongly affected by a tide-driven estuarine plume from the Huelva or adjacent Guadiana and Rio Piedras systems. This observation is partly the result of the low freshwater flow entering the Huelva estuary from the feeding rivers (October 1998, estimated flow of Tinto and Odiel: 0.1 and 0.4 m^3 s^{-1}, respectively), and also the result of the residual current structure in the Gulf of Cadiz, with currents moving in an west-east direction and estuarine plumes remaining close to the coast. Ship-board on-line voltammetric measurements of

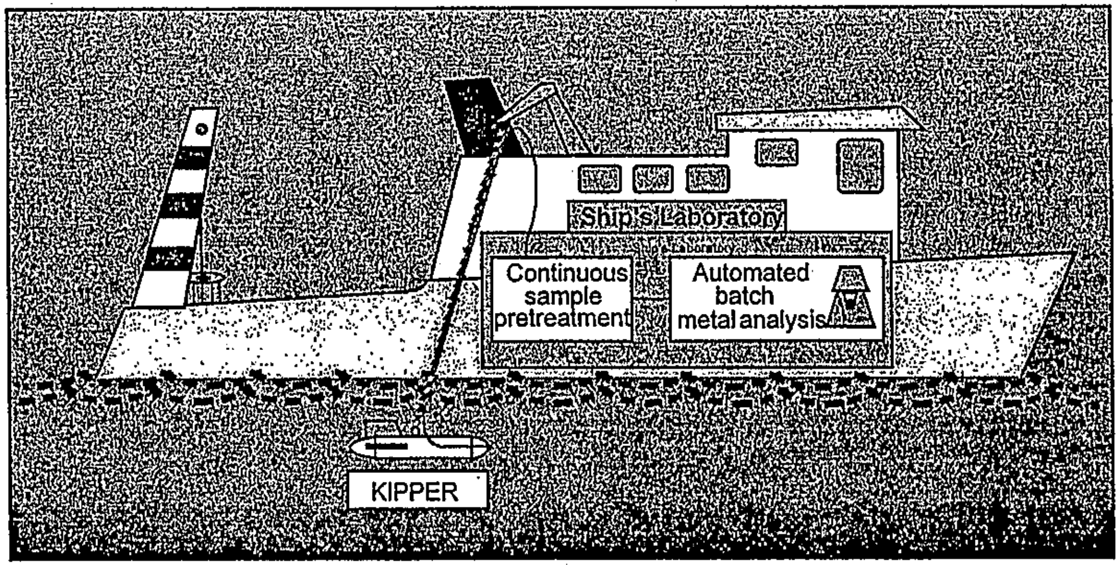


Figure 9. Drawing of ship-board continuous underway sampling and analysis system (Reproduced with permission from reference 8. Copyright 1999 Elsevier).

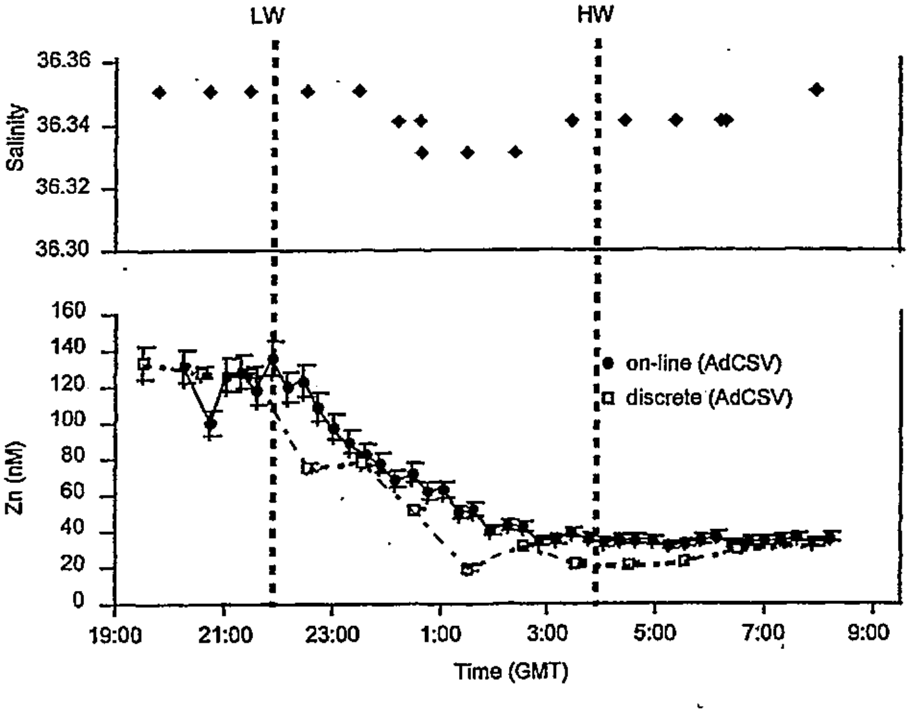


Figure 10. Time series of salinity and total dissolved Zn over a tidal cycle undertaken at a fixed position in the Gulf of Cadiz (October 1998). On-line dissolved Zn measurements were performed on-board ship using automated voltammetry with a pumped seawater supply, and discrete dissolved Zn measurements were performed in the laboratory in Plymouth in samples collected (hourly) using Niskin samplers.

dissolved Zn (8-hydroxy-quinoline as AdCSV ligand), and results of AdCSV Zn analysis in discrete samples (ammonium pyrrolidine dithiocarbamate as added AdCSV ligand) taken in parallel at hourly intervals using modified trace metal clean Niskin bottles, are presented in Figure 10b. The dissolved Zn concentrations from the on-line measurements varied between ca. 30 and 140 nM. The highest dissolved Zn concentrations (100-136 nM) were observed around the time of low tide at the mouth of Huelva estuary. The lowest concentrations (31-38 nM), which are representative of background Zn concentrations in the nearshore waters of the Gulf of Cadiz (*38*), were observed during high tide. The dissolved Zn concentrations in discrete samples agreed well with the results from the on-line analysis. The differences, with slightly lower Zn concentrations in discrete sample, can most likely be explained by differences in sampling depths. The discrete samples were obtained from a depth of ca. 7-8 m, whereas the pumped water intake was positioned at a depth of ca. 3 m. The high number of data points (n = 41) obtained during the on-line analysis (compared with 13 discrete samples), provides a good picture of the temporal trend of dissolved Zn in these coastal waters.

Coastal surveys with continuous underway sampling and near real-time on-line trace metal measurements have been undertaken in the Gulf of Cadiz in the period 1996-1998. The studies were performed using a small vessel (*Cirry Tres*, 9 m length) for surveying of shallow near coastal waters, and a larger vessel (*B/O Garcia del Cid*, 45 m length) for off-shore work. Two fully automated voltammetric systems were used on-board ship. Dissolved Cu and Zn (8 hydroxy-quinoline as AdCSV ligand) were determined simultaneously with one, and dissolved Co and Ni (dimethyl glyoxime as AdCSV ligand) with the other system. Figure 11 shows the sampling grids, with the locations of on-line determined trace metal samples for surveys in April 1998 (Figure 11a; *Cirry Tres*; >60 data points) and October 1998 (Figure 11b; *B/O Garcia del Cid*; 190 Zn, 270 Ni data points). Figure 12a shows the contour plots of salinity, total dissolved Zn and Ni for the April 1998 survey. The contour plots were generated using Surfer (Win 32), and the interpolation between the data points was carried out using Kriging as the gridding method. The results from the on-line measurements were not corrected for tidal excursion, which has to be considered when interpreting the contour plots. The salinity contour plot indicate low salinity surface water patches to the south-east of the Huelva estuary (S<34.2) and to the west of the Huelva estuary (S<34.4) and an additional pocket (S<34.2) was observed at the western fringe of the survey area. The metal concentrations were elevated in the plume coming out of the Huelva estuary, to the south-east of the estuary's mouth (≤300 nM Zn, ≤ 10 nM Ni) and in the north-west corner of the surveyed area (108 nM Zn, 5.1 nM Ni).

During the October 1998 survey (Figure 12b), areas of low salinity (≤36.0) were observed associated with the Guadiana in the west, the Huelva estuary in

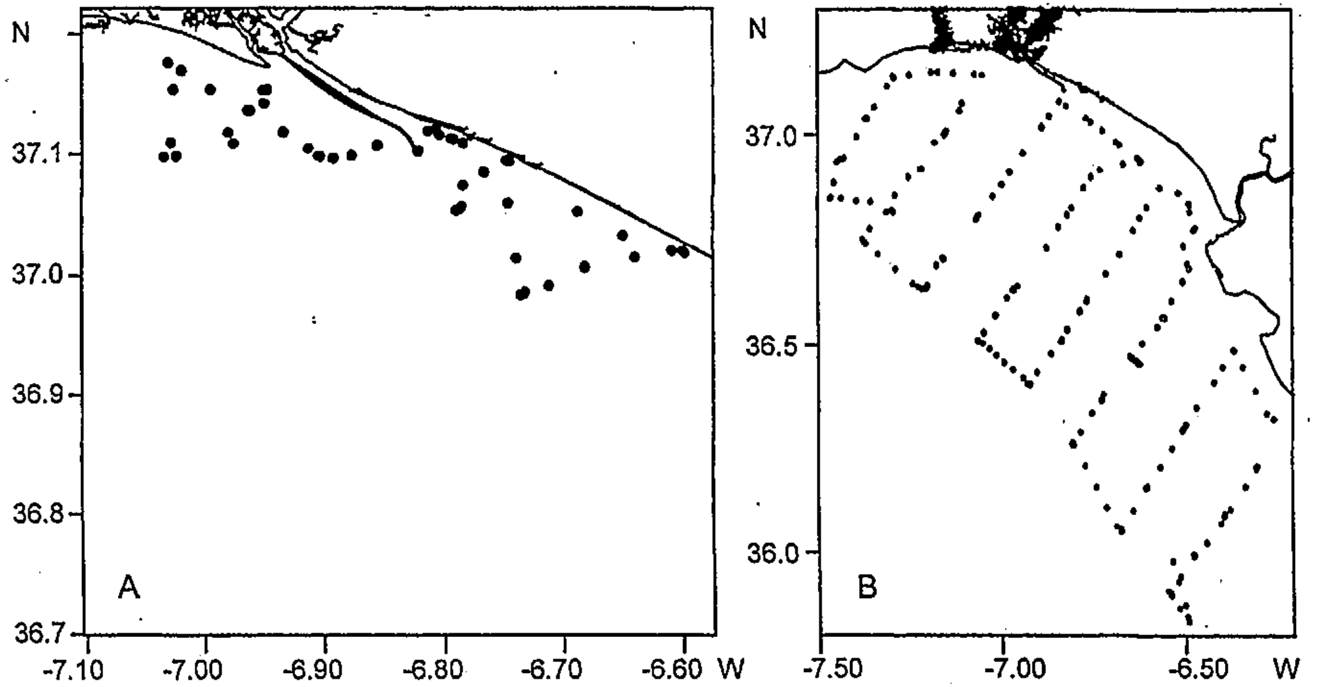


Figure 11. Locations of sampling positions in the Gulf of Cadiz where on-line trace metal measurements were undertaken in April 1998 (Figure 11a) and October 1998 (Figure 11b).

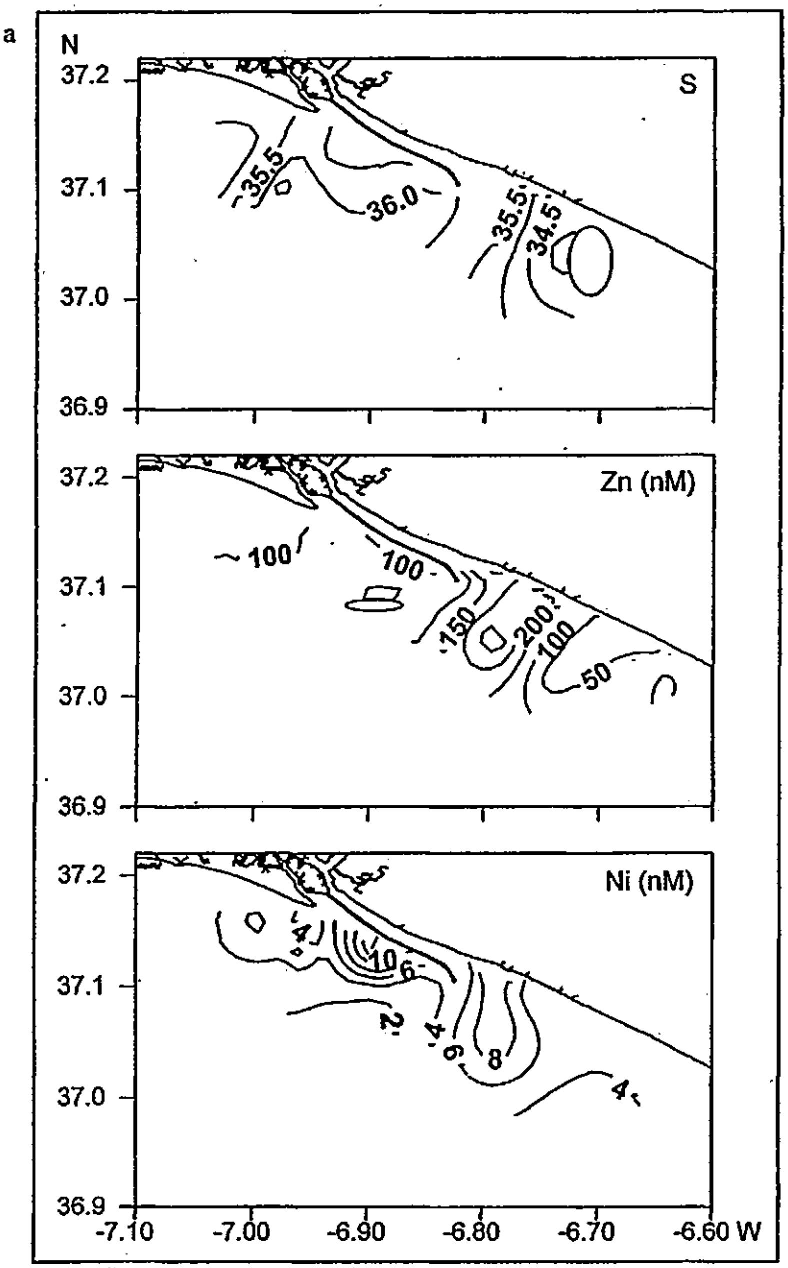


Figure 12. Contour plots of salinity and total dissolved Zn and Ni in surface waters of the Gulf of Cadiz during Cirry Tres *cruise (April 1998; Figure 12a); and* Garcia del Cid *cruise (October 1998; Figure 12b).*

b

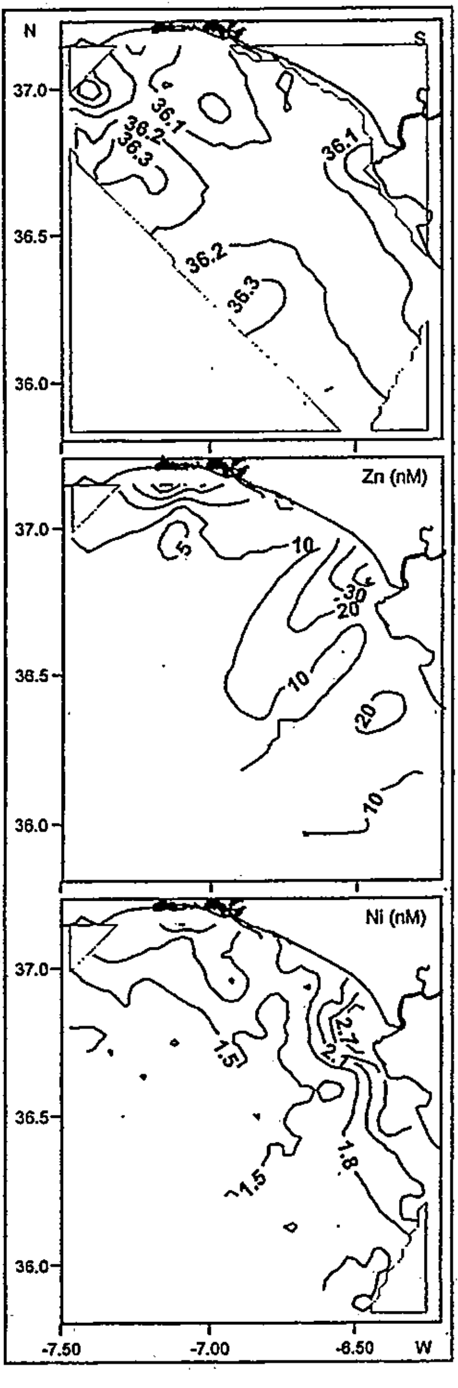


Figure 12. Continued.

the north and the Guadalquivir in the east of the Gulf of Cadiz. In surface waters of the central and offshore areas of the Gulf of Cadiz, the salinity was greater than 36.2, with maxima above 36.6. High concentrations of Zn (18-90 nM) and elevated Ni levels (2.0-6.0 nM) were observed along the shoreline. A steep concentration gradient between the outflow of the Huelva and Guadalquivir estuaries was observed. In an offshore direction, the Zn and Ni concentrations showed a decrease as a result of mixing of metal enhanced near coastal waters with more pristine Atlantic Ocean surface waters. The concentrations at offshore locations in the Gulf of Cadiz were ≤11 nM Zn and ≤2.0 nM Ni. Relative to off shore waters, Ni concentrations in the nearshore waters of the Gulf of Cadiz were not as elevated compared with Zn. This can be explained by the absence of Ni enrichment, and a high abundance of Zn, in the geology of the Iberian Pyrite Belt. The observations obtained using the near-real time ship-board monitoring approach were in good agreement with trace metal values reported in literature (*38,39*). However, the high spatial resolution of the data has allowed a more thorough interpretation of sources and behaviour of trace metals in the Gulf of Cadiz.

Distribution and behaviour of trace metals in English coastal waters

As a result of the wet climatic conditions of the British Isles, freshwater inputs into coastal waters are much more important compared with southern Spain. Major fluxes of dissolved trace elements around the coast of England are associated with inputs of freshwater by rivers, and of seawater by the English Channel and the northern North Sea (*40,41,42*). The strong influence of rivers on English Coastal Waters is exemplified by Figure 13, which shows salinity and dissolved Cu and Zn data for a coastal track undertaken by *RRS Challenger* (October 1994). The cruise started in Barry (south Wales) and finished in Hull (northeast England). Continuous sampling and analyses was undertaken during the cruise, resulting in high resolution data for the English Coastal Waters. The data in Figure 13 have been plotted in the sequence in which they were obtained using the underway sampling approach. Positions of the vessel in the vicinity of estuarine plumes have been indicated by vertical dotted lines. Low salinity values associated with river water inputs into the coastal waters are apparent in Figure 13. The cruise in October 1994 took place during a relatively dry period, with river flows below the mean monthly long-term (1961-1990) flow. Enhanced dissolved Cu and Zn concentrations were observed in the vicinity of rivers and estuaries. Important trace metal signals were observed for the Fal, Thames, Humber, Tees, Tyne and Wash. These systems are anthropogenically perturbed with relatively large mining, industrial and urban waste water discharges. Metal-salinity relationships obtained using the high resolution data showed a non-

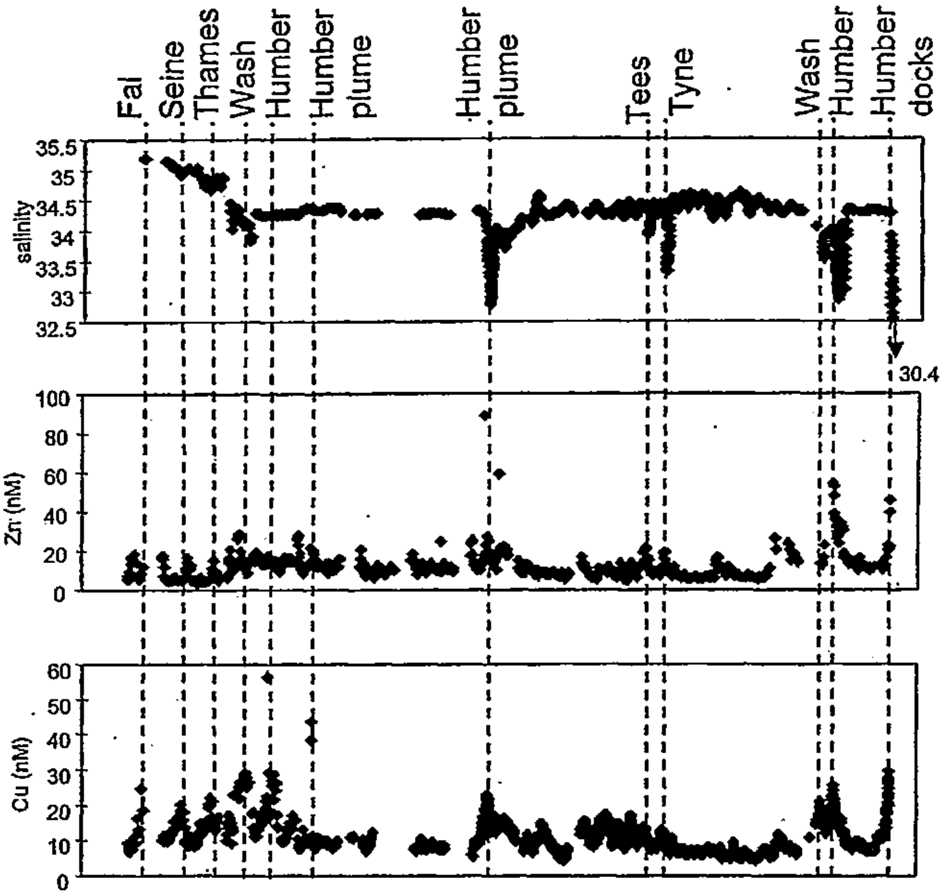


*Figure 13. Salinity and total dissolved Zn and Cu plotted against location of vessel (*RRS Challenger*) in English Coastal Waters during cruise in October 1994. Data are plotted in sequence in which they were obtained using underway sampling (Adapted with permission from reference 20. Copyright 1999 Elsevier).*

conservative behaviour for both Cu and Zn, indicating that processes other than mixing between water masses was influencing their behaviour in English Coastal Waters (*20*).

Conclusions

The application of ship-board voltammetric instrumentation for high resolution monitoring of trace metals in coastal seas has distinct advantages. The trace metal data obtained in the Gulf of Cadiz revealed a complex mixing pattern of contaminated estuarine with more pristine Atlantic waters. This data will be used in numerical models to evaluate the biogeochemical behaviour of the trace metals investigated during our studies. The enhanced spatial resolution of trace metals observed during our work in the English Coastal Waters allowed us to identify signals from fluvial sources which would possibly be missed using a traditional sampling and analysis approach. In addition, our approach aided the interpretation of trace metal behaviour in coastal waters. The high resolution measurements in coastal waters therefore result in data sets that allow a more thorough deconvolution of the complex temporal and spatial behaviour of trace metals in such waters.

A recent development in trace metal monitoring is the fully submersible voltammetric probe. One such probe, which is currently used in our laboratory, is the voltammetric in-situ profiling (VIP) system (*9,43,44*). The VIP allows the determination of Cu, Cd, Pb and Zn in seawater using ASV, with a gel integrated mercury coated microelectrode as the working electrode (*45*). The iridium microelectrode array displays an excellent sensitivity and reproducibility, and the agarose gel prevents fouling of the electrode and allows metal speciation measurements. Although a series of papers has been published on the analytical developments involving the VIP, the application of the probe for in-situ real-time trace metal measurements in estuarine and coastal waters has yet to be fully exploited. The remote deployment of the probes, with satellite communication, will allow the systems to be used in pollution control and water quality management scenarios.

Acknowledgements

The authors would like to thank the European Union (TOROS project, contract ENV4-CT96-0217; IMTEC project, contract (ENVK3-CT-2000-00036), the Natural Environment Research Council (GT 4/98/MS/234) and the University of Plymouth for funding. Dr. Whitworth is acknowledged for the

Tamar data. We thank the crew and scientist on the *Cirry Tres, Garcia del Cid* and *Challenger* for their assistance.

References

1. European Commission. Towards a European Integrated Coastal Zone (ICZM) Strategy. Office for Official Publications of the European Communities, Luxembourg, 1999.
2. Morley, N.H.; Fay, C.W.; Statham, P.J. *Adv. in Underwat. Technol., Ocean Sci. and Offshore Engin.* 1988, *16*, 283-289.
3. Howard, A.G.; Statham, P.J. Inorganic trace analysis: philosophy and practice; Wiley, 1993.
4. Johnson, K.S.; Coale, K.H.; Jannasch, H.W. *Anal. Chem.* 1992, *64*, 1065A-1075A.
5. Andrew, K.N.; Blundell, N.J.; Price, D.; Worsfold, P.J. *Anal. Chem.* 1994, *66*, 916A-922A.
6. Braungardt, C.; Achterberg, E.P.; Nimmo, M. *Anal. Chim. Acta* 1998, *377*, 205-215.
7. Wang, J.In *Laboratory techniques in electrochemical chemistry*; Kissinger, P.T., Heineman, W.R., Eds.; Marcel Dekker: New York, 1996; pp 719-738.
8. Achterberg, E. P and Braungardt, C. B. *Anal. Chim. Acta* 1999, *400*, 381-397.
9. Tercier, M.L.; Buffle, J.; Graziottin, F. *Electroanal.* 1998, *10*, 355-363.
10. Zirino, A.; Leiberman, S.H.; Healy, M.L. In *Marine Electrochemistry*; Berkowitz, B.J., Horne, R., Banus, M., Howard, P.L., Pryor, M.J., Whitnack, G.C., Weiss, H.V., Eds.; Electrochemical Soc.: Princeton, N.J., 1973; pp 319-332.
11. van den Berg, C.M.G. *Analyst* 1989, *114*, 1527-1530.
12. Paneli, M.G.; Voulgaropoulos, A. *Electroanal.* 1993, *5*, 355-373.
13. Florence, M. *J. of Electroanal. Chem.* 1970, *27*, 273-281.
14. Luther III, G.W.; Brendel, P.J.; Lewis, B.L.; Sundby, B.; Lefrancois, L.; Silverberg, N.; Nuzzio, D.B. *Limnol.Oceanogr.* 1998, *43*, 325-333.
15. de Vitre, R.R.; Tercier, M.-L.; Tsacopoulos, M.; Buffle, J. *Anal. Chim. Acta.* 1991, *249*, 419-425.
16. Herdan, J.; Feeney, R.; Kounaves, S.P.; Flannery, A.F.; Storment, C.W.; Kovacs, G.T.A.; Darling, R.B. *Environ.Sci.Technol.* 1998, *32*, 131-136.
17. Bond, A.M.; Mahon, P.J.; Schiewe, J.; VicenteBeckett, V. *Anal. Chim. Acta* 1997, *345*, 67-74.
18. Bond, A.M.; Czerwinski, W.A.; Llorente, M. *Analyst* 1998, *123*, 1333-1337.
19. Achterberg, E.P.; van den Berg, C.M.G. *Mar. Poll. Bull.* 1996, *32*, 471-479.

20. Achterberg, E.P.; Colombo, C.; van den Berg, C.M.G. *Cont.Shelf Res.* **1999,** *19*, 537-558.
21. Whitworth, D.-J.; Achterberg, E.P.; Nimmo, M.; Worsfold, P.J. *Anal. Chim. Acta* **1998,** *371*, 235-246.
22. Achterberg, E.P.; van den Berg, C.M.G. *Anal. Chim. Acta* **1994,** *284*, 463-471.
23. Achterberg, E.P.; van den Berg, C.M.G. *Anal. Chim. Acta* **1994,** *291*, 213-232.
24. Achterberg, E.P.; Braungardt, C.B.; Sandford, R.C.; Worsfold, P.J. *Anal. Chim. Acta* **2001,** in press.
25. Muller, F.L. *Mar. Chem.* **1996,** *52*, 245-268.
26. Stumm, W.; Morgan, J.J. In *Aquatic Chemistry-Chemical equilibria and rates in natural waters;* John Wiley & Sons: New York, 1996; pp 1-1022.
27. Butler, E.I.; Tibbitts, S. *J.Mar.Biol.Assoc.UK.* **1972,** *32*, 681-699.
28. Morris, A.W.; Bale, A.J.; Howland, R.J.M. *Est. Coast. Shelf Sci.* **1982,** *14*, 649-661.
29. Grabemann, I.; Uncles, R.J.; Krause, G.; Stephens, J.A. *Est. Coast. Shelf Sci.* **1997,** *45*, 235-246.
30. Apte, S.C.; Gardner, M.J.; Gunn, J.E.; Vale, J. *Mar. Poll. Bull.* **1990,** *21*, 393-396.
31. Laslett, R.E.; Balls, P.W. *Mar.Chem.* **1995,** *48*, 311-328.
32. Morris, A.W. *Sci. Tot. Env.* **1986,** *49*, 297-304.
33. Morris, A.W.; Bale, A.J.; Howland, R.J.M.; Millward, G.E.; Ackroyd, D.R.; Loring, D.H.; Rantala, R.T.T. *Wat. Sci. Technol.* **1986,** *18*, 111-119.
34. Elbaz-Poulichet, F.; Morley, N.H.; Cruzado, A.; Velasquez, Z.; Green, D.; Achterberg, E.P.; Braungardt, C.B. *Sci. Tot. Env.* **1999,** *227*, 73-83.
35. Ackroyd, D.R.; Bale, A.J.; Howland, R.J.M.; Knox, S.; Millward, G.E.; Morris, A.W. *Estuar.Coast.Mar.Sci.* **1986,** *23*, 621-640.
36. Zwolsman, J.J.G.; Van Eck, B.T.M.; Van der Weijden, C.H. *Geochim.Cosmochim.Acta* **1997** , *61*, 1635-1652.
37. Braungardt, C.B.; Achterberg, E.P.; Elbaz-Poulichet, F.; Morley, N.H.; Cruzado, A.; Velasquez, Z.; Nimmo, M. **2001,** In preparation.
38. van Geen, A.; Boyle, E.A.; Moore, W.S. *Geochim. Cosmochim. Acta* **1991,** *55*, 2173-2191.
39. van Geen, A.; Rosener, P.; Boyle, E. *Nature* **1988,** *331*, 423-426.
40. Burton, J.D.; Althaus, M.; Millward, G.E.; Morris, A.W.; Statham, P.J.; Tappin, A.D.; Turner, A. *Phil.Trans.R.Soc.London* **1993,** *343*, 557-568.
41. Tappin, A.D.; Hydes, D.J.; Burton, J.D.; Statham, P.J. *Continent. Shelf Res.* **1993,** *13*, 941-969.
42. Tappin, A.D.; Millward, G.E.; Statham, P.J.; Burton, J.D.; Morris, A.W. *Est. Coast. Shelf Sci.* **1995,** *41*, 275-323.

43. Belmont-Hébert, C.; Tercier, M.L.; Buffle, J. *Anal.Chem.* **1998**, *70*, 2949-2956.
44. Tercier, M. L.; Buffle, J.; Koudelka-Hep, M.; Graziottin, F. In *Electrochemical Methods for the Environmental Analyses of Trace Element Biogeochemistry*, Taillefert, M.; Rozan, T., Eds. American Chemical Society Symposium Series; American Chemical Society: Washington, D. C., **2001**, this volume, Chapter 2.
45. Tercier-Waeber, M.-L.; Buffle, J.; Confalonieri, F.; Riccardi, G.; Sina, A.; Graziottin, F.; Fiaccabrino, G.C.; Koudelka-Hep, M. *Meas. Sci. Technol.* 1999, *10*, 1202-1213.

FULL PAPER
THE ANALYST
www.rsc.org/analyst

The determination of trace metals in estuarine and coastal waters using a voltammetric *in situ* profiling system

Kate A. Howell,[a] Eric P. Achterberg,*[a] Charlotte B. Braungardt,[a] Alan D. Tappin,[a] David R. Turner[b] and Paul J. Worsfold[a]

[a] *School of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Plymouth, UK PL4 8AA. E-mail: Eachterberg@Plymouth.ac.uk; Fax: +44-1752-233035; Tel: +44-1752-233036*

[b] *Department of Analytical and Marine Chemistry, University of Göteborg, Göteborg 3412 96, Sweden*

Received 20th January 2003, Accepted 25th April 2003
First published as an Advance Article on the web 14th May 2003

This work presents the optimisation, validation and field deployment of a voltammetric *in situ* profiling (VIP) system for the simultaneous determinations of dynamic Cd(II), Cu(II) and Pb(II) in estuarine and coastal waters. Systematic studies in $NaNO_3$ (as a supporting electrolyte) and seawater, indicated that variations in ionic strength, pH and dissolved oxygen did not affect the response of the instrument, whereas an Arrhenius type temperature response was observed. The VIP instrument allows the determination of 2–3 samples h^{-1}, and has a detection limit (defined as 3σ) in seawater for Cd(II): 23 pM, Cu(II): 1.13 nM, and Pb(II): 23 pM. The VIP system accurately measured the total dissolved concentrations of Cd(II), Cu(II) and Pb(II) in two certified reference materials; SLRS-3, a river water, and SLEW-2, an estuarine water. Field evaluation of the instrumentation and analytical methods was achieved through a series of surveys in the Plym Estuary (Devon, UK), from which environmental data are presented.

Introduction

Trace metals, such as cadmium (Cd(II)), copper (Cu(II)) and lead (Pb(II)) play an important role in the ecological status of natural waters. Concentrations of dissolved trace metals are generally determined in the filtrate of sample waters that pass through a 0.4 or 0.45 μm pore diameter membrane filter.[1] This operationally defined dissolved fraction includes free hydrated metal ions, readily dissociable (labile) metal complexes, species adsorbed on inorganic and organic colloids and metals strongly bound to inorganic and organic complexes.[2]

The concentration of total dissolved metal is the most frequently reported parameter, but information on trace metal speciation is important because bioavailability is determined by the physico-chemical form of a metal species. This affects the mode of transport across cell membranes, the lipophilicity of the metal fraction, and the *in vivo* biochemical pathways of the exposed organisms.[3] Stripping voltammetry has become established as one of the more powerful tools for trace metal speciation investigations because of its sensitivity and metal species selectivity,[4] and anodic stripping voltammetry (ASV), in particular, is now commonly used to determine picomolar concentrations of labile metal ions in natural waters.

The measurement of dissolved metal fractions in natural waters is generally made in order to improve our understanding of the processes governing contaminant behaviour and effects. The traditional approach, based upon discrete sampling followed by land-based laboratory analysis (*e.g.* using inductively coupled plasma mass spectrometry,[5] electrothermal atomic absorption spectroscopy[6]), has a number of weaknesses. These include the cost (*i.e.* specialised sampling equipment, provision of clean rooms, time consuming analyses), the difficulty in making reliable speciation measurements (due to contamination, loss by adsorption, pressure and/or temperature changes with concomitant pH and redox changes, coagulation of colloidal matter followed by sedimentation of the aggregates and the associated trace metals, physicochemical changes due to microbial activity), the poor sampling frequency in relation to the dynamics of aquatic ecosystems and the inability to rapidly and effectively monitor unforeseen transient contaminant discharges. Automated voltammetric systems, using a pumped water supply and unattended on-line measurements on board a research vessel,[4,7] partially overcome these problems but often autonomous monitoring systems complete with data transmission are required. Thus the development of submersible probes capable of performing *in situ*, real-time monitoring of specific forms of trace elements is of prime interest. The development of such a tool is a challenging task, as it requires analytical approaches using miniaturised and automated monitoring probes that combine high sensitivity and reliability, multi-element analyses, speciation capability and unattended operation.

To date, a limited number of submersible probes for *in situ* trace metal analyses in natural waters has been reported (*e.g.* a Ni/U probe, developed by Wang and co-workers[8]). The voltammetric *in situ* profiling (VIP) system described in this paper has been developed by Buffle and co-workers since 1990 and is the first commercially available instrument package to address issues raised above. The VIP system[9–19] has been successfully applied in lake,[10,12] river[19] and fjord waters[10,15] to measure the dynamic fractions (free metal, electrochemically labile and other small, mobile species) of Cd(II), Cu(II), Pb(II), Zn(II) and Mn(II).[16]

The aim of this paper is to evaluate the analytical aspects of the dynamic trace metal measurements using the VIP system in marine waters and in particular estuarine and coastal waters, where master variables (salinity, temperature, pH, dissolved oxygen) can exhibit marked spatial and temporal variability. We have therefore conducted systematic studies, using seawater, to examine the effect of each master variable on instrumental response. In addition, these experiments were conducted in sodium nitrate for comparison, because this is a commonly used supporting electrolyte for electrochemical studies, and typically used as a matrix to calibrate and validate

DOI: 10.1039/b300712j

voltammetric instrumentation. The instrumentation was successfully deployed *in situ* during tidal cycles in the Plym Estuary (Devon, UK) and environmental data from these surveys for Cd(II), Cu(II) and Pb(II) are presented.

Experimental

Voltammetric *in situ* profiling (VIP) system

All voltammetric measurements were performed using the VIP system (Idronaut, Milan, Italy), shown schematically in Fig. 1A. The system has been described in detail elsewhere,[12,15] and only an outline is given here. The submersible probe consists of a Delrin upper case that contains electronic hardware and firmware, a voltammetric flow cell (Plexiglas) (Fig. 1B) and a lower case (Delrin) that contains a preamplifier and peristaltic pump. The voltammetric cell has an internal sample volume of 4.2 mL and consists of a three-electrode system with an internal flow-through compartment (accommodating the working micro-electrode and in-built Pt ring) and an outer compartment (containing the reference electrode). The section between the inner and outer compartments is filled with agarose/$NaNO_3$ gel. The gel acts as a pressure equaliser through a pressure compensator (see Fig. 1B) and as a double bridge between the sample and the Ag/AgCl/3 M KCl/1 M $NaNO_3$ reference electrode, *via* two ceramic junctions.[16]

An Ir working micro-electrode array (μ-AMMIA) was used in this study, which has 100 interconnected Ir discs (5 μm diameter) and is coated with a layer of agarose gel (LGL, 1.5% w/w) with a thickness of 300 μm and a pore size of a few nanometres.[14,17] The gel allows diffusion of dissolved metal ions and small complexes to the micro-electrode surface whilst acting as a barrier to colloids and macromolecules. This gives protection from organic and surfactant fouling and allows long-term deployment for dynamic trace metal measurements to be undertaken. Hemispherical Hg drops are electrochemically plated through the gel layer onto the Ir sensor surface and the array with the Hg coating are stable for up to three weeks.

The VIP system can be deployed at depths of up to 500 m and communication between the probe and a laptop computer is enabled *via* an armoured coaxial cable. The VIP system is connected to a 12 V marine battery for field trials that permits continuous deployment for 4 days.

Materials and methods

All reagents were purchased from VWR (Poole, UK) and were of AnalaR grade unless otherwise stated. Handling of reagents, solutions and samples was undertaken in a Class 100 laminar flow cabinet, using clean techniques for trace metals.[20] All aqueous reagents were prepared using water purified by reverse osmosis (Milli-RO, Millipore, Bedford, USA) followed by ion exchange (MQ water) (Millipore, 18.2 MΩ cm^{-1}). Ultrapure HNO_3 and CH_3COOH were prepared by sub-boiling (Sub) quartz distillation,[20] and ultrapure NH_4OH by isothermal distillation (Iso).

High density polyethylene (HDPE, Nalgene, Rochester, NY, USA) sample and reagent bottles were cleaned by soaking in 2% (v/v) Decon 90 for 24 h, 0.1 M NaOH for 24 h, 5 M HCl for one week, and finally 2 M HNO_3 for one week. A thorough MQ water rinse was carried out after each step. Bottles were filled with acidified MQ water (1% Sub-HNO_3, pH 2) and stored in resealable plastic bags.

The supporting electrolyte for laboratory VIP measurements was 0.1 M $NaNO_3$ (Trace select, Sigma Aldrich, Dorset, UK)

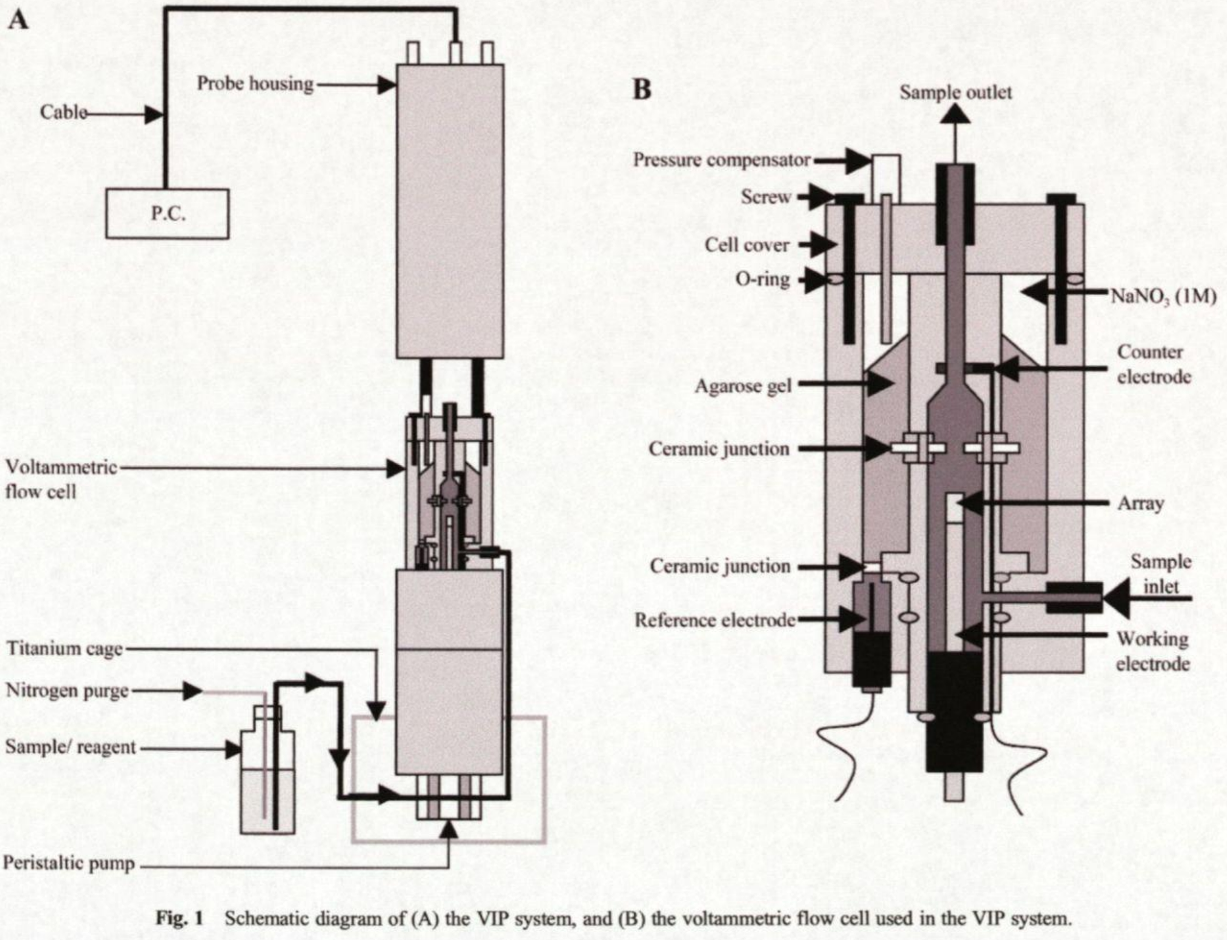


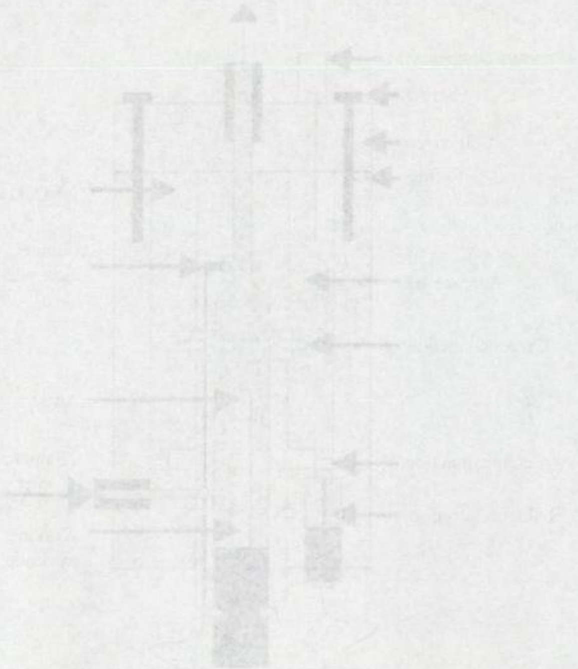
Fig. 1 Schematic diagram of (A) the VIP system, and (B) the voltammetric flow cell used in the VIP system.

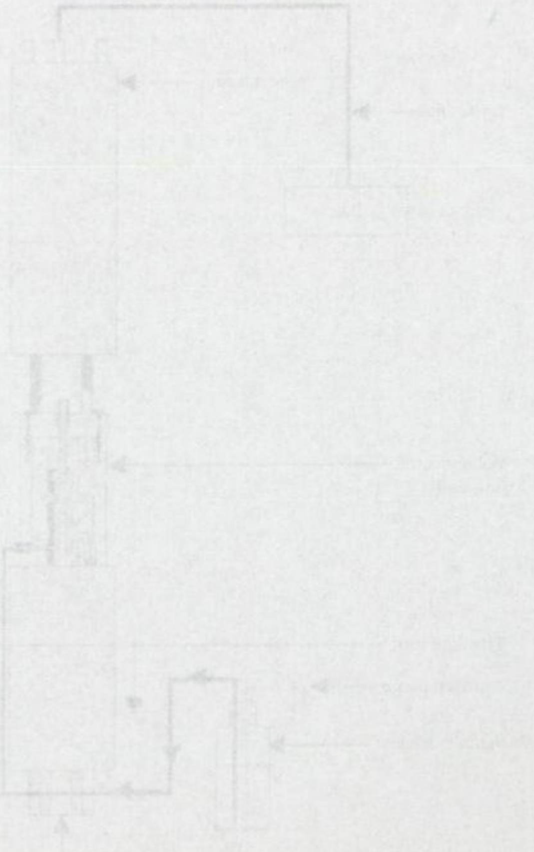
[illegible]

Microwave digestion

[illegible] Milli-RO, Millipore, Bedford, USA) followed by ion exchange on MQ water (Millipore, 18.2 MΩ cm [illegible] LOOH were prepared by sub-boiling (Milli [illegible]

[illegible]





unless otherwise stated. The commercial $NaNO_3$ is low in trace metals, and was used in the range 0.05–0.6 M to approximate the ionic strengths of seawater (in the salinity range 2.5 to 35). The relationship between conductivity and salinity for the salinity range 2.5–4 displayed a slope of 0.578 mS salinity unit^{-1} (R^2 = 0.999), and for the salinity range 4–35 displayed a slope of 0.731 mS salinity unit^{-1} (R^2 = 0.997). The relationship between conductivity and $NaNO_3$ for the concentration range 0.02–0.08 M displayed a slope of 0.013 mS M^{-1} (R^2 = 0.997), and for the concentration range 0.1–0.5 displayed a slope of 0.012 mS M^{-1} (R^2 = 0.994).

Standard solutions of 1×10^{-4} M Cd(II), Cu(II) and Pb(II) were prepared from atomic absorption spectrometry standard solutions (SpectrosoL; 1000 mg L^{-1}), acidified to pH 2 using Sub-HNO_3. Acetate buffers in the pH range 2–6 were prepared from Sub-CH_3COOH and Iso-NH_4OH, to give a final concentration of *ca.* 1 M. An aqueous solution of 1 M n-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES, pH buffer) was prepared and the pH adjusted to 7.8 using Iso-NH_4OH.

Coastal seawater (salinity 35) sampled off Plymouth and discrete samples collected in the Plym Estuary were filtered through acid washed polycarbonate membrane filters (0.4 μm, Cyclopore, Whatman, Kent, UK) and subsequently UV-digested[21] (400 W medium pressure UV lamp, Photochemical Reactors, Slough, UK) in 30 mL quartz tubes for 5 h, after the addition of H_2O_2 (8.8 mM, final concentration). Low trace metal seawater was prepared by passage through Chelex-100 resin (Bio-Rad, Hercules, CA, USA) packed into a column made of low density polyethylene tubing (Nalgene) fitted with a quartz wool plug. Prior to use, the column was conditioned with three 150 mL aliquots of Sub-HNO_3 (0.5 M) followed by six 150 mL aliquots of MQ water. The cleaned seawater was UV-digested for a second time for 5 h.

LDL agarose gel (Biofinex, Neuchâtel, Switzerland) was prepared by agitation of agarose (1.5% v/v) in MQ water at 80 °C for 10 min until a transparent gel was formed. The gel was cooled to 60 °C before coating the micro-electrode array. A Hg film was electrochemically deposited (at −400 mV for 6 min) onto the μ-AMMIA using a 5.5 mM $Hg(CH_3COO)_2$/0.1 M $HClO_4$ solution, resulting in Hg hemisphere radii in the range of 5.1–5.8 μm. The size of the Hg hemispheres was calculated from the current obtained during the electro-deposition of Hg on the μ-AMMIA.[18] Following each series of experiments, the Hg was electrochemically removed in a solution of 1 M KSCN by a scan from −300 to +300 mV (scan rate of 5 mV s^{-1}).

In the laboratory, trace metal measurements using the VIP instrument were made in acidified (pH 2), deoxygenated (using humidified N_2 (g) (BOC, Surrey, UK)) 0.1 M $NaNO_3$ or acidified (pH 2), deoxygenated seawater unless otherwise stated. Sample was pumped through the VIP flow cell for 3 min (at 7 mL min^{-1}), equilibrated with the agarose gel on the micro-electrode for 6.5 min, followed by a second pumping stage of sample for 3 min. Subsequently, a deposition stage was conducted at −1100 mV (unless otherwise stated) for a period between 5 to 40 min during which metals were pre-concentrated in the Hg. After a 10 s equilibration period, a square wave (SW) ASV scan was carried out using a frequency of 200 Hz (pulse amplitude of 25 mV, step amplitude of 8 mV, unless otherwise stated). The background scan was recorded subsequently, without prior deposition, and the background current was numerically subtracted from the sample stripping current using the VIP firmware.[22] All quoted potentials are *versus* the Ag/AgCl/3M KCl/1M $NaNO_3$ reference electrode.

Total dissolved Cd(II) and Pb(II) in discrete samples were determined using the VIP system in the laboratory. In addition, total dissolved Cu(II) was determined using adsorptive cathodic stripping voltammetry with a hanging Hg drop electrode (663 VA stand, Metrohm, Herisau, Switzerland) interfaced with a μAutolab (EcoChemie, Utrecht, The Netherlands) using 8-hydroxyquinoline (25 μM final concentration) as a complexing ligand and HEPES (0.01 M final concentration) as a pH buffer.[23]

Results and discussion

Influence of deposition potential and time, and pulse and step amplitudes

The potential applied during the deposition step in voltammetric measurements may affect analytical sensitivity. The effect of deposition potential on the peak current response over the range −800 mV to −1200 mV in $NaNO_3$ and seawater was examined to optimise voltammetric conditions; the results are shown in Fig. 2. A relative standard deviation (RSD) < 4% (n = 15) was obtained for repeat measurements of the peak currents over the whole deposition potential range for Cu(II) and Pb(II) in both matrices. Cd(II) showed a marked decrease in sensitivity for both matrices at potentials more positive than −1100 mV. These observations for Cd(II), Cu(II) and Pb(II) are in agreement with previously reported results from similar investigations using 0.1 M $NaNO_3$ (pH 2).[14,17] At potentials more negative than −1200 mV, the irreversibility of the reduction of hydrogen ions resulted in a large current response in the acidified solutions (pH 2).[24] A deposition potential of −1100 mV was considered optimal for both matrices and used during further laboratory and field studies.

The pulse and step amplitudes influence analytical sensitivity and peak shape and were optimised by systematic adjustment in the range 12.5–50 mV and 1–16 mV, respectively. A step amplitude of 8 mV and a pulse amplitude of 25 mV resulted in good sensitivity and peak shapes for all metals, and an excellent resolution of the Cd(II) and Pb(II) peaks, in both $NaNO_3$ and seawater. These conditions were used during further measurements and are consistent with previously reported optimum conditions for 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffered (pH 6.7), deoxygenated 0.1 M $NaNO_3$.[15] A good

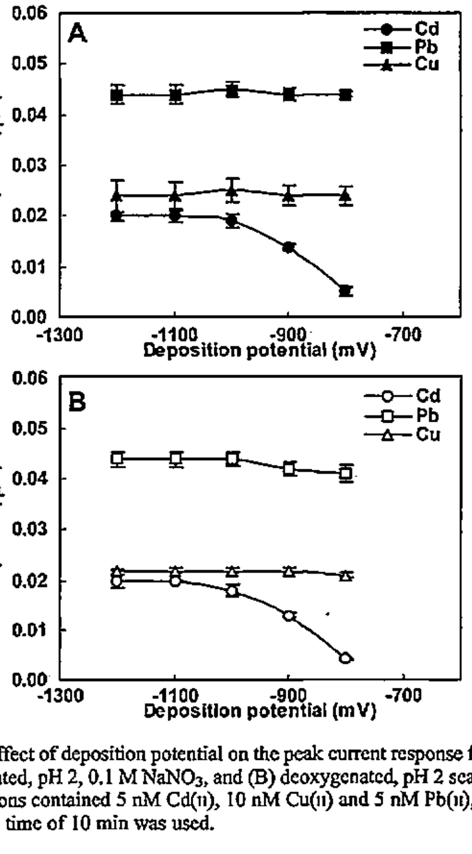


Fig. 2 Effect of deposition potential on the peak current response for (A) deoxygenated, pH 2, 0.1 M $NaNO_3$, and (B) deoxygenated, pH 2 seawater. The solutions contained 5 nM Cd(II), 10 nM Cu(II) and 5 nM Pb(II), and a deposition time of 10 min was used.

linearity ($R^2 > 0.98$ for Cd(II), Cu(II) and Pb(II)) for the relation between peak current response and deposition time was observed in seawater (Fig. 3). A deposition time typically in the range 15–30 min was used in the studies reported in this paper, yielding easily measurable peak current responses (at least 1–2 nA).

Optimisation of sample transport and gel equilibration times

Estuarine and coastal waters are subject to strong spatial and temporal changes in trace metal concentrations due to changes in tidal state, and riverine and waste water discharges. Sharp changes in trace metal concentrations may influence dynamic trace metal determinations using the VIP because of memory effects between samples when the voltammetric flow cell is insufficiently flushed, or when insufficient time is allowed for metals in the sample to equilibrate with the agarose gel on the micro-electrode prior to the deposition step. The effect on the peak current response of measurements involving solutions with significantly different metal concentrations was examined by alternate analyses of $NaNO_3$ solutions spiked with Cd(II), Cu(II) and Pb(II) in the range 10–20 nM, and a $NaNO_3$ blank (*i.e.* negligible trace metal content). With a time period of 5 min to achieve equilibration between the sample solution and the agarose gel, and a sample volume of 12.6 mL pumped through the voltammetric cell between measurements, a memory effect was observed (Fig. 4A) resulting in a RSD for the different solutions in the range 1–7% ($n = 3$). An increase in flushing volume between measurements, to 21 mL, alleviated the memory effect for the $NaNO_3$ blank by sufficiently cleaning the voltammetric cell to give RSD < 1% ($n = 3$) for all metals (see Fig. 4B). An increase in gel equilibration time from 5 to 6.5 min for the metal spiked $NaNO_3$ solutions improved the RSD < 2% ($n = 3$) for all metals (see Fig. 4B) by allowing sufficient time for the metals to diffuse through the agarose gel of the μ-AMMIA. These conditions were subsequently used for all further measurements.

Influence of pH on dynamic metal determinations

Estuaries, particularly within the low salinity regions, may be subject to marked variations in pH (*e.g.* 6.5–8.1) as a result of changes in tidal state, biological respiration, primary productivity, riverine and waste water discharges. The effect of pH on the peak current response of dynamic trace metal measurements with the VIP was investigated in metal spiked $NaNO_3$ and seawater. The pH may affect the peak potential during the electrochemical analysis as a result of the Nernstian pH behaviour (*e.g.* 60 mV pH^{-1}) of the type of Ag/AgCl reference electrode used in the VIP system, and the pH has an influence on the negative limit of the polarisation range of Hg.[25] The UV-digested seawater was subjected to an additional filtration step using an 0.1 μm pore size polycarbonate membrane filter (Whatman, Cyclopore); the reasons for which are outlined below. Fig. 5A and B show the peak current as a function of pH in $NaNO_3$ and seawater with added HNO_3 (pH 2–3.2), NH_4Ac buffer (0.01 M; pH range 4–7.1), HEPES buffer ($NaNO_3$ only; 0.01 M; pH 7.8) or no addition (seawater only; pH 8.3). Fig. 5A shows that the pH did not significantly affect the peak current response in $NaNO_3$ over a pH range between 2 and 7.8, and in seawater between pH 2.6 and 4.6 (Fig. 5B). However, a marked drop in peak current response was observed in seawater at pH ≥ 5.4 for Cd(II), Cu(II) and Pb(II).

The stable peak current response with changing pH for $NaNO_3$ is because the only speciation change in the matrices is hydrolysis. For *e.g.* Cu at higher pH values, three hydrated species will be present ($Cu(H_2O)_n{}^{2+}$, $Cu(H_2O)_{n-1}{}^{+}$, $Cu(H_2O)_{n-2}$), with $n = 6$.[26] We can expect that these species have very similar diffusion coefficients and therefore similar peak current responses. Trace metal determinations using the VIP in seawater at pH values below 5.4 yielded similar sensitivity as in $NaNO_3$ because of similar diffusion coefficients of the hydrated metal species (also see Table 1). The pH therefore does not appear to affect the electrochemical measurement of trace metals using the VIP. The decrease in the peak current response at pH ≥ 5.4 for Cd(II), Cu(II) and Pb(II) in seawater could most likely be explained by metal speciation changes in the UV-digested and metal spiked seawater solution. The additional filtration (0.1 μm pore size filter) of the seawater was undertaken in an attempt to remove metal scavenging colloids from the 0.4 μm filtered UV-digested (at pH 8.1) solution. However, at higher pH values, a fraction of the metals

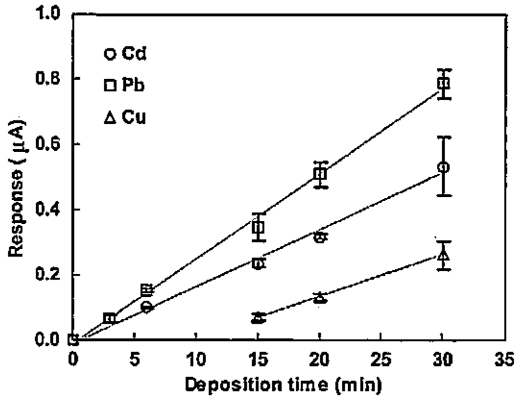


Fig. 3 Effect of deposition time on peak current response in deoxygenated, pH 2, 0.1 M $NaNO_3$ containing 5 nM Cd(II), 5 nM Cu(II) and 5 nM Pb(II).

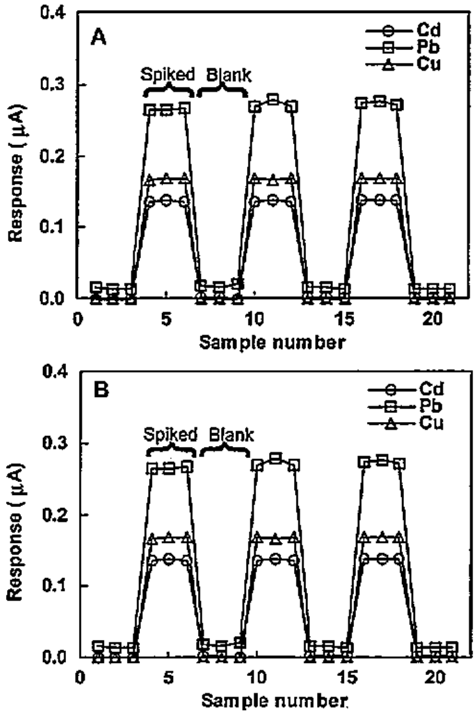


Fig. 4 Effect of repeat changes in sample metal concentrations on peak current response for (A) 5 min gel equilibration time and 12.6 mL flow cell flushing volume between measurements, with deoxygenated, pH 2, 0.1 M $NaNO_3$ containing 10 nM Cd(II), 20 nM Cu(II) and 10 nM Pb(II) (denoted spiked) and $NaNO_3$ blank (*i.e.* negligible trace metal content) (denoted blank), at a deposition time of 15 min, and (B) optimised conditions at 6.5 min gel equilibration time and 21 mL of flow cell flushing volume.

was not detected by the VIP system, probably because they were unable to pass through the agarose gel covering the micro-electrode. If metal colloids were not completely removed during the ultrafiltration step, then the observation of rapid removal at pH ≥5.4 is consistent with sorption of metal onto metal colloids (*e.g.* Mn oxides) in seawater which is free of metal buffering organic ligands.[26]

The pH experiments appear to indicate that Cd(II), Cu(II) and Pb(II) determinations using the VIP system can be undertaken over the pH range that is typically encountered in the estuarine and coastal waters and will yield high quality dynamic metal concentrations.

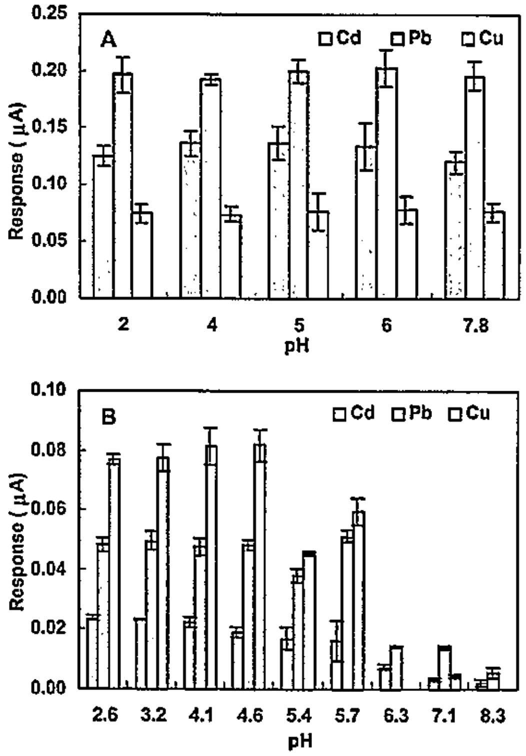


Fig. 5 Effect of pH on peak current response in (A) deoxygenated, 0.1 M $NaNO_3$ containing 10 nM Cd(II), 20 nM Cu(II) and 10 nM Pb(II), and using a 15 min deposition time, and (B) deoxygenated seawater with addition of 4 nM Cd(II), 40 nM Cu(II) and 4 nM Pb(II), and using a 5 min deposition time. Adjustment of pH in $NaNO_3$ and seawater with added HNO_3 (pH 2–3.2), NH_4Ac buffer (0.01 M; pH range 4–7.1), HEPES buffer ($NaNO_3$ only; 0.01 M; pH 7.8) or no addition (seawater only; pH 8.3).

Table 1 The effect of oxygenation and deoxygenation on the slope (peak current (nA)/concentration (nM) × deposition time (min)) of calibration graphs for current response against concentration in pH 2, 0.1 M $NaNO_3$ and seawater containing Cd(II), Cu(II) and Pb(II) in the range 1–10 nM, at a deposition time of 10 min

Matix	Oxygenation	Slope/ nA nM^{-1} min^{-1}	Correlation coefficient
$NaNO_3$	Oxygenated	Cd(II):0.58 Pb(II):1.22 Cu(II):0.45	Cd(II):0.993 Pb(II):0.993 Cu(II):0.999
	Deoxygenated	Cd(II):0.58 Pb(II):1.25 Cu(II):0.47	Cd(II):0.993 Pb(II):0.996 Cu(II):0.997
Seawater	Oxygenated	Cd(II):0.51 Pb(II):1.12 Cu(II):0.43	Cd(II):0.992 Pb(II):0.999 Cu(II):0.993
	Deoxygenated	Cd(II):0.50 Pb(II):1.10 Cu(II):0.43	Cd(II):0.993 Pb(II):0.991 Cu(II):0.995

Influence of ionic strength and salinity on dynamic metal determinations

In estuarine systems, salinity can range from negligible to fully marine (salinity 35–36), and this determines the ionic strength of a sample to which the VIP system is exposed. Variations in ionic strength and salinity may influence trace metal measurements using the VIP as they affect the conductivity of a sample and therefore possibly affect the peak current response for a metal. In addition, it is possible that enhanced ionic interactions with an increase in ionic strength influence the electrochemical response in trace metal measurements. The effect of ionic strength and salinity changes on the peak current response was investigated using solutions of $NaNO_3$ (0.05–0.60 M) and seawater (salinity 2.5–35; dilutions with MQ water) containing Cd(II), Cu(II) and Pb(II) (in the range 5–10 nM). Fig. 6 shows that for $NaNO_3$ (Fig. 6A) and seawater (Fig. 6B) the response was stable over the ionic strengths and salinities examined (RSD < 3% (n = 18) for all metals in $NaNO_3$; RSD < 6% (n = 15) for all metals in seawater). In addition, a t-test on the results showed that there was no significant difference between the peak current responses for the different ionic strength or salinities (99% confidence interval). The absence of an influence of ionic strength on the peak current response can be explained by the low ohmic drop of the µ-AMMIA, which is typical for micro-electrodes because of their very small surface area.[25] Our results demonstrate that dynamic trace metal measurements using the VIP system are unaffected by variations in ionic strength/salinity and that the probe can therefore be deployed *in situ* throughout the full range of salinities encountered in estuarine and coastal waters.

Influence of dissolved oxygen concentration on dynamic metal determinations

The dissolved oxygen (DO) concentration in estuarine waters can change as a result of variations in temperature, salinity, re-

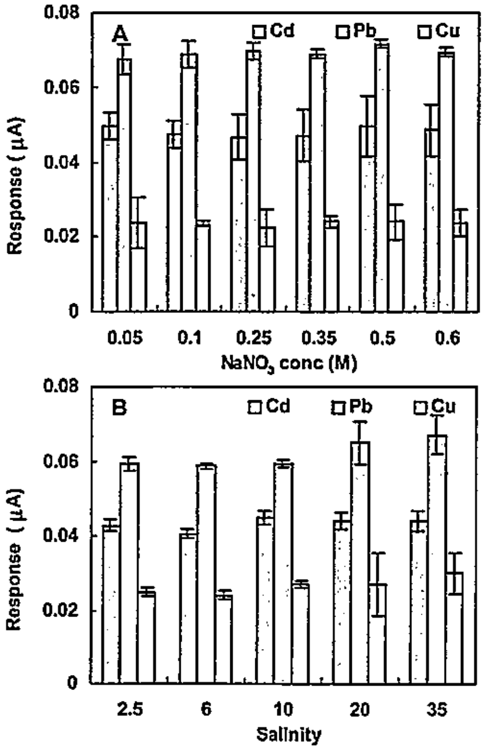


Fig. 6 Effect of ionic strength on peak current response for (A) deoxygenated, pH 2, 0.1 M $NaNO_3$, and (B) deoxygenated, pH 2, seawater. The solutions contained 5 nM Cd(II), 10 nM Cu(II) and 5 nM Pb(II), and a deposition time of 15 min was used.

aeration, photosynthesis, biological respiration and mixing of different water masses. Dissolved oxygen interferes with the voltammetric measurements due to the reduction of oxygen to hydrogen peroxide, resulting in a reduction peak at *ca.* −100 mV. The DO concentrations in saturated waters are much higher ($\sim 3 \times 10^{-4}$ M) than trace metal concentrations and consequently the oxygen reduction current is 4–6 orders of magnitude higher than that produced by trace metals of interest.[25] In addition, a pH increase (to ~11) may occur at the micro-electrode surface during the reduction of oxygen, resulting in the possible formation of insoluble metal oxy-hydroxides[27] and a decrease in sensitivity and reproducibility. In the laboratory the oxygen interference is removed by deoxygenation of the sample using N_2 (g), but this degassing procedure is not applicable to *in situ* measurements. The VIP instrument minimises oxygen interference by a combination of background subtraction and the use of SWASV at a fast frequency (200 Hz is the optimal scanning rate[15]). In the first approach the background current is subtracted from the stripping current, and the second approach makes use of the strongly reduced sensitivity of fast SWASV to irreversible processes (*e.g.* oxygen reduction).[25] However, the combined approach does not eliminate the reduction of oxygen during the voltammetric scan, with the consequent pH increase at the electrode surface. Therefore, it is applicable to well-buffered aquatic media, such as seawater,[25] but in the case of natural (unbuffered) freshwater an additional *in situ* deoxygenation step needs to be used.[10]

To examine the influence of DO on trace metal determinations by the VIP instrument, solutions of $NaNO_3$ and seawater, both oxygen saturated and deoxygenated (N_2 degassed), containing Cd(II), Cu(II) and Pb(II) in the range 5–15 nM were analysed. The peak current response was similar for oxygen saturated and deoxygenated solutions in both matrices, and no differences in sensitivity or slope of calibration graphs were observed for the metals (Table 1). The results show that the sensitivity of the VIP is not dependent on the DO concentration in $NaNO_3$ and seawater. This is critical for VIP deployments in estuarine and coastal waters where significant gradients in DO concentrations may be encountered.

Influence of temperature on dynamic metal determinations

The temperature in the water column of estuarine and coastal systems in mid-latitudinal regions typically varies between 4 and 25 °C. In order to obtain reliable trace metal determinations from *in situ* VIP measurements, the influence of the temperature on the peak current response needs to be established. Changes in the Hg drop geometry can be neglected in the temperature range between 4 and 25 °C, but the diffusion of trace metals through the agarose gel to the micro-electrode is temperature dependent,[12] as shown by the Stokes–Einstein relationship:

$$D \propto \frac{RT}{6N_A r\pi} \exp\left[\frac{-E_a}{RT}\right] \qquad (1)$$

where D is the diffusion coefficient, r is the radius of the species of interest, R is the gas constant, T is temperature, E_a is the activation energy and N_A is Avogadro's number. For the micro-electrode used in this study, the current response (i) should be proportional to D, and consequently for reversible, diffusion-controlled systems, a linear relationship is expected[12] between $\ln(i)$ and $1/T$. The quantitative relationship between the peak current response, i, and temperature therefore should follow the Arrhenius equation:

$$i = i_0 \exp\left[\frac{-\Delta G^*}{RT}\right] \qquad (2)$$

In order to verify this behaviour, the VIP system was placed in a temperature controlled room and the temperature was varied in the range 4–23 °C in oxygen saturated $NaNO_3$ and seawater, containing Cd(II), Cu(II) and Pb(II) in the range 5–10 nM. Indeed, linear responses ($R^2 > 0.98$) were observed for the trace metals by plotting $\ln(i)$ against $1/T$. Experimentally determined slopes were compared with theoretical slopes[12] for reversible systems, as shown in Table 2. For each metal in both matrices, the values for the slope were in good agreement with the theoretical values, thereby verifying that the metal ion oxidation–reduction step is completely reversible in both matrices over the range of temperatures tested. The obtained slopes are used for temperature corrections of dynamic trace metal data obtained during *in situ* deployment of the VIP probe in marine waters.

Stability test for long-term dynamic metal determinations

The long-term *in situ* deployment of the VIP system is important for biogeochemical studies in dynamic and complex estuarine and coastal systems which require high temporal and/or spatial data resolution, and for use of the probe as an early warning system against accidental waste water discharges in these waters. Probe stability may be influenced by deterioration with time of the Hg film and/or the micro-electrode array surface. To examine the stability of measurements using the VIP probe with time, determinations of Cd(II), Cu(II) and Pb(II) in the range 5–10 nM in $NaNO_3$ and seawater were undertaken. These laboratory measurements were made hourly for the first 12 h with subsequent measurements every 12 h over a period of 5 days, using a deposition time of 10 min. Precision remained good over this period to yield an RSD (n = 20 for all three metals in both matrices) of 3.1% and 10.0% for Cd(II), 4.4% and 9.9% for Cu(II) and 2.1% and 6.6% for Pb(II) in $NaNO_3$ and seawater, respectively. The good stability of peak current response for trace metal determinations using the VIP probe is an important quality with respect to the applicability of the system for *in situ* measurements over periods of days to weeks.

Analytical figures of merit

The limits of detection (LOD) for Cd(II), Cu(II) and Pb(II) were determined using low trace metal $NaNO_3$ and seawater. The LOD (n = 3), defined as 3σ, together with the precision at the LOD, and linear range and correlation coefficient for the conditions used for the LOD determination, are shown in Table 3. These LOD values are in reasonable agreement with values

Table 2 Theoretical and experimental slopes (in K) of Arrhenius plots for the temperature dependence of the diffusion coefficients calculated by eqn. (1), and experimental SWASV current responses (eqn. (2)) in oxygen saturated, pH 2, 0.1 M $NaNO_3$ and seawater containing 5 nM Cd(II) and Pb(II) and 10 nM Cu(II) at a deposition time of 5 min. Confidence interval represents 1σ at n = 3 for each metal ion in each matrix

	Cd(II)	Pb(II)	Cu(II)
Theoretical slopes for ln(*D*) *versus* 1/*T*	−2555	−2555	−2555
Experimental slopes for ln(*i*) *versus* 1/*T* in $NaNO_3$	−2245 ± 327	−2324 ± 325	−2240 ± 349
Experimental slope for ln(*i*) *versus* 1/*T* in seawater	−2581 ± 351	−2873 ± 423	−2400 ± 336

Table 3 Analytical figures of merit for analysis (n = 3) in deoxygenated, pH 2, 0.1 M $NaNO_3$ and in pH 2 seawater, using a 20 min deposition time. LOD is defined as 3σ

Matrix	Metal	LOD/pM nM^{-1}	Precision (at LOD, %)	Linear range/nM	Correlation coefficient (R^2)
$NaNO_3$	Cd(II)	58 pM	5.1	0.06 to 20.0	0.998
$NaNO_3$	Pb(II)	26 pM	8.4	0.03 to 17.6	0.999
$NaNO_3$	Cu(II)	0.75 nM	5.2	0.75 to 23.0	0.996
Seawater	Cd(II)	23 pM	4.8	0.02 to 21.3	0.995
Seawater	Pb(II)	23 pM	7.1	0.02 to 18.8	0.994
Seawater	Cu(II)	1.13 nM	7.6	1.13 to 24.0	0.989

Table 4 Results of analysis of CRMs using the VIP system, with confidence intervals for measurements quoted as $\pm 2\sigma$

Metal ion	CRM	Certified value/nM	VIP determined value/nM
Cd(II)	SLRS-3	0.116 ± 0.017	0.13 ± 0.01
	SLEW-2	0.169 ± 0.016	0.17 ± 0.02
Pb(II)	SLRS-3	0.415 ± 0.034	0.43 ± 0.001
	SLEW-2	0.130 ± 0.024	0.12 ± 0.01
Cu(II)	SLRS-3	21.3 ± 1.1	22.0 ± 1.1
	SLEW-2	25.5 ± 1.7	25.1 ± 1.2

reported by Tercier-Waeber *et al.*[12] for 0.1 M $NaNO_3$, 15 min deposition time: Cd(II) 50 pM, Cu(II) 200 pM, and Pb(II) (50 pM) (LOD based on a signal to noise ratio of 2). The LOD results for seawater indicate that the VIP system is ideally suited for dynamic trace metal measurements in estuarine and coastal waters, where trace metal concentrations are somewhat elevated compared to open ocean environments. Furthermore, if required for VIP deployments in trace metal contaminated waters, the linear range of the measurements can be extended by decreasing the deposition time.

The accuracy of the trace metal analyses using the VIP system was assessed using CRMs (National Research Council, Ottawa, Canada) for dissolved metals. For this purpose, analyses were undertaken in UV-digested CRMs of a riverine water (SLRS-3) and an estuarine water (SLEW-2). The results in Table 4 indicate a good agreement between the observed and certified values, with confidence intervals for measurements quoted as $\pm 2\sigma$.

In situ deployment of the VIP system in estuarine waters

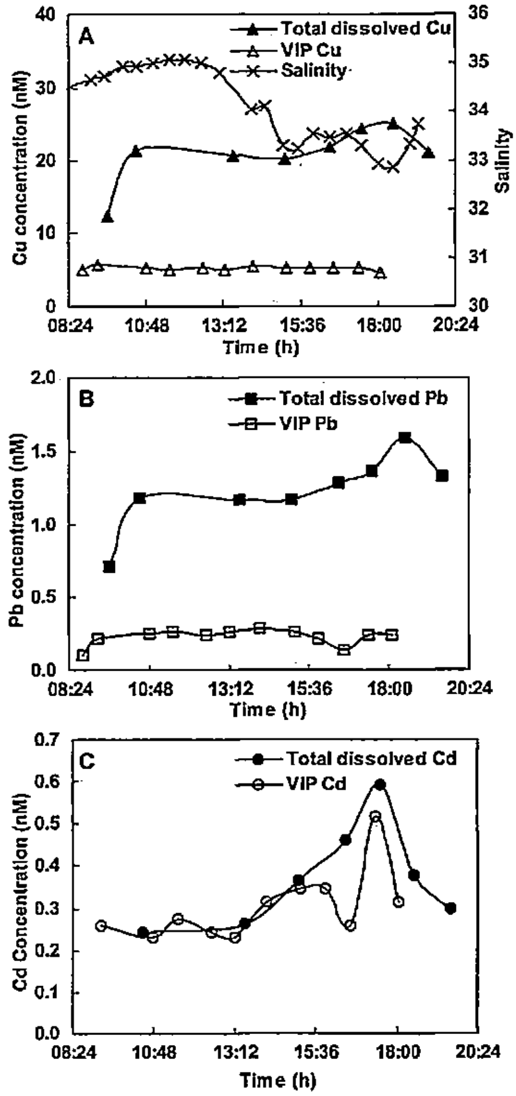


Fig. 7 Time series of dynamic Cd(II), Cu(II) and Pb(II) concentrations determined using the VIP probe over a 12 h period in the Plym Estuary on September 14, 2002. High and low water occurred at *ca.* 12:10 and 18:30 h, respectively. Concentrations of total dissolved metal concentrations determined in the laboratory in discrete samples are included, and salinity is presented in A.

Fig. 7 shows results of dynamic Cd(II), Cu(II) and Pb(II) measurements for an *in situ* deployment of the VIP system at *ca.* 2 m depth at a fixed station (from an anchored vessel) in the Plym Estuary, over a period of 12 h on September 14, 2002. In addition, Fig. 7 presents total dissolved trace metal concentrations (determined in the laboratory in discretely collected samples), and Fig. 7A shows the salinity profile for the deployment. An example of a SWASV measurement showing Cd(II), Cu(II) and Pb(II) peaks, obtained during the *in situ* deployment of the VIP system, is shown in Fig. 8. The Plym is a macrotidal estuary in the south west of England and is characterised by strong variations in water flow and turbidity, and is subjected to enhanced trace metal inputs from a variety of sources, including sewage treatment works, a landfill site, metal contaminated sediments and anti-fouling paints from boats. Concentrations of total dissolved Cd(II), Cu(II) and Pb(II) in the Plym were highest at low water (18:30 h) as a result of the influence of river waters with enhanced trace metal concentrations, in addition to the above mentioned metal sources which are all situated upstream of our VIP probe deployment station. Lower total dissolved metal concentrations were observed at high water (12:10 h), as a result of the inflow of cleaner Atlantic seawater into the estuary. Fig. 7A and B show that the dynamic Cu(II) and Pb(II) concentrations were significantly lower than the total dissolved concentrations. This dynamic fraction is considered bioavailable,[12] and therefore of importance for ecosystem functioning. The difference between these metal fractions constitutes the concentration of metals on/in colloids and small (< few nm) non-labile metal-organic complexes. The

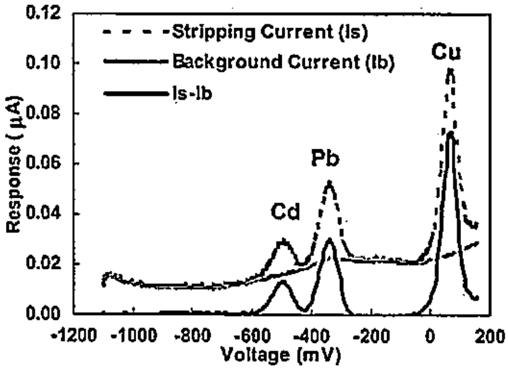


Fig. 8 Example of SWASV measurement of Cd(II), Cu(II) and Pb(II) obtained during *in situ* deployment of the VIP system in the Plym Estuary. Stripping current is depicted by 'Is', background current by 'Ib', and final background corrected current by 'Is–Ib'.

largest difference between the two fractions was observed at low water indicating the importance of freshwater (rivers and waste water discharges) as a source of Cu(II) and Pb(II) containing colloids and non-labile Cu(II)– and Pb(II)–organic complexes. In the case of Cd(II) (Fig. 7C), the differences between the dynamic and total dissolved concentrations were small. This can be explained by the weak complexation of Cd(II) with organic complexes and the dominance of Cd–chloride complexes in marine waters,[28] which are included in the VIP dynamic Cd determination.

The deployment of the VIP system during the survey resulted in high-resolution trace metal data (2 measurements h^{-1}) showing important concentration and speciation changes over a tidal cycle in an anthropogenically perturbed estuary. The VIP enabled unattended, multi-elemental measurements over the 12 h period, with no deterioration of the metal signals because of the protection of the micro-electrode surface by the agarose gel. The deployment of this submersible *in situ* probe therefore provides significant advantages in terms of metal speciation measurements and temporal resolution of the data.

Conclusions

The VIP system can be used for the determination of Cd(II), Cu(II) and Pb(II) in estuarine and coastal waters. The trace metal measurements using the VIP were not affected by changes in pH, salinity and oxygen, whereas an Arrhenius type temperature response was observed which is used for temperature correction of *in situ* dynamic trace metal data. In addition, determinations of trace metals using the VIP system are not affected by pressure in the range 1–600 bar.[14] The limits of detection (3σ) were 23 pM for Cd(II), 1.13 nM for Cu(II) and 23 pM for Pb(II). The procedures were shown to be accurate by obtaining good agreement with certificied values for riverine and estuarine CRMs. The VIP instrumentation was successfully deployed for monitoring dynamic trace metals in estuarine waters over a period of 12 h, without fouling problems of the micro-electrode surface. Field studies with longer deployment periods (1–3 weeks) are planned, to investigate biogeochemical processes in estuarine waters.

Acknowledgements

The authors wish to thank Prof. J. Buffle and Dr M.-L. Tercier-Waeber (University of Geneva), and Mr F. Graziottin (Idronaut, Milan) for their excellent advice and support. Financial support from NERC (GT 4/98/MS/234) and the European Union Framework 5 IMTEC Project (Contract EVK3-CT-2000-00036) is acknowledged.

References

1 A. J. Horowitz, K. R. Lum, J. R. Garbarino, G. E. M. Hall, C. Lemieux and C. R. Demas, *Environ. Sci. Technol.*, 1996, **30**, 954–963.
2 G. E. Millward and A. Turner, in *Trace Elements in Natural Waters*, ed B. Salbu and E. Steinnes, CRC Press, 1995, pp. 223–245.
3 P. G. C. Campbell, in *Metal Speciation and Bioavailability in Aquatic Systems*, ed A. Tessier and D. R. Turner, J. Wiley & Sons, Chichester, 1995, pp. 45–103.
4 E. P. Achterberg and C. B. Braungardt, *Anal. Chim. Acta*, 1999, **400**, 381–397.
5 M. J. Bloxham, S. J. Hill and P. J. Worsfold, *J. Anal. At. Spectrom.*, 1994, **9**, 935–938.
6 S. J. Hill, *Chem. Soc. Rev.*, 1997, **26**, 291–98.
7 C. B. Braungardt, E. P. Achterberg and M. Nimmo, *Anal. Chim. Acta*, 1998, **377**, 205–215.
8 J. Wang, J. Lu, D. Luo, J. Wang, M. Jiang, B. Tian and K. Olsen, *Anal. Chem.*, 1997, **69**, 2640–2645.
9 M.-L. Tercier-Waeber, J. Buffle, M. Koudelka-Hep and F. Graziottin, *Abstr. Pap. Chem. S.*, 2001, **221**, 188–189.
10 M.-L. Tercier-Waeber and J. Buffle, *Environ. Sci. Technol.*, 2000, **34**, 4018–4024.
11 J. Pei, M.-L. Tercier-Waeber and J. Buffle, *Anal. Chem.*, 2000, **72**, 161–171.
12 M.-L. Tercier-Waeber, J. Buffle, F. Confalonieri, G. Riccardi, A. Sina, F. Graziottin, G. C. Fiaccabrino and M. Koudelka-Hep, *Meas. Sci. Technol.*, 1999, **10**, 1202–1213.
13 M.-L. Tercier-Waeber, J. Buffle, F. Graziottin and M. Koudelka-Hep, *Sea Technol.*, 1999, **40**, 74–79.
14 C. Belmont-Hebert, M.-L. Tercier, J. Buffle, G. C. Fiaccabrino, N. F. de Rooij and M. Koudelka-Hep, *Anal. Chem.*, 1998, **70**, 2949–2956.
15 M.-L. Tercier, J. Buffle and F. Graziottin, *Electroanalysis*, 1998, **10**, 355–363.
16 M.-L. Tercier-Waeber, C. Belmont-Hebert and J. Buffle, *Environ. Sci. Technol.*, 1998, **32**, 1515–1521.
17 M.-L. Tercier and J. Buffle, *Anal. Chem.*, 1996, **68**, 3670–3678.
18 C. Belmont, M.-L. Tercier, J. Buffle, G. C. Fiaccabrino and M. Koudelka-Hep, *Anal. Chim. Acta*, 1996, **329**, 203–214.
19 M.-L. Tercier, N. Parthasarathy and J. Buffle, *Electroanalysis*, 1995, **7**, 55–63.
20 A. G. Howard and P. J. Statham, in *Inorganic Trace Analysis: Philosophy and Practice*, ed. A. G. Howard and P. J. Statham, J. Wiley & Sons, Chichester, 1993, pp. 182.
21 E. P. Achterberg, C. B. Braungardt, R. C. Sandford and P. J. Worsfold, *Anal. Chim. Acta*, 2001, **440**, 27–30.
22 M.-L. Tercier and J. Buffle, *Electroanalysis*, 1993, **5**, 187–200.
23 C. M. G. van den Berg, *J. Electroanal. Chem.*, 1986, **215**, 111–121.
24 C. M. G. van den Berg, *Anal. Chim. Acta*, 1991, **250**, 265–276.
25 J. Buffle and M.-L. Tercier-Waeber, in *In situ Monitoring of Aquatic Systems*, ed J. Buffle and G. Horvai, J. Wiley & Sons, Chichester, 2000, pp. 279–406.
26 W. Stumm and J. J. Morgan, in *Aquatic Chemistry; Chemical Equilibria and Rates in Natural Waters*, ed W. Stumm and J. J. Morgan, J. Wiley & Sons, Chichester, 1996, pp. 516–608.
27 J. Buffle, *J. Electroanal. Chem.*, 1981, **125**, 273–278.
28 F. Elbaz-Poulichet, J. M. Martin, W. W. Huang and J. X. Zhu, *Mar. Chem.*, 1987, **22**, 125–136.

Voltammetric *in situ* measurements of trace metals in coastal waters

Kate A. Howell, Eric P. Achterberg*, Alan D. Tappin, Charlotte B. Braungardt, Paul J. Worsfold and David R. Turner[1]

School of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Plymouth, PL4 8AA, UK

[1]Department of Analytical and Marine Chemistry, University of Göteborg, S-412 96 Göteborg, Sweden

*Tel: 44-1752-233036; Fax: 44-1752-233035.

E-mail:

The introduction of contaminant monitoring programmes in coastal waters requires sensitive, reliable and robust analytical methods for trace metal measurements. Developments in instrument automation and miniaturisation have resulted in the manufacture of portable electrochemical instrumentation. The instrumentation has been used for continuous trace metal measurements from the banks of estuaries and on-board ships. The most recent developments in flow-through cells with a gel-coated Ir micro-electrode array have resulted in submersible *in situ* voltammetric probes which allow long-term trace metal measurements at subnanomolar concentrations in coastal waters. Such *in situ* probes hold great promise for water quality monitoring in anthropogenically perturbed coastal ecosystems.

Keywords: Trace metals, In situ monitoring, Stripping voltammetry, Coastal waters

1 Introduction

Trace metals play an important role in biological processes, with a range of metals (including Co, Cu, Fe, Zn) being essential for efficient growth and functioning of aquatic organisms [1]. Anthropogenic inputs of trace metals to coastal waters from domestic and industrial waste waters can substantially increase natural background concentrations. At enhanced concentrations, most trace metals become toxic to aquatic organisms [2;3], resulting in perturbations to ecosystem functioning and potentially posing human health risks through seafood consumption.

In Western Europe, legislative measures (Water Framework Directive (WFD) [4] and the Oslo-Paris (OSPAR) Convention [5]) have been implemented to assess and reduce anthropogenic inputs and improve the quality of marine environments. The implementations of the WFD and OSPAR Convention require long term monitoring programs of pollutants (including trace metals). Generally, monitoring activities in coastal waters involve the collection of discrete samples using survey vessels. This approach results in a limited number of collected samples. Estuarine and coastal waters are dynamic, with complex current structures and potentially numerous pollutant sources. Consequently, high frequency or more beneficially continuous pollutant monitoring activities are required commensurate with the marked spatial and temporal variability, necessitating the use of the reliable, accurate, and sensitive analytical techniques.

Stripping voltammetry is such a technique and allows for the direct determination of trace metals in marine waters. The technique is very sensitive (typical detection limits 10^{-9} to 10^{-12} M), has a wide linear dynamic range (typically two orders of magnitude) and can measure several elements simultaneously. Furthermore, the instrumentation can easily be taken into the field as it is small and readily automated.

This article highlights recent advances, trends and applications of stripping voltammetry for the determination of trace metals in coastal waters. There is a discussion of the progression of voltammetric instruments and methods from laboratory to shipboard and to proper *in situ* measurements, illustrating the advantages of this electrochemical approach. Readers are referred to comprehensive text books for detailed information on electrochemical systems [6].

2 Laboratory instrumentation and methods

The monitoring of trace metals in coastal waters typically involves the collection of discrete samples with subsequent land-based laboratory analysis. This process is time consuming and hence expensive. Land-based laboratory techniques for trace metal analyses in seawater include electrothermal atomic absorption spectroscopy [7] and inductively coupled plasma mass spectrometry [8] after matrix removal and sample preconcentration. Anodic and cathodic stripping voltammetric techniques have also been applied in the laboratory, and allow determination of trace metals in seawater with minimal sample handling [9]. The development of automated voltammetric systems has enhanced sample analysis throughput in the laboratory. Voltammetric systems have been described using automated batch methods with a hanging mercury drop electrode (HMDE) as the working electrode [10]. In this approach, using peristaltic and syringe pumps, sample, reagents and standards are pumped in and out of a standard voltammetric cell, which typically has a volume of 10 ml. The advantage of the automated measurements is that each sample is fully calibrated with standard metal additions, thereby enhancing the accuracy of the method. The implementation of flow through cells has resulted in faster sample processing and reduced sample volumes. A flow cell based on a wall jet cell design with an HMDE has been described by Colombo et al. [11], resulting in the measurement of ca. 60 sample h^{-1}.

3 Field instrumentation and methods

The automation of voltammetric trace metal analyses in land-based laboratories has reduced the risk of sample contamination due to the minimisation of sample handling. However, the risk of contamination may still occur during sample collection, handling and storage. An important development has been the use of the continuous sampling of surface waters with subsequent automated voltammetric trace metal determination on the bank of an estuary or bay [12;13] or on-board ship [14;15]. The earliest application of automated shore based monitoring has been reported by Zirino et al. [12], who used anodic striping voltammetry (ASV) on a mercury film electrode (MFE; glassy carbon surface). The continuous sampling approach requires a peristaltic or Teflon bellows pump, acid-cleaned Teflon or PVC tubing, and a float and anchor for positioning the sample inlet during monitoring from an estuarine bank at a fixed point [13]. On a survey vessel, the float and anchor are replaced by a 'fish' which is towed at a depth of ca. 2-4 m [14]. Figure 1 shows the instrumental set-up used during continuous sampling and automated voltammetric analysis. On-line filtration (using 0.4 μm pore size membrane filters) followed by on-line organic matter removal by UV digestion are used to obtain total dissolved trace metal concentrations in marine waters. The HMDE has been used as working electrode in the automated batch [13-15] and flow cell [16] approaches. The closed seawater and reagent flow approach has greatly reduced the risk of sample contamination, and the automation of the method has resulted in an enhanced sample throughput yielding high resolution environmental data. This sampling and analytical approach is valuable in the monitoring of dynamic estuarine and coastal waters, where important temporal and spatial changes in metal concentrations occur. Interactive surveys, through near real-time measurements, can be used to ascertain point and diffuse metal pollution sources. Figure 2 shows the distribution of total dissolved Cu in surface waters of the Gulf of Cadiz, obtained using ship-board voltammetric measurement with continuous underway sampling. This coastal system receives large trace metal inputs from the river-estuarine systems of the Rio Tinto and Odiel and Guadalquivir which are subjected to acid mine drainage [17]. Cathodic stripping voltammetry (CSV) with automated batch analyses using an HMDE was used during this survey, with 8-hydroxyquinoline as the added CSV ligand, and HEPES as pH buffer (final concentrations 15 μM and 10 mM, respectively; pH 7.8). Figure 2 indicates that the highest Cu concentrations were observed near the outflows of the estuarine systems. The high frequency of measurements (4 samples h^{-1}) obtained with the ship-board monitoring approach has resulted in a high spatial resolution of the data, allowing a thorough interpretation of the input and removal mechanisms affecting trace metals in the Gulf of Cadiz.

4 *In situ* electrochemical instrumentation: component considerations

Advances in flow cell and electrode design, and in the manufacturing of novel working electrodes and miniaturisation of instrumentation, has resulted in the development of submersible voltammetric probes during the last decade. These *in situ* voltammetric probes allow unattended deployment in coastal waters, result in a further minimisation of sample handling, allow depth profiles to be undertaken in coastal waters, yield spatial and temporal data sets that allow a more thorough interpretation of biogeochemical cycles, and facilitate rapid detection of pollution incidents when combined with satellite telemetry. A further advantage of the submersible probes is that the *in situ* approach, allows unperturbed trace metal speciation measurements to be undertaken.

A range of *in situ* voltammetric probes have been described in the literature [18-21;21-25], with the majority of the systems using ASV as the preferred electrochemical method. The choice of working electrode has been crucial for the successful deployment of these systems. Probes using MFEs have been superior, and the application of both Hg-film macro ($r > 100$

μm) and micro (r <10 μm) electrodes has been reported. The current trend is towards the use of micro-electrodes as they have a number of advantages over macro-electrodes, including spherical diffusion that permits trace metal measurements in quiescent solutions (i.e. not sensitive to convection in the sample medium), a low ohmic drop, rapid mass transport rates, low capacitive currents and a high signal to noise ratio resulting in an excellent sensitivity [26]. Substrates used for macro- and micro-electrodes include C, Ag, Au, Pt and Ir. Carbon and Au based Hg-film macro-electrodes have been shown to be stable for at least one day, whereas Ir based Hg-film micro-electrodes are stable for more than ten days [27]. The advantages of Ir based Hg-film micro-electrodes originate from the superior stability of the Hg-film on the Ir surface due to the good wetability of Ir, and the low solubility in Hg combined with high resistance to oxidation [28]. Ir based Hg-film micro-electrodes are also stable in open circuit and long-term measurements (3-4 weeks) can be undertaken in natural waters using the same Hg-film [26].

Recent advances in both thin film technology on chips and photolithographic techniques have allowed the mass manufacturing of Ir based micro-electrode arrays, featuring interconnected Ir micro-disc electrodes [29]. A Hg-film is electrochemically coated on the Ir micro-discs (5-10 μm diameter), resulting in Hg hemispheres. The low cost and disposable nature of the Ir micro-electrode array provide advantages over the single Ir micro-electrode, which requires careful mechanical polishing on a regular basis, unlike the array electrodes. However, current micro-electrode array designs have shown no gain in sensitivity compared with the single Ir micro-electrode.

5 *In situ* electrochemical instrumentation: design and applications

The first fully submersible *in situ* voltammetric monitor was deployed for measurements of trace metals in the marine waters of San Diego Bay [18]. The probe consisted of a flow-through

Plexiglas voltammetric cell, a Plexiglas submersible housing and a control box with its communication cord. The use of a glassy carbon based Hg-film macro-electrode in the probe allowed for continuous measurements using ASV of Cu, Pb, Cd and Zn, with limits of detection (LODs) of 3-4 pM for each metal.

Wang and co-workers have reported the deployment of *in situ* probes for trace metal (Cr, Cu, Hg, Ni, U) measurements in natural waters (river, sea and ground water), utilising both stripping potentiometry [19-21] and stripping voltammetry [21;22]. Hg-film coated macro-electrodes [20;22] and bare gold micro-electrodes [19;21] have been used as working electrodes in these electrochemical probes. The probes form a major advance in *in situ* trace metal measurements in natural waters, but most of the systems lack the sensitivity required for many coastal waters (nanomolar to picomolar concentrations).

An early environmental application of a voltammetric probe with an Ir based micro-electrode array was reported by Herdan *et al.* [23]. An electrode consisting of 20 mercury plated interconnected Ir micro-discs (10 μm diameter) [30] was used for the determination of Cu and Pb in groundwater. The micro-electrode and electrochemical hard and firmware were housed in a PVC tube. *In situ* Cu and Pb measurements showed an excellent correlation with laboratory analysis of filtered groundwater samples. However, interferences from dissolved organic matter were reported during *in situ* analyses, leading to a reduced sensitivity.

The voltammetric *in situ* probe used in the laboratories of the authors is the VIP (voltammetric *in-situ* profiling) system. This probe is the first commercially available system for trace metal measurements in natural waters and was developed by Buffle and co-workers [24;25]. The submersible probe consists of an upper housing (manufactured from Delrin) containing electronic hardware and firmware, a flow-through voltammetric cell (Plexiglas) and a lower housing (Delrin) containing a preamplifier and peristaltic pump (Fig. 3). The voltammetric cell holds a three electrode system which is contained in an internal flow-through cell (working micro-electrode and built-in Pt ring) and an outer cell (reference electrode), both are held together by a cell cover (see Fig. 4). The compartment between the inner and outer cells is filled with agarose/$NaNO_3$ gel, and acts as a pressure equaliser through the pressure compensator, as a double bridge between the sample and the agarose gel reference electrode (through two ceramic junctions) and as a shield for the micro- and counter electrodes [24].

The micro-electrode is the heart of the VIP, and the use of both an Ir disc micro-electrode of a few μm diameter (μ-AMMIE) [24;27], and an Ir micro-electrode array (μ-AMMIA) [29] have been reported. The micro-electrode array consists of 100 interconnected Ir micro-discs with a diameter of 5 μm and a centre to centre spacing of 150 μm. Both types of microelectrodes are coated with a layer of agarose gel (LGL, 1.5%) with a pore size of a few nanometres [31;32]. The gel thickness is between 400 and 600 μm for the μ-AMMIE and 300 μm for the μ-AMMIA. The gel acts as a semi-permeable membrane, permitting diffusion of dissolved metal ions and small complexes whilst hindering the diffusion of colloids and macromolecules to the micro-electrode surface. The gel provides protection against organic and surfactant fouling, thereby allows long-term VIP deployment, but importantly allows trace metal speciation measurements. Hg semi-drops are electrochemically plated through the gel layer onto the Ir sensor surface and the Hg-coating is stable for up to three weeks [33]. The life-time of the gel and μ-AMMIA is up to 1 month.

The VIP system can be deployed at depths of up to 500 m. An armoured coaxial cable is used for communication between the probe and a laptop computer. The VIP system is connected to an 12 V marine battery that enables continuous deployment for 4 days. A calibration deck unit (see Fig. 3) is used in the laboratory and on-board ship for renewal of the Hg-layer, calibration of the VIP system and analysis of discrete samples and standard solutions.

Trace metal measurements with the VIP involve pumping sample through the voltammetric cell, followed by an equilibration period (between 5 and 30 min) during which the preconcentration of the metals in the Hg takes place, with subsequently a square wave anodic voltammetric stripping step inside the gel. Simultaneous determinations of Cd, Cu, Pb, and Zn can be undertaken, with LODs of 50 pM, 200 pM, 50 pM [24] and 400 pM (unpublished), respectively, with a 15 min preconcentration time. The sensitivity of the system for seawater measurements remains unperturbed by salinity, pH, dissolved oxygen and pressure [33]. However, the transport of the analyte to the Hg-film and the reaction kinetics are influenced by temperature, and hence an Arrhenius temperature correction must be made for *in situ* measurements [33].

VIP systems have been employed in coastal waters by the research groups of the Universities of Plymouth and Göteborg. Figure 5a, b show *in situ* measurements of trace metals and salinity undertaken at ca. 2 m depth at a fixed station (from an anchored vessel) in the Tamar Estuary (UK), over a period of 7 h on 15/11/2001. The Tamar is subject to run-off from disused metalliferous mines and consequently has enhanced trace metal concentrations [34]. Concentrations of Cd, Cu and Pb were highest at low water (ca. 13:00 h) as a result of the influence of freshwaters with enhanced trace metal concentrations. The lowest concentrations for all metals were observed at high water (ca. 17:00 h), as a result of the inflow of cleaner Atlantic seawater into the Tamar Estuary.

The trace metal fraction determined by the VIP is termed 'dynamic', and includes free ions and small complexes with a size smaller than a few nm (passing through the agarose gel) [25]. This dynamic fraction is considered bioavailable [25], and therefore of importance for ecosystem functioning. The agarose gel on the micro-electrodes excludes the permeation of colloids and larger particles. The concentration of metals on/in colloids and small (< few nm) non-labile metal-organic complexes [26] can be obtained as the difference between measurements of VIP 'dynamic' metal and total filterable metal from discretely collected samples which have been filtered (0.4 μm pore size membrane filter), acidified (pH 2) and UV-digested [35] prior to analysis. Figure 5b shows total filterable Cu and VIP 'dynamic' Cu concentrations for the study in the Tamar Estuary. The 'dynamic' Cu concentrations were much lower than the total filterable Cu concentration. The largest difference between the two fractions was observed at low water indicating the importance of freshwater (rivers and mine run-off) as a source of metal colloids and small non-labile metal-organic complexes.

The application of the VIP during the survey resulted in high-resolution trace metal data (2 measurements h^{-1}) which show the dynamic changes in concentrations as a result of tidal variations. The VIP allowed unattended, multi-elemental measurements for a 7 h period and the deployment of this submersible *in situ* probe provides significant advantages in terms of metal speciation measurements and the temporal resolution of the data.

6 Conclusions and future trends

Large progress has been made with the development of electrochemical probes for *in situ* trace metal measurements in natural waters. The use of new materials and designs for flow cells and micro-electrodes components has greatly improved the reliability, sensitivity and stability of the analytical systems. The automation and miniaturisation of the probes has facilitated their use in fieldwork programmes, providing high quality and high temporal resolution trace metal data. The *in situ* probes can now make an impact on the monitoring programs required for the WFD and the OSPAR Convention.

Further miniaturisation of electrochemical *in situ* probes through 'Lab-on-a-Chip' technology is being undertaken. Recent advances in micro-machining technology have enabled complete laboratory analysis systems to be built on a chip. The group of Wang at

New Mexico University are testing these miniaturised systems for fully submersed and remote deployment with continuous trace metal analysis [36].

Extending the range of metals that can be determined in seawater using ASV (Cd, Cu, Pb and Zn), will improve the monitoring capabilities of the electrochemical *in situ* probes. Recent work has described *in situ* probes for measurement of Cr using CSV [22], and Ni and U using stripping potentiometry [20] with the use of a dialysis membrane to prevent electrode fouling. Further improvements to these systems, resulting in lower LODs will allow their application in dynamic coastal waters experiencing a range of metal concentrations.

In the near future we can expect sophisticated *in situ* voltammetric probes which will undertake simultaneous, unperturbed speciation measurements of separate trace metal fractions and hence allow an improved interpretation of geochemical cycling and biological effects of these elements in coastal waters.

Acknowledgements

The authors wish to thank Prof. J. Buffle and Dr. M.-L. Tercier-Waeber (University of Geneva), and Mr. F. Graziottin (Idronaut Srl) for their first-rate advice and support. Financial support from NERC (GT 4/98/MS/234) and the European Union IMTEC Project (Contract EVK3-CT-2000-00036) is acknowledged.

Figure Captions

Figure 1. Diagram of the continuous underway pumping system with sample pre-treatment (left box), linked to the computer controlled, automated voltammetric metal monitor operating in batch mode (right box).

Figure 2. Total dissolved Zn (in nM) distribution in the Gulf of Cadiz, October 1998. The contour plot was created from ca. 280 on-line measurements, performed during three days of steaming with the vessel *Garcia del Cid.* Axes on the graph depict latitude (y-axis; degrees N) and longitude (x-axis; degrees W).

Figure 3. Schematic diagram of the VIP system.

Figure 4. Schematic diagram of the voltammetric flow-through cell of the VIP system.

Figure 5a. Time series of 'dynamic' Cd and Pb concentrations measured using the VIP probe over a 7 h period in the Tamar Estuary. Low and high water occurred at ca 13:00 and 17:00 h, respectively.

Figure 5b. Time series of salinity, and concentrations of total filterable (measured in lab in discrete samples), and 'dynamic' Cu measured *in situ* using the VIP probe, over a 7 h period in the Tamar Estuary.

Reference List

1, A.Butler, Science 281 (1998) 207.

2, L.E.Brand, W.G.Sunda, and R.R.L.Guillard, J.Exper.Mar.Biol.Ecol. 96 (1986) 225.

3, P.G.C.Campbell, in: Interactions between trace metals and aquatic organisms: A critique of the free ion activity model, eds. A.Tessier and D.R.Turner, Metal speciation and bioavavailibility in aquatic systems (John Wiley & Sons, Chichester, 1995) pp. 45-103.

4, European Commission, Official Journal of the European Commission L327 (2002) 1.

5, OSPAR Commission. Quality Status Report 2000. 111, 1-108. 2000. London, OSPAR Commission. Assessment and Monitoring.

6, P.T.Kissinger and W.R.Heineman, Laboratory techniques in electroanalytical chemistry (Marcel Dekker, New York, 1995).

7, L.G.Danielsson, B.Magnusson, and S.Westerlund, Analytical Chemistry 98 (1978) 47.

8, M.J.Bloxham, S.J.Hill, and P.J.Worsfold, Journal of Analytical Atomic Spectrometry 9 (1994) 935.

9, Achterberg, E. P and Braungardt, C. B. Analytica Chimica Acta (400), 381 1999.

10, E.P.Achterberg and C.M.G.van den Berg, Analytica Chimica Acta 284 (1994) 463.

11, C.Colombo, C.M.G.vandenBerg, and A.Daniel, Analytica Chimica Acta 346 (1997) 101.

12, A.Zirino, S.H.Lieberman, and C.Clavell, Envir.Sci.Technol. 12 (1978) 73.

13, D.-J.Whitworth, E.P.Achterberg, M.Nimmo, and P.J.Worsfold, Anal.Chim.Acta 371 (1998) 235.

14, C.Braungardt, E.P.Achterberg, and M.Nimmo, Anal.Chim.Acta 377 (1998) 205.

15, E.P.Achterberg, C.Colombo, and C.M.G.van den Berg, Cont.Shelf Res. 19 (1999) 537.

16, E.P.Achterberg, C.Colombo, and C.M.van den Berg, Continental Shelf Research (2002), in press.

17, F.Elbaz-Poulichet, N.H.Morley, A.Cruzado, Z.Velasquez, D.Green, E.P.Achterberg, and C.B.Braungardt, The science of the total environment 227 (1999) 73.

18, M.L.Tercier, J.Buffle, A.Zirino, and R.R.de Vitre, Anal.Chim.Acta 237 (1990) 429.

19, J.Wang, N.Foster, S.Armalis, D.Larson, A.Zirino, and K.Olsen, Analytica Chimica Acta 310 (1995) 223.

20, J.Wang, J.Lu, D.Luo, J.Wang, M.Jiang, and B.Tian, Anal.Chem. 69 (1997) 2640.

21, S.Daniele, C.Bragato, M.A.Baldo, J.Wang, and J.Lu, Analyst 125 (2000) 731.

22, J.Wang, J.Wang, J.Lu, B.Tian, D.MacDonald, and K.Olsen, Analyst 124 (1999) 349.

23, J.Herdan, R.Feeney, S.P.Kounaves, A.F.Flannery, C.W.Storment, G.T.A.Kovacs, and R.B.Darling, Environ.Sci.Technol. 32 (1998) 131.

24, M.L.Tercier, J.Buffle, and F.Graziottin, Electroanalysis 10 (1998) 355.

25, M.-L.Tercier-Waeber, J.Buffle, F.Confalonieri, G.Riccardi, A.sina, F.Graziottin, G.C.Fiaccabrino, and M.Koudelka-Hep, Meas.Sci.Techno. 10 (1999) 1202.

26, J.Buffle and M.-L.Tercier-Waeber, in: In situ monitoring of aquatic systems: chemical analysis and speciation, eds. J.Buffle and G.Horvai, In situ monitoring of aquatic systems: chemical analysis and speciation, vol. 6 (John Wiley & Sons, Chichester, 2000) pp. 279-405.

27, M.L.Tercier, N.Parthasarathy, and J.Buffle, Electroanalysis 7 (1995) 55.

28, S.P.Kounaves and W.Deng, Journal of Electroanalytical Chemistry 301 (1991) 77.

29, C.Belmont, M.L.Tercier, J.Buffle, G.C.Fiaccabrino, and M.Koudelka-Hep, Analytica Chimica Acta 329 (1996) 203.

30, S.P.Kounaves, W.Deng, P.R.Hallock, G.T.A.Kovacs, and C.W.Storment, Analytical Chemistry 66 (1994) 418.

31, M.L.Tercier and J.Buffle, Analytical Chemistry 68 (1996) 3670.

32, C.Belmont-Hébert, M.L.Tercier, J.Buffle, G.C.Fiaccabrino, N.F.de Rooij, and M.Koudelka-Hep, Anal Chem 70 (1998) 2949.

33, C.Belmont-Hébert, M.L.Tercier, and J.Buffle, Anal.Chem. 70 (1998) 2949.

34, D.R.Ackroyd, A.J.Bale, R.J.M.Howland, S.Knox, G.E.Millward, and A.W.Morris, Estuar.Coast.Mar.Sci. 23 (1986) 621.

35, E.P.Achterberg, C.B.Braungardt, R.C.Sandford, and P.J.Worsfold, Anal.Chim.Acta 440 (2001) 27.

36, J.Wang, B.Tian, J.Wang, J.Lu, C.Olsen, C.Yarnitzky, K.Olsen, D.Hammerstrom, and W.Bennett, Analytica Chimica Acta 385 (1999) 429.

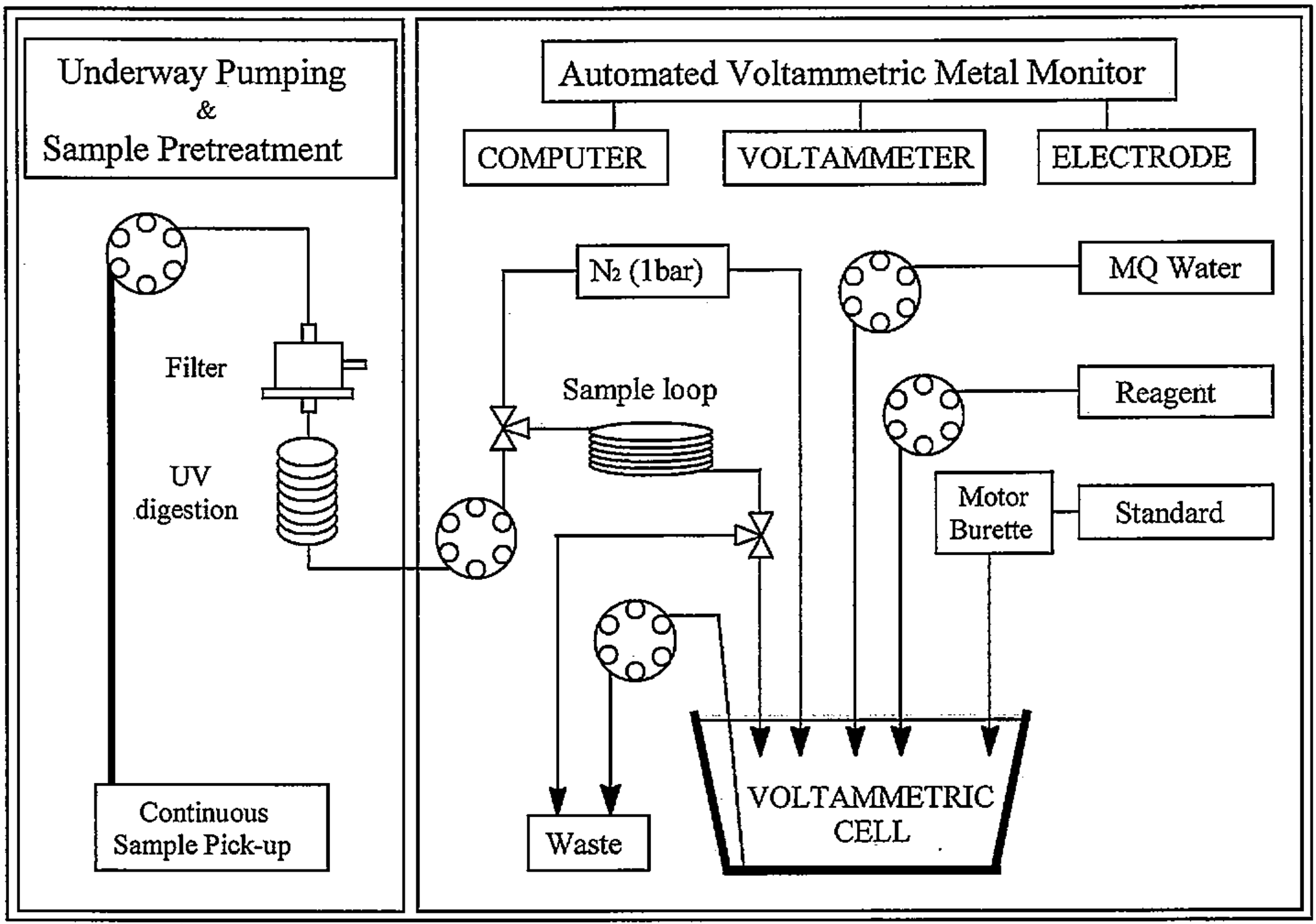

Underway Pumping
&
Sample Pretreatment
Filter
UV
digestion
Continuous
Sample Pick-up
Automated Voltammetric Metal Monitor
COMPUTER
VOLTAMMETER
ELECTRODE
N₂ (1bar)
MQ Water
Sample loop
Reagent
Motor
Burette
Standard
Waste
VOLTAMMETRIC
CELL

Fig. 1

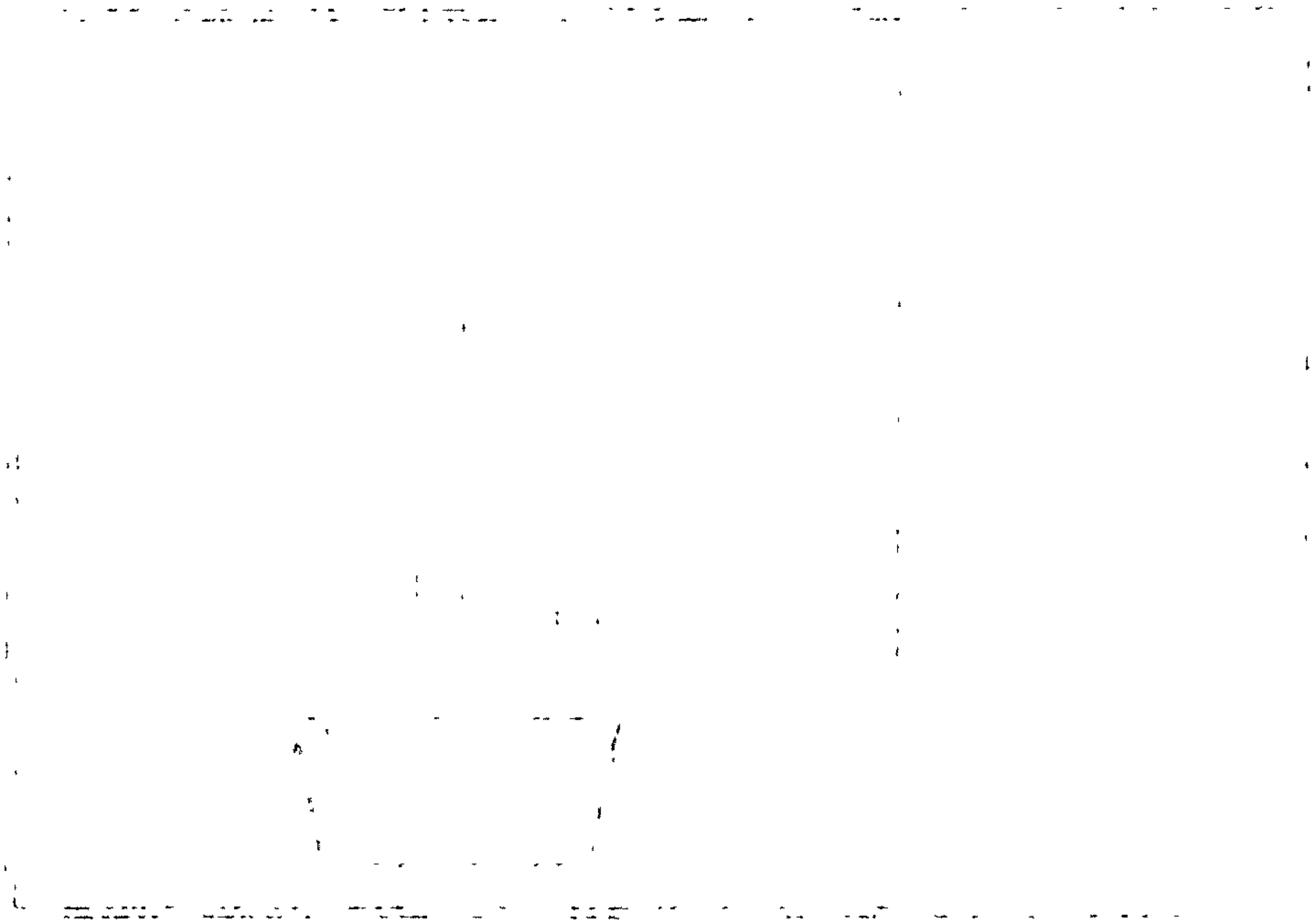
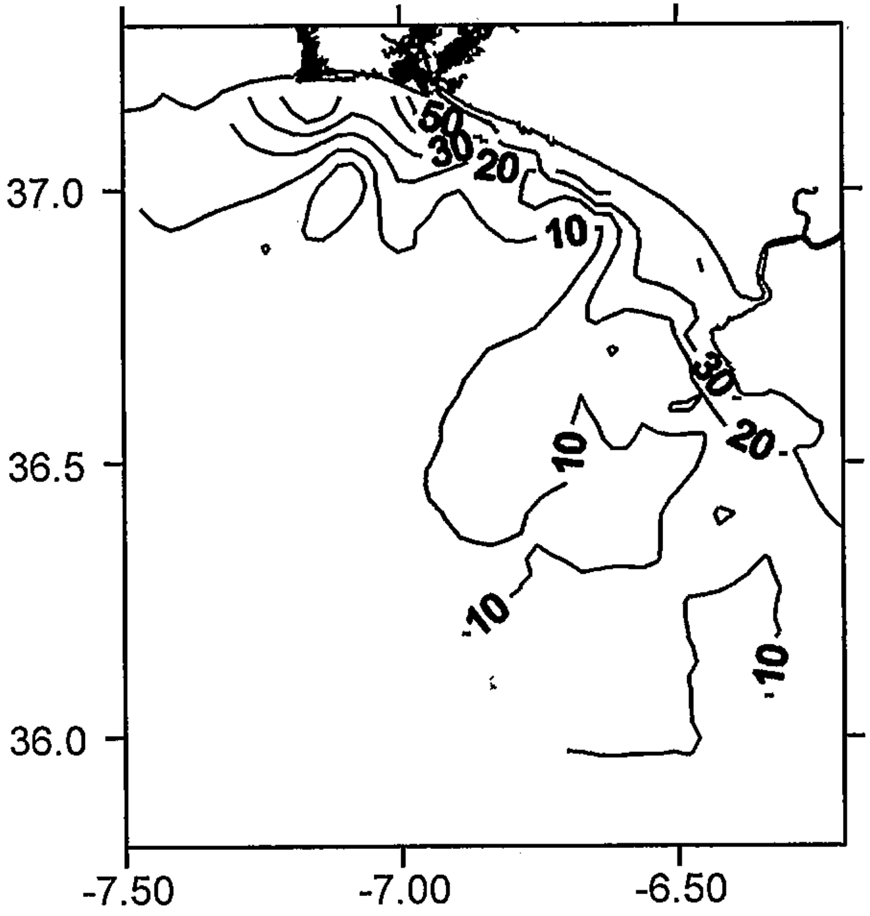


Fig. 2.



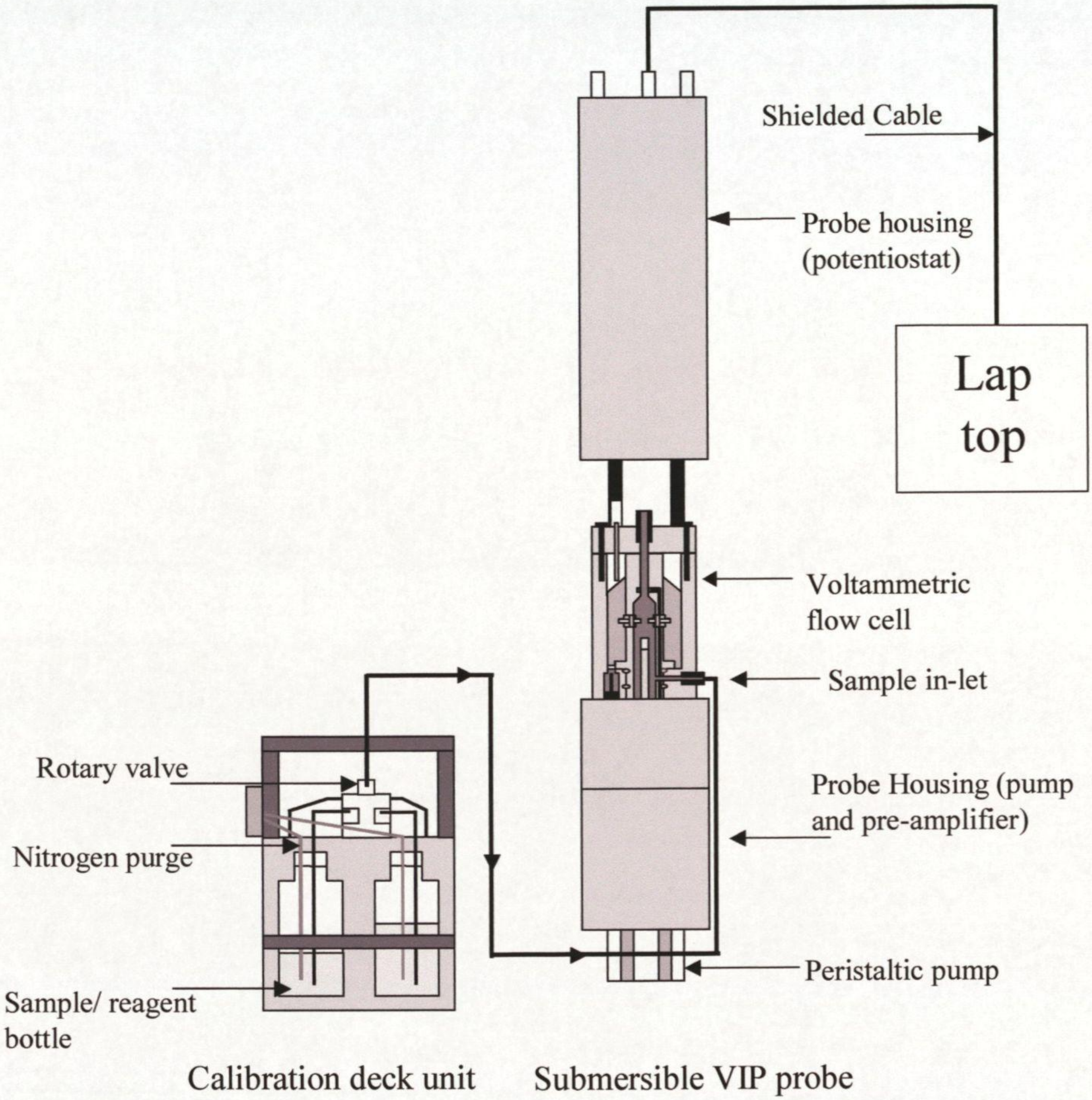
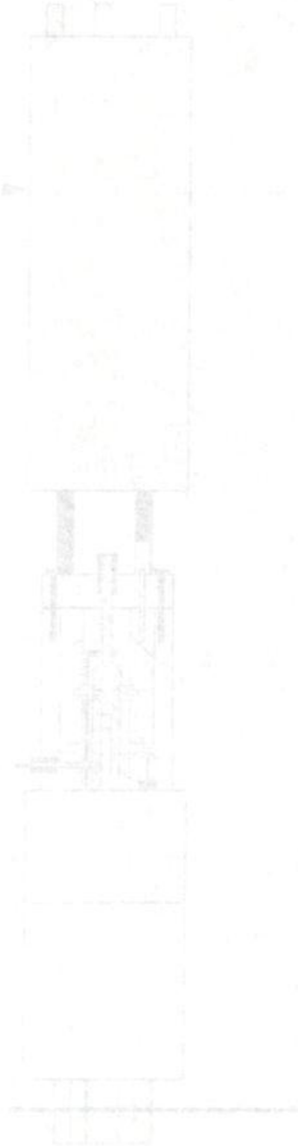
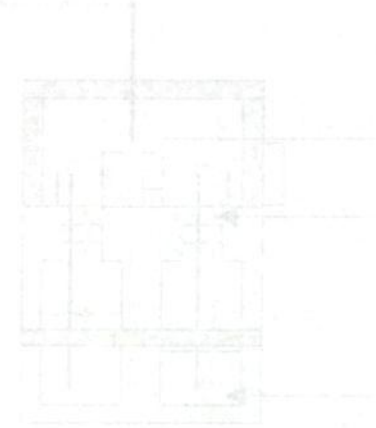

Shielded Cable
Probe housing
(potentiostat)
Lap
top
Voltammetric
flow cell
Sample in-let
Rotary valve
Probe Housing (pump
and pre-amplifier)
Nitrogen purge
Peristaltic pump
Sample/ reagent
bottle
Calibration deck unit
Submersible VIP probe

Fig. 3





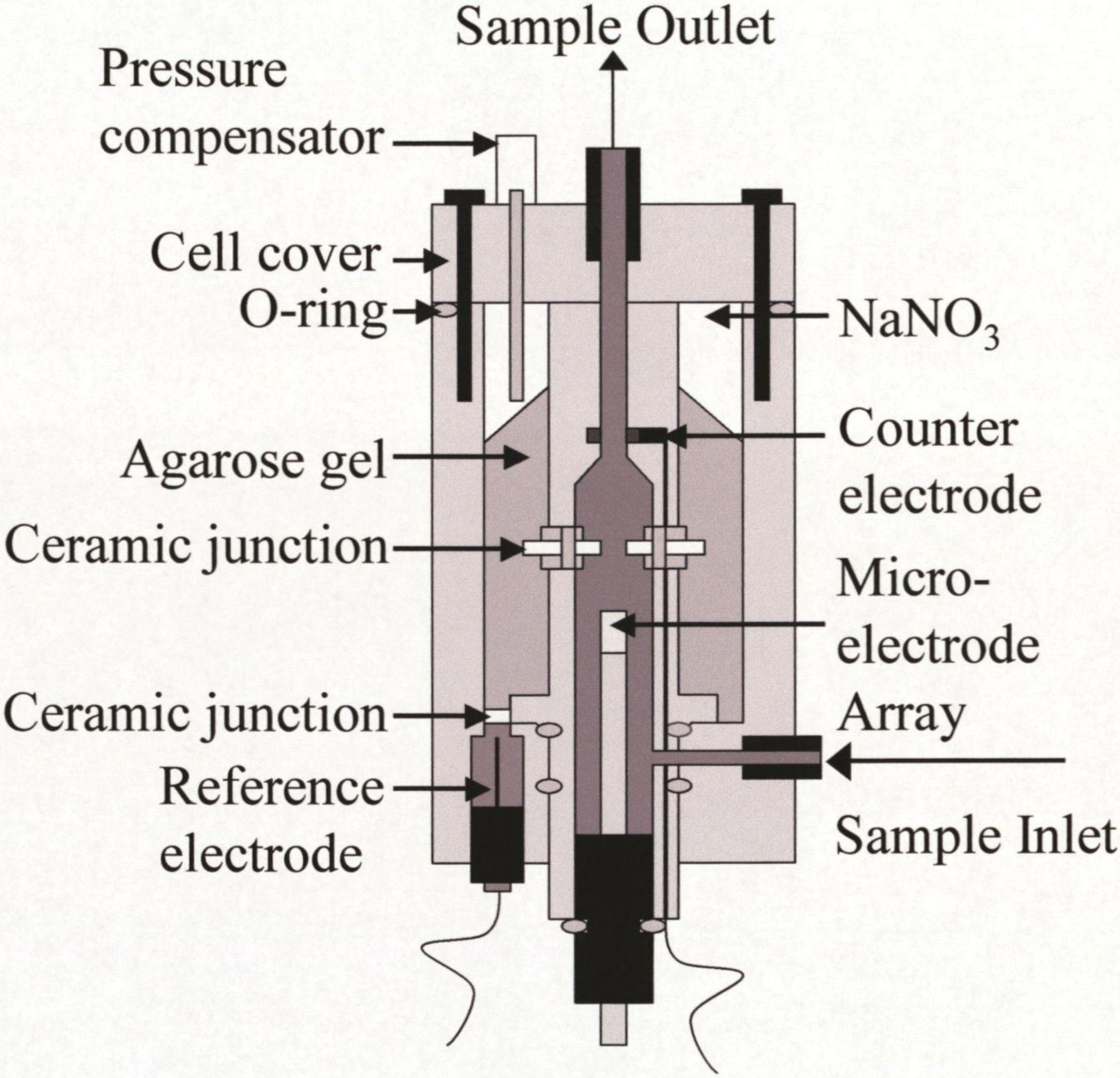

Sample Outlet
Pressure compensator
Cell cover
O-ring
NaNO$_3$
Agarose gel
Counter electrode
Ceramic junction
Micro-electrode Array
Ceramic junction
Reference electrode
Sample Inlet

Fig. 4

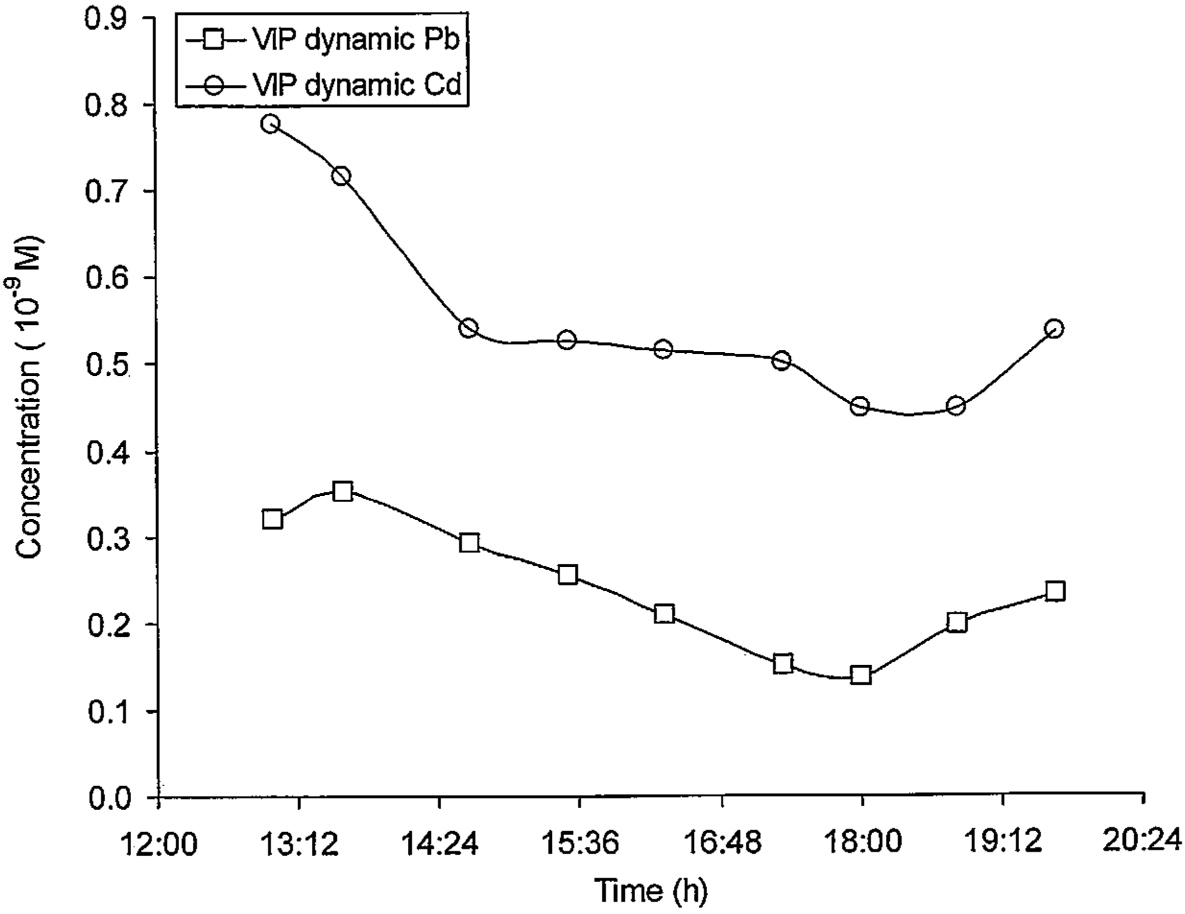
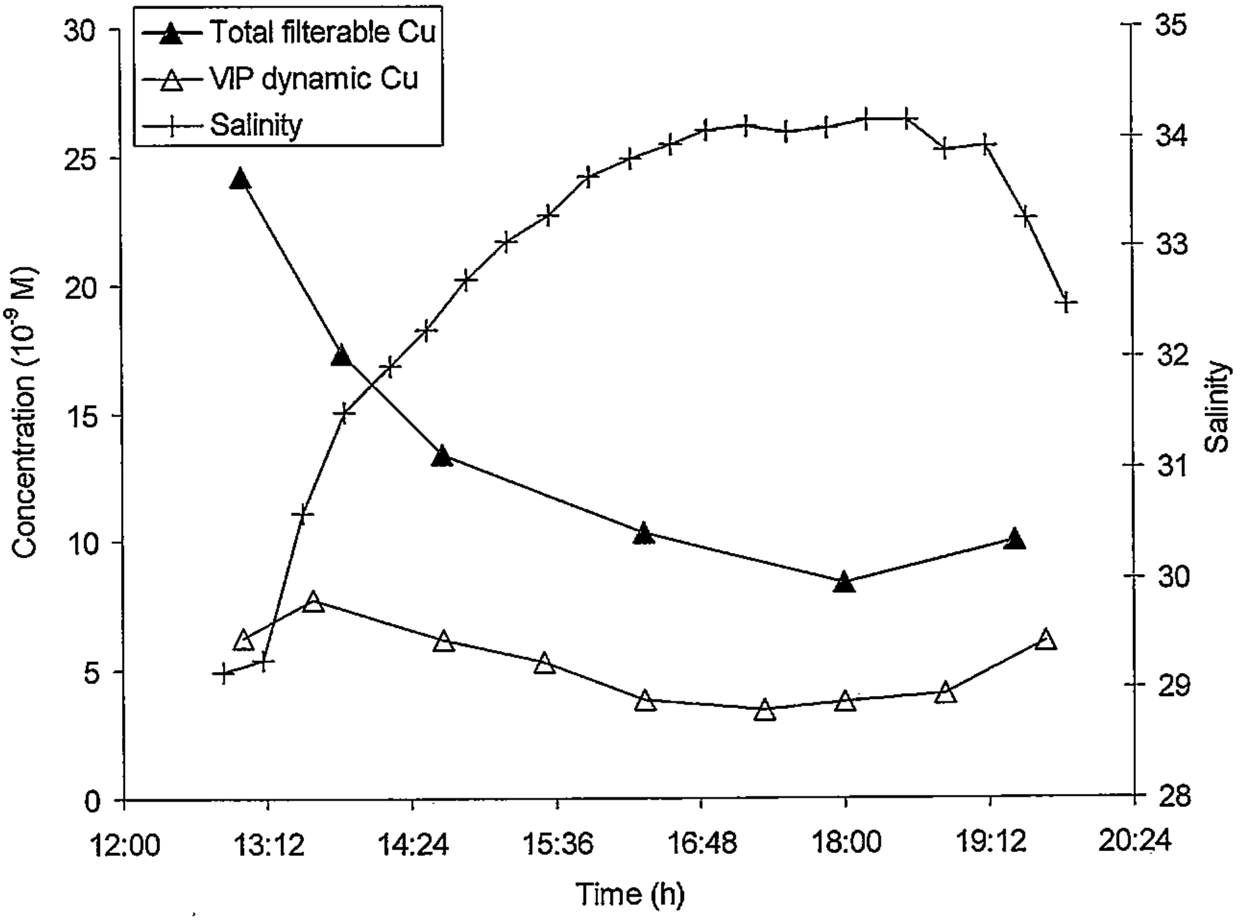

VIP dynamic Pb
VIP dynamic Cd
Concentration (10^{-9} M)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12:00
13:12
14:24
15:36
16:48
18:00
19:12
20:24
Time (h)

Fig. 5a



Total filterable Cu
VIP dynamic Cu
Salinity
Concentration (10^{-9} M)
0
5
10
15
20
25
30
Salinity
28
29
30
31
32
33
34
35
12:00
13:12
14:24
15:36
16:48
18:00
19:12
20:24
Time (h)

Fig. 5b