



Temporal variability in dynamic and colloidal metal fractions determined by high resolution *in situ* measurements in a UK estuary

Charlotte B. Braungardt^{*}, Kate A. Howell¹, Alan D. Tappin, Eric P. Achterberg²

School of Geography, Earth and Environmental Sciences, University of Plymouth, Devon PL4 8AA, UK

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ABSTRACT

In recent environmental legislation, such as the Water Framework Directive in the European Union (WFD, 2000/60/EC), the importance of metal speciation and biological availability is acknowledged, although analytical challenges remain. In this study, the Voltammetric *In situ* Profiler (VIP) was used for high temporal resolution *in situ* metal speciation measurements in estuarine waters. This instrument simultaneously determines Cd, Cu and Pb species within a size range (ca. <4 nm) that is highly relevant for uptake by organisms. The colloidal metal fraction can be quantified through a combination of VIP measurements and analyses of total dissolved metal concentrations.

VIP systems were deployed over tidal cycles in a seasonal study of metal speciation in the Fal Estuary, southwest England. Total dissolved concentrations were 4.97–315 nM Cu, 0.13–8.53 nM Cd and 0.35–5.75 nM Pb. High proportions of Pb ($77 \pm 17\%$) and Cu ($60 \pm 25\%$) were present as colloids, which constituted a less important fraction for Cd ($37 \pm 30\%$). The study elucidated variations in the potentially toxic metal fraction related to river flow, complexation by organic ligands and exchanges between dissolved and colloidal phases and the sediment. Based on published toxicity data, the bioavailable Cu concentrations (1.7–190 nM) in this estuary are likely to severely compromise the ecosystem structure and functioning with respect to species diversity and recruitment of juveniles. The study illustrates the importance of *in situ* speciation studies at high resolution in pursuit of a better understanding of metal (bio)geochemistry in dynamic coastal systems.

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1. Introduction

The European coastal zone has a substantial ecological, recreational and economic value (European Environment Agency, 2005). Nevertheless, contamination of European coastal seas is widespread as a result of the urbanisation of coastal margins, industrialisation and intensification of agriculture. Although land-based inputs of metals (e.g. Cd, Cu, Pb) to marine waters are reported to have been reduced recently, this has not necessarily resulted in lower concentrations in marine biota (European Environment Agency, 2003). Furthermore, emerging pressures may add to the metal burden in coastal waters. First, the marked increase in mariculture over the last few decades has been accompanied by a rise in the use of copper-based anti-fouling paints (Braithwaite et al., 2007). Secondly, 20% of Europe's coastline is

being actively eroded and this may increase as a result of projected sea level rise and increased storminess, whereby medium to long-term impacts include the remobilisation of historically metal-contaminated coastal sediments (European Environment Agency, 2005). Thirdly, increasing atmospheric CO₂ concentrations may result in a reduction in seawater pH and enhanced release of metals from sediments. Increasing acidity may also alter metal speciation, with unpredictable consequences, particularly with respect to metal bioavailability and toxicity (The Royal Society, 2005).

The biogeochemical role of trace metals is determined by their physical and chemical speciation, encompassing particulate, colloidal and truly dissolved forms. Colloids are generally viewed as organic-rich solid-in-liquid dispersions with a size in the range 1–1000 nm (Stolpe et al., 2010). Truly dissolved metals include free metal ions and simple inorganic and organic metal complexes that are considered to be readily assimilated by (micro)biota via transport across cellular plasma membranes (Buffle and Terrier-Waeber, 2005; Slaveykova and Wilkinson, 2005). In estuaries, colloids play an important role in the exchange processes of metals between the sediment and the water column and in controlling metal speciation. For example, Powell et al. (1996) reported colloidal Cu as the dominant fraction of this metal in

^{*} Corresponding author. Tel.: +44 1752 584577; fax: +44 1752 58471.

E-mail addresses: A.Tappin@plymouth.ac.uk, C.Braungardt@plymouth.ac.uk (C.B. Braungardt), Kate.Howell@nanotechnology.co.uk (K.A. Howell), eric@noc.soton.ac.uk (E.P. Achterberg).

¹ Nanotechnology Ltd., Epsilon House, Southampton Science Park, Southampton SO16 7NS, UK.

² School of Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, SO14 3ZH, UK.

low salinity estuarine waters. Complexes of Cu with dissolved organic ligands have been widely reported to dominate the total dissolved (0.45 μm pore size filtrate) Cu fraction at enhanced salinities, whereby a high proportion (40–94%) of the organically complexed Cu is associated with colloids (Powell et al., 1996; Wen et al., 1999; Waeles et al., 2008). Copper speciation varies widely between estuaries and within estuaries with tides and seasons, depending on the nature and concentration of organic matter present (Waeles et al., 2008). In saline systems featuring low dissolved organic matter concentrations, copper carbonate species are likely to be the most important form of dissolved Cu (Chester, 2003). In rivers, Cd is mostly present as aqueous Cd(II), although significant colloidal fractions can occur (Powell et al., 1996; Wen et al., 1999; Howell et al., 2006). In more saline waters, chloro-complexed Cd typically dominates the dissolved speciation of the metal (Chester, 2003), but colloidal Cd can be important at mid- to high salinities (34–82% of total dissolved), where interaction of Cd with Fe/Mn (oxy)hydroxide and/or organic colloids may occur (Wells et al., 1998; Wen et al., 1999; Waeles et al., 2008). Dissolved Pb in fresh waters strongly associates with carbonates (Chester, 2003) and also with large iron-, and to a lesser extent, carbon-rich colloids greater than 10 kDa (ca. 10 nm) (Lyvén et al., 2003). Colloidal iron oxides, hydroxides and sulphides may be the dominant absorbent phases for Pb in saline waters, as indicated by the high colloidal Pb fraction of total dissolved Pb observed in some estuaries (59–99%; Braungardt et al., 2009; Waeles et al., 2008).

Metal-colloid associations are studied typically by employing a range of separation techniques (e.g. ultrafiltration, centrifugation, field flow fractionation) that require the collection of samples and their subsequent processing in the laboratory. However, in order to provide a more detailed understanding of metal behaviour, toxicity and fate in dynamic coastal systems, their speciation profile should be studied at high temporal resolution and preferably in the field. Recent developments in *in situ* analytical instrumentation for trace metal speciation monitoring allow such field measurements of metal speciation. *In situ* simultaneous measurements of dynamic Cd(II), Cu(II) and Pb(II) species in coastal marine waters using the Voltammetric *In situ* Profiler (VIP, Idronaut, Milan) system (the term 'dynamic' is defined below) have been reported by, e.g. Howell et al. (2003), Tercier-Waeber et al. (2005) and Braungardt et al. (2009).

The aims of this study were to (i) investigate the speciation of Cd, Cu and Pb in an estuarine system contaminated by historic metalliferous mining and continued inputs of metal-rich acid mine waters, (ii) elucidate temporal differences in metal speciation and (iii) relate speciation information to biological availability. To this end, VIP instruments were deployed in a seasonal study in estuarine waters for high resolution monitoring of Cu, Cd and Pb speciation over full tidal cycles.

2. Experimental

2.1. VIP instrumentation

The VIP is designed for short and medium-term (up to 3 weeks) monitoring tasks and comprises of fully submersible (500 m) components for sample transport (peristaltic pump), sample analysis (3-electrode voltammetric system) and data management (hardware, firmware, data memory). The flow-through cell houses within the sample pathway a working electrode and a platinum counter electrode. The working electrode is a gel-integrated micro-electrode (GIME), which consists of an array of 100 interconnected Ir-based micro-disc electrodes onto which mercury is electroplated prior to use. The reference electrode is a miniaturised

Ag/AgCl/KCl saturated gel electrolyte electrode and it is electrically connected to the sample pathway via a system of two zirconium oxide ceramic bridges embedded into bridge electrolyte gel (1 M NaNO₃ in agarose 1.5% w/v LGL). A gel membrane (1.5% (w/v) agarose LGL, 300 μm thick) prevents fouling of the working electrode surface during deployment in natural waters. A full description of the system and its preparation for use is detailed elsewhere (Tercier-Waeber et al., 2002; Braungardt et al., 2009).

The VIP quantifies a fraction of the conventional total dissolved trace metal pool using size separation, whereby the metal flux towards the working electrode, measured as an electrical current, is directly proportional to the diffusion coefficient of the analyte metal species and negligible for metal species of a size >4 nm. This enables the direct voltammetric analysis of what is termed the dynamic metal fraction, which includes free metal ions as well as sufficiently labile and mobile inorganic and organic metal complexes of a few nanometres in size. The dynamic fraction may be readily biologically available because dynamic metal complexes can dissociate within the time it takes to diffuse from the bulk medium to the cell surface receptor sites of organisms (Slaveykova and Wilkinson, 2005). The non-dynamic fraction, comprising metals associated with biopolymers, macromolecules and inorganic and organic colloids, is excluded from analysis (Buffle and Tercier-Waeber, 2005). The concentration of metal in the non-dynamic, or colloidal, fraction was calculated as the difference between the dynamic concentration and the total dissolved metal concentration, defined by conventional filtration (0.4/0.45 μm pore size) of discrete samples. Here, the dynamic dissolved metal is termed Me_{dyn} , the total dissolved metal Me_{tot} and the colloidal metal Me_{coll} .

2.2. Reagents and apparatus

The preparation and handling of reagents, samples and apparatus was carried out using trace metal clean techniques in protected environments, such as a covered bench on board ship and Class-100 laminar flow cabinets in laboratories. Water was purified by ion exchange (de-ionised water, Milli-Q system, Millipore, 18.2 $\text{M}\Omega\text{ cm}^{-1}$) and used for the preparation of all aqueous solutions. Reagents were AR grade (Alpha Aesar, VWR, Poole, UK) unless stated otherwise. Nitric acid and hydrochloric acid were purified by sub-boiling distillation (sub-HNO₃, sub-HCl). Clean ammonium hydroxide was prepared by isothermal distillation (iso-NH₄OH). Metal standards (10 μM Cu(II), 1 μM Cd(II) and Pb(II)) were prepared from Spectrosol stock solutions (10 g L^{-1} , Merck Chemicals) by serial dilution, and acidified to pH < 2 with either sub-HNO₃ (for voltammetric analysis with the VIP and a glassy carbon rotating disc electrode on a VA 663 Stand (Metrohm, Switzerland) interfaced with an μ -Autolab, Ecochemie) or with sub-HCl for analysis with a hanging mercury drop electrode on a VA 663 Stand. Solutions of mercuric acetate (5 mM) in perchloric acid (10 mM), aqueous mercuric nitrate (2 mM), potassium thiocyanate (1 M), sodium nitrate (0.05 and 1.0 M, Trace select, Sigma Aldrich, UK), 8-hydroxyquinoline stock solution (0.01 M, 99% grade, Merck Chemicals), HEPES pH buffer solution (1 M, biochemical grade, Merck Chemicals) and ammonium acetate pH buffer (1 M) were prepared, purified and the pH adjusted where required (Braungardt et al., 2009).

Glass- and plastic-ware were decontaminated (2% Decon, >24 h; NaOH pH10, >24 h), acid-washed (1.2 M HCl, >24 h; 1.4 M HNO₃, >4 h) and thoroughly rinsed between steps with de-ionised water. Glass-ware was combusted (450 °C, 6 h) as a final step. All glass fibre filters (GF/F, nominal pore size 0.7 μm , Whatman) were combusted (450 °C, 4 h) and polycarbonate membrane filters (0.4 μm pore size, Whatman) were acid-washed (1.2 M HCl, >24 h).

2.3. Study area

Restronguet Creek is located within the larger macro-tidal Fal Estuary, which extends 18 km inland from its mouth to its tidal limit (Fig. 1). The River Carnon is the main freshwater input to Restronguet Creek and water from the Creek is tidally exchanged with water from the outer Fal Estuary, which is known as Carrick Roads. The extensive inter-tidal mudflats (Fig. 1A) and saltmarshes of the area are heavily contaminated with As, Cu, Fe, Pb and Zn as a result of long-term run-off of acid mine drainage and a history of tin streaming. Average total dissolved concentrations of Cd and Cu in the River Carnon are ca. 40 and 3800 nM, respectively (Langston et al., 2003), while sediments in the Creek contain up to 80 $\mu\text{mol g}^{-1}$ Cu, 48 nmol g^{-1} Cd and 2.8 $\mu\text{mol g}^{-1}$ Pb (Langston et al., 2003; Pirrie et al., 2003). These concentrations exceed the Interim Marine Sediment Quality Guidelines (ISQG; 249 nmol g^{-1}

Cu, 6.25 nmol g^{-1} Cd and 144 nmol g^{-1} Pb). Continued adverse effects on the ecology of Restronguet Creek, as well as adaptation to contamination by biota, have been widely reported (Warwick, 2001; Burlinson and Lawrence, 2007).

2.4. Sampling

Measurements over a single tidal cycle were undertaken at Restronguet Point (RP), at the junction of Restronguet Creek and Carrick Roads (Fig. 1), in June, August and December 2002 and March, April and July 2003. Surveys coincided with spring tides (tidal range 3.7–4.7 m), except for August 2002 (tidal range 2.3 m). During each period, two VIP instruments were deployed simultaneously *in situ*; one to determine Cu_{dyn} concentrations and the other to quantify Cd_{dyn} and Pb_{dyn} . At the same time, discrete samples were collected using a manually deployed sampler or a

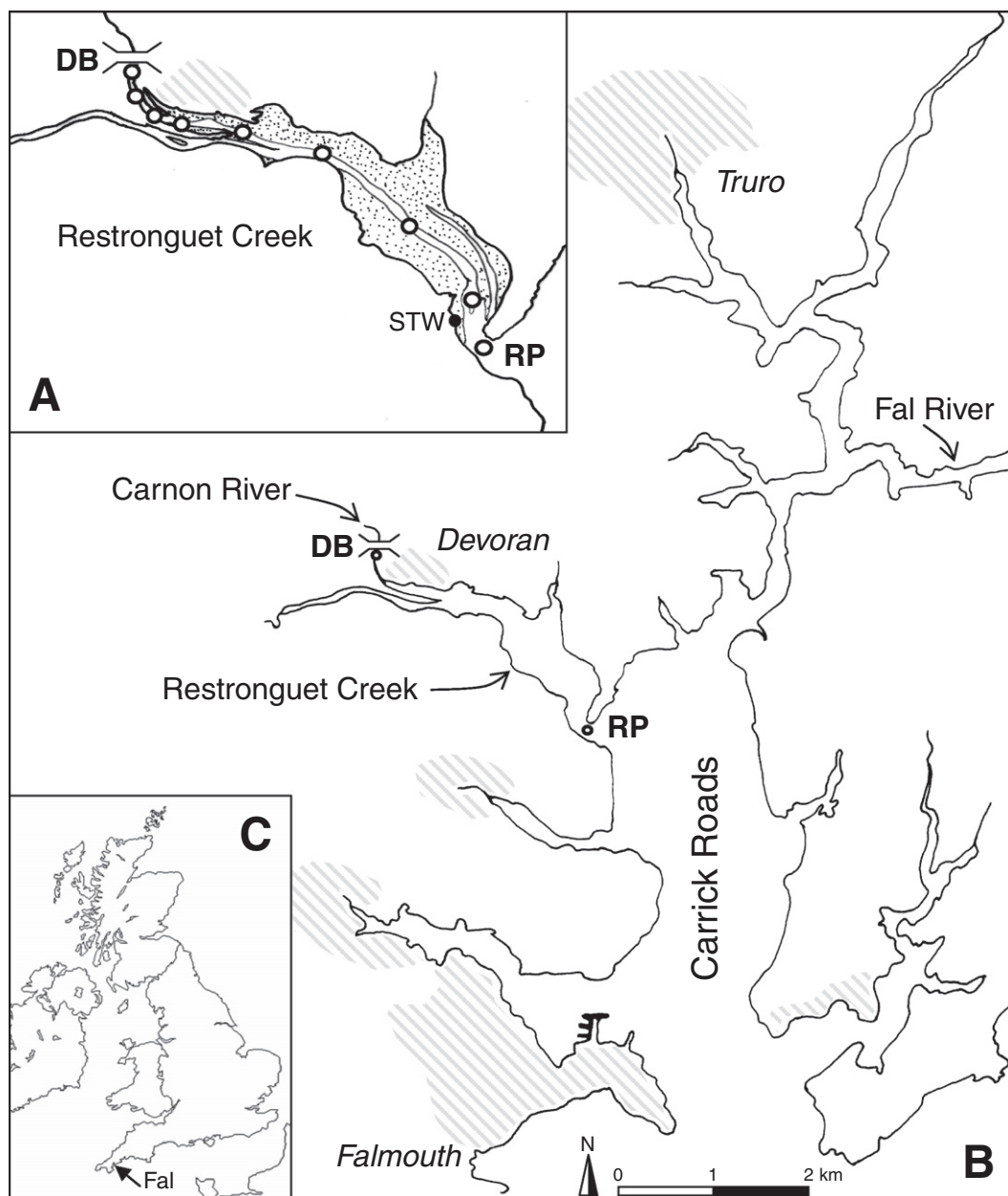


Fig. 1. Location of Restronguet Creek (A) within the larger Fal Estuary (B) in the southwest of England (C). RP: sampling location off Restronguet Point during the tidal cycles, DB: location of the freshwater end-member at Devoran Bridge. The stippled areas within Restronguet Creek mark areas above datum predominantly covered by mudflats. Open circles show additional sampling points within Restronguet Creek. STW: location of a discharge consent for treated sewage effluent ($>7.3 \text{ m}^3 \text{ d}^{-1}$, Langston et al., 2003).

high-volume peristaltic pump for the determination of total concentrations of Cu_{tot} , Cd_{tot} and Pb_{tot} , salinity, suspended particulate matter (SPM), nitrate + nitrite, ortho-phosphate, dissolved organic carbon (DOC) and suspended chlorophyll *a*. Discrete samples were collected at 1.5 m below surface, the same depth at which the *in situ* sample inlet of the VIP was located. At Restronguet Point, the water column was well mixed. Master variables (salinity, pH (calibrated using NBS buffers), dissolved oxygen saturation, turbidity) were also determined, using calibrated hand-held or CTD instrumentation. A sample representing the freshwater end-member was collected from the River Carnon at Devoran Bridge (Fig. 1) during the latter five surveys. In April and July 2003, discrete samples were also collected along an axial transect of the Restronguet Creek (Fig. 1A).

2.5. Quantification

2.5.1. Dynamic dissolved metals

Laboratory and *in situ* determinations of Cu(II), Cd(II) and Pb(II) concentrations by VIP were carried out as described in Braungardt et al. (2009). The analysis included a pre-concentration step (5–20 min), during which a specific deposition potential was applied (e.g. –1270 mV in de-oxygenated NaNO_3 electrolyte, between –1300 and –1100 mV in seawater). Subsequently, two square wave anodic scans (pulse amplitude, 25 mV; step amplitude, 8 mV; frequency, 50 Hz) to a more positive potential (e.g. +150 mV in NaNO_3 or –80 mV in seawater) were carried out to determine the stripping and background currents, respectively. Calibrations were carried out in the laboratory before and after each measurement series (laboratory or *in situ*) using de-oxygenated mixed standards (0.75–2.5 nM Cd and Pb, 3–20 nM Cu) in NaNO_3 (0.1 M). The sensitivity of the signal to changes in pre-concentration time, temperature and ionic strength have been discussed elsewhere (Howell et al., 2003), and appropriate adjustments were made during the quantification of the current peak for each analyte. The adjusted peak height is proportional to the concentration. The analytical limit of detection in seawater at 20 min pre-concentration time (defined as 3σ) was 23 pM for Cd, 23 pM for Pb and 1.13 nM for Cu.

2.5.2. Total dissolved metals

Samples for total dissolved metals were filtered (0.4 μm pore size filters) into 250 mL LDPE bottles and stabilised at pH 2 with sub-HCl (250 μL). In order to remove potentially interfering dissolved organic matter prior to analysis the samples were UV-irradiated for 5 h (in 30 mL quartz tubes) after addition of H_2O_2 (8.8 M, final concentration) using a 400 W medium pressure Hg vapour lamp (Photochemical Reactors, Slough). The pH of the acidified samples was raised (using iso- NH_4OH) to a level appropriate for the electrochemical method employed. Concentrations of Cd_{tot} , Cu_{tot} and Pb_{tot} were determined using anodic stripping voltammetry (Cd: thin-film mercury electrode; Cd, Pb and Cu: VIP) and by adsorptive cathodic stripping voltammetry (Cu: hanging mercury drop electrode). The accuracy of the Cd_{tot} , Cu_{tot} and Pb_{tot} analyses by the VIP was verified using certified reference materials for river, estuarine and coastal waters (Braungardt et al., 2009).

2.5.3. Salinity, nitrate + nitrite and ortho-phosphate, DOC and chlorophyll *a*

Salinity in the discrete samples was determined using a salinometer (Guildline, Autosal). Suspended particulate matter was collected on pre-weighed glass fibre filters (GF/F), rinsed with a few mL of de-ionised water to remove sea salts, and stored at –20 °C. Filters were subsequently air-dried and SPM determined by gravimetry. Nitrate + nitrite and ortho-phosphate were deter-

mined on filtered (GF/F) samples. Filtrates (25 mL) were transferred to 30 mL LDPE sealable bottles and stored at –20 °C until analysis by colorimetry using a segmented flow analyser (Skalar SANplus). Water samples for DOC analysis were filtered using ashed GF/F filters and the filtrates (20 mL) transferred to 40 mL glass vials (TOC Free, Nalgenunc). Filtrates were acidified to ca. pH 2 using 6 M HCl (20 μL) and stored at –20 °C until analysis by high temperature catalytic combustion using a Shimadzu TOC 5000A total carbon analyser (Badr et al., 2003). DOC measurements in certified reference material (CRM; US National Science Foundation/University of Miami, deep Sargasso Seawater, Batch 2, 2000) were $46.7 \pm 6.8 \mu\text{M}$, and in good agreement with the range in consensus values (44–47 μM). Suspended particles for chlorophyll *a* determination, collected on GF/F filters, were wrapped in Al foil and stored at –20 °C. The plant pigment was extracted into acetone (90% v/v) and determined by fluorescence, following Parsons et al. (1984).

3. Results and discussion

3.1. Master variables, nutrients, DOC and chlorophyll *a*

Summary data ($x \pm 1\sigma$) for master variables, nutrients, DOC and chlorophyll *a* for each tidal cycle survey at Restronguet Point are shown in Fig. 2. Also included are the daily river flows for the River Fal (the nearest gauged river; Fig. 1); these data (Fig. 2A) show that surveys were undertaken during contrasting hydrological conditions, with mean flows ranging from 0.72–4.93 $\text{m}^3 \text{s}^{-1}$. The greatest salinity range was observed in December 2002 ($S = 30.3 \pm 3.5$; Fig. 2B), when the highest river flow was encountered at the maximum tidal range (4.7 m), while the most saline waters were observed during spring/summer surveys (up to $S = 34.6$). Throughout, the lowest salinity values coincided with low water. The pH, at around 8.2 (Fig. 2D), was typical for high-salinity estuarine waters, although lowest values occurred during winter (7.97 ± 0.08) and highest values during summer (8.52 ± 0.13). The higher summer values coincided with super-saturation of dissolved oxygen ($116 \pm 11\%$; Fig. 2E), indicating that phytoplankton production was important at this time and yielded increases in pH. However, the oxygen super-saturation did not coincide with highest concentrations of chlorophyll *a* (Fig. 2F). Concentrations of nitrate + nitrite were highest in the winter ($33 \pm 13 \mu\text{M N}$) and lowest in summer ($3.1 \pm 1.7 \mu\text{M N}$) and were linked to relative changes in catchment run-off and algal growth (Fig. 2G and F). Ortho-phosphate concentrations were low (less than $0.31 \pm 0.05 \mu\text{M P}$), but with a seasonal cycle more complex than nitrate + nitrite, probably due to the influence of effluent inputs from sewage treatment works (Fig. 1A) (Langston et al., 2003).

3.2. Concentrations and behaviour of Me_{tot} and Me_{dyn}

Concentration data ($x \pm 1\sigma$) for Me_{tot} and Me_{dyn} for each tidal cycle at Restronguet Point are summarised in Fig. 3. Mean Cu_{tot} concentrations showed relatively little variation throughout the sampling period, at 78 ± 45 – $126 \pm 100 \text{ nM}$ (Fig. 3A), with a minimum to maximum range of 4.97–315 nM for all data. Mean concentrations of Cu_{dyn} showed more variability (13.3 ± 6.3 to $64.2 \pm 30.5 \text{ nM}$; range 1.7–190 nM) and values were generally higher during the winter high river flow periods. Mean concentrations of Cd_{tot} varied between 0.53 ± 0.34 and $2.69 \pm 2.49 \text{ nM}$ (Fig. 3B), with a range of 0.13–8.53 nM Cd_{tot} for all data. Concentrations of Cd_{dyn} ranged from 0.55 ± 0.81 to $1.75 \pm 1.47 \text{ nM}$, with a range of 0.17–5.06 nM and, as for Cu_{dyn} , mean values were higher during the winter. These total dissolved metal concentrations are in good agreement with recent data reported for Restronguet Point

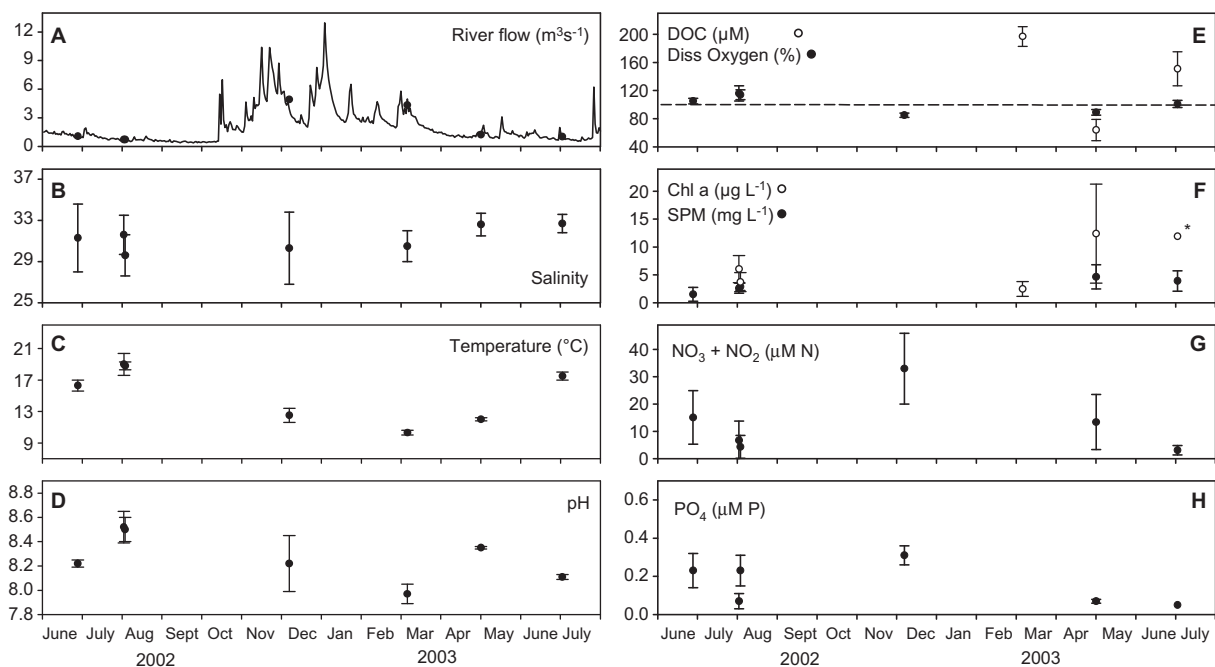


Fig. 2. Summary data (mean \pm 1 standard deviation) of determinands for each of the sample collection periods at Restronguet Point. (A) daily flows ($\text{m}^3 \text{s}^{-1}$) in the nearest gauged river (Fal), the sampling time and associated mean flow during the previous 7 d (\bullet); (B) salinity; (C) temperature ($^{\circ}\text{C}$); (D) pH; (E) dissolved oxygen saturation (%) (dashed line shows 100% saturation), dissolved organic carbon (DOC, μM); (F) suspended particulate matter (SPM, mg L^{-1}), chlorophyll *a* (Chl *a*, $\mu\text{g L}^{-1}$) (*single data point); (G) nitrate + nitrite ($\mu\text{M N}$); (H) ortho-phosphate ($\mu\text{M P}$). Tidal range at Falmouth: 2002: June 3.8 m, August 2.3 m, December 4.7 m; 2003: March 4.6 m, April 3.9 m, July 3.7 m.

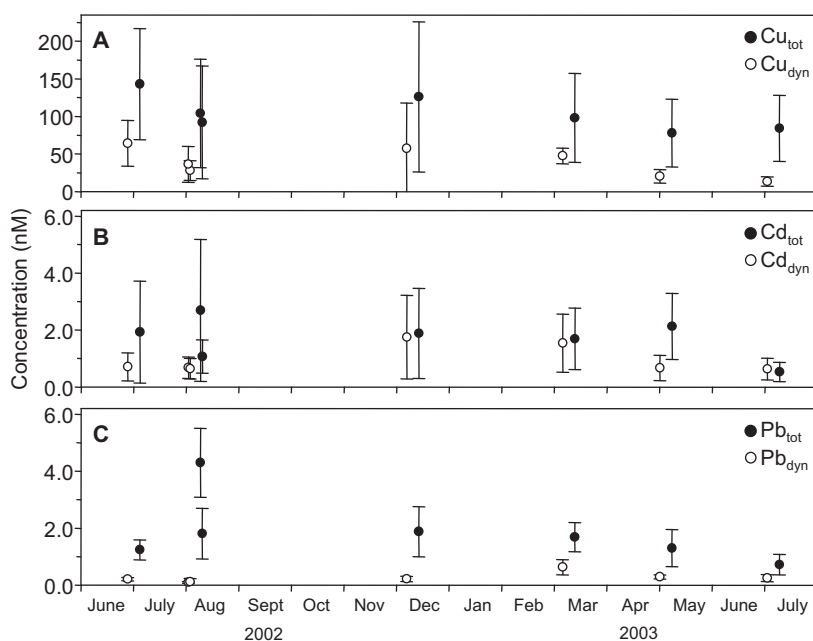


Fig. 3. Summary concentration data (mean \pm 1 standard deviation) of dynamic and total dissolved metals for each of the sample collection periods at Restronguet Point. (A) copper, (B) cadmium, (C) lead (all nM). The data points for the dynamic and total fractions are offset to aid clarity.

by Langston et al. (2003); in this study the median metal concentrations were 110 nM Cu_{tot} (range 30–415 nM) and 1.8 nM Cd_{tot} (range <1.8–80 nM). Mean concentrations of Pb_{tot} (Fig. 3C) were 1.24 ± 0.35 – 4.30 ± 1.21 nM (range 0.35–5.74 nM), and as for Cd_{tot} , were lowest during July 2003 (0.72 ± 0.36 nM). In contrast to Cu_{dyn} and Cd_{dyn} , mean concentrations of Pb_{dyn} were relatively uniform during the sampling period (0.10 ± 0.03 – 0.29 ± 0.07 nM), except during March 2003, when higher concentrations were observed

(0.63 ± 0.27 nM Pb_{dyn}). The range in concentrations of Pb_{dyn} was 0.038–1.30 nM for all data.

Concentrations of dynamic and total dissolved Cu and Cd were generally highest near low water (± 1 h) and hence coincided with the lowest salinity during all surveys (e.g. Fig. 4A–D for June and December 2002). Freshwater endmember concentrations of Cu (355–3310 nM) and Cd (15.4–20 nM) in the River Carnon were higher than concentrations of Cd and Cu at Restronguet Point,

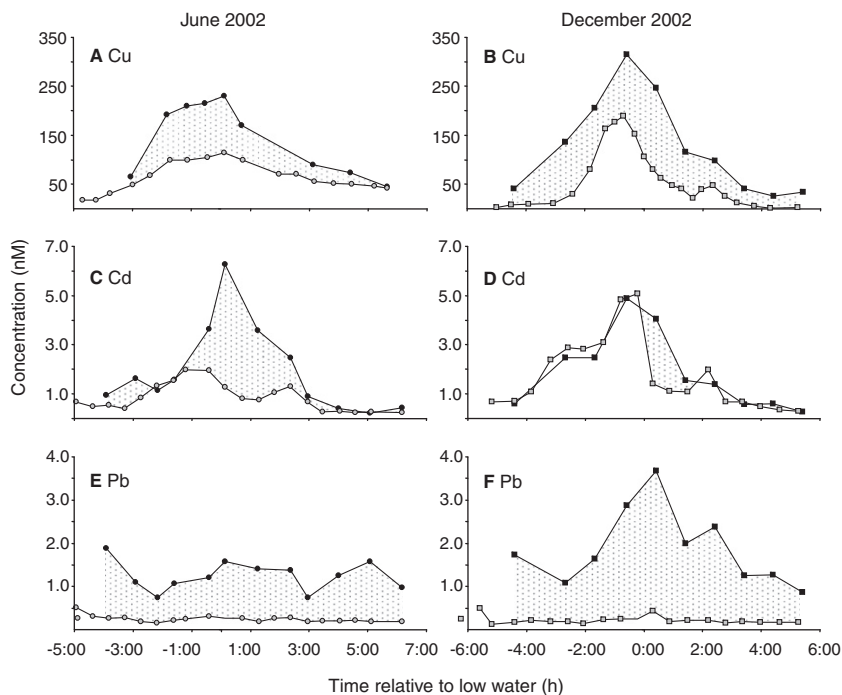


Fig. 4. Speciation of dissolved Cu, Cd and Pb during a summer (○: June 2002) and a winter (□: December 2002) survey. Open and filled symbols represent the dynamic and total concentrations, respectively. Total concentrations were determined in discrete samples, dynamic concentrations were determined *in situ*. The patterned area represents the non-dynamic, or colloidal, metal fraction, calculated by subtraction.

providing a strong source of dissolved metals to the estuary. Property-salinity analyses of the tidal cycle data indicate that concentrations of Cd_{tot} and Cd_{dyn} were largely controlled by the mixing of metal-rich water from the River Carnon, at the head of Restronguet Creek, with marine water low in dissolved metals (Cd_{tot} : $r^2 = 0.64$, $p < 0.0001$, $n = 63$; Cd_{dyn} : $r^2 = 0.69$, $p < 0.0001$, $n = 39$). Copper also correlated with salinity, although there was more scatter in the data (Cu_{tot} : $r^2 = 0.54$, $p < 0.0001$, $n = 51$; Cu_{dyn} : $r^2 = 0.43$, $p < 0.0001$, $n = 69$). Approximately linear inverse relationships between total dissolved Cu and Cd, and salinity were observed along transects in Restronguet Creek in April and July 2003; similar patterns have also been reported by Langston et al. (2003). Concentrations of Cu and Cd in the surface bed sediments (<100 μm fraction) of Restronguet Creek are very high (21–30 $nmol g^{-1}$ DW Cd; 25×10^3 – 71×10^3 $nmol g^{-1}$ DW Cu) due to the mine waste inputs and the scatter in the property-salinity relationships may be explained, in part, by benthic remobilisation of dissolved metals (Langston et al., 2003). Although reliable quantitative data on Cu release from the sediment have not been reported for this system, Skrabal et al. (2000) and references therein have directly measured fluxes of dissolved metals out of the sediment where pore water concentrations of metals are higher than in the overlying waters.

The concentrations of Pb_{dyn} and Pb_{tot} exhibited no obvious pattern related to the tidal state during most of the deployments (Fig. 4E/F), although there was a weak negative correlation between Pb_{dyn} and salinity ($r^2 = 0.15$, $p = 0.02$, $n = 40$). However, Pb_{tot} concentrations observed at low water at Restronguet Point (Fig. 4F) were always higher, sometimes markedly so, than the concentrations of Pb_{tot} (0.65–1.1 nM) determined at the tidal limit of the River Carnon during the same period. These elevated concentrations of Pb_{tot} at Restronguet Point indicate that there were additional sources of Pb in the estuary. Concentrations of Pb in the sediments of Restronguet Creek are in the range 1000–2700 $nmol g^{-1}$ DW, making it one of the most lead contaminated estuaries studied (Pirrie et al., 2003). Therefore the Creek's extensive inter-tidal

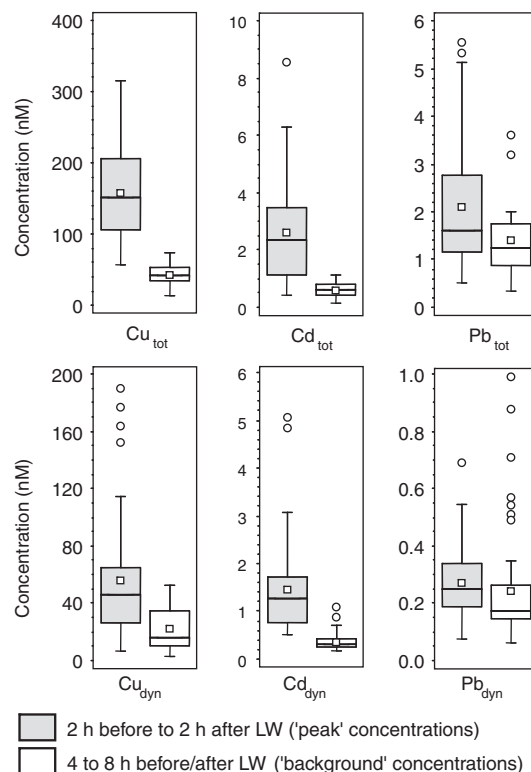


Fig. 5. Total and dynamic Cu, Cd and Pb concentrations from seven tidal cycle surveys at Restronguet Point. Grey box: concentrations observed within 2 h either side of low water. White box: concentrations observed 4–6 h before or after low water. The central box covers the central 50% of data, the horizontal line is the median and the square is the mean.

mudflats are a reservoir, from which dissolved (including colloidal) lead may be released into the water column.

The mobilisation of colloidal Pb and Cu from sediments has been reported as an important contributor to dissolved metal concentrations in other estuaries (e.g. Waeles et al., 2008). Furthermore, Boyden et al. (1979) reported a several-fold increase in SPM concentrations in bottom waters at Restronguet Point at low water, compared to high water, with concomitant increase in the particulate concentrations of Fe, Cu and Pb. Such a source of truly dissolved and/or colloidal metals would contribute to the scatter in the Pb and Cu-salinity relationships observed in this study.

The distinctly tidally influenced profiles of Cu and Cd enabled the calculation of 'background' concentrations dominated by the marine end-member from data points obtained 4–6 h before or after LW, as well as 'peak' concentrations influenced by the inputs from the River Carnon (2 h either side of LW, Fig. 5). The average peak values for dynamic and total dissolved Cu and Cd were significantly higher than their respective background values (z -test, $p \ll 0.05$). There was no statistically significant difference between concentrations of Pb_{dyn} or Pb_{tot} determined during the different time windows.

3.3. Speciation of dissolved Cu, Cd and Pb

The size of the colloidal pool, as a fraction of the total dissolved pool, was significantly different for each metal (z -test, $p < 0.05$; all data) and followed the sequence $77 \pm 17\% Pb_{col} > 60 \pm 25\% Cu_{col} > 37 \pm 30\% Cd_{col}$. Both the magnitude of the colloidal fraction and the sequence is comparable with other coastal and estuarine systems (see Section 1.2). During the spring and summer surveys, the fraction of Cu_{col} dominated with an average of $67 \pm 14\%$ and $56 \pm 26\%$ of Cu_{tot} during the low water and non-low water periods of the tidal cycles, respectively (Fig. 4A). In December, however, the main increase in Cu_{tot} at the time of low water was in the dynamic fraction (Cu_{dyn}), which formed 50% of Cu_{tot} (Fig. 4B), compared to a mean fraction of 20% Cu_{dyn} at background concentrations at this time. This suggests that in winter, when river flows were relatively high, dynamic Cu was more important, while in spring and summer, Cu in Restronguet Creek was largely present as non-dynamic complexes. The importance of terrestrially-derived DOC in determining Cu complexation behaviour has been reported for other estuaries (Shank et al., 2004a; Waeles et al., 2009). However, in these studies, the complexing ligands were hydrophobic, non-polar entities extractable by C_{18} resins and likely to form Cu complexes too strong (i.e. not sufficiently mobile and labile) to be detected by the VIP. Therefore, the increase in the dynamic fraction observed in Restronguet Creek during winter may have been due mainly to inorganic Cu complexes mobilised in a catchment markedly influenced by acid mine drainage. In the spring and summer of 2003 (no DOC data for 2002), there was no significant negative correlation between DOC and salinity (salinity range 0.43–34.4), nor a positive correlation between chlorophyll *a* and DOC. This suggests that the river was not an important source of terrestrial DOC during these periods and that suspended phytoplankton did not markedly contribute to the DOC pool in the Creek. There was, however, a weak positive linear correlation between Cu_{tot} and DOC concentrations ($r^2 = 0.23$, $p = 0.03$, $n = 20$). Furthermore, Kawakami (2004) reported that dissolved Cu was strongly complexed in the Creek ($\log K'_{CuL} = 10.8$ – 12.8) during April and July 2003 (and August 2002). Potential sources of Cu-complexing DOC to the water column include the benthic sediment (Skrabal et al., 2000). In the Cape Fear River estuary, Shank et al. (2004a) reported that the benthic sediment was an important source of strong Cu-binding ligands ($\log K'_{CuL} > 11$) during the summer months, although their influence on Cu complexation was dependent on ambient concentrations of strong-binding ligands in the water column. While the fluxes of strongly complexing ligands and DOC from estuarine sediments are not always well correlated (Shank et al., 2004b), the positive

correlation between Cu_{tot} and DOC in the current study is consistent with the sediment as a source of these moieties. The effluent from sewage treatment works may have also been a source of strong-binding ligands (van Veen et al., 2002), particularly during the low river flow periods, when effluent water makes up a larger proportion of surface water flow in this region (Langston et al., 2003). It is also possible that dissolved Cu is complexed by plankton-derived ligands (Dryden et al., 2007). However, Kawakami (2004) showed for August 2002, April and July 2003 that the ligands (phytochelatins and glutathione) produced by autochthonous plankton growth constituted a minor component (<7%) of the total ligand concentration (45–620 nM) that contributed the strong Cu complexation observed. In summary, the data suggest that relatively high concentrations of bioavailable Cu are present during low water in the winter, while in spring and summer an important colloidal phase reduces Cu bioavailability and potential toxicity.

Dissolved Cd exhibits a complex geochemistry in river estuaries. In both, freshwater and brackish-to-saline estuarine water, dissolved Cd can have a significant colloidal component, or very little. Furthermore, dissolved Cd appears to undergo dynamic changes in size distributions (e.g. <1 kDa, 1–10 kDa, 10 kDa–0.45 μ m) along the salinity gradient (Powell et al., 1996). Presumably, changes in size distribution will exert an effect on the strength of ligand binding. In June and August 2002 and in April 2003, Cd_{col} increased with Cd_{tot} , for example constituting a shift from 31% to 42% Cd_{col} at background concentrations to over 81% Cd_{col} at maximum concentrations at low water in June (Fig. 4C). In contrast, peak concentrations of Cd_{tot} resulted mainly from an increase in the dynamic fraction in December 2002 (Fig. 4D) and March and July 2003. The lack of a seasonal trend and the strong interaction between Cd and colloids in estuaries suggests that the estuarine speciation of Cd may have been controlled by a complex interplay of several processes: riverine Cd_{dyn} inputs, a redistribution of riverine colloidal Cd from larger to smaller size fractions at increasing salinity in the estuary (Powell et al., 1996), Cd release from decomposing organic matter in the benthic compartment and/or release of Cd_{col} from (re)-suspended inorganic colloids (Waeles et al., 2009). As the total Cd concentration in the fresh water input of the River Carnon was constant (15–20 nM) during all surveys, the flux into the estuary would have been lower during periods of low river flow, which coincided with surveys when the increase in Cd around low water was mainly colloidal (June/August 2002 and April 2003). During the December and March surveys, high river flow and riverine Cd flux may have partially masked estuarine processes, and riverine Cd_{dyn} dominated the Cd increase at Restronguet Point at low water. This is consistent with the prevalence of free hydrated Cd ions in acidic river water and the tendency of Cd to form chloro-complexes at higher salinities (Chester, 2003).

The colloidal fraction of Pb ranged from 30% to 99% of Pb_{tot} (mean 80%) and minima or maxima in this fraction were not associated with particular tidal stages (z -test, $p \gg 0.05$ for difference between different times of the tidal cycle). The association of Pb with colloidal iron hydroxide phases, rather than with organic colloids has been shown to be important in its estuarine speciation (Waeles et al., 2008; Stolpe et al., 2010) and the presence of precipitated Fe phases and primary sulphide minerals in the sediment in the Restronguet Creek has been reported (Pirrie et al., 2003). The evolution of Pb speciation during individual tidal cycle studies (e.g. June and December 2002, Fig. 4E/F) indicates that the changes in Pb_{tot} resulted largely from variations in the colloidal fraction not associated with high/low water cycles. Furthermore, the riverine concentration of Pb was consistently below Pb_{tot} at Restronguet Point (see Section 3.2). These data suggest that Pb inputs to the water column resulted from the mobilisation of Pb associated with colloidal Fe phases in the sediment, induced by disturbances of surface sediments through tidal hydrodynamics and boating

activities at the mouth of the Creek. While it is likely that oxidative release of metals from primary and secondary metal sulphides as a result of resuspension or bioturbation contributes to the overall metal load in the water column, oxidation rates of PbS have been shown to be too low (PbS is not affected by oxidation after 8 h suspension in oxic seawater, Simpson et al., 1998) to be significant on the time scale of a tidal cycle. This supports the suggestion that colloidal Fe oxide/hydroxide phases play the dominant role in the sediment/water interaction for Pb.

3.4. Impacts on biota

The Environmental Quality Standard (EQS) for dissolved copper in saltwater/estuarine waters (78.7 nM annual average, European Commission (EC) Dangerous Substances Directive, DSD) was exceeded during all tidal cycle studies at Restronguet Point by peak concentrations of Cu_{tot} (2 h either side of low water, Fig. 5) and within Restronguet Creek itself (up to 3.30 μM Cu_{tot}). Concentrations of Cd_{tot} and Pb_{tot} remained below the respective EQS values (120 nM Pb and 22.3 nM Cd, both annual averages EC DSD) at Restronguet Point and in the Creek (up to 20 nM Cd_{tot} and 4.2 nM Pb_{tot}). During all surveys, freshwater endmember concentrations of Cu_{tot} and Cd_{tot} in the River Carnon exceeded the relevant EQS values (1.6–440 nM Cu, depending on total hardness and 8.9 nM Cd, both annual averages, EC DSD). This indicates that detrimental effects on species susceptible to contamination in the water column, such as phytoplankton, macroalgae and some macroinvertebrate species, arise throughout Restronguet Creek from dissolved copper alone, particularly during the ebb tide.

More specific information on toxicity is provided by the dynamic metal concentrations obtained during the tidal cycle studies at Restronguet Point, as dynamic metal species approximates the biologically available fraction (Buffle and Tercier-Waeber, 2005). However, there is a paucity of published data on the toxicity of the dynamic fraction. Therefore, assuming a salinity of 30, pH 8.2 and that the dynamic Cu, Cd and Pb fractions are made up entirely of inorganic complexes, minimum and maximum free metal ion concentrations were calculated for June and December 2002 surveys using the thermodynamic equilibrium modelling software MINEQL+ (Version 4.5, Schecher and McAvoy, 2003).

The calculated free cupric ion concentration varied between 0.12 and 14 nM Cu^{2+} in December and between 1.3 and 8.5 nM Cu^{2+} in June. These results show that cyanobacteria and many plankton species are likely to be highly compromised in the Creek, as studies showed that their viability declined at concentrations in the range 0.001–0.1 nM Cu^{2+} (Brand et al., 1986; Sunda et al., 1987). Furthermore, larvae bioassays of higher organisms, such as sea urchin (*Strongylocentrotus purpuratus*), mussel (*Mytilus galloprovincialis*) and oyster (*Crassostrea gigas*), exhibited sensitivity toward Cu^{2+} at $EC_{50} \leq 0.90$ nM, $EC_{50} \leq 0.06$ nM and $EC_{50} = 0.23 \pm 0.08$ nM, respectively (Rivera-Duarte et al., 2005; Money, 2008), and hence the development of juveniles of such organisms is likely to be severely impaired at the study location.

The calculated minimal and maximal concentrations of Cd^{2+} and Pb^{2+} were in the sub-nanomolar range for both surveys (0.009–0.18 nM Cd^{2+} and 0.008–0.028 nM Pb^{2+}). Phytoplankton species are less sensitive to Pb and Cd than to Cu. For example, reduced growth rates have been reported for a range of coastal phytoplankton species at a free ion concentration of $Cd^{2+} > 23$ nM (Payne and Price, 1999), suggesting that Cd_{dyn} in Restronguet Creek was below the effect threshold by at least two orders of magnitude. Money (2008) reported toxicity to *C. gigas* (abnormal larva development) with an $EC_{50} = 88.0 \pm 34.7$ nM for Cd^{2+} and $EC_{50} = 362 \pm 178$ nM for Pb^{2+} , indicating that on their own neither Cd nor Pb present environmental hazards to macroinvertebrates in the Fal Estuary.

The data presented here suggest that the dynamic concentration of Cu (2.8–190 nM) in the Restronguet Creek is of the order of magnitude likely to have toxic effects on juveniles of coastal macroinvertebrate species, as well as negatively affect the even more sensitive phytoplankton community. Moreover, bioassay studies of metal mixtures have shown synergistic toxic effects (e.g. Kobayashi and Okamura, 2005) between metals, and therefore, the combination of enhanced Cu_{dyn} , Cd_{dyn} and Pb_{dyn} at low water is of concern. Furthermore, high total dissolved Zn and As concentrations (76–22900 nM Zn, 40–1250 nM As) have been reported for the Restronguet Creek (Langston et al., 2003), adding to the toxic burden.

4. Conclusions

A unique set of data has been presented that resolved the dynamic changes of Cu, Cd and Pb speciation in an estuary impacted by the legacy of metal mining in its catchment. The information on dynamic metal species obtained *in situ* at high temporal resolution highlighted the enhanced concentrations of potential toxic forms of Cu and Cd when the influence of the riverine contamination arising from mine drainage is highest, i.e. during times of low water and/or high river water discharge. Changes in dynamic and colloidal metal concentrations in the water column highlighted the different behaviour of Cu, Cd and Pb in this estuary. This data set combined total metal concentrations along the salinity gradient, speciation data for tidal cycles and auxiliary data, which allowed to infer that important interactions between dissolved Pb and Cd and the benthic compartment took place. These data show that important fluctuations in metal toxicity can occur in estuaries over a range of time scales, from minutes to seasons. Furthermore, these fluctuations are more complex than simple relations to river inputs, the stage of the tide or seasonal factors. This study illustrates that in order to understand the potential impact of contamination within the dynamic coastal environment, biologically relevant fractions of metals have to be studied *in situ* and at sufficiently high resolution.

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