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Multi Physical–Chemical profiler for real-time in situ monitoring of trace metal speciation and master variables: Development, validation and field applications

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Abstract

This paper describes the development and the application of a submersible Multi Physical–Chemical profiler (MPCP) for in situ measurements of priority trace metal pollutant speciation and master bio-physicochemical variables. It has been designed using advanced voltammetric microsensors, microprocessor, telemetry and “smart” data acquisition and transfer technology. The MPCP allows simultaneous reliable monitoring, down to 150 m over extended period of time, of several major fractions of Cu(II), Pb(II), and Cd(II): (i) the free metal ions which are known to be related to biological uptake, (ii) the dynamic metal species which are potentially also available for organisms, (iii) the total extractable metals which represent the reservoir of metal in the test medium, and (iv) the particulate and colloidal species, by subtracting (ii) of (iii), which play important role in transport properties and residence time. In addition, it measures also pressure, temperature, pH, oxygen, conductivity, salinity, redox E, turbidity and chlorophyll *a*. The MPCP can be controlled either by an operator on board, in automatic mode following pre-programmed parameters, or remotely from a land station when connected to a remote controller installed in a buoy profiler or on a platform.

A detailed description of the system is given. A summary of the laboratory and on-field tests performed to characterize and validate the MPCP measurements is presented. Finally, examples of attended and remote in situ MPCP monitoring/profiling in estuaries and coastal sea waters are reported. The results indicate that the system is reliable and that in situ simultaneous measurements of the three distinct fractions of trace metals can be achieved down to concentrations at the ppt level using square wave anodic stripping voltammetry (SWASV). They also demonstrated the significant advantages of the MPCP in terms of spatial and temporal resolution of data and thus its usefulness for cost effective, more efficient environmental monitoring and pollution control.

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Keywords: Trace metals; Speciation; Microsensors; In situ voltammetric probe; Remote monitoring; Aquatic systems

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1. Introduction

The man-induced contamination of the environment by trace metal ions is widespread all over the world (Nriagu and Pacyna, 1988). These metal ions may form a severe hazard to the normal functioning of these ecosystems as they are not biodegradable and are involved in biogeochemical cycles and distributed in various physicochemical forms. Toxicological impact, linked to bioavailability and bioaccumulation in the food chain, mobility and persistence will depend on the proportion of these different forms which may vary continuously in space and time (Salbu and Steinnes, 1996; Tessier and Turner, 1995; Ure and Davidson, 1995). The measurement of relevant fractions of trace metals in natural waters, in order to improve our understanding of the natural processes governing their contaminant behavior and long-term impact on the aquatic ecosystems, is currently restricted by the nature of the available measurement techniques. The traditional monitoring approach, based upon discrete sampling followed by laboratory analysis, is inadequate due to various reasons: (i) it is very costly (i.e., specialized sampling equipment and expensive facilities such as clean rooms are needed) and time consuming; (ii) reliable speciation measurements are almost impossible due to the numerous possible artifacts that may occur during sampling and sample handling (contamination, loss by adsorption, pressure and/or temperature changes with concomitant pH and redox conditions changes, coagulation of colloidal matter followed by sedimentation of the aggregates and the associated trace metals, physicochemical changes due to microbial activity, etc.); (iii) it does not provide the data frequency required for the study of trace metals in dynamic land/coastal ecosystems and (iv) it does not allow effective monitoring of the pollution discharges into the systems with rapid remedial action. The development of new analytical tools capable of performing in situ, real-time, autonomous monitoring of specific forms of trace elements is thus of prime importance. Such development is a challenging task as it requires techniques that combine high sensitivity and reliability, multi-element analyses, speciation capability, integrity of the samples and unattended operation. Specific technical criteria such as miniaturization and robustness of the equipment, ease of handling and flexibility, rapidity

of data acquisition and transmission as well as low energy consumption are also important factors which have to be taken into account during the development. Voltammetric techniques, amongst a few others recently reviewed (Buffle and Horvai, 2000; Taillefert and Rozan, 2002), fulfill the above requirements.

The feasibility and the usefulness of submersible voltammetric probes for in situ trace metal monitoring in the water column have been reported by several authors (Tercier et al., 1990, 1998; Wang et al., 1995, 1998; Herdan et al., 1998). Most of them were prototypes for which applications have been limited to short-term (typically 1 day) in situ measurements in surface water, i.e., depth < 20 m (Tercier et al., 1990; Wang et al., 1995, Herdan et al., 1998), or even to laboratory measurements (Wang et al., 1998). Their use for long-term monitoring at greater depth was limited in particular by the following problems (Buffle and Tercier-Waeber, 2000): (i) insufficient reliability of the voltammetric sensors, (ii) the use of conventional-sized electrodes (typically electrode with diameter $\geq 100 \mu\text{m}$) which are sensitive to convection in the test media and applicable only in high ionic strength waters ($> 10^{-2} \text{ M}$ such as sea water), (iii) fouling of the sensor surface due to adsorption of natural organic or inorganic matter, (iv) interference from the dissolved oxygen, and/or (v) the use of standard commercially available laboratory equipment (i.e., electrodes, pumps, valves, etc.) which do not withstand pressure. These problems were largely overcome with the commercially available (Idronaut, Milan, Italy) voltammetric in situ profiling system (VIP) developed by Tercier et al. (Tercier et al., 1998; Tercier-Waeber et al., 1999) for autonomous, continuous monitoring down to 500 m. The VIP has been successfully applied in freshwater (Tercier-Waeber et al., 1998, 2002; Tercier-Waeber and Buffle, 2000), groundwater (Pauwels et al., 2002), fjord water (Tercier-Waeber et al., 1999), estuarine and coastal marine waters (Tercier et al., 1998; Howell et al., 2003), in some case up to 1 week. Detection limits at the ppt level for the dynamic fraction of Cu(II), Pb(II), Cd(II), Zn(II) and ppb level for Mn(II), with standard deviation $\leq 10\%$, were reported.

In this paper, we report the development, characterization, validation and environmental applications of a new generation of submersible system, with

extended capability compared to the VIP, called: Multi Physical–Chemical Profiler (MPCP). The MPCP has been designed to allow simultaneous *in situ*, autonomous monitoring of several major fractions (speciation) of priority trace metal pollutants (at its present state Cu, Pb and Cd) as well as master variables (pressure, temperature, pH, oxygen, conductivity, salinity, redox E, turbidity and chlorophyll *a*) down to 150 m. It is based on gel integrated voltammetric microsensors and a multi-channel voltammetric probe as well as advanced microprocessor, telemetry and software technology leading to smart data acquisition, transmission and processing. The characteristics of the microsensors and main components of the probe are summarized. Laboratory tests, and attended and remote field applications of the MPCP for biogeochemical studies in estuaries and sea water are reported. The key features of the MPCP for more effective environmental monitoring and pollution control of priority metal pollutants are discussed.

2. Experimental section

2.1. Description of the MPCP

A picture of the MPCP and the schematic diagrams of its main components are given in Fig. 1a and b–c respectively. The MPCP is based on the VIP System (Tercier et al., 1998). The heart of the VIP probe is a gel integrated microelectrode (GIME), which consists of 100 interconnected Hg-plated Ir-based microelectrode array covered with a 300 μm thick agarose antifouling gel (Fig. 1b; Belmont-Hébert et al., 1998), integrated in a specially designed pressure compensated Plexiglas mini flow-through voltammetric cell (Tercier et al., 1998). The VIP allows the direct measurement of the concentrations of the dynamic fraction of trace metals, defined as the sum of the free metal ions and the small labile complexes with size of few nanometers (Buffle and Tercier-Waeber, 2000). To extend the capability of the VIP to *in situ* trace metal speciation and master variable monitoring, the following work has been performed: (1) development of (i) a complexing gel integrated microelectrode (CGIME; Fig. 1b) for voltammetric measurements of free metal ion concentrations; (ii) a

submersible flow-injection analysis (FIA) system for automatic, on-line sample pre-treatment prior GIME voltammetric measurements of total extractable metal concentrations (Fig. 1c); (iii) an improved probe based on three independent voltammetric measuring channels (Fig. 1c); (2) integration of all these components together with commercially available multiparameter (Idronaut, Milan-Italy) and fluorescence (Seapoint Inc., USA) probes into a single monitoring package, the MPCP, protected by a titanium cage (Fig. 1a). A remote controller has also been developed. Coupling of the MPCP to this controller, installed either in a moored buoy or on a platform, allows MPCP remote monitoring with control from a shore station. The characteristics and performance of the main components of the MPCP are summarized below.

The CGIME consists of 100 interconnected Hg-plated Ir-based microelectrode array covered by a thin 3.5 μm layer of a Microchelex chelating resin (polystyrene immobilized iminodiacetate chelating resin with average bead size of 0.2 μm purchased from CETAC Technology, USA), which in turn is covered by a thick 300 μm antifouling agarose gel layer (Fig. 1b). The CGIME measurements are performed in three steps: (1) equilibration of the sensor with the sample, during this step metals accumulate on the chelating resin in proportion to free metal ion concentrations as the site number of the resin is very small compared to the reservoir of the metal ions in the test natural water; (2) exchange of the sample by an acidic solution, the metals fixed on the resin are released by the acid and immediately electrochemically reduced and pre-concentrated at the Hg plated microsensor array; (3) detection by electrochemical reoxidation performed by square wave anodic stripping voltammetry (SWASV). The automatic control of the CGIME measurements with the MPCP probe was achieved by integrating the sensor in the channel 2 flow-through voltammetric cell coupled to the following fluidic components: a soft bag for medical use (MMS Medical Shop Service, Switzerland) containing the acidic reagent, a two-way rotary valve and a one channel sampling peristaltic pump (Fig. 1c). Detailed descriptions of the CGIME development and principle as well as of its laboratory characterization, optimization and validation in model complexing solutions are reported elsewhere (Nöel et al., submitted for publi-

cation). Examples of characterization of direct CGIME measurements in natural samples are given in the Section 3.2.

The submersible FIA. The schematic diagram of the submersible FIA is given in Fig. 1c. It is comprised of mixing, heating and cooling coils, reagent and waste soft bag containers, a submersible heater

and a three channel sampling peristaltic pump. It was designed based on an analytical procedure involving the coupling of two chemical sample pre-treatment techniques, the competitive ligand exchange and acidification techniques, proposed in the literature to release, at room temperature, metal ions bound to colloids and particles or present as inert complexes.

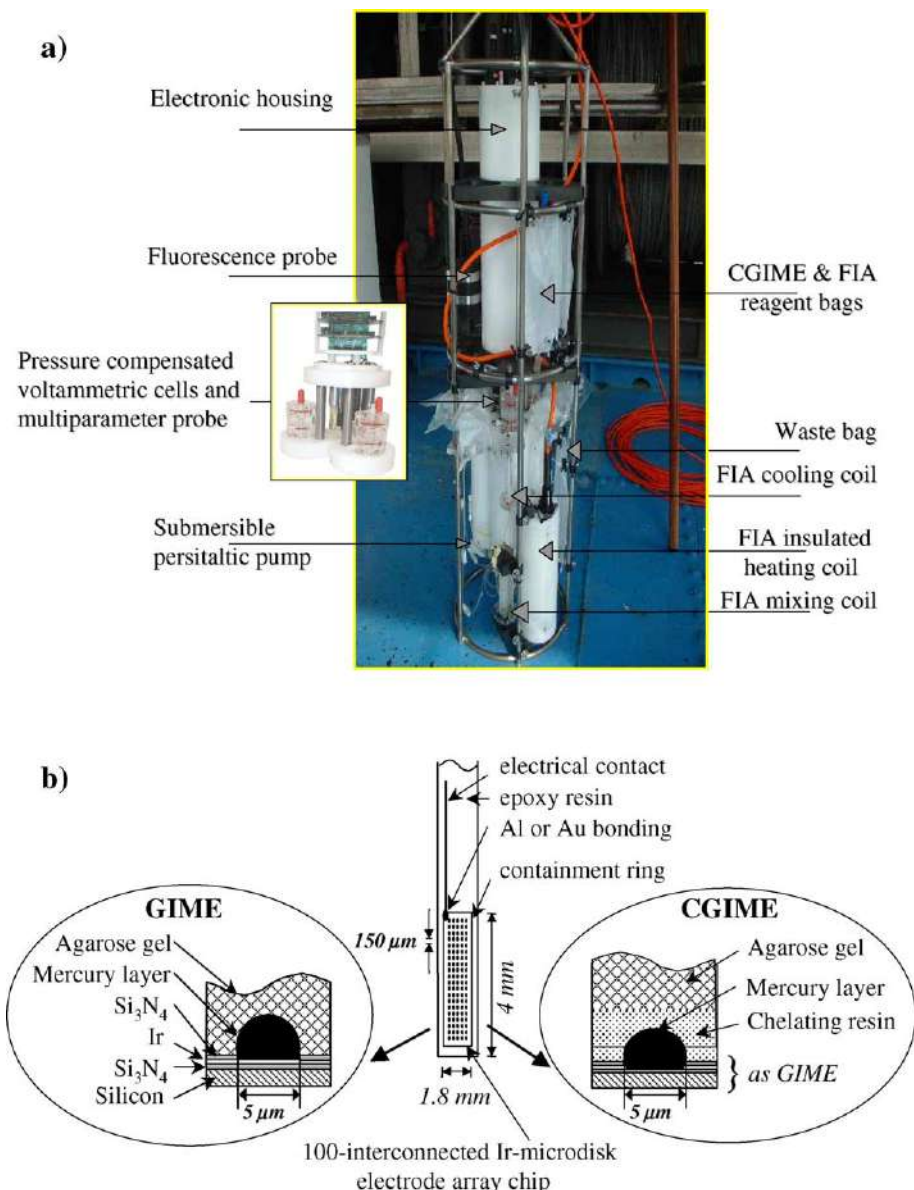


Fig. 1. (a) Photo of the MPCP ready for deployment; (b) schematic diagram of the GIME and CGIME microsensors; (c) schematic diagram of MPCP three individual channel voltammetric probe and its fluidic system.

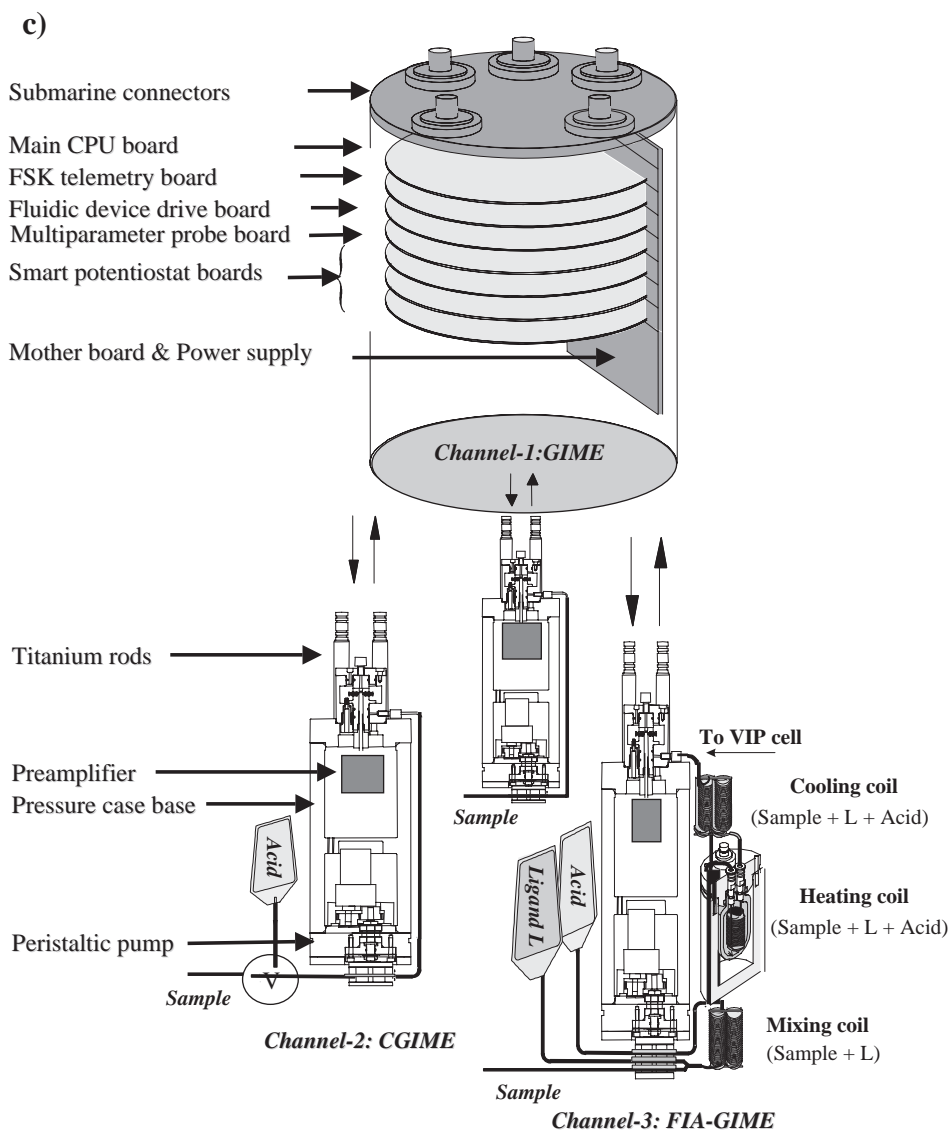


Fig. 1 (continued).

The main steps and principles of this procedure are (1) addition of triethylenetetramine (TRIEN) for competitive exchange with natural ligands, and in particular those which form unstable complexes in acidic media such as humic acid complexes; (2) acidification at pH 2 (HNO_3) to release metal ions bound or complexed; (3) controlled mixing and/or heating of the pre-treated sample to enhance the reaction rate and the efficiency of the metal ion exchange/release. Coupled to the channel 3 of the MPCP voltammetric probe (see

below), it allows in situ, automatic, on-line pre-treatment of the sample prior to the SWASV GIME measurements of total extractable metal concentrations. Detailed descriptions of the analytical and technical developments of the submersible FIA system are reported in (Tercier-Waeber et al., in preparation), and of its characterization and optimization in (Tercier-Waeber et al., in preparation) and the Section 3.2.

The MPCP probe is comprised of distinct specific modules (Fig. 1a, c): an electronic housing; three

pressure compensated flow-through voltammetric cells (Tercier et al., 1998), each one screwed on a pressure case base incorporating a preamplifier for the working microsensor and the motor of a submersible sampling peristaltic pump; integrated standard physical, chemical and optical sensors. The electronic housing contains all the hardware and firmware necessary to manage (i) the simultaneous control of the fluidic system and the SWASV measurement of each cell, (ii) the interfacing of the integrated multiparameter probe and the external fluorescence probe for the measurements of the master variables independently or simultaneously to the voltammetric monitoring of the three metal species, (iii) the data acquisition, storage (into a non-volatile memory having its own battery which guaranty high data retention and protection) and transfer via either RS232 (depth \leq 100 m) or high speed FSK telemetry system (depth $>$ 100 m). The pressure compensated flow-through voltammetric cells are based on a three electrode system: a GIME (channels 1 and 3) or a CGIME (channel 2) working electrode described above; a built-in platinum ring as auxiliary electrode and a home-made manufactured Ag/AgCl/KCl sat. gel/1.5% LGL agarose – 1 M NaNO₃ suprapur gel reference electrode (Tercier et al., 1998). The submersible sampling pumps are based on the specially built VIP peristaltic pump (Tercier et al., 1998) in which optical encoders, for a precise detection of the pump rotor position, and computer control speed of the pump head were added. These technical improvements allow precise control of the sample and reagent flow-rates which, in turn, insures reliable automatic CGIME measurements, FIA on-line sample pre-treatments and FIA sample and reagent dilution factor determination. The tubing used for the fluidic systems is in Teflon (PTFE—polytetrafluoro-ethylene or FET—fluorinated-ethylene-propylene Teflon) or Tygon and the fittings are standard 1/4 · 28 thread fittings made of inert, metal free PEEK (poly-ether-ether-ketone) or Teflon materials (Upchurch Scientific). Power supply of the MPCP and communication between the system and a laptop computer is performed via a single armoured coaxial cable. The system can be connected to either ship board stabilized 220 V or 24 V battery during field trials. The interface between the laptop computer and the MPCP probe is accomplished via a custom-written user friendly Win-

dows management software. This software allows the user, through menus and memo-technique commands, to control and set up the MPCP voltammetric probe operating parameters and functions, such as (i) fluidic and electrochemical parameters, (ii) three channel independent or simultaneous measurements, (iii) calibration, (iv) data “smart” processing (i.e., graphical display, curve smoothing, metal peak current and potential searching, correction of temperature effect (Tercier-Waeber et al., 1999), calculation of metal concentration using pre-configured calibration curves), (v) diagnostic and maintenance operations of the probe components.

The remote controller is comprised of a buoy controller module (BCM), a computer-driven motorized winch and a control land station based on a cellular phone link. The control land station, through its custom-written Windows management software, allows the user to control the MPCP from a shore station. Three different autonomous pre-defined time-scale monitoring/profiling options are available: (i) at fixed depth; (ii) at programmed depths (up to 6); (iii) linear profiling at constant depth step. For the last two measuring options, the motorized winch acts as a lift, moving the MPCP up and down to the next monitoring position. The transfer of the data from the MPCP to the land station can be performed on the user request or automatically at a pre-defined time interval. The land station management software also allows the user to modify the MPCP operating parameters and the remote measuring option as well as to check the status of the MPCP and remote controller components via diagnostic commands. The BCM contains all the hardware and firmware necessary to (i) supervise the monitoring activities of the MPCP, (ii) collect and store the data of the voltammetric, multiparameter and fluorescence probes in a non-volatile memory; (iii) send stored data to the control land station; (iv) up-grade operating and measuring cycle parameters received from the control land station; (v) diagnose and report failures of the MPCP or remote controller components.

2.2. Materials and methods

Certified reference materials (CRMs—National Research Council, Ottawa, Canada) of river water (SLRS-4) and near-shore seawater (CASS-4) were

purchased from LGL Promochem, Germany. Standard stock solutions of 10 μM Cu(II), 5 μM Pb(II) and 6 μM Cd(II) were prepared from atomic absorption spectroscopy 1 g l⁻¹ metal standard solutions (Merk) and acidified to pH 2 using suprapur concentrated HNO₃. All other chemicals were purchased from Merck and were of analytical-reagent grade unless otherwise stated. Water purified with a Milli-Q system (Millipore, 18 $\mu\text{S cm}^{-1}$) was used for the preparation of all solutions. Handling of reagents, solutions and samples was performed in a Class-100 laminar flow hood.

Low density polyethylene (LDPE, Nalgene, Fisher Scientific, Switzerland) reagent and sample bottles and polypropylene volumetric flasks (PP, Nalgene, Fisher Scientific, Switzerland) for solution preparations were cleaned by soaking in 0.1 M HNO₃ suprapur for 24 h, Milli-Q water for 12 h, then two times successively in 10⁻² M HNO₃ suprapur and Milli-Q water for 12 h. After the cleaning steps, the bottles and flasks were filled with 10⁻² M HNO₃ suprapur and stored in plastic bags. Before use, they were thoroughly rinsed and then filled with Milli-Q water and left to equilibrate for at least 1 h. The sampling bottles were rinsed three times with the water sample before filling up.

Discrete samples for complementary laboratory measurements were collected using peristaltic pumps or GoFlo bottles deployed on a Kevlar rope. Filtration through acid washed 0.4 μm pore size polycarbonate membrane was performed on-field. Unfiltered and /or filtered samples, for the measurements of total and/or total dissolved metal concentrations in the laboratory by GIME-VIP/MPCP, were acidified at pH 2 on-board using suprapur concentrated HNO₃ and subsequently UV-digested in laboratory. UV-digestion of the samples, after addition of H₂O₂ (8.8 mM final concentration), was performed in acid pre-cleaned 30 ml quartz tubes for 5 h at 70 °C (400 W medium pressure Hg lamp–Photochemical Reactors, UK) or 12 ml quartz tubes for 2 h at 85 °C (500 W medium pressure Hg lamp–Methrohm UV irradiator 705, Switzerland).

The Hg deposition on the Ir substrates of the gel VIP/MPCP microsensor arrays (see (Belmont-Hébert et al., 1998; Tercier and Buffle, 1996) for gel sensor preparation) was performed at a constant potential of -400 mV in a N₂ deoxygenated 5 mM Hg(CH₃COO)₂, 10⁻² M HClO₄ for the GIMES and 10⁻¹ M HClO₄

for the CGIME solutions using a deposition time of 7 and 8 min respectively. The Hg reoxidation for all sensors was performed by scanning the potential from -300 mV to +300 mV in a N₂ degassed 1 M KSCN solution. The principles and conditions used for the measurements of the distinct trace metal species with the MPCP three individual voltammetric channels are summarized below. *Channel 1-GIME* voltammetric measurements of the dynamic fraction of trace metals are performed in two steps: equilibration of the agarose gel with the sample followed by SWASV metal measurements inside the gel (Tercier et al., 1998). In this latter technique, the target metal ions are first reduced at a constant potential and pre-concentrated in the Hg (deposition step), than their reoxidation peaks are measured by scanning the potential toward more positive values using a square wave modulation. The conditions used were as follows: sample pumping time=3 min (at 7 ml min⁻¹); equilibration time of the agarose gel with the sample=6 min; second pumping stage of 1 min; specific SWASV settings: deposition E =initial scanning E =-1200 to -1100 mV; deposition t =10 to 30 min; final scanning E =-50 to +120 mV. *Channel 2-CGIME* measurements of free metal ion concentrations were performed in three steps: (1) equilibration of the sensor with the sample to accumulate trace metal in proportion to free metal ions on the Microchelex resin; (2) exchange of the sample by a 0.1 M NaNO₃+0.1 M HNO₃ suprapur solution to release trace metal accumulated and (3) SWASV detection of the trace metal released. The conditions used were as follows: Me accumulation time=1 to 2 h with a renewal of the sample in the cell every 15 min; specific SWASV settings: deposition E =initial scanning E =-800 to -900 mV; deposition t =5 min; final scanning E =+80 to +120 mV. *Channel 3-FIA-GIME* measurements of total extractable metal concentration are performed in three steps: (1) on-line sample pre-treatment, based on ligand exchange followed by acidification and heating of the sample, to release trace metal complexed/adsorbed; (2) equilibration of the sensor agarose gel with the pre-treated sample; (3) SWASV detection of total extractable metal concentrations. Two set of conditions, defined as (a) and (b), were used for the on-line sample pre-treatment step: ligand stock solution=(a) 150 μM , (b) 180 μM TRIEN; acid stock solution=(a) and (b)

3.3×10^{-2} M HNO₃ suprapur; sample, ligand and acid flow-rates: 3.5 ml/min, 2.2 ml/min and 3 ml/min corresponding to dilution factors of 2.5, 4, and 2.9 for the sample, ligand and acid respectively (i.e., final reagent concentrations in the pre-treated samples: (a) 37.5 μM and (b) 45 μM TRIEN, (a) and (b) 1.1×10^{-2} M HNO₃); equilibration time sample+ligand=(a) and (b) 2 min; equilibration time sample+ligand+acid at 50 °C=(a) 2 min, (b) 4 min; total FIA pre-treatment time: (a) 6 min, (b) 8 min. Other conditions used were as follows: equilibration time of the agarose gel with the pre-treated sample=6 min; specific SWASV settings: deposition E =initial scanning E =−950 mV; deposition t =5 to 15 min; final E =0 to +120 mV. SWASV conditions common for the three channels were pulse amplitude=25 mV; step amplitude=8 mV; frequency=200 Hz; a second potential scan, performed under conditions identical to those used for the stripping ramp, to allow background current measurement and subtraction (Tercier et al., 1998); a pre-cleaning step of 1 min at final scanning E and an equilibrium step of 30 s at initial scanning E before each stripping (except for CGIME measurements) and background measurements. Calibrations of the three channels were performed in 0.1 M NaNO₃ suprapur electrolyte and synthetic sea water (Instant Ocean, Aquarium Systems, France) spiked with various concentrations of Cu, Pb and Cd.

Total metal concentrations in pH 2 and UV-digested discrete samples was measured in the laboratory using a VIP System. Conditions used for (i) the flushing of the cell and the equilibration of sensor gel prior each measurement, and (ii) the SWASV measurements were as reported above for the MPCP channel 1 and channel 3 respectively.

3. Characterization and validation of the MPCP components

3.1. Reliability and accuracy of the voltammetric multi-channel probe

The reliability and the accuracy of the SWASV measurements of Cu, Pb and Cd using the three individual channels of the MPCP probe were first assessed by installing one GIME sensor in each flow-through voltammetric cell and performing, in

laboratory, the following tests simultaneously on the three channels: (i) calibration of the metal ions in N₂ deoxygenated 0.1 M NaNO₃ suprapur (Me concentration ranges of standard solutions: 1 to 8 nM for Pb(II) and Cd(II); 2 to 20 for Cu(II)); (ii) measurements of total metal concentrations in UV-digested certified reference materials (CRMs) of riverine (SLRS-4) and sea water (CASS-4).

For the former test, linear calibration curves ($R^2 \geq 0.99$) with reproducible slopes were observed for each channel and between the channels using several GIME sensors. Excellent linearity ($R^2 > 0.99$) was also observed for trace metal peak currents as a function of the deposition time in a range of 5 to 45 min. The average normalized calibration slopes (i.e., calibration slopes divided by the deposition time), obtained from two replicate calibrations for three GIME sensors are reported in Table 1 for each channel ($N=6$) and all the channels ($N=18$), together with the limit of detection (LOD) computed based on a signal to noise ratio of 2 and a deposition time of 15 min. These results are in good agreements with those previously reported for the VIP (Tercier et al., 1998; Tercier-Waeber et al., 1999; Howell et al., 2003). The results obtained for test (ii) are reported in Table 2. The values measured by the three channels were found to be in a good agreement with the certified ones, excepted for Pb in CASS-4. The higher Pb concentrations in CASS-4 obtained from the three channel measurements are presumably due to a contamination during the CRMS sample handling or UV-digestion.

The MPCP probe integrating the three GIME sensors was then used for in situ measurements of the dynamic fractions of Cu, Pb and Cd in Gullmar Fjord (Kristineberg Marine Station, Sweden, August 18–29, 2002). The probe was first deployed from the pontoon of the station (1.5 m depth) and autonomous measurements were performed every 1 h over a total time of 5 h. Relative standard deviations $\leq 12\%$ were found for the average concentrations of the three metals determined from (i) each individual channel and (ii) over the three channels. The probe was then deployed from the *RV Arne Tiselius* vessel at various stations and depths together with several VIP probes. Concentrations of the dynamic fraction of the three metals monitored with the three MPCP individual channels and the VIP probes were found to be in good agreement.

Table 1

GIME-MPCP average normalized calibration slopes and detection limits determined from two replicate calibrations of the trace metals using three different GIME sensors in each channel (i.e., $N=6$ for each channel and $N=18$ for the average values over the three channels)

Metal ion	Normalized slopes (nA/nM min)				LOD (pM) ($t_{\text{dep}}=15$ mn)
	Channel 1	Channel 2	Channel 3	Av. channels 1 to 3	
Cu(II)	0.59 ± 0.04	0.55 ± 0.05	0.55 ± 0.04	0.57 ± 0.05	250
Pb(II)	1.25 ± 0.07	1.29 ± 0.06	1.32 ± 0.06	1.28 ± 0.06	30
Cd(II)	0.58 ± 0.03	0.61 ± 0.06	0.60 ± 0.05	0.59 ± 0.05	55

The metal calibration was performed in N_2 degassed 0.1 M $NaNO_3$ suprapur electrolyte using a deposition time of 15 min.

All these results demonstrated the high reliability, accuracy and sensitivity of the voltammetric three individual channels for the simultaneous measurements of one distinct metal fraction. This was a prerequisite condition before the integration of the CGIME sensor and the submersible FIA, to insure further reliable measurements and interpretation of trace metal speciation.

3.2. Characterization and optimization of the CGIME sensor and FIA system

The CGIME was applied for the first time to direct measurements of free metal ions in real samples during a fieldwork at the Kristineberg Marine station (Sweden, August 18–29, 2002). As the validation of new technique measurements in complex media is not straightforward, hollow fiber permeation liquid membrane (HF-PLM) coupled to ICP-MS detection was used in parallel to CGIME measurements for comparison purpose. This technique allows the measurements of free metal ions and some lipophilic complexes (Parthasarathy et al., 1997, 2004). The contribution of lipophilic complexes for in particular

Pb and Cd is expected to be negligible due to the low concentrations of these metals in the studied aquatic system and the HF-PLM accumulation time used (typically 2 h). Laboratory measurements with CGIME, made using a standard Metrohm cell (Herisau–Switzerland) and a computer-controlled Amel 433A potentiostat (Milan-Italy), and with HF-PLM were performed in filtered and/or raw, non-acidified samples. These samples were collected, using GoFlo bottles, at different stations and depths in Gullmar fjord, stored at 4 °C directly after sampling, and measured as soon as possible (i.e., maximum of 24 h later). In parallel, laboratory measurements of the total extractable metal concentrations were performed in the same samples using a prototype version of the FIA system coupled to the MPCP GIME-channel 3. The total extractable metal concentrations obtained, after an on-line FIA pre-treatment time of 6 min using the conditions (a) of the experimental section, were compared to (i) pH 2 extractable metal concentrations and (ii) total metal concentrations measured in laboratory by GIME-VIP in pH 2 acidified (equilibration time at room T to reach a maximum metal release: 1 h for sea water and ≤ 60 h for estuarine

Table 2

Results obtained for analysis of CRMs performed simultaneously using the three MPCP probe voltammetric channels integrating a GIME sensor

Metal ion	CRM	Certified value (nM)	GIME-MPCP measured value (nM)		
			Channel 1	Channel 2	Channel 3
Cu(II)	CASS-4	9.31 ± 0.87	9.68 ± 0.70	8.98 ± 0.90	9.35 ± 0.62
	SLRS-4	28.49 ± 1.26	27.48 ± 1.40	27.90 ± 1.12	28.10 ± 0.90
Pb(II)	CASS-4	0.05 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	0.08 ± 0.02
	SLRS-4	0.41 ± 0.03	0.38 ± 0.02	0.48 ± 0.04	0.42 ± 0.03
Cd(II)	CASS-4	0.23 ± 0.03	0.20 ± 0.02	0.25 ± 0.03	0.22 ± 0.05
	SLRS-4	0.11 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.09 ± 0.02

Confidence intervals for measurements taken as two times the standard deviation obtained for 5 replicate measurements.

and river samples (Pei et al., 2000)) and pH 2+UV digested samples respectively.

Typical results of the concentrations of the target metal species obtained with the different techniques in samples collected at two different stations and depths (i.e., above and below the main pycnocline) of Gullmar fjord are given in Fig. 2a. Cu, Pb and Cd concentrations measured using CGIME sensors were found to be lower or relatively similar to those of the HF-PLM/ICP-MS in most of the samples analysed. Concerning the FIA-GIME MPCP measurements, the results showed that Cu and Pb total

extractable concentrations are similar to Cu and Pb pH 2 extractable concentrations; Pb total extractable concentrations similar to Pb total concentrations, and Cu total extractable concentrations < Cu total concentrations (typically 65 to 75%; see Fig. 3a). Significant contaminations were observed for the Cd FIA-GIME MPCP measurements performed in the first three samples (Fig. 2a), but data obtained in the last sample (Ingela's 30 m; Figs. 2a, 3a) suggest similar trends for Cd to those observed for Pb.

The CGIME, together with its fluidic system, and the submersible version of the FIA system were then

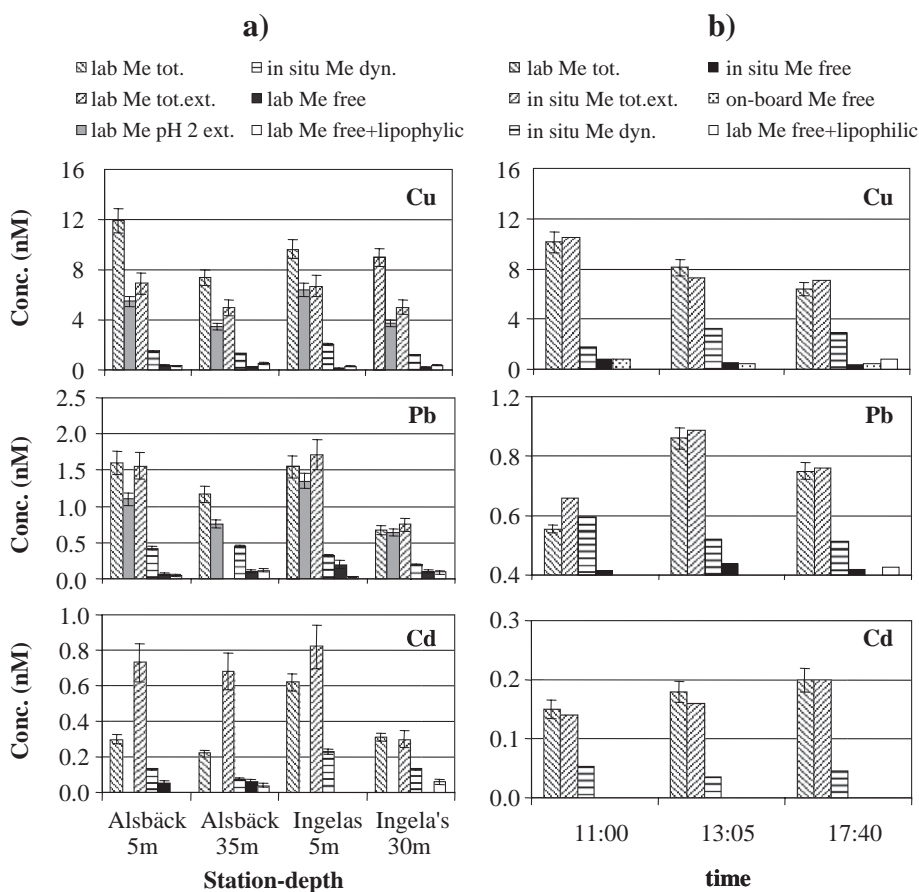


Fig. 2. Comparison between metal species concentrations measured using the MPCP and complementary laboratory techniques in (a) Gullmar fjord (Kristineberg marine station, Sweden, 18–29 August 2002); (b) breakwater marine coastal area (Plymouth, UK, 26 April 2003). Me tot.—total metal concentrations measured in pH 2+UV digested samples by GIME-VIP; Me tot. ext.—total extractable metal concentrations measured by FIA-GIME-MPCP; Me pH 2 ext.—pH 2 extractable metal concentrations at room T measured by GIME-VIP; Me dyn.—dynamic metal concentrations measured by GIME-MPCP; Me free—free metal ion concentrations measured by CGIME-Amel (measurements in laboratory), CGIME-VIP (on-board measurements) and CGIME-MPCP (in situ measurements); Me free+lipophilic—concentrations of free metal ions+some lipophilic complexes measured by HF-PLM coupled to ICP-MS.

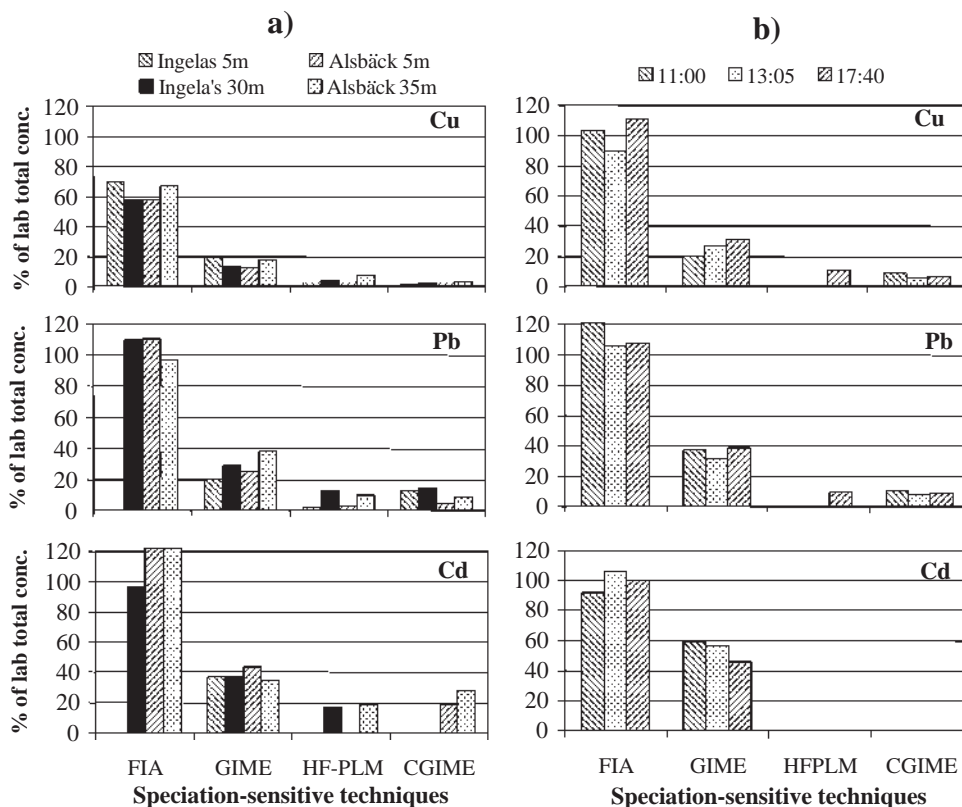


Fig. 3. Speciation sensitive measurements of copper, lead and cadmium at (a) various stations and depths in the Gullmar Fjord (Kristineberg marine station, Sweden, 18–29 August 2002); (b) one station and three different times in Breakwater marine coastal area (Plymouth, UK, 26 April 2003).

integrated in the MPCP channels 2 and 3 respectively (Fig. 1c) and applied for in situ measurements in coastal sea water (Breakwater–Plymouth, UK, April 26, 2003). The MPCP was deployed at 2.5 m depth, from anchored boat, and in situ measurements performed over a period of ca. 7 h.

In situ CGIME measurements were performed using a metal accumulation time on the chelating resin of 2 h and compared with on-board CGIME-VIP measurements, performed in on-line collected samples at the same depth, using an accumulation time of 1 to 2 h, and with laboratory HF-PLM/ICP-MS measurements performed in a discrete sample. Examples of results obtained at three different times of the monitoring period are given in Fig. 2b. Cd free ion concentrations were found to be below detection limit for both CGIME and HF-PLM techniques, and Pb concentration from on-board CGIME measure-

ments could not be detected due to signal noise problems. Cu^{2+} measured in situ and on-board using CGIME sensors was found to be in good agreement and relatively similar to the free+lipophilic Cu concentration measured in laboratory using HF-PLM/ICP-MS. Similar concentration of Pb was also observed between in situ CGIME and laboratory HF-PLM/ICP-MS measurements. These results demonstrate the proper functioning of the CGIME fluidic system at depth.

In situ total extractable metal concentration measurements were performed with a time interval of typically 30 min, using a FIA pre-treatment time of 8 min and the conditions (b) of the experimental section. Results obtained were compared with the VIP laboratory measurements of total metal concentrations in pH 2 acidified and UV-digested discrete samples. Typical examples of results are given in

Fig. 2b. Under the FIA pre-treatment conditions (b) used, an higher efficiency for the release of Cu were observed with a percentage of in situ total extractable to total metal concentrations typically in the range 90 to 110% for Cu (Fig. 3b). Increase in efficiency under FIA conditions (b) was confirmed by a percentage of Cu recovery $\geq 88\%$ obtained for other measurements performed in Southwest England macrotidal estuaries, Northwest Adriatic microtidal estuaries and sea costal area (see example in Fig. 8b). Percentage of recovery for Pb and Cd was found to be typically $100 \pm 8\%$ (Fig. 3b). These results show that only a small proportion of metals, and in particular of Cu, present as refractive organic complexes, i.e., complexes which can be dissociate only under drastic chemical conditions, are not detected. These findings demonstrated the usefulness of the FIA as it allows fast, in situ voltammetric measurements of the maximum amount of metals which may be release in case of drastic variation of the physicochemical conditions of the media (e.g., problem of acidification).

3.3. Evaluation of the MPCP speciation capability

The data collected during the two field preliminary tests reported in Section 3.2 provided a first opportunity to evaluate a set of different speciation-sensitive techniques, i.e., GIME, CGIME and HF-PLM and to assess the potentiality of the MPCP as new analytical tool for in situ trace metal analysis and speciation. For this purpose, the data obtained with the above techniques and with the FIA-GIME were reported as percentage of laboratory total metal concentrations measured in pH 2 and UV-digested discrete samples (Fig. 3).

Similar trends were mostly observed for the measurements at the two stations and depths of Gullmar fjord (Fig. 3a) and the temporal measurements at a given site of Breakwater coastal sea area (Fig. 3b). The measured fraction decreases in the order $GIME > HF-PLM \geq CGIME$ for each metal monitored. This is what one would expect from the theory and the validation in laboratory of the different analytical techniques (Buffle and Horvai, 2000; Nöel et al., submitted for publication; Parthasarathy et al., 1997, 2004). In particular, CGIME which has the aim to measure free metal ions yields similar values or slightly lower than HF-PLM which measures free

metal ions and some lipophilic complexes; CGIME and HF-PLM data are significantly lower than GIME for free metal ions and small labile complexes with size of few nanometers, but greater than the value reported using an indirect determination of free metal ion concentrations by competitive ligand-exchange voltammetric methods (Xue and Sigg, 2002); GIME data considerably lower than the values of the total extractable fraction obtained with FIA-GIME which, under optimum FIA pre-treatment conditions (Fig. 3b), are similar, or slightly lower for Cu, to total metal concentrations measured in pH 2 and UV-digested samples using traditional laboratory techniques. Within each technique, the measured metal fractions increase in the order $Cu < Pb < Cd$ (Fig. 3a and b), which is in agreement with the theoretical expected complexation behavior of the three target metals in sea water. These findings demonstrate the capability of the MPCP as a tool for simultaneous in situ monitoring of environmental relevant distinct fractions of metal species.

4. Environmental applications

The MPCP has been applied for attended, unattended and remote monitoring of trace metal speciation and master variables in various estuarine and coastal sea waters. The preparation of the sensors and the calibration of the system were performed in laboratory the day before the first field deployment. Two Me standard solutions were then measured every 2 to 3 days during the field surveys. Typical examples of voltammograms, calibration curves and on-board Me standard solution measurements are reported in Fig. 4. Standard deviations obtained for the three channel metal average calibration slopes were found to be $< 10\%$ ($N=5$) for the three metals. Variations of maximum -10% were observed between the metal signals obtained for the standard solution measurements during field surveys and the calibration in laboratory, even for a continuous application of the MPCP, without renewal of the sensor mercury layers, up to 8 days (maximum time tested, see examples in Fig. 4b). These results confirm those previously reported for the reliability and the long-term stability of the GIME and CGIME sensors (Tercier-Waeber and Buffle, 2000; Nöel et al., submitted for publica-

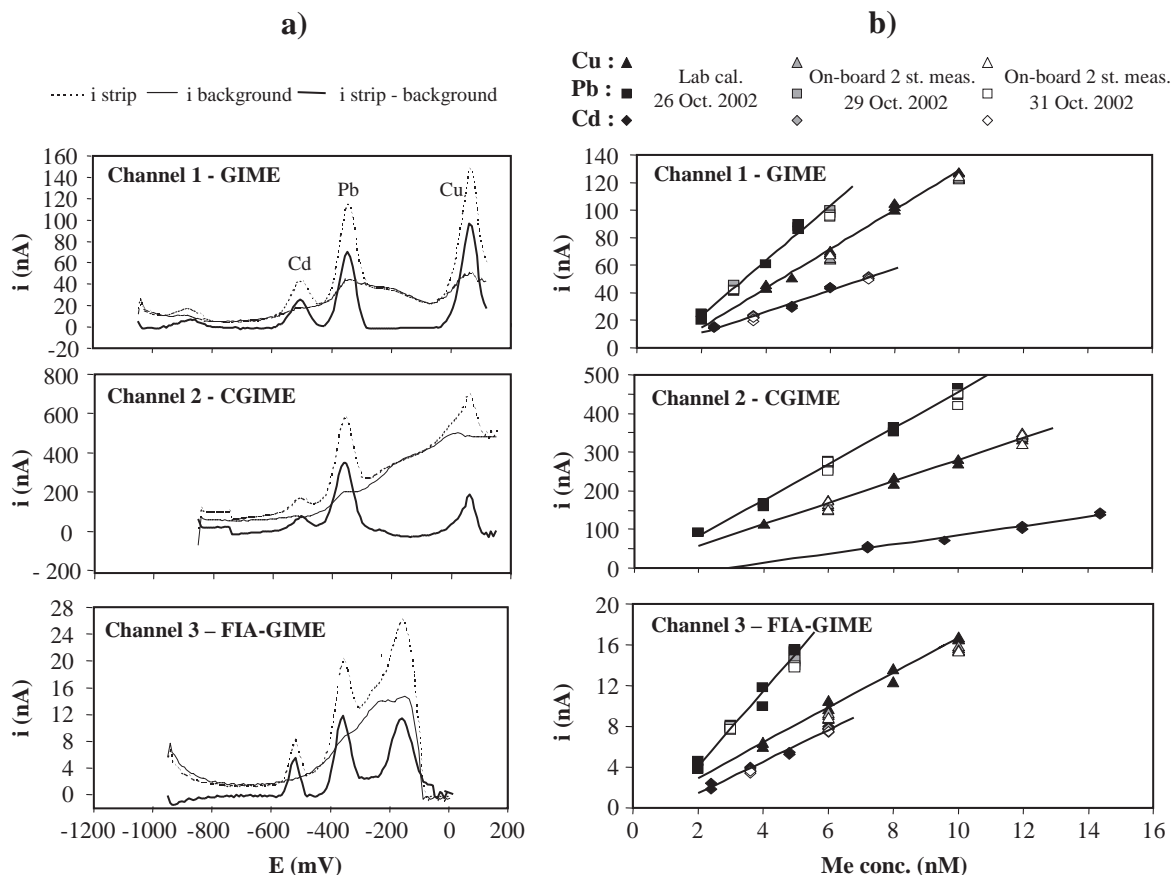


Fig. 4. (a) Examples of voltammograms obtained for GIME, CGIME and FIA-GIME measurements performed with the MPCP in non-deoxygenated: pH 3.5, 0.1 M NaNO₃ spiked with 8 nM Cu, 4 nM Pb and 4.8 nM Cd; 0.1 M NaNO₃ spiked with 8 nM Cu and Pb, 12 nM Cd; synthetic sea water spiked with 8 nM Cu, 4 nM Pb, 4.8 nM Cd, respectively. FIA sample dilution factor=2.5. (b) GIME, CGIME and FIA-GIME calibrations curves and metal standard solution measurements obtained in laboratory and on-board ship respectively before and during an Adriatic cruise (Italy, 27 October to 2 November 2002).

tion) and the absence of memory effect from in situ measurements (Tercier et al., 1998). Typical examples of MPCP results obtained from field surveys are presented below.

4.1. Temporal variation of trace metal speciation in macrotidal estuaries

The MPCP was applied to monitor trace metal speciation and master variables during a tidal cycle at one geographical point of the Fal estuary. The Fal estuary, located in Cornwall in south west of England, is a complex of creeks and tidal rivers extending 17 km inland in an area having a long mining

history. It is characterized by strong variations in flow and turbidity and subject to enhanced inputs of trace metals from mining waste discharge and run-off of the surrounding contaminated land. The MPCP was deployed at 2 m depth at the entrance of the Restronguet creek, from an anchored boat, and in situ, continuous measurements performed over a period of ca 10 h on April 30, 2003.

The MPCP results obtained for the concentrations of the distinct fractions of Cu, Pb and Cd together with the salinity and turbidity data are given in Fig. 5. These speciation data show that freshwater is an important source of Cu and Pb associated to particulate and colloidal species (i.e., difference between

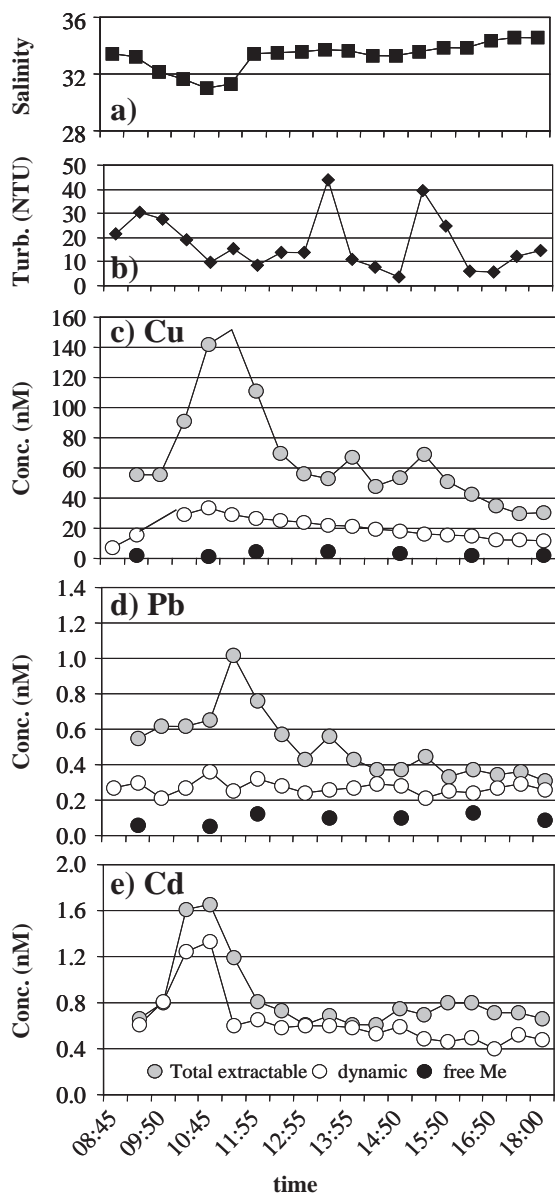


Fig. 5. In situ MPCP monitored (a) salinity, (b) turbidity and (c) to (e) trace metal distinct fractions at the Restronguet point of the Fal Estuary during a tidal cycle (UK—30 April 2003).

total extractable and dynamic species at low water) in the estuary water. Comparison with turbidity data shows that there is another temporal source of these species, in particular for Cu and Pb (i.e., peaks at 13:25 and 15:25), linked to a re-suspension of sediment by tidal and wind forces, and/or small boat

activity in this shallow estuary. Freshwater is found to be also an important source of Cu and Cd dynamic species, with a potential toxicity impact for coastal biota at long-term. In contrast, the free Pb and Cu concentrations were found to be lower at low water compared to high water (free Cd concentrations were found to be lower than the detection limit of the CGIME sensor) while the dynamic Pb concentrations were found to be relatively constant over the complete tidal cycle. The ratios of particulate/colloidal species, dynamic species and free metal ion concentrations to total extractable concentrations were found to vary in various proportions for the three metals within the tidal cycle. These findings clearly confirm the necessity of continuous, real-time monitoring of trace metal speciation (versus total metal concentrations), simultaneously to other master variables, to interpret the biogeochemical cycles of the trace elements and to evaluate their long-term impacts.

4.2. Monitoring of trace metal speciation gradients in the Po estuary and its plume

The MPCP was also applied to measure Cu and Pb concentrations and speciation gradients together with master variables in the Po estuary and its plume, during an Adriatic oceanographic cruise carried out on board *G. Dallaporta* vessel (IRPEM-CNR, Ancona, Italy) from October 27 to November 2, 2002. The Adriatic Sea is characterized by low tidal range and important pollution inputs, concentrated mainly in the northwest area where the river Po enters the sea. The Po is the longest river in Italy and it crosses the entire northern part of the country, one of the most industrialized areas in Europe. The river flows to the sea through a delta of 400 km², contributing to about 50% of the total freshwater discharge load to the shallow Northern Adriatic basin (average depth ~30 m). Due to the prevailing marine currents (Northward along the Croatian coast and Southward along the Italian coast) pollutants discharged by the river Po mainly affect the Italian coast to approximately the city of Ancona.

The MPCP was deployed at various stations and depths along the Po plume toward the open sea, direction East–South East. The concentrations of the three distinct fractions of Cu and Pb obtained from MPCP in situ measurements are reported in Fig. 6. A

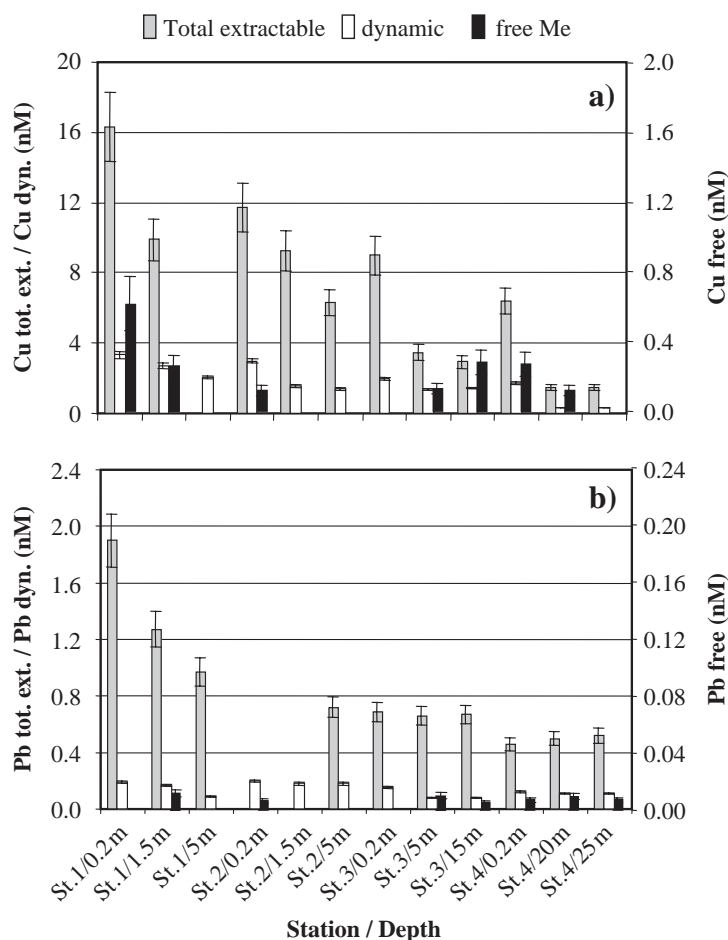


Fig. 6. (a) Cu and (b) Pb speciation monitored in situ with the MPCP at various stations and depths in the Po river plume (Adriatic cruise—Italy, 27 October to 2 November 2002). Concentrations of total extractable and dynamic Me concentrations reported are average of three FIA-GIME and GIME respectively replicate measurements. Concentrations of free metal ions are average of two replicate CGIME measurements.

decrease in the Cu and Pb total extractable and dynamic concentrations as a function of both the distance from the Po mouth and the depth (in particular between the 0.2 m surface water and the deepest layers) were observed. Comparison with the data of the master variables, measured simultaneously, showed that the decrease of these metal species is mainly correlated to the variation in salinity (Fig. 7a–b, d–e), i.e., to the mixing of the Po river and the Adriatic sea waters. The Cu and Pb free metal ion concentrations do not show the same trends. The Cu free ion concentrations were found to be more correlated to Chlorophyll *a*, i.e., to the primary productivity than to the salinity (Fig. 7c), whereas Pb free ion

concentrations were found to be very low (typically 0.01 nM) and relatively constant (Fig. 7f). These results suggest that a significant proportion of Cu^{2+} is either assimilated by the phytoplankton or complexed by their exudates, or even both. The role of biota is supported by the fact that Pb^{2+} , which is known to be not easily assimilated, does not follow the same trend as Cu^{2+} .

4.3. Remote MPCP monitoring and profiling in marine coastal area

The MPCP coupled to the remote controller was used in marine coastal area during a fieldwork at the

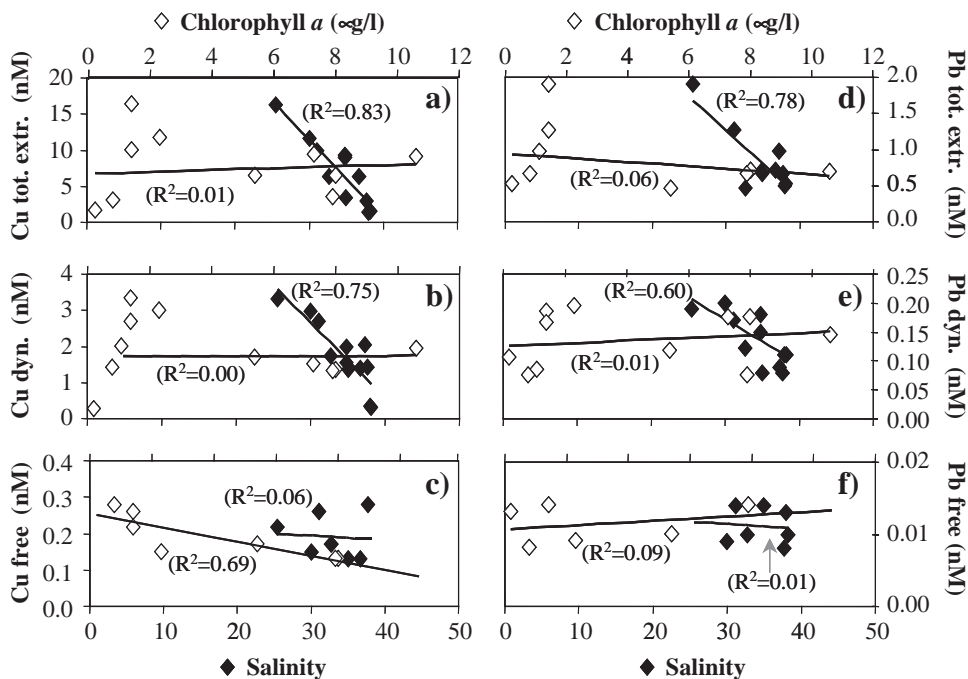


Fig. 7. Concentrations of Cu (a to c) and Pb (d to f) species as a function of salinity and chlorophyll *a* monitored in situ, simultaneously, with the MPCP at various stations and depths in the Po plume (Adriatic cruise, Italy, 27 October to 1 November 2002).

CNR Acqua Alta Platform, Venice, from June 30 to July 3, 2003. The Acqua Alta Platform is located in the Northern Adriatic Sea (latitude: $45^{\circ} 18' 51''$ North, longitude: $12^{\circ} 30' 30''$ East), 16 km of the coastline of Venice, on 16 m of depth. Two MPCPs were deployed. Remote MPCP monitoring/profiling with automatic data transfer were done with the MPCP 1 from 1 to 3 July using the three autonomous measurement options, i.e., at constant depth (3 m—July 1, 10:30 to 15:30), linear profiling using a constant depth step (2, 8 m—July 1–2, 19:00 to 07:00; 4, 8, 12 m—July 2, 09:30 to 17:30; 2, 4, 6 m—July 2–3, 19:30 to 03:00) and at programmed depths (10, 12, 9, 2 m—July 3, 06:30 to 14:00). The time interval between each measurement cycle was 2 h 30. At each cycle, one CGIME measurements and two replicate GIME and FIA-GIME measurements, with a time interval of 30 min between the replicates, were made. For comparison purpose, attended profiling (1.5, 4, 6.5, 9, 12 m), based on three replicate GIME and FIA-GIME and one CGIME measurements at each depth, and surface monitoring at 4 m from a minimum to a maximum tide were made with

the second MPCP system (MPCP 2) on July 2 and 3 respectively. GoFlo discrete samples were collected for complementary laboratory voltammetric total metal concentration measurements in pH 2, UV-digested samples.

The range of values obtained with both systems over the complete period for profiling and surface monitoring of the Cu fractions and the master variables is given in Figs. 8 and 9 respectively. Significant variations of all the parameters as a function of both time and depth were observed within experimental errors. The ranges of the three metal fraction concentrations (see example for Cu in Fig. 8b) and of the master variable values (Fig. 8a) monitored at the various depths with both MPCP systems were similar demonstrating the reliability of the MPCP probes as well as of the unattended measurements performed with the remote controller supported MPCP. By examining in more detail the surface data, the salinity values monitored at 4 m depth during a minimum to a maximum tidal cycle using the MPCP 2 (Fig. 9B–a) and of the MPCP 1 at 2 m depth measured at various time over the 3 days of

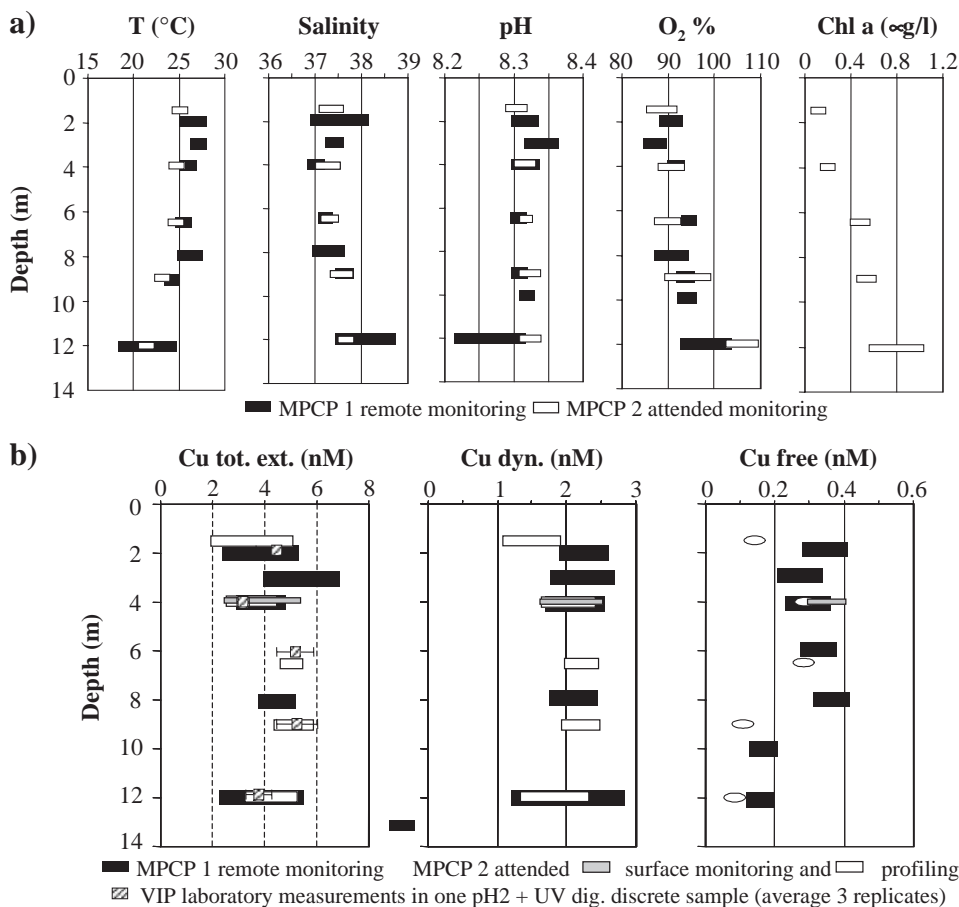


Fig. 8. Data windows of (a) master variables and (b) Cu distinct fractions obtained from remote MPCP 1 profiling and attended MPCP 2 surface monitoring and profiling in the Northern Adriatic sea water (CNR Acqua Alta Platform, Venice, Italy, 1 to 3 July 2003). Note: 1 measurement at each depth for CGIME MPCP 2 Cu free ion profiling.

unattended deployment period (Fig. 9A–a) show that this marine site is still influenced by freshwater input; and that the temporal variations of trace metal speciation and bio-physicochemical conditions are, at least partially, related to this freshwater advection. This was supported by the significant increase of the three element total extractable concentrations as well as of the Pb and Cd dynamic concentrations as a function of the decrease in salinity observed on the plots of the three target element fractions as a function of salinity (see examples for Cu in Fig. 9A–b,c and B–b). This trend is very similar to the one observed for the spatial variations of the above species in the Po plume. Similar trend was also observed for Cu and Pb free metal ions,

i.e., Cu²⁺ correlated to chlorophyll *a* (see examples in Fig. 9B–c) and Pb²⁺ remained relatively constant. The only significant difference observed was for the variation of the concentration of the Cu dynamic fraction, which, in this case, was found to be more correlated to chlorophyll *a* (Fig. 9B–c) than to the salinity (Fig. 9A–c). This suggests a potential bio-availability of some small labile Cu complexes, which is supported by a larger decrease in the Cu dynamic concentrations compared to the Cu free ion concentrations at the higher chlorophyll *a* concentrations. However, more systematic studies, like in the case of coastal lake water (Tercier-Waeber and Buffle, 2000), have to be made to confirm this. For this purpose, remote seasonal MPCP monitoring of trace

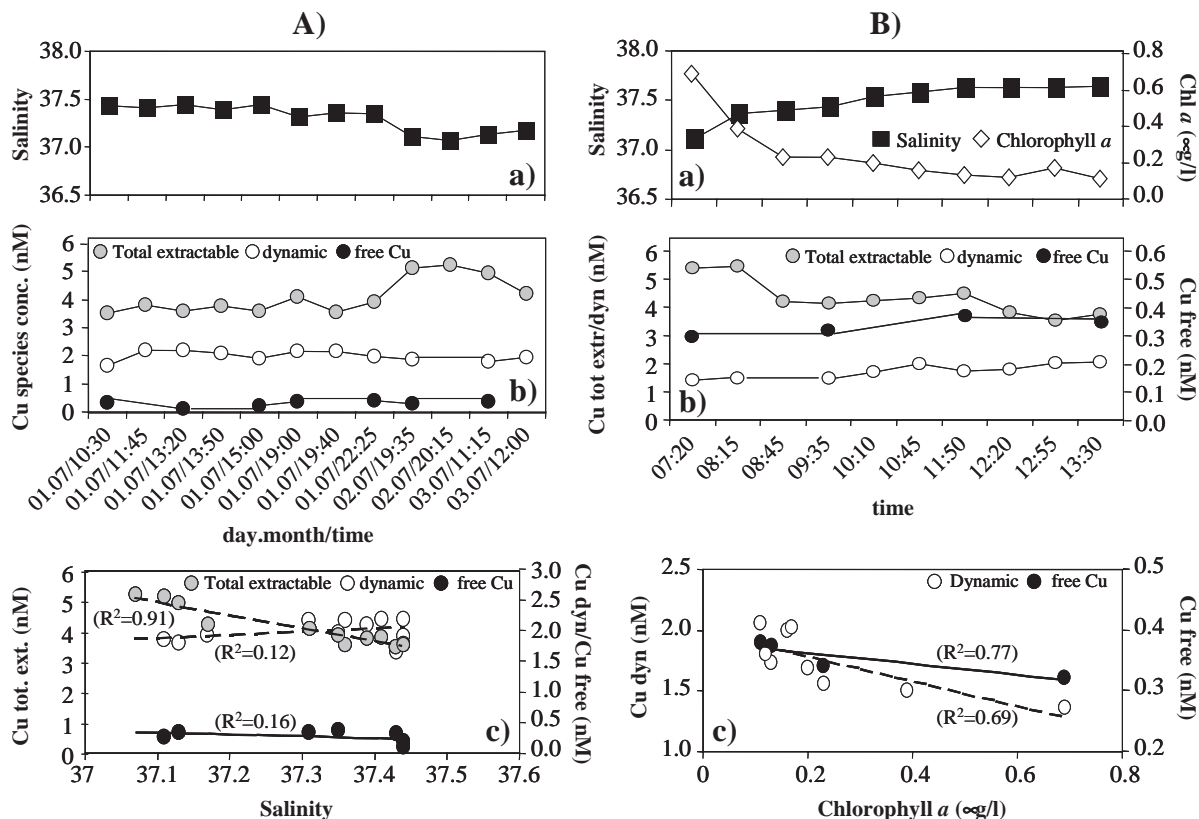


Fig. 9. (A) Remote MPCP 1 monitored (a) salinity and (b) Cu speciation at 2 m depth at various date (1 to 3 July 2003) and times; and (B) attended MPCP 2 monitored (a) salinity and chlorophyll *a*, (b) Cu speciation at 4 m during a minimum to a maximum tidal cycle on 3 July 2003 in the Northern Adriatic sea (CNR Acqua Alta Platform, Venice, Italy). Concentrations of the Cu distinct species as a function of (A–c) salinity and (B–c) chlorophyll *a* monitored with the MPCP 1 and the MPCP 2 respectively.

metal speciation and chlorophyll *a* is of particular interest.

5. Summary and perspective

It has been demonstrated that the recent improvements in voltammetric sensors, cell and probe coupled to breakthrough in electronic, micromechanic, fluidic automation and data processing allow the development of remote submersible analytical systems allowing reliable long-term, simultaneous measurements of distinct relevant fractions of priority trace metal pollutants as well as master variables. Even if the MPCP is at its infancy, its field applications in various estuarine and marine coastal systems have shown its capability for high-resolution, sensi-

tive trace metal speciation and master variable measurements. These studies have led to important findings demonstrating the potentiality of such system for cost effective, efficient environmental monitoring and pollution control of priority trace metal pollutants.

In particular, the results of the work presented in this paper have shown that the concentrations of the so-called total dissolved metals, obtained from measurements in 0.4 µm pore size membrane filtered, pH 2 acidified and UV-digested samples, are significantly different from the Me fractions measured in situ with the CGIME and GIME-MPCP (e.g., Fig. 2b). In addition, the proportion of these two later fractions are significantly influenced by the physical and biogeochemical processes (e.g., Figs. 5, 7, 9). All this strongly suggests that the conventional mon-

itoring approach, based on total dissolved metal concentrations, is inadequate for accurate assessment of the potential toxicological impact of trace metal pollutants.

Important variations in the metal concentrations and their speciation as well as in the master variables, on time scales of hours, were observed not only in highly polluted macrotidal estuaries (e.g., Fig. 5) but also in a microtidal marine area located at 16 km from the coast (e.g., Fig. 8—MPCP 2). This demonstrates the importance of continuous, simultaneous, near real-time measurements of a large number of parameters to identify the predominant parameter(s) that influence the spreading of trace metals and their speciation (e.g., Figs. 5, 9), and thus to better understand the behavior and fate, and ultimately the long-term impact of these priority pollutants.

Remote continuous monitoring/profiling is also important for cost effective pollution control as it allows to follow spatial and temporal variation (e.g., Figs. 8, 9) under appropriate time scale and thus to give timely appropriate warning/alarm in case of significant/sudden increase of priority pollutant levels.

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