

2019-04-04

# In-situ trace metal (Cd, Pb, Cu) speciation along the Po River plume (Northern Adriatic Sea) using submersible systems

Illuminati, S

<http://hdl.handle.net/10026.1/13708>

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10.1016/j.marchem.2019.04.001

Marine Chemistry

Elsevier

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## Manuscript Details

<b>Manuscript number</b>	MARCHE_2018_153
<b>Title</b>	In-situ trace metal (Cd, Pb, Cu) speciation along the Po River plume (Northern Adriatic Sea) using submersible systems.
<b>Article type</b>	Research Paper

### Abstract

Information on the distribution and speciation of trace metals is of critical importance for our ability to interpret the links between the bioavailability and uptake of an element, and its biogeochemical cycle in coastal environments. Within the framework of the European Project "In-situ automated Monitoring of Trace metal speciation in Estuaries and Coastal zones in relation with the biogeochemical processes (IMTEC)", the chemical speciation of Cd, Pb and Cu was carried out along the Po River plume in the period 27 October – 2 November 2002. During the cruise five Voltammetric In-situ Profiling systems and one Multi Physical Chemical Profiler, as well as conventional voltammetric instruments, were successfully applied in order to evaluate the distribution of Cd, Pb and Cu between different fraction (free ion, dynamic, colloidal, dissolved and particulate fractions) and to assess the evolution of these fractions during the estuarine mixing and in the water column. Dynamic concentrations were 0.05-0.2 nmol L<sup>-1</sup> Cd, 0.02-0.2 nmol L<sup>-1</sup> Pb, and 0.15- 4.0 nmol L<sup>-1</sup>Cu. Cd was mainly present as dynamic fraction (40-100% of the dissolved Cd). High proportions of Pb (~70%) and Cu (~80%) were presents as colloids of probably biogenic origin. Principal components analysis reveals a strong influence of the Po River discharge on the spatial and vertical distributions of metal species. Almost all the metal fractions globally decreased following the salinity gradient. Metal concentrations are far below (at least one order of magnitude lower) the Environmental Quality Standard established by the Italian law. However, the Cu dynamic fraction showed concentrations likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal macroinvertebrate species (toxicity data extracted from literature).

<b>Keywords</b>	metals; speciation; estuary; voltammetry
<b>Taxonomy</b>	Marine Pollution, Metal Biogeochemistry, Environmental Chemistry Substances, Marine Chemistry, Environmental Science
<b>Corresponding Author</b>	Cristina Truzzi
<b>Corresponding Author's Institution</b>	Departement of Life and Environmental Sciences, Università Politecnica delle Marche
<b>Order of Authors</b>	Silvia Illuminati, Anna Annibaldi, Cristina Truzzi, Marylou Tercier-Waeber, Charlotte Braungardt, Eric Achterberg, David Turner, mauro marini, Tiziana Romagnoli, Cecilia Maria Totti, Flavio Graziottin, Jacques Buffle, Giuseppe Scarponi
<b>Suggested reviewers</b>	Roko Andricevic, Matthieu Waeles, Sabina Susmel, Clara Turetta, Ana-Marija Cindric

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Dear Sirs,

I enclose a manuscript entitled  
“In-situ trace metal (Cd, Pb, Cu) speciation along the Po River plume (Northern Adriatic Sea) using submersible systems”

by

Silvia Illuminati, Anna Annibaldi, Cristina Truzzi, Mary-Lou Tercier-Waeber, Stéphane Noël, Charlotte B. Braungardt, Eric P. Achterberg, Kate A. Howell, David Turner, Mauro Marini, Tiziana Romagnoli, Cecilia Totti, Fabio Confalonieri, Flavio Graziottin, Jacques Buffle, Giuseppe Scarponi

to be considered for publication as Research Paper in “Marine Chemistry”.

The manuscript describes and discusses the results on the chemical speciation of Cd, Pb and Cu carried out along the Po River plume, Adriatic Sea

It presents some interesting news: (1) the application of voltammetric sensors (Voltammetric In-situ Profiler and Multi Physico-Chemical Profiler) allowing the evaluation in-situ of Cd, Pb and Cu specie distribution; (2) the assessment of the evolution of metal fractions during the estuarine mixing and in the water column; (3) the multivariate statistical analysis applied to the overall dataset revealed a strong influence of the Po River discharge on the spatial and vertical distributions of metal species; (4) the metal concentrations are one order of magnitude lower than the Environmental Quality Standard established by the Italian law; (5) the Cu dynamic fraction showed concentrations likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal macroinvertebrate species.

Please, note that two authors (Fabio Confalonieri and Flavio Graziottin) have the same e-mail addresses, the only available. One of the authors, Kate A. Howell, is dead. Anyway, we would like to include her name in the author’s list, in memory of her strong contribution to the project and...of her smile.

Thanking you in advance for your attention,  
Yours sincerely,

Dr. Silvia Illuminati  
Polytechnic University of Marche – Ancona,  
Department of Life and Environmental Sciences,  
Via Brece Bianche, 60131, Ancona, Italy  
E-mail: [s.illuminati@univpm.it](mailto:s.illuminati@univpm.it)

## **Research Highlights**

- First speciation study on trace metals in the Adriatic Sea
- In-situ speciation by submersible, reliable voltammetric sensors
- Metal species distribution mainly affected by Po River outflow
- Metal dynamic concentrations below legal limits
- Cu dynamic concentrations toxic to sensitive phytoplankton

1 **In-situ trace metal (Cd, Pb, Cu) speciation along the Po River plume (Northern Adriatic Sea)**  
2 **using submersible systems.**

3  
4 Silvia Illuminati<sup>a1</sup>, Anna Annibaldi<sup>a1</sup>, Cristina Truzzi<sup>\*a</sup>, Mary-Lou Tercier-Waeber<sup>b</sup>, Stéphane Noël<sup>b</sup>, Charlotte B.  
5 Braungardt<sup>c</sup>, Eric P. Achterberg<sup>d</sup>, Kate A. Howell<sup>c,e</sup>, David Turner<sup>f</sup>, Mauro Marini<sup>g</sup>, Tiziana Romagnoli<sup>a</sup>, Cecilia Totti<sup>a</sup>,  
6 Fabio Confalonieri<sup>h</sup>, Flavio Graziottin<sup>h</sup>, Jacques Buffle<sup>b</sup>, Giuseppe Scarponi<sup>a</sup>

7  
8 <sup>a</sup>Department of Life and Environmental Sciences, Università Politecnica delle Marche, Italy

9 <sup>b</sup>CABE, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland

10 <sup>c</sup>School of Earth, Ocean and Environmental, University of Plymouth, UK

11 <sup>d</sup>GEOMAR, Helmholtz Centre for Ocean Research Kiel, Germany

12 <sup>e</sup>Nanotecture Ltd. Epsilon House, Southampton Science Park, UK

13 <sup>f</sup>Department of Marine Sciences, University of Gothenburg, Sweden

14 <sup>g</sup>Institute of Marine Science, National Research Council (CNR), Ancona, Italy

15 <sup>h</sup>Idronaut Srl, Brughiero (MI), Italy

16  
17 <sup>1</sup>Both authors contributed equally to this manuscript

18 **Contact information for Corresponding Author**

19 <sup>\*</sup>Cristina Truzzi, Department of Life and Environment Sciences, Università Politecnica delle Marche, Via Breccie  
20 Bianche, 60131 Ancona, Italy. Phone: +390712204981 Fax: +390712204650. E-mail: [c.truzzi@univpm.it](mailto:c.truzzi@univpm.it)

21  
22  
23 **Abstract**

24 Information on the distribution and speciation of trace metals is of critical importance for our ability to interpret the links  
25 between the bioavailability and uptake of an element, and its biogeochemical cycle in coastal environments. Within the  
26 framework of the European Project “In-situ automated Monitoring of Trace metal speciation in Estuaries and Coastal  
27 zones in relation with the biogeochemical processes (IMTEC)”, the chemical speciation of Cd, Pb and Cu was carried out  
28 along the Po River plume in the period 27 October – 2 November 2002. During the cruise five Voltammetric In-situ  
29 Profiling systems and one Multi Physical Chemical Profiler, as well as conventional voltammetric instruments, were  
30 successfully applied in order to evaluate the distribution of Cd, Pb and Cu between different fraction (free ion, dynamic,  
31 colloidal, dissolved and particulate fractions) and to assess the evolution of these fractions during the estuarine mixing  
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34 Cu (~80%) were presents as colloids of probably biogenic origin. Principal components analysis reveals a strong influence  
35 of the Po River discharge on the spatial and vertical distributions of metal species. Almost all the metal fractions globally  
36 decreased following the salinity gradient. Metal concentrations are far below (at least one order of magnitude lower) the  
37 Environmental Quality Standard established by the Italian law. However, the Cu dynamic fraction showed concentrations

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39 macroinvertebrate species (toxicity data extracted from literature).

40

41

## 42 **Keywords**

43 Metals; Speciation; Estuary; Voltammetry

44

## 45 **1. Introduction**

46 The Adriatic Sea is a semi-enclosed shelf basin located in the northeast part of the Mediterranean Sea and it is strongly  
47 influenced by several riverine inputs. In its northern sub-basin, the freshwater plume of the Po River plays a fundamental  
48 role in driving the coastal dynamics and the physical and biogeochemical processes of the whole basin. Crossing the  
49 entire northern part of the Italian country, which is one of the most industrialized areas in Europe, the Po river discharges  
50 in the Adriatic Sea a remarkable load of pollutants, which in turns affect the Italian coasts even to approximately the city  
51 of Ancona (following the prevailing southward marine currents).

52 The Northern Adriatic Sea has been intensely investigated, studying the physical structure of the water column, as  
53 well as the circulation patterns (Russo and Artegiani, 1996; Falcieri et al., 2014), the plankton community (Totti et al.,  
54 2005; Cabrini et al., 2012; Godrijan et al., 2013), the nutrient (Boldrin et al., 2005; Grilli et al., 2013) and trace element  
55 distribution in seawater (Annibaldi et al., 2009, 2011, 2015; Cindrić et al., 2015; Tankere and Statham, 1996; Zago et al.,  
56 2000, 2002).

57 Nowadays, following the enactment of specific EU Directives, there is a growing need to control the quality of coastal  
58 waters, which are affected by several anthropogenic activities (building of infrastructure for human settlement, habitat  
59 modification, tourism, transport by sea and disposal of industrial and domestic effluents). Combined efforts are requested  
60 to develop monitoring and assessment programs in order to survey and guarantee a good chemical and ecological status  
61 of the coastal ecosystems, including heavy metals. Heavy metals are ubiquitous in the environment and their  
62 concentrations are increased with respect to natural pre-industrial values due to anthropogenic activities, which have  
63 modified at the same time, metal biogeochemical cycles on a regional and global scale (Sadiq, 1992). It is well known  
64 that the toxicity, bioavailability and mobility of trace metals in seawater depend on their speciation rather than on the only  
65 total concentration (Allen and Hansen, 1996). Dissolved trace metals in seawater can exist in different chemical species  
66 (forms), such as free hydrated ions, organic and inorganic complexes. The study of the metal species distribution in  
67 seawater gives insights on their cycles, as each metal species is characterized by different reactivity and different  
68 interactions with organisms. Free hydrated ions are the most reactive and highly bioavailable, but their reactivity, as well

69 as their toxicity can be greatly modified by the presence of organic and inorganic compounds. Thus, to better understand  
70 the biogeochemical cycle of a trace metal and its overall mobility in the water column, it is essential to identify and  
71 quantify the various species that make up its total concentration.

72 Across much of the world, regulatory limit values (e.g., Environmental Quality Standards in the EU, Water Quality  
73 Criteria in the US, Australia, Canada, etc.) for metals in water bodies do not take metal speciation into account. They are  
74 mainly based on dissolved metal, since this fraction more closely approximates the bioavailable fraction of the metal in  
75 the water column. Recently, thanks to a major refinement in the scientific understanding of the behavior, fate and  
76 toxicology of metals in the environment, and to a series of statutory and voluntary risk assessments performed under the  
77 existing regulations on pollutant monitoring program, several public administrations started to implement the in-force  
78 water quality assessments by introducing a speciation-based approach. The revised Priority Substances Daughter  
79 Directive, 2013/39/EU (2013) includes now annual average Environmental Quality Standards (EQS) for nickel and lead  
80 in the freshwater environment that refer to bioavailable concentrations. These bioavailable EQS are based on Biotic  
81 Ligand Models (BLM), but, at the time of the development of the latest draft of the Water Framework Directive, there  
82 was no validated and accepted BLM for some metals, therefore complimentary availability-based approaches were  
83 adopted to define the EQS bioavailable. For example, the EQS for lead are based on the availability correction for the  
84 dissolved organic carbon (SCHEER-EC, 2017).

85 Very few studies reported chemical speciation of trace metals in the Adriatic Sea, the most of them dealing with the  
86 horizontal and vertical partitioning between dissolved and particulate phases (Tankere and Statham, 1996; Tankere et al.,  
87 2000; Zago et al., 2000, 2002; Annibaldi et al., 2011, 2015; Cindrić et al., 2015). Some authors (Scarponi et al., 1995,  
88 1998), through voltammetric titration, studied the distribution of dissolved trace metals between the free fraction (mainly  
89 ionic and inorganic complexed metal) and bound fraction (organically complexed metal), as well as the content of ligands  
90 complexing metal and the related conditional stability constants.

91 Within the framework of the European Project “In-situ automated Monitoring of Trace metal speciation in Estuaries  
92 and Coastal zones in relation with the biogeochemical processes (IMTEC)”, several studies on metal speciation were  
93 carried out in different European coastal areas by the partners of the project (University of Geneva, University of  
94 Göteborg, University of Plymouth, University of Ancona, University of Neuchatel).

95 This paper focuses on the chemical speciation of Cd, Pb and Cu along the Po River plume, which is particularly  
96 significant for the study of physical, hydrological and biological processes that affect trace metal contents and  
97 distributions during the estuarine mixing, and for the better understanding of the behaviour and fate of the different metal  
98 species. The speciation studies were carried out by applying submersible voltammetric probes, that were developed and  
99 tested within the IMTEC project for the trace element monitoring in natural environments.



100 The Voltammetric In-situ Profiling (VIP) probe is the first commercially available instrument (Idronaut, Italy)  
101 developed by Tercier and co-workers (Tercier et al., 1998) that combines trace metal speciation analysis of high sensitivity  
102 and resolution with automated in-situ operation and options for remote deployment. The VIP allows the long-term, real-  
103 time and simultaneous determination of a fraction of the conventional total dissolved trace metal, the so-called “dynamic  
104 fraction”, of several trace metals, directly in the water column, with no or minimum pretreatments. The dynamic fraction  
105 is defined as the sum of the free metal ions and the sufficiently labile (high dissociation rate) and mobile (high diffusion  
106 rate) inorganic and organic complexes of a few nanometers in size (Buffle and Tercier-Waeber, 2005). This metal fraction  
107 is of great importance, as it represents the concentration of metals potentially bioavailable, i.e. the dynamic metal  
108 complexes may dissociate within the time it takes to diffuse from the bulk medium to an organism’s cell surface receptor  
109 site, and hence be readily available (Buffle and Tercier-Waeber, 2005). This is possible due to a specifically designed  
110 gel-integrated microelectrode (GIME) that consists of an array of 100 interconnected Ir-based micro-disc electrodes,  
111 coated with an antifouling gel membrane of 300  $\mu\text{m}$  thickness (for more details see Belmont-Hebert et al., 1998). The  
112 non-dynamic fraction, comprising metals associated with colloidal material cannot be determined by the VIP system  
113 (Buffle and Tercier-Waeber, 2005; Braungardt et al., 2011). Generally, colloids are micro particles and macromolecules  
114 in the size range of 1 nm to 1  $\mu\text{m}$  with  $1\text{nm} \cong 1\text{ kDa}$  for globular macromolecules. They can have an inorganic (clays,  
115 metal oxides, metal hydroxides, and metal carbonates) or organic (detrital matter, e.g. fulvic and humic acids, and living  
116 organism, such as algae, bacteria, virus) nature. Because of their high capacity of adsorbing considerable amounts of trace  
117 metals, due to a large specific area and, consequently, of a high number of active sites (Vignati and Dominik, 2003),  
118 colloids play a critical role in controlling metal speciation and the cycling of many elements in natural waters. The  
119 colloidal fraction of metals can be deduced from the difference between the total dissolved metal concentration and the  
120 dynamic concentration, according to Braungardt et al., 2011. A more sophisticated system, called Multi Physical  
121 Chemical Profiler (MPCP), has also been developed to extend the capability of the VIP to *in situ* monitoring of trace  
122 metal speciation (Tercier-Waeber et al., 2005). The MPCP allows the simultaneous *in situ*, autonomous monitoring and  
123 profiling (down to 150 m) of three major metal fractions: i) the free metal ion concentration, (i.e. the species related to  
124 biological uptake) by means of a particular GIME sensor, the complexing gel integrated micro-electrode (CGIME), which  
125 is covered by a thin 3.5  $\mu\text{m}$  layer of a Microchelex chelating resin which in turns is covered by a thick 300  $\mu\text{m}$  antifouling  
126 agarose gel (details of CGIME measurement principles and preparation in Noel et al., 2006); ii) the dynamic metal species  
127 by a GIME sensor; and iii) the total acid-extractable metal concentration by means of a GIME sensor coupled to a  
128 submersible flow-injection analysis (FIA) system (Tercier-Waeber et al., 2005, 2008). The MPCP is coupled with a CTD  
129 probe for the measurement of the master variables (pressure, temperature, pH, oxygen, conductivity, salinity, redox  
130 potential, turbidity and chlorophyll-a). Both the VIP and the MPCP have been successfully applied in several

131 environments, i.e. freshwater (Tercier-Waeber et al., 1998, 2002; Tercier-Waeber and Buffle, 2000), groundwater, fjord  
132 water (Tercier-Waeber et al., 1999), macro-tidal estuaries (Tercier-Waeber et al., 2005; Braungardt et al., 2009) and  
133 coastal marine waters (Tercier-Waeber et al., 1999, 2005; Howell et al., 2003; Braungardt et al., 2009).

134 In the autumn 2002 a joint oceanographic cruise was carried out along the Po river plume in order i) to evaluate the  
135 metal distribution between different fractions (free ion, dynamic, colloidal, dissolved and particulate fractions) and their  
136 relationship with the total content; ii) to assess the evolution of the different metal fractions during the estuarine mixing  
137 and in the water column; and iii) to evaluate the effect of the hydrological characteristics of the water column, as well as  
138 of phytoplankton, on metal speciation. Moreover, preliminary tests of the CGIME sensor on discrete samples were also  
139 carried out. As the validation of new technique measurements in complex media is not straightforward, hollow fiber  
140 permeation liquid membrane (HF-PLM) coupled to an inductively coupled plasma mass spectrometer (ICP-MS) were  
141 used in parallel to CGIME measurements for comparison purposes. This technique allows the measurements of free metal  
142 ions and some lipophilic contribution (Parthasarathy et al., 1997).

143

144

## 145 **2. Materials and Methods**

146

### 147 2.1. Laboratory, apparatus and reagents

148 A clean room laboratory ISO 14644-1 Class 6 (or US Fed. Std. 209e Class 1000), with areas at ISO Class 5 (or US F.  
149 S. 209e Class 100) under laminar flow cabinets, was available for trace metal clean working conditions. A portable  
150 laminar flow hood was available on board for sample treatments and analyses carried out on-site. Clean room garments,  
151 masks and gloves were worn by the personnel, who strictly followed clean room procedures during the most critical  
152 analytical steps.

153 Seawater sampling bottles were 8-L and 10-L GO-FLO from General Oceanics (Florida, USA). CTD probes, mod.  
154 Ocean Seven 316 CTD and Ocean Seven 301 were from Idronaut (Milan, Italy). The Handheld Salinity, Conductivity  
155 and Temperature System, Mod. 30, was from YSI (Yellow Springs, OH, USA). The Portable Turbidimeter Model 966  
156 was from Orbeco-Hellige (New York, USA).

157 Filtration systems were the Sulfoflo from Nalgene (Rochester, New York) equipped with 0.45  $\mu\text{m}$  pore size membrane  
158 filters (cellulose mixed esters  $\varnothing$  47 mm, Schleicher & Schuell, Dassel, Germany) and the glassware apparatus with pre-  
159 weighted filter membranes. The UV digestion apparatus was the Mod. 705 UV-digester from Metrohm, (Herisau,  
160 Switzerland), equipped with twelve 12-mL quartz vessels and a 500 W mercury lamp. The Mod. 290A pH-meter,  
161 equipped with an Orion epoxy pH electrode Sure-Flow (Mod. 9165BN) was from Orion (Beverly, MA, USA). For

162 phytoplankton analyses, the inverted microscope, Mod. Axiovert 135, and the epifluorescence microscope Mod. Axioplan  
163 were from Zeiss (Milan, Italy). For nutrients, the Technicon Autoanalyzer Traacs 800 system was from Seal Analytical  
164 (Norderstedt, Germany). Plastic containers were of low-density polyethylene material (Kartell, Italy). The polyethylene  
165 bottles, the quartz vessels, the sampling equipment, the filtration apparatus and all other plastic containers were  
166 decontaminated following the procedure reported elsewhere (Illuminati et al., 2015). Details of the decontamination  
167 procedure are briefly described in Supporting Information, text S1.

168 The instrumentation for metal speciation consisted of (i) two Metrohm (Herisau, Switzerland) 746 VA Trace Analyser  
169 and two 747 VA Stand, each one equipped with a Teflon PFA (perfluoroalkoxy copolymer) cell and a three-electrode  
170 system, which includes an epoxy-impregnated graphite rotating disk working electrode (as a support for the thin mercury  
171 film electrode, TMFE), an Ag/AgCl, KCl 3 mol L<sup>-1</sup> reference electrode (to which all potentials are referred throughout)  
172 and a glassy carbon rod counter electrode; (ii) five Voltammetric *In-situ* Profiling (VIP) systems (Idronaut, Italy),  
173 equipped each one with a pressure compensated mini flow-through Plexiglas voltammetric cell comprising a Gel  
174 Integrated Mercury-plated Microelectrode (GIME sensor), an Ag/AgCl, KCl saturated gel reference electrode and a built-  
175 in platinum ring auxiliary electrode; (iii) one Multi Physical-Chemical Profiler (MPCP) equipped with two VIP systems  
176 and one submersible Flow-Injection Analysis (FIA) system; (iv) one Amel potentiostat equipped with a Metrohm cell  
177 based on a three-electrode configuration which includes a CGIME sensor, an Ag/AgCl/KCl<sub>(saturated)</sub> reference electrode and  
178 a platinum rod counter electrode; and (v) one Hollow Fiber Permeation Liquid Membrane (HF-PLM) device coupled to  
179 an ICP-MS. Transferpette variable volume micropipettes from Brand (Wertheim, Germany) and neutral tips were used.

180 Ultrapure water was Milli-Q from Millipore (Bedford, MA, USA). Ultrapure HCl (34.5%), HNO<sub>3</sub> (70%), H<sub>2</sub>O<sub>2</sub> (ca.  
181 30%), and superpure HClO<sub>4</sub> (65%) were from Romil (Cambridge, England; UpA grade). Superpure KCl, Hg(CH<sub>3</sub>COO)<sub>2</sub>,  
182 hexadistilled mercury, KSCN and NaNO<sub>3</sub> were from Merck (Darmstadt, Germany). 1.5% LGL agarose gel was from  
183 Biofinex (Neuchâtel, Switzerland). Atomic absorption standards of Cd (II), Pb (II) and Cu (II) were from Carlo Erba  
184 (Milan, Italy). Research-grade nitrogen, purity ≥ 99.999%, was from Sol (Monza, Italy). The estuarine water reference  
185 material SLEW-3, the nearshore-seawater reference material CASS-4, the seawater reference material NASS-5 for trace  
186 metals were from the National Research Council of Canada.

187

188

## 189 2.2. Study area

190 The Adriatic Sea can be divided in three regional basins (North, Central and South), differing in bathymetry,  
191 hydrology, morphology and biogeochemical features (Russo and Artegiani, 1996). The northern basin, with a width of  
192 about 75 nautical miles and a mean depth of 30 m (maximum depth about 70 m), is the shallower part of the Adriatic

193 epicontinental shelf. It is characterized by large inputs of freshwaters from several rivers mainly concentrated along the  
194 northern and the north-western coast of Italy. The Po River is the Italy's largest river (with a length of 673 km and drainage  
195 basin of 71,000 km<sup>2</sup>) and it flows through one of the most industrialized regions of the country. It has an annual outflow  
196 rate of 1500 – 1700 m<sup>3</sup> s<sup>-1</sup>, accounting for about one third of the total riverine freshwater input into the Adriatic Sea  
197 (Marini et al., 2010). Discharge peaks of about 2000 m<sup>3</sup> s<sup>-1</sup> are generally observed in spring (May-June) following the  
198 snow melting, and in autumn (October-November) when intense rainfall contributes to Po river discharge (Boldrin et al.,  
199 2005; Marini et al., 2008).

200 The oceanographic cruise in the Northern Adriatic Sea was carried out on board of *G. Dallaporta* vessel (CNR,  
201 Ancona, Italy) from October 27<sup>th</sup> to November 4<sup>th</sup> 2002, along a transect of six stations from the Po mouth toward the  
202 open sea, in direction East – South East (Fig. 1). About five stations were located in the plume while the remaining one  
203 was outside. For a better representation of data, the study area was divided into three different zones. According to Falcieri  
204 et al., 2014 (who carried out a 8-year simulation of the Po plume mean spatial variability based on data of temperature,  
205 salinity and currents collected during September 2002 as initial conditions), we marked the limit for the front between the  
206 plume and the sea water at the surface salinity of ~36. Here, we called “pro-delta area” the first segment of the transect,  
207 which was 10-km away from the Po mouth. This segment was characterized by high fluctuations of salinity (from ~18 to  
208 ~29) and it included Stns. 1 and 2. The second segment, called “frontal area”, extended from 10 km to ~40 km from the  
209 Po mouth. It was characterized by more stable salinity values (from ~33 to ~36) and it includes Stns. from 3 to 5. Finally,  
210 the third segment was called “marine area”, which included the Stn. 6 and it was characterized by marine water masses  
211 not influenced by the Po River (Fig. 1). To be noted that Stn. 5 had surface salinity around 36 and it should be part of the  
212 marine area, nevertheless, it was included in the second segment of the transect, because at this station most of all the  
213 variables studied had a behaviour comparable to the frontal area instead of the marine one.

214

### 215 2.3. Sample collection and treatments

216 During the oceanographic cruise, two kinds of speciation were carried out. The first type dealt with the *in situ*-based  
217 speciation using several VIPs and one MPCP (equipped with two VIPs and one GIME-FIA system) for the determination  
218 of the dynamic ( $Me_{dyn}$ ) and the total acid-extractable ( $Me_{ac.extr}$ ) fractions of Cd, Pb and Cu in the water column. The  
219 second type regarded the on board/laboratory-based speciation carried out on discrete samples collected at the same depths  
220 of the VIP and MPCP deployment, by means of the conventional Metrohm instrumentations for the total and dissolved  
221 metal concentration measurements. Discrete samples were also collected for immediate on-board analysis of both  
222 dynamic and total extractable fractions on surface samples, since the VIP and MPCP probes, due to their manufacture  
223 characteristics, cannot be used in depths < 1 m (Tercier et al., 1998; Tercier-Waeber et al., 2005).

224 Surface samples were collected manually in polyethylene bottles by a rubber dinghy at ~1 km from the oceanographic  
225 vessel. Immediately after collection, the samples were divided in various aliquots that were subjected to different  
226 treatments in order to obtain the different metal fractions. The first was acidified (raw sample) with ultrapure HCl (2:1000,  
227 pH ~2) for the determination of the total metal concentration ( $Me_{tot}$ ). An aliquot was filtrated through 0.45- $\mu$ m pore-size  
228 membrane filter and then acidified with ultrapure HCl (2:1000, pH ~2) for the determination of the dissolved metal  
229 contents ( $Me_{diss}$ ). Before analysis, all the acidified samples (both raw and filtered) were subjected to photo-oxidative  
230 digestion, by UV irradiation for 12 h after the addition of 1:1000 ultrapure  $H_2O_2$ , in order to destroy organic matter, to  
231 leach bound metals, and to avoid possible adverse effect on the voltammetric determination (Kolb et al., 1992). Total  
232 particulate metal concentration ( $Me_{part}$ ) was obtained by difference between the total and the dissolved concentrations,  
233 while the metal concentration in the colloidal fraction ( $Me_{coll}$ ) was computed as difference between the dynamic and the  
234 total dissolved concentration.

235 Aliquots of seawater samples were acidified with ultrapure HCl (2:1000, pH ~2) and then analysed on board without  
236 UV digestion for the determination of the total HCl-extractable metal fraction to compare it with that measured by the  
237 GIME-FIA system. Other seawater aliquots were used, without any treatments, for the determination on board of the free  
238 metal ion concentration ( $Me_{free}$ ) using both CGIME sensor and the PLM technique. Finally, other seawater aliquots were  
239 used to measure turbidity, DOC and nutrients.

240

#### 241 2.4. Metal speciation methodology

242 Cd, Pb and Cu were determined simultaneously using the square-wave anodic stripping voltammetry (SWASV) that  
243 was implemented both on conventional laboratory instrumentation and on voltammetric probes for the in-situ  
244 measurements.

245

##### 246 2.4.1. *In situ*-based speciation

247 The dynamic fraction and the in-situ total acid extractable concentration of Cd, Pb and Cu were determined by using  
248 the VIP and the MPCP systems.

249

250 *VIP system.* The voltammetric determination of the metal dynamic fractions required the initial Hg deposition on the Ir  
251 substrates of the GIME sensors, by applying a constant potential of -400 mV for 6 min in a  $N_2$  de-oxygenated solution  
252 of  $Hg(CH_3COO)_2$  (5 mmol  $L^{-1}$ ) and  $HClO_4$  ( $10^{-2}$  mol  $L^{-1}$ ) (Belmont-Hebert et al., 1998). The Hg film remained in place  
253 for the entire period of the oceanographic cruise. The Hg film was removed by scanning the potential from -300 mV to  
254 +300 mV at 5  $mV s^{-1}$  in a  $N_2$  de-oxygenated solution of KSCN (1 mol  $L^{-1}$ ). The voltammetric analyses were performed

255 in successive steps: (i) sample pumping through the cell for 3 min at 7 mL min<sup>-1</sup>; (ii) equilibration of the agarose gel with  
256 the sample for 5-6 min; (iii) SWASV measurements inside the gel, using the background subtraction technique (Belmont-  
257 Hebert et al., 1998), and the following instrumental parameters: deposition potential,  $E_{\text{dep}} = -1100$  mV; deposition time,  
258  $t_{\text{dep}} = 10$  to 45 min (as a function of the metal concentrations); final potential,  $E_{\text{fin}} = -50$  mV in sea water, +120 mV in de-  
259 oxygenated NaNO<sub>3</sub> (0.1 mol L<sup>-1</sup>) electrolyte during laboratory calibration; equilibration potential,  $E_{\text{equil}} = -1100$  mV;  
260 equilibration time,  $t_{\text{equil}} = 20$  s; pre-cleaning potential,  $E_{\text{precl}} = -50$  mV (in sea water), +120 mV (in NaNO<sub>3</sub>); pre-cleaning  
261 time,  $t_{\text{precl}} = 20$  s; pulse amplitude,  $E_{\text{SW}} = 25$  mV; step amplitude,  $\Delta E_{\text{step}} = 8$  mV; frequency,  $f = 200$  Hz.

262 *MPCP system.* The three-channel system of the MPCP constituted of two GIME sensors for the determination of the  
263 dynamic fractions (instrumental parameters as for other VIP probes), and a GIME-FIA system for the determination of  
264 the in-situ total extractable metal concentrations. The latter were measured in three steps: (1) on-line pre-treatments based  
265 on ligand exchanges followed by acidification and heating of the sample to release trace metals complexed and/or  
266 adsorbed; (2) equilibration of the sensor agarose gel with the pre-treated sample; (3) SWASV measurements of total  
267 extractable metal concentration (Tercier-Waeber et al., 2005). The conditions used for the sample pre-treatment were as  
268 follows: ligand stock solution = 150  $\mu\text{mol L}^{-1}$  of triethylenetetramine (TRIEEN); acid stock solution =  $3.3 \times 10^{-2}$  mol L<sup>-1</sup>  
269 superpure NaNO<sub>3</sub>; sample, ligand and acid flow-rates = 4.2 mL min<sup>-1</sup>, 2.4 mL min<sup>-1</sup> and 3.2 mL min<sup>-1</sup> corresponding to  
270 dilution factors of 2.3, 4.1, and 3.1 for the sample, ligand and acid, respectively (i.e. reagents concentration in the pre-  
271 treated samples; 36.6  $\mu\text{mol L}^{-1}$  TRIEN,  $9.7 \times 10^{-3}$  mol L<sup>-1</sup> superpure NaNO<sub>3</sub>); equilibration time sample+ligand = 2 min;  
272 equilibration time sample+ligand+acid at 50 °C = 2 min. The SWASV parameters used were as follows: equilibration  
273 time of the agarose gel with the pre-treated sample = 6 min;  $E_{\text{dep}} = -950$  mV;  $t_{\text{dep}} = 5$  to 15 min (as a function of the metal  
274 concentrations, during *in situ* deployments);  $E_{\text{fin}} = -50$  mV in sea water or +120 mV in laboratory calibration in superpure  
275 NaNO<sub>3</sub>;  $E_{\text{equil}} = -1100$  mV;  $t_{\text{equil}} = 20$  s;  $E_{\text{precl}} = -50$  mV to +120 mV;  $t_{\text{precl}} = 20$  s;  $E_{\text{SW}} = 25$  mV;  $\Delta E_{\text{step}} = 8$  mV;  $f = 200$   
276 Hz.

277

278 The quantification of both the dynamic and the total extractable concentration was obtained using the calibration curve  
279 method, in N<sub>2</sub> degassed 0.1 mol L<sup>-1</sup> NaNO<sub>3</sub> standard solutions spiked with various concentrations of Cd, Pb and Cu. The  
280 resulting slopes were normalized (Tercier-Waeber et al., 1999) after: (1) smoothing of the currents (using Golay-Savitzky  
281 moving average); (2) subtraction of the background current from the current signal; (3) peak height quantification; and,  
282 where necessary, (4) temperature effect correction on peak current measured *in situ* (Tercier-Waeber et al., 1998; Howell  
283 et al., 2003). The calibration procedure was performed before and at end of the oceanographic campaign, while the validity  
284 of slopes was tested during the cruise, on board, by analysing a N<sub>2</sub>-degassed standard solution previously used in the  
285 calibration curve procedure.

286

287 2.4.2. On board/laboratory-based speciation

288 *Metrohm system.* Total and the dissolved metal fractions were determined in land-based laboratory (within 1-3 months  
289 after the sample collection) by conventional Metrohm instrumentation. The procedure involved the pouring of a 10-mL  
290 UV-digested sample aliquot into a pre-cleaned voltammetric cell, where the thin mercury film electrode (TMFE) was  
291 already prepared and tested (Annibaldi et al., 2007; Illuminati et al., 2013, 2015). The  $\text{Hg}(\text{NO}_3)_2$  solution ( $2.5 \times 10^{-2}$  mol  
292  $\text{L}^{-1}$ ) for the TMFE was obtained by oxidation of hexadistilled mercury with ultrapure nitric acid. The voltammetric  
293 analyses were carried out in the background subtraction technique, using the following instrumental parameters:  
294  $E_{\text{dep}} = -975$  mV;  $t_{\text{dep}} = 3$  to 10 min (as a function of the metal concentrations, during *in situ* deployments);  $E_{\text{fin}} = 0$  mV;  
295  $E_{\text{equil}} = -975$  mV;  $t_{\text{equil}} = 7.5$  s;  $E_{\text{precl}} = -50$  mV;  $t_{\text{precl}} = 5$  min;  $E_{\text{SW}} = 25$  mV;  $f = 100$  Hz;  $\Delta E_{\text{step}} = 8$  mV;  $t_{\text{step}} = 150$  ms.  
296 Two-three replicates were carried out with the sample in the cell, after which quantification was obtained using the  
297 multiple standard addition method.

298 *CGIME sensor.* Preliminary tests for the determination of the free metal ion concentration with the complexing gel  
299 integrated microelectrode (CGIME) were carried out on discrete samples on board, by using a standard Amel potentiostat  
300 with a three-electrode Metrohm cell. First, mercury films were deposited on CGIME sensor, by applying a constant  
301 potential of  $-400$  mV for 8 min in a  $\text{N}_2$  de-oxygenated  $5$  mmol  $\text{L}^{-1}$   $\text{Hg}(\text{CH}_3\text{COO})_2$  and  $10^{-1}$  mol  $\text{L}^{-1}$   $\text{HClO}_4$  solution  
302 (Tercier et al., 1995). Removal of the mercury film was carried out with the same procedure used for GIME sensors (see  
303 above). The measurement of free metal ion concentrations was performed in two steps: (1) equilibration of the sensor  
304 with the sample to accumulate trace metals on the Microchelex resin in proportion to free metal ion concentrations in  
305 seawater; (2) transfer of the CGIME sensor in a glass cell filled with an acid solution ( $0.1$  mol  $\text{L}^{-1}$   $\text{NaNO}_3$  +  $0.1$  mol  $\text{L}^{-1}$   
306 superpure  $\text{HNO}_3$ ). Here, the accumulated metals were released by acid and then, immediately detected by SWASV using  
307 the following instrumental parameters (Noel et al., 2006; Tercier-Waeber et al., 2005): accumulation time = 1 to 2 h, with  
308 a renewal of the sample in the cell every 15 min;  $E_{\text{dep}} = -900$  mV;  $t_{\text{dep}} = 10$  to 30 min;  $E_{\text{fin}} = +100$  mV;  $E_{\text{SW}} = 25$  mV;  
309  $\Delta E_{\text{step}} = 8$  mV;  $f = 50$  Hz.

310 *HF-PLM technique.* Hollow Fiber Permeation Liquid Membrane (HF-PLM) coupled to ICP-MS detection was used for  
311 laboratory measurements performed in some discrete samples for comparison purposes with the CGIME measurements  
312 of the free metal ion concentrations. The analytical procedure used for the HF-PLM preparation and measurements  
313 included different steps (Parthasarathy et al., 1997, 2001): (1) preparation of the hollow fiber which was a loosely, coil,  
314 single polypropylene hollow fiber with an inner diameter of  $600$   $\mu\text{m}$ ; (2) impregnating of hollow fiber with the organic  
315 metal carrier ( $0.1$  mol  $\text{L}^{-1}$  1,10-didecyl diaza 18-crown-6 and  $0.1$  mol  $\text{L}^{-1}$  lauric acid dissolved in a mixture of  
316 phenylhexane and toluene); (3) metal separation and pre-concentration, in which the impregnated hollow fiber membrane

317 was immersed in the sample solution placed in a plastic beaker and held vertical by means of a clamp; (4) filling of the  
318 lumen side of the hollow fiber with  $5 \times 10^{-4}$  mol L<sup>-1</sup> CDTA (trans-cyclohexanediamine tetra-acetic acid) strip solution  
319 adjusted to pH 6.4 with NaOH; (5) collection of the strip solution by pushing the solution by means of a peristaltic pump;  
320 (6) laboratory metal determination by ICP-MS in both the sample and strip solutions.

321

## 322 2.5. Accuracy tests

323 To ascertain accuracy and to assure comparability of data produced during the cruise, analytical quality control of Cd,  
324 Pb and Cu measurements were carried out by analysing several certified reference materials (the SLEW-3 for estuarine  
325 water, the CASS-4 for nearshore seawater, and the NASS-5 for open seawater) with all the instrumentations available.  
326 Certified reference materials were UV irradiated prior to analysis, as for the seawater samples collected.

327 The results of the systematic measurements carried out on reference materials during the entire period of work, using  
328 all the instrumentations available on board and in laboratory, gave ( $n = 5-7$ ) mean values ( $\pm$  SD) of SLEW-3, Cd  
329  $0.43 \pm 0.01$  nmol L<sup>-1</sup>; Pb  $0.046 \pm 0.010$  nmol L<sup>-1</sup>; Cu  $20 \pm 2$  nmol L<sup>-1</sup>; CASS-4, Cd  $0.24 \pm 0.02$  nmol L<sup>-1</sup>; Pb  $0.045 \pm 0.008$   
330 nmol L<sup>-1</sup>; Cu  $9.3 \pm 0.8$  nmol L<sup>-1</sup>; NASS-5 Cd,  $0.21 \pm 0.02$  nmol L<sup>-1</sup>; Pb  $0.038 \pm 0.006$  nmol L<sup>-1</sup>; Cu  $4.6 \pm 0.3$  nmol L<sup>-1</sup>,  
331 against certified mean values ( $\pm$  95% confidence interval) of SLEW-3 Cd  $0.43 \pm 0.04$  nmol L<sup>-1</sup>; Pb  $0.043 \pm 0.007$  nmol  
332 L<sup>-1</sup>; Cu  $24 \pm 2$  nmol L<sup>-1</sup>; CASS-4 Cd  $0.23 \pm 0.03$  nmol L<sup>-1</sup>; Pb  $0.047 \pm 0.017$  nmol L<sup>-1</sup>; Cu  $9.3 \pm 0.9$  nmol L<sup>-1</sup>; NASS-5  
333 Cd  $0.20 \pm 0.03$  nmol L<sup>-1</sup>; Pb  $0.040 \pm 0.003$  nmol L<sup>-1</sup>; Cu  $4.7 \pm 0.7$  nmol L<sup>-1</sup>. Results were in good agreement with certified  
334 reference values within the experimental errors, showing a good accuracy of all the measurements.

335

## 336 2.6. Inter-comparison exercises

337 During the cruise, laboratory and field-based inter-comparison exercises of all the analytical techniques available were  
338 carried out, in order to validate the developed analytical tools with common analytical procedures. Three principal types  
339 of field-based inter-comparisons were carried out: (i) the comparison between the different dynamic fractions determined  
340 by the GIME sensors available on board; (ii) the comparison between the total and the total HCl-extractable concentrations  
341 measured by conventional instrumentations, on one side, and the in-situ total acid extractable concentration measured by  
342 the GIME-FIA system, on the other; and (iii) the comparison between the free metal ion concentrations determined by  
343 the CGIME sensor and the HF-PLM technique. Some results of the inter-comparison exercises were reported in  
344 Braungardt et al. (2009) and Tercier-Waeber et al. (2005). Braungardt et al. (2009) compared the metal dynamic  
345 concentrations measured at 5-m depth of the first two stations of the study transect. Tercier-Waeber et al. (2005) compared  
346 the results obtained by the in-situ application of the GIME-FIA and the total content measured by the conventional  
347 Metrohm instrumentation. Here we extended the comparison between the different voltammetric instrumentations to all



348 the samples collected during the cruise. Results are fully reported in the Supporting Information (Text S2; Tables S1 and  
349 S2).

350 As shown in Table S1, a general good agreement was observed between the dynamic fraction data measured by  
351 different VIPs available on board. Cd dynamic fraction determined by the VIP-A represented the only exception, showing  
352 at the surface very high values, probably related to laboratory contamination. Concerning the total concentrations (see  
353 Table S2 in the Supporting Information), although some high values due to possible contamination problems, the in-situ  
354 total extractable fraction measured by the GIME-FIA procedure was often consistent with the total metal concentration  
355 for Pb. On the contrary, the in-situ total extractable fraction of Cu was comparable to the total acid-extractable fraction  
356 measured by conventional Metrohm instrumentation. The few data of GIME-FIA available for Cd were higher than the  
357 total concentrations, probably due to contamination problems.

358 The very few data obtained for the free metal ion concentration by the CGIME sensor (values available only for Cu)  
359 seemed to be in agreement with values measured by the HF-PLM technique.

360

## 361 2.7. Ancillary measurements

362 At each station of the 2002-Adriatic cruise transect, continuous water column profiles (vertical resolution of 0.5 m),  
363 were sampled for the master hydrographic variables (i.e. temperature, salinity, dissolved oxygen, oxygen saturation, pH,  
364 redox potential) using a CTD probe fitted with a fluorometer for the chlorophyll-a vertical profile.

365 Temperature and salinity data obtained from the CTD measurements were used to calculate the density ( $\rho$ ), and the  
366 water column stability. The latter is proportional to the Brunt-Värsälä buoyancy frequency  $N^2(z)$ , which represents the  
367 strength of density stratification (Agusti and Duarte, 1999). The depth at which the Brunt-Värsälä buoyancy frequency  
368 was estimated to be maximal, which also corresponds approximately to the middle of the pycnocline, represents the Upper  
369 Mixed Layer (UML). The value of  $N^2(z)$  was also taken as a stability index of the water column (i.e. what it is commonly  
370 called pycnocline strength).

371 At each station, several discrete samples were also collected at different depths for the determination of turbidity,  
372 dissolved organic carbon (DOC), dissolved inorganic nutrients (nitrate- $\text{NO}_3^-$ , nitrite- $\text{NO}_2^-$ , ammonium- $\text{NH}_4^+$ ,  
373 orthophosphate- $\text{PO}_4^{3-}$  and orthosilicate- $\text{Si}(\text{OH})_4$ ), and phytoplankton abundance and composition. Nutrient  
374 concentrations were measured following modified procedures developed by Strickland and Parsons (1972). Detailed  
375 description of the nutrient analysis is reported elsewhere (Campanelli et al., 2012; Marini et al., 2008). Total dissolved  
376 inorganic nitrogen (DIN) was calculated as the sum of the  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  concentrations.

377 The analysis of phytoplankton was carried out following the Utermöhl method (Edler and Elbrachter, 2010).  
378 Immediately after collection seawater samples were preserved in dark glass bottles by adding 0.8% formaldehyde

379 neutralized with hexamethylenetetramine and stored at 4 °C. In laboratory, 40-100 mL sub-samples were homogenized,  
380 settled in a cylinder-chamber complex and then observed using the inverted microscope. During counting procedure, 30  
381 random fields were examined at 400-x magnification. All phytoplankton cells larger than 2 µm were identified and  
382 counted. Then the entire chamber was observed at 200-x to assess the larger and less frequent organisms. Bio-volume  
383 was measured to evaluate the biomass (expressed in µg C L<sup>-1</sup>) following Menden-Deuer and Lessard (2000).

384

## 385 2.8. Statistical analysis

386 Countered sections are plotted using master variable and nutrient data that were gridded using the Data-interpolating  
387 Variational Analysis (DiVA) coupled to the software Ocean Data View 4.7.10 (Schiltzer, 2017).

388 Experimental data were elaborated by principal component analysis (PCA), which was carried out on standardized  
389 data; significant components were obtained through the Wold cross-validation procedure (Wold, 1978). The 5 m samples  
390 of Stns. 1 and 2 were excluded from the dataset, because only the dynamic fractions were determined at this depth. Only  
391 three depths of Stn. 6 (0.2 m, 5m and 25 m) were considered, since metal dynamic fractions were measured only at those  
392 depth The high (contaminated) values of dynamic Cd were substituted by the HCl-extractable dissolved fraction (obtained  
393 by filtering seawater samples on 0.45 µm pore membranes, acidifying with superpure HCl 2:1000 and analysing without  
394 UV pre-treatment) which was very close to the Cd dynamic fraction (see data in Supplementary Information, Table S3),  
395 and when this fraction was not available (we determined HCl-extractable dissolved fraction only in surface samples), the  
396 dissolved Cd values (10- and 15-m depth values of Stn. 3) were used. The free metal ion concentrations were also  
397 excluded, because of the very few and sporadic results obtained. The statistical analysis and the PCA were carried out  
398 using Statistica package (StatSoft; vers. 8.0), and the differences were deemed statistically significant at  $p < 0.05$ .

399

400

401

## 402 3. Results

403

### 404 3.1. Hydrography, dissolved nutrients and phytoplankton

405 Data on the master hydrographic variables, dissolved organic carbon (DOC), dissolved nutrients and phytoplankton  
406 obtained during the cruise in the 2002-Adriatic Sea cruise are shown in Figures 2-4, S1 and Table S4.

407 Temperature and salinity varied from 16.28°C to 19.74°C and from 25.50 to 38.90, respectively (Fig. 2). Density  
408 anomalies varied from 18.12 kg m<sup>-3</sup> to 28.66 kg m<sup>-3</sup> (Fig. S1). Temperature varied within a narrow range (up to 18%  
409 variability), thus density variations were essentially due to salinity variations (also confirmed by the high positive

410 correlation coefficient,  $r = 0.9798$ , Tab. S4). Within the Po River plume, a stratification of the water column was observed  
411 with a very shallow UML (up to 5-m depth) and with a sharp salinity gradient. At this depth, the pycnocline showed a  
412 high strength ( $N^2(z) = 557 \times 10^{-4} \text{ s}^{-2}$ ), which progressively decreased seawards. In the marine area (Stn. 6) water column  
413 became quite homogeneous, the UML extended deeper (~50 m), the halocline disappeared, while the thermocline still  
414 persisted and was responsible of a very weak pycnocline ( $N^2(z) = 15 \times 10^{-4} \text{ s}^{-2}$ ) (Fig. 2).

415 Dissolved oxygen showed concentrations in the range  $3.5 - 11 \text{ mg L}^{-1}$ , slightly over saturation levels (100%) with  
416 percentages ranging between 100 and 138% within the stratified layer (except for Stn.1, Fig. 2). Then, it gradually  
417 decreased down to the bottom reaching saturation values of ~60%. Values slightly lower than 50%, indicative of hypoxia,  
418 were observed only at the Stn. 5 close to the bottom, while in the marine area, dissolved oxygen showed a homogenous  
419 vertical distribution and well-oxygenated waters (~100% of saturation). Along the longitudinal profile, dissolved oxygen  
420 values (as well as saturation percentages) showed a surface maximum at Stn. 4, after which it decreased seawards to  
421 values very close to those of the pro-delta stations.

422 pH ranged from 7.94 to 8.39 and showed a longitudinal profile similar to that observed for dissolved oxygen (Fig. 2).  
423 In the pro-delta area, pH slightly decreased with depth, except in Stn.1 where it showed an opposite trend in the water  
424 column. In the frontal and marine areas, pH remained almost constant within the mixed layer, and then it sharply decreased  
425 with depth, even though at the Stn. 6 this decrease was less marked (Fig. S1).

426 Turbidity was high (~16 NTU) near the Po mouth, but sharply decreased seawards reaching values close to the  
427 detection limit of the instrument. In all the stations studied, turbidity showed a similar trend in the water column; it  
428 decreased with depth, but proceedings seawards, higher values (sometimes 2-3 times higher than the surface) were  
429 recorded, in the proximity of the bottom (Fig. S1).

430 Chlorophyll-a (Chl-a) concentration measured *in situ* by the CTD probe ranged between  $0.3$  and  $13.3 \text{ mg L}^{-1}$  and  
431 sharply decreased seawards. A clearly defined deep chlorophyll maximum (DCM) of  $\sim 10 \text{ mg m}^{-3}$  was observed, closely  
432 following the pycnocline. In the marine area, Chl-a vertical distribution was homogeneous with very low values,  $\sim 0.3 \text{ mg}$   
433  $\text{L}^{-1}$  (Fig. 3).

434 The total phytoplankton abundance ranged between  $8.3 \times 10^6 \text{ cell L}^{-1}$  and  $3.4 \times 10^5 \text{ cell L}^{-1}$  (Tab. S4). Spatial  
435 distribution of micro-phytoplankton showed a general seaward-decreasing trend, even if a peak (principally due to  
436 diatoms) of abundance was observed in the frontal area, with densities similar to those of the stations closer to the Po  
437 mouth. The most abundant groups were represented by diatoms and phytoflagellates (ranging from  $0.014$  to  
438  $4.8 \times 10^6 \text{ cells L}^{-1}$  and from  $0.25$  to  $3.8 \times 10^6 \text{ cells L}^{-1}$ , respectively, Tab. S4) in agreement with several previous studies  
439 on phytoplankton composition in the northern Adriatic Sea (Totti et al., 2005).

440 DOC concentrations decreased along the Po plume, with values that ranged between  $\sim 140 \mu\text{mol L}^{-1}$  at the Po mouth  
441 and  $\sim 60 \mu\text{mol L}^{-1}$  in open sea, with a variation of  $\sim 60\%$  (Fig. 3). This horizontal decreasing trend of DOC was also  
442 observed in the water column, with bottom values that were 40-60% lower than those of the surface. This distribution  
443 changed in the marine area, where after a slightly increase within the first 15-m depth, it was quite homogeneous down  
444 to the bottom.

445 DIN (ranging from  $\sim 1 \mu\text{mol L}^{-1}$  to  $\sim 90 \mu\text{mol L}^{-1}$ ) and orthosilicates,  $\text{Si}(\text{OH})_4$  (ranging from 0.02 to  $42 \mu\text{mol L}^{-1}$ )  
446 showed a decreasing trend ( $\sim 98\%$ ) seawards (Fig. 4). DIN was represented for the most part by nitrates (60-100% of the  
447 total). A very small fraction (ranging from 0.1% to  $\sim 15\%$ ) of the DIN was represented by ammonium, while nitrites  
448 represented a more remarkable quote (1-40% of the total). Both DIN and orthosilicates were characterized by a general  
449 drawdown of concentrations within the stratified layer. This decreasing trend was more marked in the stations closer to  
450 the Po, because of the river influence. Afterward, concentrations remained almost constants with depth, while at the  
451 bottom they arose again (the anomalous high seafloor value of nitrates at Stn. 6 was probably due to contamination  
452 problems, and thus, it was not considered in the computation of DIN) (Fig. 4).

453 Orthophosphates were generally low, with values ranging from  $0.04 \mu\text{mol L}^{-1}$  to  $\sim 0.12 \mu\text{mol L}^{-1}$ . They showed an  
454 opposite trend with respect to nitrogen species and silicates, concentrations increasing of  $\sim 50\%$  along the transect (Fig.  
455 4). Phosphate distribution in the water column varied greatly from station to station. In Stns. 1 and 3 it increased gradually  
456 with depth, while at Stn. 2 a gradual decrease was observed. From Stn. 4 to the open sea, phosphates showed a maximum  
457 at the pycnocline; afterwards they slightly decreased down to the bottom.

458

459

### 460 3.3. Metal speciation

461 The distribution of the different metal fractions along the study transect is reported in Figures 5-8, divided for each metal  
462 and for each sampling station. Total, dissolved, particulate, dynamic and colloidal concentrations are given for each  
463 element. Also free-ion metal concentrations are discussed, even if these data are reported only in Tab. S2, due to the  
464 paucity of the values measured. Data on dynamic fraction are the mean of measurements carried out by each of the VIP  
465 systems available on board, with the exception of contaminated samples. It is to be noted that preliminary results on free  
466 metal ion concentrations have been previously reported in Tercier-Waeber et al. (2005). Here, we provide a more  
467 exhaustive description which includes such data presented previously, as well as the comparison with the other metal  
468 fractions and master variables. The metal concentrations here measured are also compared to literature data obtained from  
469 several estuaries, deltas, coastal and oceanic waters, worldwide (Table 1).

470

### 471 3.3.1. Cadmium

472 The Cd content in the waters of the transect was very low with an overall mean (interquartile range) of 0.14 (0.11 –  
473 0.16) nmol L<sup>-1</sup>. Our values are lower than those recorded in previous surveys in the same area and in other Italian rivers,  
474 while they are similar to Cd concentrations measured in the whole basin of the Adriatic Sea and in general in the  
475 Mediterranean Sea. Further, our values are as in the same order of magnitude as those found elsewhere in European  
476 estuaries and, in general, as those measured in oceanic waters worldwide (Tab. 1).

477 A general longitudinal decreasing trend can be observed seaward for all the Cd fractions detected (Fig. 5a). Dissolved  
478 Cd (from 0.068 nmol L<sup>-1</sup> to 0.25 nmol L<sup>-1</sup>) constituted the most important fraction of the total (concentrations ranging  
479 between 0.080 nmol L<sup>-1</sup> and 0.26 nmol L<sup>-1</sup>). The only exception was present in the stations closer to the Po mouth, where  
480 a more marked contribution by particulate fraction can be detected, though Cd<sub>diss</sub> percentages remained still high (70-90%  
481 of the total). The vertical profile of dissolved Cd showed an almost homogeneous behaviour over the transect, with a  
482 maximum (excepting in the pro-delta area) at the DCM (5-10 m depth).

483 Apart from the high values at the surface of Stns. 2, 4 and 6 and at the bottom of Stn. 3, due to possible contamination  
484 problems, the Cd dynamic fraction accounted for ~40% to ~100% of Cd<sub>diss</sub> (values ranging from 0.05 nmol L<sup>-1</sup> to 0.20  
485 nmol L<sup>-1</sup>). The percentage of this fraction with respect to the dissolved concentration was high (90-100%) in the pro-delta  
486 area, while it decreased seawards within the UML, reaching in the open sea values about half to the Cd<sub>diss</sub> (Fig. 6). Below  
487 the pycnocline, the concentration of Cd<sub>dyn</sub> increased down to the seafloor, where its percentage was again 90-100% of  
488 Cd<sub>diss</sub>. In the open sea, a quite homogeneous distribution of Cd<sub>dyn</sub> with depth was observed, with a contribution to the  
489 dissolved fraction of about 60-70%. An opposite trend was observed for the colloidal Cd fraction, that increased seaward,  
490 reaching values ~40-50% of Cd<sub>diss</sub>, and sharply decreased with depth to values close to zero (Fig. 6).

491 Free Cd-ion [Cd<sup>2+</sup>] concentrations were found to be below the detection limit (~60 pmol L<sup>-1</sup>, as reported in Noël et  
492 al., 2006) for both the CGIME sensor and the HF-PLM system (Tab. S-2).

493

### 494 3.3.2. Lead

495 Lead speciation changed greatly along the transect (Fig. 5b and Fig. 7). The total concentration of Pb was generally  
496 high at the Po river mouth (~1.4 nmol L<sup>-1</sup>) and then decreased seaward, to reach asymptotically an open sea value of  
497 ~0.2 nmol L<sup>-1</sup>, with an 85% decreasing trend. At the surface of the pro-delta and frontal areas, Pb content was dominated  
498 by the particulate fraction (accounting for ~70% of the total), since dissolved Pb showed low concentrations (~0.40 nmol  
499 L<sup>-1</sup>). Pb<sub>diss</sub> initially decreased in the frontal area, passing from ~0.4 nmol L<sup>-1</sup> at the Po River mouth to ~0.09 nmol L<sup>-1</sup> up

500 to ~30 km off shore from the river. Proceeding seaward, it increased both in absolute and in relative terms reaching values  
501 close to those of the  $Pb_{tot}$  (~0.2 nmol L<sup>-1</sup>). Therefore,  $Pb_{part}$  greatly decreased along the Po plume, reaching values close  
502 to zero in the marine area. Both the total and the dissolved Pb concentrations were in good agreement with data reported  
503 in the literature for the same area and for other rivers worldwide (Tab. 1). Obviously, our data are higher than those  
504 referred to oceanic waters (sometimes about one order of magnitude) with the exception of the seawater end-member  
505 values, which fall within the same order of magnitude of literature data.

506 In the water column, Pb was mainly present in its dissolved form (70 – 100% of the total) which was also responsible  
507 of the maximum of  $Pb_{tot}$  at the DCM (5-10 m depths). The particulate fraction returned significant at the seafloor, where  
508 its contribution to the total increased up to ~70%.

509 The dynamic fraction of Pb ranged between ~0.02 nmol L<sup>-1</sup> and ~0.2 nmol L<sup>-1</sup>. In general,  $Pb_{dyn}$  represented a very  
510 small and constant fraction of the  $Pb_{diss}$  (~15-20%) within the first 10 km far from the Po mouth, further offshore it greatly  
511 increased in both its concentration and percentage reaching at the surface values up to the 50% of the dissolved  
512 concentration. The contribution of  $Pb_{dyn}$  with respect to the dissolved concentrations increased with depth in the stations  
513 close to the Po mouth, while it decreased in the water column with the increasing of the distance from the river. Hence,  
514 the colloidal fraction of Pb was dominant (~80% of the  $Pb_{diss}$ ) in the pro-delta area and then decreased in the open sea to  
515 values half of those of  $Pb_{diss}$  (Fig. 7).

516 The free-ion Pb fraction (no CGIME data are available) showed very low concentration, varying from about 2 pmol L<sup>-1</sup>  
517 near the Po river to ~10 pmol L<sup>-1</sup> in the open sea (Tab. S-2). It represented a few percentages of the  $Pb_{diss}$  (from ~1% to  
518 ~10%), but the contribution to  $Pb_{dyn}$  was much higher (from ~5% to ~40%). Due to the very few data available for this  
519 fraction, we are not able to describe in details the behaviour of  $[Pb^{2+}]$  in the study area. We can only observe an increasing  
520 trend of  $[Pb^{2+}]$ , both in absolute and in relative terms with the distance from the river and with depth.

521

522

### 523 3.3.3. Copper

524 Copper showed a marked decreasing trend of all the fractions along the study transect (Fig. 5c, 8).

525 Total Cu concentration varied from ~3 nmol L<sup>-1</sup> to ~20 nmol L<sup>-1</sup>, and it mainly constituted of the dissolved fraction  
526 that ranged from 2 nmol L<sup>-1</sup> to ~11 nmol L<sup>-1</sup>. The contribution of  $Cu_{diss}$  to  $Cu_{tot}$  at the surface increased along the study  
527 transect,  $Cu_{diss}$  being ~100% of the total in the open sea. Both total and dissolved Cu values are similar to those recorded  
528 in previous surveys for the northern and central Adriatic Sea (Tab. 1), with the only exception of Tankere et al., 2000 who  
529 reported Cu higher values. For the Southern Adriatic Sea and the rest of the Mediterranean Sea (Tankere and Statham,

530 1996) lower values have been reported as well as for the oceanic waters (Tab. 1). Dissolved Cu concentrations were  
531 generally lower than those measured in other European estuaries and than the average value of the rivers worldwide (Tab.  
532 1).

533 A vertical decreasing trend of  $Cu_{tot}$  was also observed within the Po plume. At the boundary of the frontal area (Stn.  
534 5) and in the marine area,  $Cu_{tot}$  showed a maximum in the upper 10-15 m of the water column, and then it remained almost  
535 constant with depth (Fig. 8). Moreover,  $Cu_{diss}$  decreased within the stratified layer; and below it remained almost constant  
536 down to the seafloor with values close to  $Cu_{tot}$ . Therefore, the  $Cu_{diss}$  proportion increased with depth reaching values up  
537 to ~90% of the total. Total particulate Cu was high at the surface in the stations of the pro-delta and decreased seaward  
538 and with depth reaching low values (up to ~30% in the open sea) (Fig. 8).

539 As for Pb, the dynamic fraction of Cu represented a small fraction (up to ~50% Fig. 5c) of the dissolved concentration,  
540 with values ranging between ~0.15 and ~4.0 nmol L<sup>-1</sup>. The fraction of dissolved Cu associated to colloidal material was  
541 much more significant (~70-100% of the  $Cu_{diss}$ ). Both  $Cu_{dyn}$  and  $Cu_{coll}$  concentrations decreased seaward, but the  
542 contribution of the  $Cu_{coll}$  to the dissolved Cu slightly increased along the transect (Fig. 5c).

543 Considering the water column,  $Cu_{dyn}$  decreased at all the stations within the stratified layer (with the exception of Stn.  
544 5 where a maximum at 5-m depth was observed); afterwards it remained almost constant down to the seafloor (Fig. 8).  
545 The proportion of  $Cu_{dyn}$  vs.  $Cu_{diss}$  was ~30% within the stratified layer, for all the stations, and then it drastically fell to  
546 ~7% near the seafloor. Also  $Cu_{coll}$  decreased within the stratified layer, but contrary to  $Cu_{dyn}$ , below the pycnocline it  
547 increased with depth. Its percentage to  $Cu_{diss}$  was generally higher than  $Cu_{dyn}$  and slightly increased in the water column.  
548 In the marine area (Stn. 6), although the very few data available on dynamic Cu, a sub-surface decrease and a subsequent  
549 homogeneous distribution with depth can be recognized (Fig. 8).

550 The Cu free-ion fraction measured by the CGIME sensor showed very low values ranging between ~0.03 nmol L<sup>-1</sup> to  
551 ~0.62 nmol L<sup>-1</sup> (Tab. S-2).  $[Cu^{2+}]$  decreased by ~95% along the transect, as well as the proportion of this fraction with  
552 respect to the dissolved concentration decreased by ~90% seawards. Data available for this fraction are scarce, thus a  
553 proper description of  $Cu_{free}$  variation in the water column cannot be defined, even if an apparent decrease can be noted.

554

555

### 556 3.4. Multivariate statistical data analysis

557 The correlation matrix (Pearson's linear coefficients) is reported in Table S5.

558 Among nutrients, only  $NO_3^-$  and  $Si(OH)_4$  co-varied positively (highly significant correlation), whereas  $PO_4^{3-}$  showed  
559 no correlations with the other nutrients, even in the surface layers. Chl-a was associated with  $O_2$ , pH, total phytoplankton

560 abundance and biomass and negatively correlated with phosphates. Total phytoplankton abundance and biomass were in  
561 contrast with the main hydrographic parameters (depth, temperature, salinity and density) and with the distance from the  
562 Po, while they were positively correlated with turbidity and DOC.

563 Nearly, all the metal fractions were in contrast with salinity (although some of the correlations were not significant),  
564 indicating the influence of the river waters on their distribution, as subsequently confirmed by PCA (see below). Only  
565  $Pb_{dyn}$  and  $Cd_{coll}$  showed a different behaviour, co-varying (but not significantly) with salinity. Both Cd and Cu (excepting  
566  $Cd_{part}$  and  $Cd_{coll}$ ) showed significant correlations with phytoplankton abundance and biomass.  $Pb_{tot}$  was positively  
567 correlated with phytoplankton community, as well. Moreover,  $Cd_{diss}$  was correlated positively only with nitrogen species,  
568 while  $Cd_{dyn}$  co-varied with silicates and not with nitrates.  $Pb_{dyn}$  showed no significant correlations with any physical,  
569 chemical or biological variable, while  $Pb_{tot}$  was statistically correlated with  $NO_2^-$  and  $Si(OH)_4$ , and  $Pb_{diss}$  with  $NH_4^+$ . Cu  
570 was strong correlated with  $Si(OH)_4$  and, partly (only  $Cu_{tot}$  and  $Cu_{part}$ ) with  $NO_3^-$ . No significant correlations were found  
571 between any of the metals and  $PO_4^{3-}$ , overall. All Cu fractions were correlated with total, dissolved and dynamic Cd  
572 (excepting  $Cu_{part}$  which was not correlated with  $Cd_{tot}$  and  $Cd_{diss}$ ), and with the total and particulate fractions of Pb,  
573 indicating that they are affected by the same factor.

574 To better understand the effect of the Po River on the metal species distribution, a multivariate analysis (PCA) was  
575 performed to reduce the dimensionality of the dataset to few components that summarize the information contained in  
576 the overall dataset.

577 The PCA applied to the standardized variables, led to the identification of three significant, cross-validated principal  
578 components (PCs) counting for about 68% of the total variation. Figure 9 shows the results of the PCA in terms of loading  
579 plot (Fig. 9a) and score plot (Fig. 9c) of PC1 vs. PC2 and in terms of loading plot (Fig. 9b) and score plot (Fig. 9d) of  
580 PC2 vs. PC3.

581 The first principal component (explained variance 42%, Fig. 9a, 9c) is strongly associated to the main hydrographic  
582 parameters (temperature, salinity, density) and to the distance from the Po mouth (positive loadings), which are in contrast  
583 with  $NO_3^-$ ,  $Si(OH)_4$ , turbidity, DOC and the main phytoplanktonic groups (diatoms and phytoflagellates). This group of  
584 variables (nutrients and phytoplankton community) includes also all the metals and almost all the metal fractions (even if  
585 not all have high loads on PC1), with the exception of  $Pb_{dyn}$  and  $Cd_{coll}$ , that showed positive loadings on PC1. Thus, the  
586 PC1 expresses the effect of the river outflow on the distribution of metal species, nutrients and main phytoplankton  
587 communities. Quite surprisingly, phosphates were associated to salinity gradient and in contrast with the general trend of  
588 other nutrients and metals. PC2 (explained variance 16%, Fig. 9a) is associated to  $O_2$ , pH, dinoflagellates,  $Cd_{coll}$ ,  
589 coccolithophorids, Chl-a and  $NH_4^+$  (positive loadings). All these variables are in contrast with  $NO_2^-$  and, to a lesser extent



590 with silicates,  $Pb_{part}$  and turbidity. Hence, the second principal component expresses the biological activity and  
591 regeneration processes (see the score plots, Fig. 9c). The third component (explained variance 10%, Fig. 9b, 9d) is  
592 dominated by the speciation of dissolved Pb with the contrast between  $Pb_{diss}$ , and  $Pb_{coll}$ , on one side, and  $Pb_{dyn}$  (together  
593 with DOC) to the other side. The third principal component expresses the role of the organic matter on the dissolved Pb  
594 speciation.

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## 598 4. Discussion

599

### 600 4.1. Environment

601 The oceanographic cruise along the Po plume (October-November 2002) was carried out after a particularly rainy  
602 period. The mean daily discharge measured at the hydrometric station of Pontelagoscuro (located at 80-km before the Po  
603 River mouth) was around  $1300 \text{ m}^3 \text{ s}^{-1}$  (ARPA Emilia-Romagna, 2002), similar to the long-term average for the period of  
604  $1828 \text{ m}^3 \text{ s}^{-1}$  (Boldrin et al., 2005; Campanelli et al., 2012). However, the cruise was preceded by a particularly rainy  
605 period, where the Po river discharge reached values up to  $3000 \text{ m}^3 \text{ s}^{-1}$  (ARPA Emilia-Romagna, 2002). Consequently, the  
606 influence of the Po River, in terms of reduced salinity at the surface, was evident about 50-km offshore from the mouth.  
607 This resulted in a marked horizontal stratification of the water column, highlighted by the presence of thermocline, strong  
608 halocline and pycnocline ( $N_2(z) \sim 400 \times 10^{-4} \text{ s}^{-2}$ ) throughout most part of the transect. In the open sea, the effect of the Po  
609 outflow disappeared, as well as the stratification of the water column sharply reduced ( $N_2(z)$  up to  $15 \times 10^{-4} \text{ s}^{-2}$ , at 50-m  
610 depth of Stn. 6).

611 Principal component analysis applied to the whole dataset revealed that surface waters of the frontal area were affected  
612 by other factors than the Po river runoff (Fig. 9). These stations were associated with oxygen super-saturation levels (up  
613 to  $\sim 140\%$ ), high value of pH and  $\text{NH}_4^+$ , corresponding to phytoplankton abundance and biomass peaks. Although diatoms  
614 were mainly responsible of total phytoplankton abundance and biomass distribution (see below), a dramatic increase  
615 ( $\sim 80\%$ ) of both dinoflagellates and coccolithophorids occurred in the frontal area, while in the other stations these groups  
616 were practically absents (especially coccolithophorids). We hypothesize that saltier, well-oxygenated and high productive  
617 waters originated by the northern part of the Adriatic Sea (and maybe moved by winds) intrude in the frontal area and  
618 proceeded southward. Unfortunately, wind data of that period are not available, hence we are not able to corroborate this  
619 hypothesis.

620 DOC concentrations were similar to those typical of the region during the autumn. They were positively correlated  
621 with both phytoplankton abundance and biomass, highlighting that phytoplankton significantly contributed to DOC rather  
622 than the Po River. In the water column, DOC sharply decreased within the stratified layer, then below the pycnocline it  
623 showed a quite uniform profile, reaching values very close to the background levels ( $76 \pm 10 \mu\text{mol L}^{-1}$ ) found by Pettine  
624 et al. (2001) in this area and typical of the surface oceanic waters (Guo et al., 1995).

625 Nutrient distributions along the transect highlighted the role of the Po River discharge and that of the biological  
626 processes in controlling the nutrient levels in the Northern Adriatic Sea as already observed in previous surveys (Boldrin  
627 et al., 2005; Grilli et al., 2005; Campanelli et al., 2012). Nutrient concentrations are typical of the autumn season for the  
628 Northern Adriatic Sea (Boldrin et al., 2005; Campanelli et al., 2012) and showed a clearly seaward decreasing gradient.  
629 The nutrient vertical profiles were generally characterized by high surface concentrations (especially in the pro-delta and  
630 frontal areas), decreasing down to a depth of about 5-10 m (within the stratified layer), a trend opposite to that of the  
631 salinity, highlighting the influence of the Po river input. Below the 10-m depth concentrations were vertically uniform or  
632 increased with depth.

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634

635

#### 636 4.2. Metal speciation along the Po River plume

637 Several processes, both internal (i.e. trace metal removal from the dissolved phase by adsorption, precipitation and  
638 co-precipitation of solutes, or by flocculation and net sedimentation with suspended matter, or by biological uptake;  
639 production of trace metals in the dissolved phase by desorption or solubilisation of particulate matter; transformation and  
640 migration of trace metals at the sediment/water interface) and external (mainly anthropogenic activities, such as, pollution,  
641 discharging of pollutants or cooling waters) control the speciation and the distribution patterns of metals in the mixing  
642 zone between salt and fresh waters. These processes are affected, in turn, by the strong longitudinal variations in physical,  
643 chemical and biological parameters that develop in estuarine systems.

644 Principal component analysis reveals a strong influence of the Po River outflow on the spatial and vertical distribution  
645 of Cd, Pb and Cu species. Almost all the metal fractions linearly decreased with salinity gradient, consequently to dilution  
646 effect (i.e. all the metal showed conservative behaviours). Although all the metal fraction contents globally decreased  
647 with salinity, speciation distribution patterns significantly differed from one metal to another.

648 Cd was mainly present in its dissolved form (particulate fraction up to 20% of the total). The contribution of Cd  
649 particulate fraction increased within the stratified layer, following the maximum phytoplankton abundance. Below the

650 pycnocline, dissolved/particulate fractionation changed again, due to remineralisation processes from dead phytoplankton  
651 cells, that released Cd in seawater (Bruland and Lohan, 2003).

652 Concerning Pb and Cu, the particulate fractions of the two metals were dominant within the pro-delta area (accounting  
653 for ~70% of the  $Pb_{tot}$  and for ~55% of the  $Cu_{tot}$ ), while the dissolved fractions were mainly presents in the open sea.  
654 Nevertheless (with the exception of the pro-delta area), Cu partitioning was dominated by the dissolved fraction  
655 throughout the study transect. Contrary to its oceanic “hybrid” distribution in the water column (e. g. surface depletion,  
656 linear increase with depth and deep scavenging by particles) (Bruland and Lohan, 2003; Jacquot and Moffet, 2015),  
657 dissolved Cu remained elevate all the way down to the bottom. Only at the seafloor of the frontal area, an increase of Cu  
658 content occurred, suggesting additional inputs from sediments (such as re-suspended sediments), as observed in open  
659 ocean waters (Tankere et al., 2000; Waeles et al., 2008; Jacquot and Moffett, 2015).

660 Depth profile of Pb confirmed its nature of particle-reactive metal. Because of desorption processes from the  
661 particulate matter, dissolved Pb concentrations increased along the water column, while the particulate fraction became  
662 again significant in proximity of the seafloor. In fact, sediment can be considered as an additional source of particulate  
663 Pb due to several biogeochemical processes within the sediments themselves and at the sediment/water interface (Tankere  
664 et al., 2000; Cobelo-Garcia and Prego, 2004).

665 Differences between Cd, on one hand, and Pb-Cu on the other hand, were observed also for the dissolved-fraction  
666 speciation. Cd was mainly present as dynamic fraction in the pro-delta area. In the remaining part of the transect, the  
667 contribution of the colloidal fraction to the dissolved Cd became more and more important within the stratified layer, up  
668 to 40-50% of  $Cd_{diss}$  in the marine area. As observed by Baeyens et al. (1998) in the Scheldt estuary (Netherlands), it seems  
669 likely that the production of dissolved Cd contributed to the dynamic fraction (potentially bio-available) near the Po  
670 mouth, afterwards it decreased in favour of less dynamic species, i.e. colloidal species. Martin et al. (1995) in the Venice  
671 Lagoon, also found similar colloidal contribution (30-40%) to the dissolved Cd, as well as in the Ochlockonne estuary  
672 (Powell et al., 1996) or in the Scheldt estuary (Baeyens et al., 1998). As reported by Comans and Van Dijk (1988), the  
673 speciation of Cd is generally dominated in estuaries by stable and soluble chlorocomplexes, but in some systems, organic  
674 complexation could be important, as well. As showed in the PCA (Fig. 9),  $Cd_{coll}$  is significantly associated with small  
675 algal cells, the dinoflagellates and the coccolithophorids, which increased in the frontal area of a percentage much higher  
676 (80-98%, respectively) than diatoms (~40%) or phytoflagellates (see in Section 4.1). Thus, the increase of colloidal Cd  
677 seawards could be ascribed to the complexation with algal cells or with exudates produced by phytoplankton, as well.  
678 Below the pycnocline and down to the bottom,  $Cd_{diss}$  returned to be mainly constituted by the dynamic fraction (90-100%  
679 of the  $Cd_{diss}$ ), following the release of this fraction from phytoplankton dead cells that sedimented in the water column.

680 Dissolved Pb and Cu speciation was dominated by colloidal fraction (40 – 95% of the dissolved fraction for both  
681 metals). The low proportion of  $Pb_{dyn}$  and the consequently strong association of Pb with colloidal material was in  
682 agreement with previous studies (Martin et al., 1995; Waeles et al., 2008; Braungardt et al., 2011). For example, Martin  
683 et al. (1995) reported that colloidal Pb accounted for up to ~90% of the total dissolved Pb in the Venice Lagoon (northern  
684 Adriatic Sea). Scarponi et al. (1995) during speciation studies carried out through voltammetric titration procedures in  
685 seawater of different areas (e.g. Adriatic Sea, Antarctic Ocean, and North Eastern Pacific) found that the ASV-labile  
686 fraction (similar to our dynamic fraction) accounted for about 15% of the total Pb at the surface.

687 When considering the contribution of colloidal copper in various estuarine or delta systems disparate results were  
688 obtained. As example, in the Danube and in the Loire estuaries (Waeles et al., 2004, 2009),  $Cu_{coll}$  accounted for only 10-  
689 40% of the total dissolved metal. In the Penzé estuary (Waeles et al., 2008) or in the Restronguet Creek (Braungardt et  
690 al., 2011) the colloidal fraction showed percentages similar to our results. The high presence of colloidal Cu confirmed  
691 results obtained in previous speciation studies carried out by Zago et al. (2002) in the Northern Adriatic Sea. The authors  
692 did not detect labile metal concentrations of the two studied metals (Cu and Zn), but, computing the ligand concentrations,  
693 they concluded that the total dissolved metal concentration of Cu was exclusively present as organically complexed  
694 metals. Several speciation studies worldwide (Capodaglio et al., 1994; L'Her Roux et al., 1998; Waeles et al., 2004, 2008,  
695 2009; Buck et al., 2007) suggested a model with two classes of ligand for both dissolved Pb and Cu, the strongest  
696 consisting of detrital matter (e.g. fulvic and humic acids) and living biogenic material (living organisms, such as algae,  
697 bacteria, virus, or organic exudates).

698 Since the phytoplankton highly contributed to the DOC pool (see section 4.1), it is plausible to hypothesise an organic  
699 origin for Pb and Cu ligands in the northern Adriatic Sea. Further studies, including a better spatial resolution and  
700 dissolved organic matter speciation are, therefore, necessary to gain more insight into the speciation of the two dissolved  
701 metals and the nature of Pb-Cu ligands in the Adriatic Sea.

702 The impact of the different processes controlling trace metal specie distributions along the Po plume can be established  
703 also by investigating metal–nutrient relationships. In open ocean water, bioactive trace metals (Cd, Cu, Fe, Ni, Zn) have  
704 positive relationships with limiting nutrients ( $PO_4^{3-}$  and  $Si(OH)_4$ ), following the assumption that the metal:P slopes in  
705 seawater should be equal to the ratios in which these elements are present in the cells; the so-called “extended Redfield  
706 ratio” (Morel and Hudson, 1985). Deviations from this linear metal–nutrient relationship may be indicative of additional  
707 inputs not observed in open ocean environments (Martin et al., 1980). In the present work, all the metal fractions did not  
708 show significant positive correlations with  $PO_4$  (Tab. S5). Similar ratios were observed also if surface values (which are  
709 more affected by river discharge) were excluded (data not shown). The opposite gradients observed in the PCA between  
710 metals and P (Fig. 9) cause the negative Me:P slopes recorded in these waters (Tab. S5), highlighting the influence of

711 other factors than biological cycles on metal distribution, i.e. the effect of the Po River discharge that masked, for  
712 examples, the typical nutrient-like profile of Cd and Cu.

713

714

#### 715 4.3. Significance of metal speciation

716 The dynamic metal concentrations here measured could offer some interesting insights on the toxic or limiting effects  
717 of specific trace metals, since its peculiar characteristic to approximate the bio-available fraction (Buffle and Tercier-  
718 Waeber, 2005) more than the dissolved fraction. Several studies have been carried out on assessing the toxicity of trace  
719 metals on marine organisms, most of them dealing with the toxicity of the free metal ions. Very few is known about the  
720 toxicity effect of the dynamic fraction. Studies based on laboratory cultures (Verweij et al., 1992; Perez et al., 2010)  
721 reported that adverse effects on cyanobacteria and many marine phytoplankton species occurred for concentrations in the  
722 range 0.001 – 0.1 nmol L<sup>-1</sup> for Cu<sup>2+</sup>, 1 – 5 nmol L<sup>-1</sup> for Cd<sup>2+</sup> and <0.1 nmol L<sup>-1</sup> for Pb<sup>2+</sup>.

723 Our dynamic Cd and Pb concentrations did not represent a risk to marine organisms in the Northern Adriatic Sea,  
724 even if Pb showed concentrations very close or slightly below the thresholds previously reported. However, the Pb<sup>2+</sup>  
725 concentrations measured by the PLM technique (no data available from CGIME sensor) were few tens of pico-molar per  
726 L (from 1 to ~15 pmol L<sup>-1</sup>), far below the toxic levels measured in laboratory cultures. On the contrary, the dynamic  
727 fraction of Cu showed values of the pro-delta (~4.0 nmol L<sup>-1</sup>) being potentially toxic to 21 marine phytoplankton species  
728 examined in laboratory cultures. Moreover, studies (Verweij et al., 1992; Beiras and Albentosa, 2004; Rivera-Duarte et  
729 al., 2005) on the embryogenesis success of bivalves (*Mytilus galloprovincialis*, *Ruditapes decussatus*), sand dollar  
730 (*Dendraster excentricus*), and sea urchin (*Strongylocentrotus purpuratus*) showed sensitivity towards Cu<sup>2+</sup> at values of  
731 EC<sub>50</sub> (effective concentration of metal causing a 50% inhibition of embryos development with respect to the control)  
732 ranged from ~0.06 nmol L<sup>-1</sup> to ~0.16 nmol L<sup>-1</sup>. Therefore, Cu dynamic fraction along Po plume showed concentrations  
733 likely to be toxic to sensitive phytoplankton community and to have negative effects on larva development of coastal  
734 macroinvertebrate species. Also Cu<sup>2+</sup> concentrations (~0.3 nmol L<sup>-1</sup>) at the Po mouth were higher than the EC<sub>50</sub> values  
735 provided for coastal macroinvertebrate larvae sensitivity to copper (Beiras and Albentosa, 2004), and that showed the  
736 same order of magnitude of the toxic threshold range reported for plankton (Brand et al., 1986). In the case of copper,  
737 hydroxy complexes are also believed to be toxic, with toxicity decreasing in the order, Cu<sup>+</sup> and Cu<sup>2+</sup> > inorganic  
738 copper > organic copper (Allen and Hansen, 1996).

739 Moreover, culture studies have demonstrated that several metals may act synergistically or antagonistically to  
740 influence growth limitation or toxicity to marine organisms (Annett et al., 2008; Semeniuk et al., 2009). In force of these  
741 metal:metal antagonisms, elevated levels of free ions of one metal (e.g., Mn<sup>2+</sup>) may alleviate the toxic effects of Cu<sup>2+</sup>

742 (Sunda and Huntsman, 1983) or  $Zn^{2+}$  (Twining et al., 2011). Therefore, further studies including other metals in addition  
743 to those of the present work are necessary in order to gain more insight into metal speciation and potentially harmful  
744 effects of metal species to marine organisms in the Northern Adriatic Sea.

745

746

## 747 **5. Conclusions**

748 The speciation of Cd, Pb and Cu was studied for the first time along the Po River plume. The oceanographic cruise  
749 (Autumn 2002) was carried out after a particularly rainy period. Consequently, the influence of the river, in terms of  
750 reduced salinity at the surface, was evident about 50-km offshore from the mouth. The distribution and speciation of Cd,  
751 Pb and Cu in the Po River plume is mainly controlled by the river outflow.

752 Almost all the metal fractions linearly decreased with salinity gradient, consequently to dilution effect (i.e. all the  
753 metal showed conservative behaviours). Cadmium was mainly present as dynamic fraction in the pro-delta area, while in  
754 the remaining part of the transect, the contribution of the colloidal fraction to the dissolved Cd became more and more  
755 important. On the contrary, the Pb and Cu partitioning was dominated by particulate fraction in the stations closer to the  
756 Po mouth and by the dissolved fraction in the open sea. The colloidal fraction of these two metals constituted a significant  
757 proportion of the dissolved concentration and it seems to be related to a biogenic origin. On the basis of speciation studies  
758 on the dissolved organic matter in the Northern Adriatic Sea, we hypothesize a biogenic origin (small algae, or  
759 phytoplankton exudates) of these colloidal fractions.

760 Metal concentrations measured in the present work are far below (at least one order of magnitude lower) the EQS  
761 established by the Italian law (Italian DPR 172/15, 2015) in the enactment of the revised Priority Substances Daughter  
762 Directive of the European Union, 2013/39/EU (2013), even if these Directives do not take copper into account at all (the  
763 only national legal limit existing for Cu dates back to the Italian DPR 152/06 (2006), that established a threshold  
764 concentration of  $16 \mu\text{mol L}^{-1}$  referring to waters suitable to the life of salmonid and cyprinid organisms). However, our  
765 Cu dynamic fraction along the Po plume showed concentrations likely to be toxic to sensitive phytoplankton community  
766 and to have negative effects on larva development of coastal macroinvertebrate species. Also  $Cu^{2+}$  concentrations at the  
767 Po mouth were higher than the  $EC_{50}$  values provided for coastal macroinvertebrate larvae sensitivity to copper.

768 The present work allows a better comprehension of the processes regulating marine biogeochemical cycles of trace  
769 metals, it improves our insights on transport and fate of contaminants in the marine environment, giving valuable tools for  
770 more reliable predictions of future changes and following actions to protect it, as recommended by the Marine Strategy  
771 Framework Directive (descriptor 8<sup>th</sup>), (European Directive 2008/56/EC, 2008).

772

773 **Acknowledgements**

774 The authors gratefully acknowledge financial support from the European IMTEC Project (In-situ Monitoring of Trace  
775 metal speciation in Estuaries and Coastal zones in relation with the biogeochemical processes, contract no. EVK3-CT-  
776 2000-00036). Many thanks are also due to Dr Luca Lambertucci for his technical assistance.

777

778 **References**

- 779 Agusti, S., Duarte, C.M., 1999. Phytoplankton chlorophyll a distribution and water column stability in the central Atlantic  
780 Ocean. *Oceanol. Acta*, 22, 193–203.
- 781 Ahner, B.A., Morel, F.M.M., 1995. Phytochelatin production in marine algae. 2. Induction by various metals. *Limnol.*  
782 *Oceanogr.*, 40, 658–665.
- 783 Allen, H.E., Hansen, D.J., 1996. The importance of trace metal speciation to water quality criteria. *Water Environ. Res.*,  
784 68, 42–54.
- 785 Annett, A.L., Lapi, S., Ruth, T.J., Maldonado, M.T., 2008. The effects of Cu and Fe availability on the growth and Cu:C  
786 ratios of marine diatoms. *Limnol. Oceanogr.*, 53, 2451–2461.
- 787 Annibaldi, A., Truzzi, C., Illuminati, S., Bassotti, E., Scarponi, G., 2007. Determination of water-soluble and insoluble  
788 (dilute-HCl-extractable) fractions of Cd, Pb and Cu in Antarctic aerosol by square wave anodic stripping voltammetry:  
789 distribution and summer seasonal evolution at Terra Nova Bay (Victoria Land). *Anal. Bioanal. Chem.*, 387, 977–998.
- 790 Annibaldi, A., Truzzi, C., Illuminati, S., Scarponi, G., 2009. Recent sudden decrease of lead in Adriatic coastal seawater  
791 during the years 2000-2004 in parallel with the phasing out of leaded gasoline in Italy. *Mar. Chem.*, 113, 238–249.
- 792 Annibaldi, A., Illuminati, S., Truzzi, C., Scarponi, G., 2011. SWASV speciation of Cd, Pb and Cu for the determination  
793 of seawater contamination in the area of the Nicole shipwreck (Ancona coast, Central Adriatic Sea). *Mar. Pollut. Bull.*,  
794 62, 2813–2821.
- 795 Annibaldi, A., Illuminati, S., Truzzi, C., Libani, G., Scarponi, G., 2015. Pb, Cu and Cd distribution in five estuary systems  
796 of Marche, central Italy. *Mar. Pollut. Bull.*, 96, 441–449.
- 797 Aparicio-González, A., Duarte, C.M., Tovar-Sánchez, A., 2012. Trace metals in deep ocean waters: A review. *J. Mar.*  
798 *Syst.*, 100, 26–33.
- 799 ARPA Emilia-Romagna, 2002. *Annali idrologici*, Pt II.
- 800 Baeyens, W., Goeyens, L., Monteny, F., Elskens, M., 1998. Effect of organic complexation on the behavior of dissolved  
801 Cd, Cu and Zn in the Scheldt estuary. *Hydrobiologia* 366, 81–90.
- 802 Baeyens, W., Leermakers, M., Gieter, M.D., Nguyen, H.L., Parmentier, K., Panutrakul, S., Elskens, M., 2005. Overview  
803 of trace metal contamination in the Scheldt estuary and effect of regulatory measures. *Hydrobiologia*, 540, 141–154.
- 804 Beiras, R., Albetosa, M., 2004. Inhibition of embryo development of the commercial bivalves *Ruditapes decussatus* and  
805 *Mytilus galloprovincialis* by trace metals; implications for the implementation of seawater quality criteria.  
806 *Aquaculture*, 230, 205–213.
- 807 Belmont-Hebert, C., Tercier, M.L., Buffle, J., Fiaccabrino, G.C., de Rooij, N.F., Koudelka-Hep, M., 1998. Gel-Integrated  
808 Microelectrode Arrays for Direct Voltammetric Measurements of Heavy Metals in Natural Waters and Other Complex  
809 Media. *Anal. Chem.*, 70, 2949–2956.
- 810 Bernardi Aubry, F.B., Gianpiero Cossarini, Acri, F., Bastianini, M., Bianchi, F., Camatti, E., De Lazzari, A., Pugnetti,  
811 A., Solidoro, C., Socal, G., 2012. Plankton communities in the northern Adriatic Sea: Patterns and changes over the  
812 last 30 years. *Estuar. Coast. Shelf. Sci.*, 115, 125–137.
- 813 Boldrin, A., Langone, L., Miserocchi, S., Turchetto, M., Acri, F., 2005. Po River plume on the Adriatic continental shelf:  
814 Dispersion and sedimentation of dissolved and suspended matter during different river discharge rates. *Mar. Geol.*,  
815 222–223, 135–158.
- 816 Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1986. Reduction of marine phytoplankton reproduction rates by copper and  
817 cadmium. *J. Exp. Mar. Biol. Ecol.*, 96, 225–250.
- 818 Braungardt, C.B., Achterberg, E.P., Axelsson, B., Buffle, J., Graziottin, F., Howell, K.A., Illuminati, S., Scarponi, G.,  
819 Tappin, A.D., Tercier-Waeber, M.L., Turner, D., 2009. Analysis of dissolved metal fractions in coastal waters: An  
820 inter-comparison of five voltammetric in situ profiling (VIP) systems. *Mar. Chem.*, 114, 47–55.
- 821 Braungardt, C.B., Howell, K.A., Tappin, A.D., Achterberg, E.P., 2011. Temporal variability in dynamic and colloidal  
822 metal fractions determined by high resolution in situ measurements in a UK estuary. *Chemosphere*, 84, 423–431.
- 823 Buck, K.N., Ross, J.R.M., Flegal, A.R., Bruland, K.W., 2007. A review of total dissolved copper and its chemical  
824 speciation in San Francisco Bay, California. *Environ. Res.*, 105, 5–19.
- 825 Buffle, J., Perret, D., Newman, M., 1992. The use of filtration and ultrafiltration for size fractionation of aquatic particles,  
826 colloids, and macromolecules. In: *Environmental Particles*. Buffle, J.; van Leeuwen, H.P. (Eds.), Boca Raton, pp.  
827 171–230.
- 828 Buffle, J., Tercier-Waeber, M.-L., 2005. Voltammetric environmental trace-metal analysis and speciation: from  
829 laboratory to in situ measurements. *Trace-Met. Anal.*, 24, 172–191.

830 Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a coastal  
831 northern Adriatic site during the last 25 years. *Estuar. Coast. Shelf Sci.*, 115, 113–124.

832 Campanelli, A., Grilli, F., Paschini, E., Marini, M., 2012. The influence of an exceptional Po River flood on the physical  
833 and chemical oceanographic properties of the Adriatic Sea. *Dyn. Atmos. Oceans*, 52, 284–297.

834 Capodaglio, G., Toscano, G., Scarponi, G., Cescon, P., 1994. Copper complexation in surface seawater of Terra Nova  
835 Bay (Antarctica). *Int. J. Environ. Anal. Chem.*, 55, 129–148.

836 Cindrić, A.-M., Garnier, C., Oursel, B., Pižeta, I., Omanović, D., 2015. Evidencing the natural and anthropogenic  
837 processes controlling trace metals dynamic in a highly stratified estuary: The Krka River estuary (Adriatic, Croatia).  
838 *Mar. Pollut. Bull.*, 94, 199–216.

839 Cobelo-Garcia, A., Prego, R., 2004. Chemical speciation of dissolved copper, lead and zinc in a ria coastal system: the  
840 role of resuspended sediments. *Anal. Chim. Acta*, 524, 109–114.

841 Comans, R.N.J., Van Dijk, C.P.J., 1988. Role of complexation processes in cadmium mobilization during estuarine  
842 mixing. *Nature*, 336, 151–154.

843 Donat, J.R., Lao, K.A., Bruland, K.W., 1994. Speciation of dissolved Copper and Nickel in South San Francisco Bay: a  
844 Multi-Method Approach. *Anal. Chim. Acta*, 284, 547–571.

845 Dorten, W.S., Elbaz-Poulichet, F., Mart, L.R., Martin, J.-M., 1991. Reassessment of the river input of trace metals into  
846 the Mediterranean Sea. *Ambio*, 20, 2–6.

847 Edler, L., Elbrachter, M., 2010. The Utermöhl method for quantitative phytoplankton analysis. In: Karlson, B., Cusak, C.,  
848 Bresnan, E. (Eds.), *Microscopic and molecular methods for quantitative phytoplankton analysis*. Intergovernmental  
849 Oceanographic Commission of UNESCO, Paris, pp. 13–20.

850 European Parliament and Council, 2008. Directive 2008/56/EC of the 17 June 2008 establishing a framework for  
851 community action in the field of marine environmental policy (Marine Strategy Framework Directive).

852 European Parliament and Council, 2013. Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and  
853 2008/105/EC as regards priority substances in the field of water policy.

854 Falcieri, F.M., Benetazzo, A., Sclavo, M., Russo, A., Carniel, S., 2014. Po River plume pattern variability investigated  
855 from model data. *Oceanogr. Coast. Scales*, 87, 84–95.

856 Gaillardet, J., Viers, J., Dupré, B., 2003. Trace Elements in River Waters A2 - Holland, Heinrich D., In: Turekian, K.K.;  
857 Heinrich, D.H.; Karl, K.T. (Eds.), *Treatise on Geochemistry*, Oxford, pp. 225–272.

858 Godrijan, J., Marić, D., Tomažić, I., Precali, R., Pfannkuchen, M., 2013. Seasonal phytoplankton dynamics in the coastal  
859 waters of the north-eastern Adriatic Sea. *J. Sea Res.*, 77, 32–44.

860 Grilli, F., Marini, M., Degobbis, D., Ferrari, C.R., Fornasiero, P., Russo, A., Gismondi, M., Djakovac, T., Precali, R.,  
861 Simonetti, R., 2005. Circulation and horizontal fluxes in the northern Adriatic Sea in the period June 1999–July 2002.  
862 Part II: Nutrients transport. *Sci. Total Environ.*, 353, 115–125.

863 Grilli, F., Marini, M., Book, J.W., Campanelli, A., Paschini, E., Russo, A., 2013. Flux of nutrients between the middle  
864 and southern Adriatic Sea (Gargano-Split section). *Mar. Chem.*, 153, 1–14.

865 Guo, L., Santschi, P.H., Warnken, K.W., 1995. Dynamics of dissolved organic carbon (DOC) in oceanic environments.  
866 *Limnol. Oceanogr.*, 40, 1392–403.

867 Harper, D.J., 1991. The distribution of dissolved cadmium, lead and copper in the Bristol Channel and the outer Severn  
868 estuary. *Mar. Chem.*, 33, 131–43.

869 Howell, K.A., Achterberg, E.P., Braungardt, C.B., Tappin, A.D., Turner, D.R., Worsfold, P.J., 2003. The determination  
870 of trace metals in estuarine and coastal waters using a voltammetric in situ profiling system. *Analyst*, 128, 734–741.

871 Illuminati, S., Annibaldi, A., Truzzi, C., Finale, C., Scarponi, G., 2013. Square-wave anodic-stripping voltammetric  
872 determination of Cd, Pb and Cu in wine: Set-up and optimization of sample pre-treatment and instrumental parameters.  
873 *Electrochim. Acta*, 104, 148–161.

874 Illuminati, S., Annibaldi, A., Truzzi, C., Libani, G., Mantini, C., Scarponi, G., 2015. Determination of water-soluble,  
875 acid-extractable and inert fractions of Cd, Pb and Cu in Antarctic aerosol by square wave anodic stripping voltammetry  
876 after sequential extraction and microwave digestion. *J. Electroanal. Chem.*, 755, 182–196.

877 Italian Republic President, 2006. Legislative Decree of the Italian Republic President, n. 152 of the 3 April 2006 on  
878 Environmental regulations.

879 Italian Republic President, 2015. Legislative Decree of the Italian Republic President, n. 172 of the 13 October 2015 on  
880 the implementation of Directive 2013/39 / EU, amending the directives 2000/60 / EC as regards priority substances  
881 in the sector of water policy.

882 Jacquot, J.E., Moffett, J.W., 2015. Copper distribution and speciation across the International GEOTRACES Section  
883 GA03. *Deep Sea Res. Part II*, 116, 187–207.

884 Kolb, M., Rach, P., Schaefer, J., Wild, A., 1992. Investigations of oxidative UV photolysis. I. Sample preparation for the  
885 voltammetric determination of zinc, cadmium, lead, copper, nickel, and cobalt in waters. *Fresenius J. Anal. Chem.*  
886 342, 341–349.

887 L’Her Roux, L., Le Roux, S., Appriou, P., 1998. Behavior and speciation of metallic species Cu, Cd, Mn and Fe during  
888 estuarine mixing. *Mar. Pollut. Bull.*, 36, 56–64.

889 Marini, M., Jones, B.H., Campanelli, A., Grilli, F., Lee, C.M., 2008. Seasonal variability and Po River plume influence  
890 on biochemical properties along western Adriatic coast. *J. Geophys. Res. Oceans*, 113, 1–18.



891 Marini, M., Grilli, F., Guarnieri, A., Jones, B.H., Klajic, Z., Pinardi, N., Sanxhaku, M., 2010. Is the southeastern Adriatic  
892 Sea coastal trip an eutrophic area? *Estuar. Coast. Shelf Sci.* 88, 395–406.

893 Martin, J.H., Knauer, G.A., Flegal, A.R., 1980. Cadmium in, natural waters. In: *Cadmium in the Environment, Part 1:*  
894 *Ecological Cycling*. Nriagu, J. O. (Ed.), New York, pp. 141–145.

895 Martin, J.-M., Dai, M.-H., Cauwet, G., 1995. Significance of colloids in the biogeochemical cycling of organic carbon  
896 and trace metals in the Venice Lagoon (Italy). *Limnol. Oceanogr.*, 40, 119–31.

897 Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist  
898 plankton. *Limnol. Oceanogr.*, 45, 569–579.

899 Michel, P., Boutier, B., Chiffolleau, J.F., 2000. Net fluxes of dissolved arsenic, cadmium, copper, zinc, nitrogen and  
900 phosphorus from the Gironde Estuary (France): seasonal variations and trends. *Estuar. Coast. Shelf Sci.*, 51, 451–462.

901 Migon, C., Nicolas, E., 1998. Effects of antipollution policy on anthropogenic lead transfers in the Ligurian Sea. *Mar.*  
902 *Pollut. Bull.*, 36, 775–779.

903 Morel, F.M.M., Hudson, R.J.M., 1985. The geobiological cycle of trace elements in aquatic systems: Redfield revisited.  
904 In: *Chemical Processes in Lakes*. Stumm, W. (Ed.), New York, pp. 251–81.

905 Noel, S., Tercier-Waerber, M.L., Lin, L., Buffle, J., Guenat, O., Koudelka-Hep, M., 2006. Integrated microanalytical  
906 system for simultaneous voltammetric measurements of free metal ion concentrations in natural waters. *Electroanal.*,  
907 18, 2061–2069.

908 Ollivier, P., Radakovitch, O., Hamelin, B., 2011. Major and trace element partition and fluxes in the Rhone River. *Chem.*  
909 *Geol.*, 285, 15–31.

910 Oursel, B., Garnier, C., Durrieu, G., Mounier, S., Omanovic, D., Lucas, Y., 2013. Dynamics and fates of trace metals  
911 chronically input in a Mediterranean coastal zone impacted by a large urban area. *Mar. Pollut. Bull.*, 69, 137–149.

912 Owens, R.E., Balls, P.W., 1997. Dissolved trace metals in the Tay Estuary. *Estuar. Coast. Shelf Sci.*, 44, 421–434.

913 Parthasarathy, N., Pelletier, M., Buffle, J., 1997. Hollow fiber based supported liquid membrane: a novel analytical system  
914 for trace metal analysis. *Anal. Chim. Acta*, 350, 183–195.

915 Parthasarathy, N., Pelletier, M., Tercier-Waerber, M.L., Buffle, J., 2001. On-line coupling of flow through voltammetric  
916 microcell to hollow fiber permeation liquid membrane device for subnanomolar trace metal speciation measurements.  
917 *Electroanal.*, 13, 1305–1314.

918 Perez, P., Beiras, R., Fernandez, E., 2010. Monitoring copper toxicity in natural phytoplankton assemblages: application  
919 of Fast Repetition Rate fluorometry. *Ecotoxicol. Environ. Saf.*, 73, 1292–1303.

920 Pettine, M., Capri, S., Manganelli, M., Patrolecco, L., Puddu, A., Zoppini, A., 2001. The Dynamics of DOM in the  
921 Northern Adriatic Sea. *Estuar. Coast. Shelf Sci.*, 52, 471–489.

922 Powell, R.T., Landing, W.M., Bauer, J.E., 1996. Colloidal trace metals, organic carbon and nitrogen in a southeastern  
923 U.S. estuary. *Mar. Chem.*, 55, 165–176.

924 Rivera-Duarte, I., Rosen, G., Lapota, D., Chadwick, D.B., Kear-Padilla, L., Zirino, A., 2005. Copper toxicity to larval  
925 stages of three marine invertebrates and copper complexation capacity in San Diego Bay, California. *Environ. Sci.*  
926 *Technol.*, 39, 1542–1546.

927 Russo, A., Artegiani, A., 1996. Adriatic Sea hydrography. *Sci. Mar.*, 60, 33–43.

928 Sadiq, M., 1992. *Toxic Metal Chemistry in Marine Environments*. Marcel Dekker, Inc., New York.

929 Scarponi, G., Capodaglio, G., Toscano, G., Barbante, C., Cescon, P., 1995. Speciation of lead and cadmium in antarctic  
930 seawater: comparison with areas subject to different anthropic influence. *Microchem. J.*, 51, 214–230.

931 Scarponi, G., Turetta, C., Capodaglio, G., Toscano, G., Barbante, C., Moret, I., Cescon, P., 1998. Chemometric studies  
932 in the Lagoon of Venice, Italy. 1. The environmental quality of water and sediment matrixes. *J. Chem. Inf. Comput.*  
933 *Sci.*, 38, 552–562.

934 Schiltzer, R., 2017. Ocean Data View, <http://odv.awi.de>.

935 Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), European Commission, 2017. Scientific  
936 advice on Guidance Document n.27: Technical guidance for deriving Environmental Quality Standards. European  
937 Union.

938 Semeniuk, D.M., Cullen, J.T., Johnson, W.K., Gagnon, K., Ruth, T.J., Maldonado, M.T., 2009. Plankton copper  
939 requirements and uptake in the subarctic Northeast Pacific Ocean. *Deep-Sea Res. I*, 56, 1130–1142.

940 Sunda, W.G., Huntsman, S.A., 1983. Effect of competitive interactions between manganese and copper on cellular  
941 manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. *Limnol. Oceanogr.*, 28, 924–34.

942 Tankere, S.P.C., Statham, P.J., 1996. Distribution of dissolved Cd, Cu, Ni and Zn in the Adriatic Sea. *Mar. Pollut. Bull.*,  
943 32, 623–630.

944 Tankere, S.P.C., Price, N.B., Statham, P.J., 2000. Mass balance of trace metals in the Adriatic Sea. *J. Mar. Syst.*, 25, 269–  
945 286.

946 Tercier, M.-L., Parthasarathy, N., Buffle, J., 1995. Reproducible, reliable and rugged Hg-plated Ir-based microelectrode  
947 for in situ measurements in natural waters. *Electroanal.*, 7, 55–63.

948 Tercier, M.-L., Buffle, J., Graziottin, F., 1998. A novel voltammetric in situ profiling system for continuous real-time  
949 monitoring of trace elements in natural waters. *Electroanal.*, 10, 355–363.

950 Tercier-Waerber, M.-L., Belmont-Hebert, C., Buffle, J., 1998. Real-time continuous Mn(II) monitoring in lakes using a  
951 novel Voltammetric in situ Profiling System. *Environ. Sci. Technol.*, 32, 1515–1521.

952 Tercier-Waeber, M.-L., Buffle, J., Confalonieri, F., Riccardi, G., Sina, A., Graziottin, F., Fiaccabrino, G.C., Koudelka-  
953 Hep, M., 1999. Submersible voltammetric probes for in situ real-time trace element measurements in surface water,  
954 groundwater and sediment-water interface. *Meas. Sci. Technol.*, 10, 1202–1213.

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

957 Tercier-Waeber, M.-L., Buffle, J., Koudelka-Hep, M., Graziottin, F., 2002. Submersible voltammetric probes for real-  
958 time continuous monitoring of trace elements in natural aquatic systems. In: *Environmental Electrochemistry: Analysis of Trace Element Biogeochemistry*. American Chemical Society, Washington DC, pp. 16–39.

960 Tercier-Waeber, M.-L., Confalonieri, F., Riccardi, G., Sina, A., Noel, S., Buffle, J., Graziottin, F., 2005. Multi physical-  
961 chemical profiler for real-time in situ monitoring of trace metal speciation and master variables: development,  
962 validation and field applications. *Mar. Chem.*, 97, 216–235.

963 Tercier-Waeber, M.-L., Confalonieri, F., Koudelka-Hep, M., Dessureault-Rompere, J., Graziottin, F., Buffle, J., 2008. Gel-  
964 integrated voltammetric microsensors and submersible probes as reliable tools for environmental trace metal analysis  
965 and speciation. *Electroanal.*, 20, 240–258.

966 Totti, C., Cangini, M., Ferrari, C., Kraus, R., Pompei, M., Pugnetti, A., Romagnoli, T., Vanucci, S., Socal, G., 2005.  
967 Phytoplankton size-distribution and community structure in relation to mucilage occurrence in the northern Adriatic  
968 Sea. *Sci. Total Environ.*, 353, 204–217.

969 Twining, B.S., Baines, S.B., Bozard, J.B., Vogt, S., Walker, E.A., Nelson, D.M., 2011. Metal quotas of plankton in the  
970 equatorial Pacific Ocean. *Deep-Sea Res. Part II*, 58, 325–341.

971 Verweij, W., Glazewski, R., De Haan, H., 1992. Speciation of copper in relation to its bioavailability. *Chem. Speci.*  
972 *Bioavail.*, 4, 43–51.

973 Vignati, D., Dominik, J., 2003. The role of coarse colloids as a carrier phase for trace metals in riverine systems. *Aquat.*  
974 *Sci.*, 65, 129–142.

975 Waeles, M., Riso, R.D., Maguer, J.F., Le Corre, P., 2004. Distribution and chemical speciation of dissolved cadmium and  
976 copper in the Loire estuary and North Biscay continental shelf, France. *Estuar. Coast. Shelf Sci.*, 59, 49–57.

977 Waeles, M., Tanguy, V., Lespes, G., Riso, R.D., 2008. Behaviour of colloidal trace metals (Cu, Pb and Cd) in estuarine  
978 waters: An approach using frontal ultrafiltration (UF) and stripping chronopotentiometric methods (SCP). *Estuar.*  
979 *Coast. Shelf Sci.*, 80, 538–544.

980 Waeles, M., Riso, R.D., Cabon, J.-Y., Maguer, J.-F., L’Helguen, S., 2009. Speciation of dissolved copper and cadmium  
981 in the Loire estuary and over the North Biscay continental shelf in spring. *Estuar. Coast. Shelf Sci.*, 84, 139–146.

982 Wold, S., 1978. Cross-validatory estimation of the number of components in factor and principal components models.  
983 *Technometrics*, 20, 397-405.

984 Zago, C., Capodaglio, G., Ceradini, S., Ciceri, G., Abelmoschi, L., Soggia, F., Cescon, P., Scarponi, G., 2000. Benthic  
985 fluxes of cadmium, lead, copper and nitrogen species in the northern Adriatic Sea in front of the River Po outflow,  
986 Italy. *Sci. Total Environ.*, 246, 121–137.

987 Zago, C., Capodaglio, G., Barbante, C., Giani, M., Moret, I., Scarponi, G., Cescon, P., 2002. Heavy metal distribution  
988 and speciation in the northern Adriatic Sea. *Chem. Ecol.*, 18, 39–51.

989

**Tab. 1.** Concentrations of trace metals along the Po River plume, selected Mediterranean rivers and open Mediterranean Sea.

Site	Dissolved and (total) concentrations (nmol L <sup>-1</sup> )			Reference
	Cd	Pb	Cu	
Po plume 2002	0.12 ± 0.04 (0.14 ± 0.05)	0.33 ± 0.21 (0.52 ± 0.35)	5.3 ± 2.5 (7.1 ± 4.6)	This study
Po river				(Tankere et al., 2000)
summer 1994		1.2 ± 0.92	41 ± 20	
winter 1995		1.5 ± 0.48	57 ± 18	
Po river 1991	0.58	0.72	25.7	(Dorten et al., 1991)
<i>European estuaries</i>				
Marche estuaries (Italy)	0.089-0.18 (0.18-0.36)	0.34-0.72 (0.48-9.7)	6.3-31 (7.9-47)	(Annibaldi et al., 2015)
Arno (Italy)	0.89	1.01	27.5	(Dorten et al., 1991)
Krka (Croatia)	0.015 (0.020)	0.03 (0.14)	4.44 (6.51)	(Cindrić et al., 2015)
Aber-Wrac'h (France)	~0 – 0.36 (~0 – 0.44)		6.3 – 9.4 (6.3 – 14)	(L'Her Roux et al., 1998)
Gironde (France)	0.04 – 0.84		2.2 – 20	(Michel et al., 2000)
Loire (France)	0.08 – 0.29		8.0 – 21	(Waeles et al., 2004)
Rhone (France)		0.33	32.7	(Ollivier et al., 2011)
Huveaunne (France)	0.07	0.64	27	(Oursel et al., 2013)
Jarret (France)	0.07	0.37	28	(Oursel et al., 2013)
Ebro (Spain)	1.07	0.75	15.3	(Dorten et al., 1991)
Severn (UK)	0.98 – 3.6	~1.9	63 – 79	(Harper, 1991)
Tay (UK)	0.11	0.65	13	(Owens and Balls, 1997)
Scheldt (Netherlands)	0.03 – 1.35	0.2 – 2.6	8 – 42	(Baeyens et al., 2005)
World average river	0.71	0.38	23	(Gaillardet et al., 2003)
Northern Adriatic Sea 1994	0.083		7.14	(Tankere and Statham, 1996)
Northern Adriatic Sea, 1996			5.4 ± 2.5	(Zago et al., 2002)
Northern Adriatic Sea, 1997			6.4 ± 2.8	(Zago et al., 2002)
Central Adriatic Sea, 2000-2004	0.14 ± 0.06	0.24 ± 0.14	7.1 ± 3.6	(Annibaldi et al., 2009)
Southern Adriatic Sea 1994	0.076		2.95	(Tankere and Statham, 1996)
Ligurian Sea		0.12 ± 0.04		(Migon and Nicolas, 1998)
Mediterranean Sea	0.062		1.7	(Tankere and Statham, 1996)
<i>Worldwide oceans</i>				
Arctic Ocean	~0.4	-	~3.0	(Aparicio-González et al., 2012)
Atlantic Ocean	~0.1	~0.1	~1.5	(Aparicio-González et al., 2012)
Indian Ocean	~0.2	~0.04	~1.5	(Aparicio-González et al., 2012)
Pacific Ocean	~0.3	~0.04	~1.4	(Aparicio-González et al., 2012)
Southern Ocean	~0.6	~0.03	~2.0	(Aparicio-González et al., 2012)

## FIGURE CAPTIONS

**Fig. 1.** Study area and location of the sampling stations from the Po mouth to the open sea.

**Fig. 2.** Contour plots of temperature, salinity, dissolved oxygen concentration, and oxygen saturation percentage during the 2002-Adriatic Sea cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

**Fig. 3.** Contour plots of chlorophyll-a and DOC during the 2002-Adriatic cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

**Fig. 4.** Contour plots of DIN, nitrates, nitrites, ammonium, phosphates, and silicates during the 2002-Adriatic cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

**Fig. 5.** Horizontal profiles of Cd (a), Pb (b), and Cu (c) speciation during the 2002-Adriatic cruise. The figures report the total concentration (—●—), the dissolved (—◇—) and the dynamic (---◆---) fraction distribution in the water column, for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations.

**Fig. 6.** Vertical profiles of Cd speciation during the 2002-Adriatic cruise. The figure reports the total concentration (—●—), the dissolved (—◇—) and the dynamic (---◆---) fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

**Fig. 7.** Vertical profiles of Pb speciation during the 2002-Adriatic cruise. The figure reports the total concentration (—●—), the dissolved (—◇—) and the dynamic (---◆---) fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

**Fig. 8.** Vertical profiles of Cu speciation during the 2002-Adriatic cruise. The figure reports the total concentration (—●—), the dissolved (—◇—) and the dynamic (---◆---) fraction distribution in the water column for each sampling station. The dark-grey patterned area represents the particulate fraction calculated by subtraction between the total and the dissolved concentrations, while the light-grey filled area represents the non-dynamic, or colloidal, fraction, computed by subtraction between dissolved and dynamic concentrations. The transparent grey stripes represent the pycnocline zones.

**Fig. 9.** PCA plots: (a) plot of the loadings of PC1 vs. PC2; (b) plot of the loadings of PC2 vs. PC3; (c) plot of the scores of PC1 vs. PC2; (d) plot of the scores of PC2 vs. PC3. The following abridgements were used for the variables: temperature (T), salinity (S), density ( $\rho$ ), dissolved oxygen ( $O_{2diss}$ ), oxygen saturation percentage ( $O_2\%$ ), turbidity (Turb), chlorophyll-a (Chl-a), Coccolithophorid abundances (Cocco\_Ab), Coccolithophorid biomass (Cocco\_B), Diatom abundances (Diat\_Ab), Diatom biomass (Diat\_B), Dinoflagellate abundances (Dinof\_Ab), Dinoflagellate biomass (Dinof\_B), Phytoflagellate abundances (Phytof\_Ab), Phytoflagellate biomass (Phytof\_B), total phytoplankton abundance (TotPh\_Ab), total phytoplankton biomass (TotPh\_B). For metal fraction abridgements see the text in Section 2.3. The objects code refers to the sampling station and to the depth.

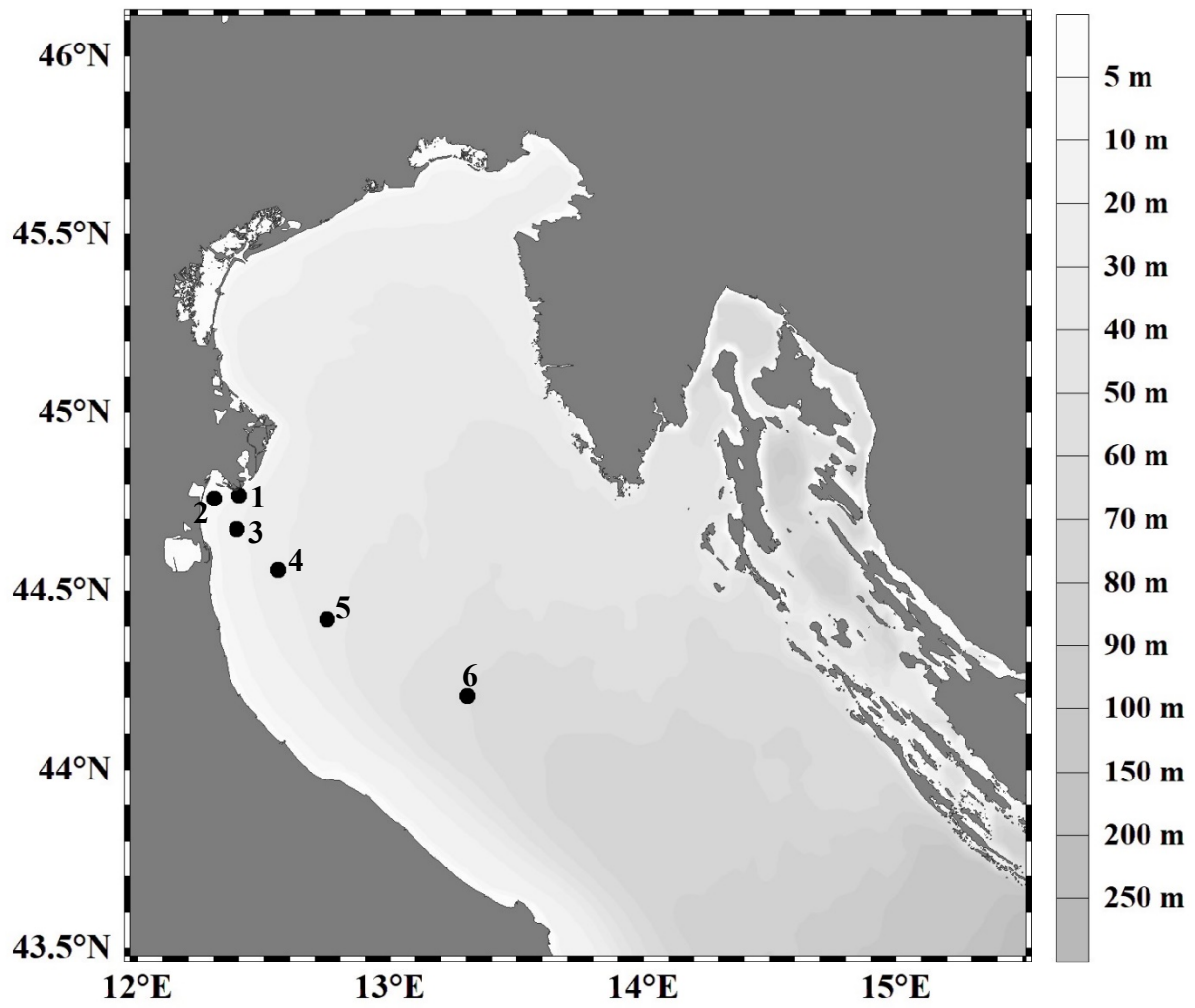


Fig. 1.

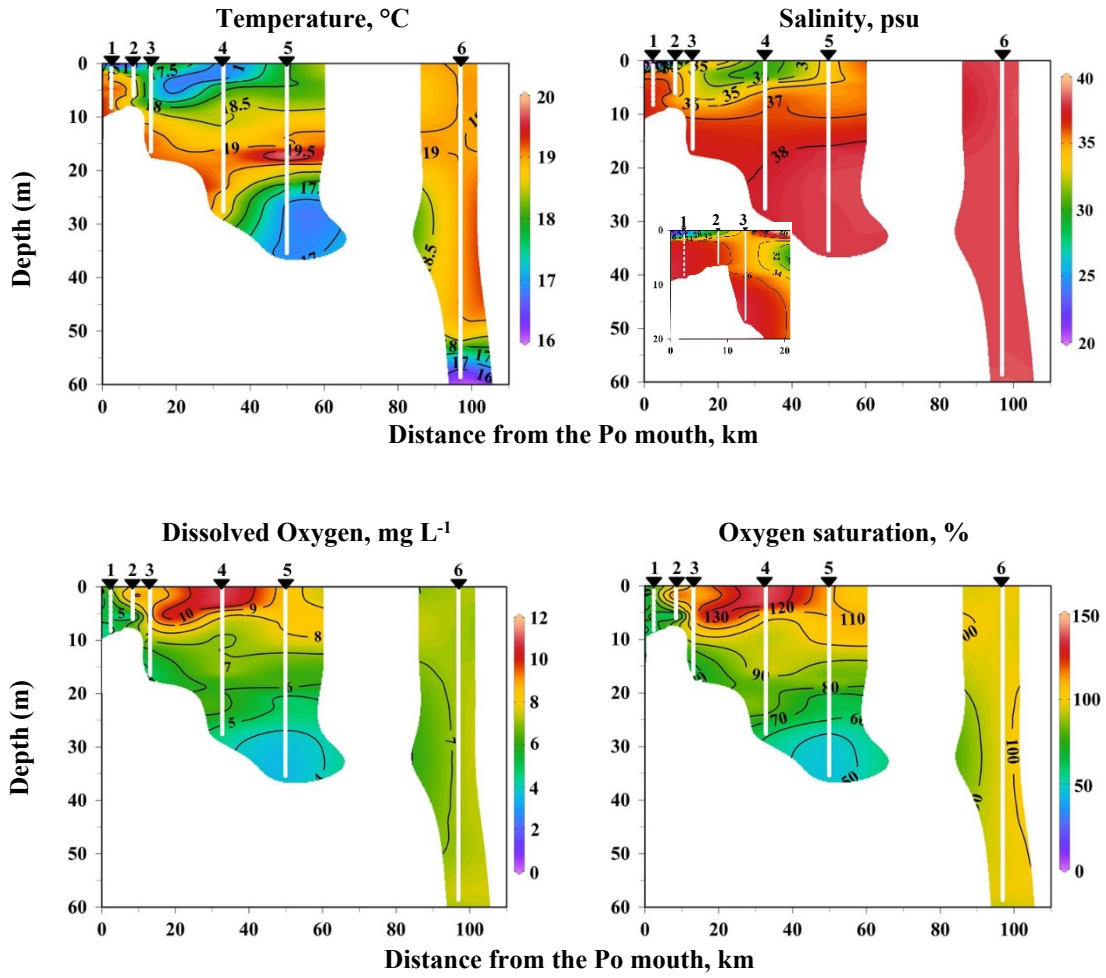


Fig. 2.

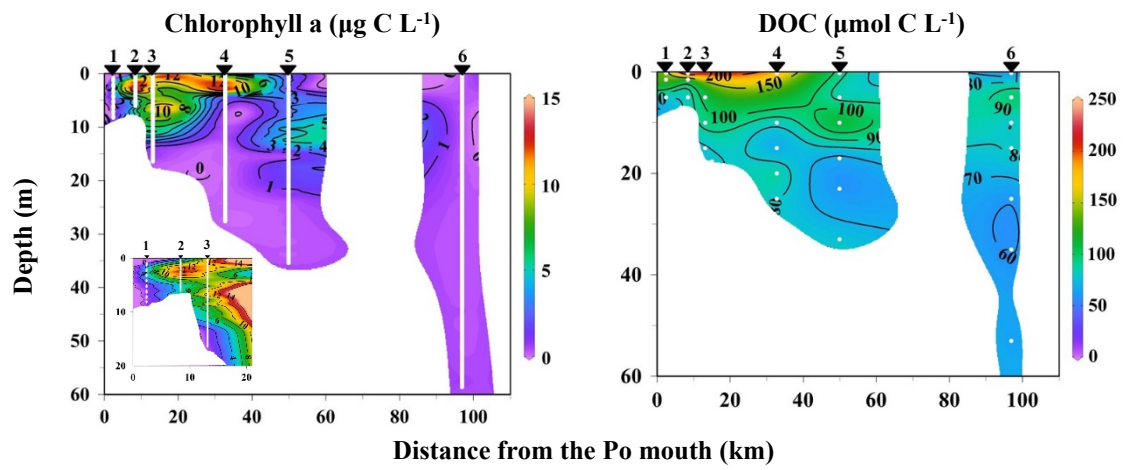


Fig. 3.

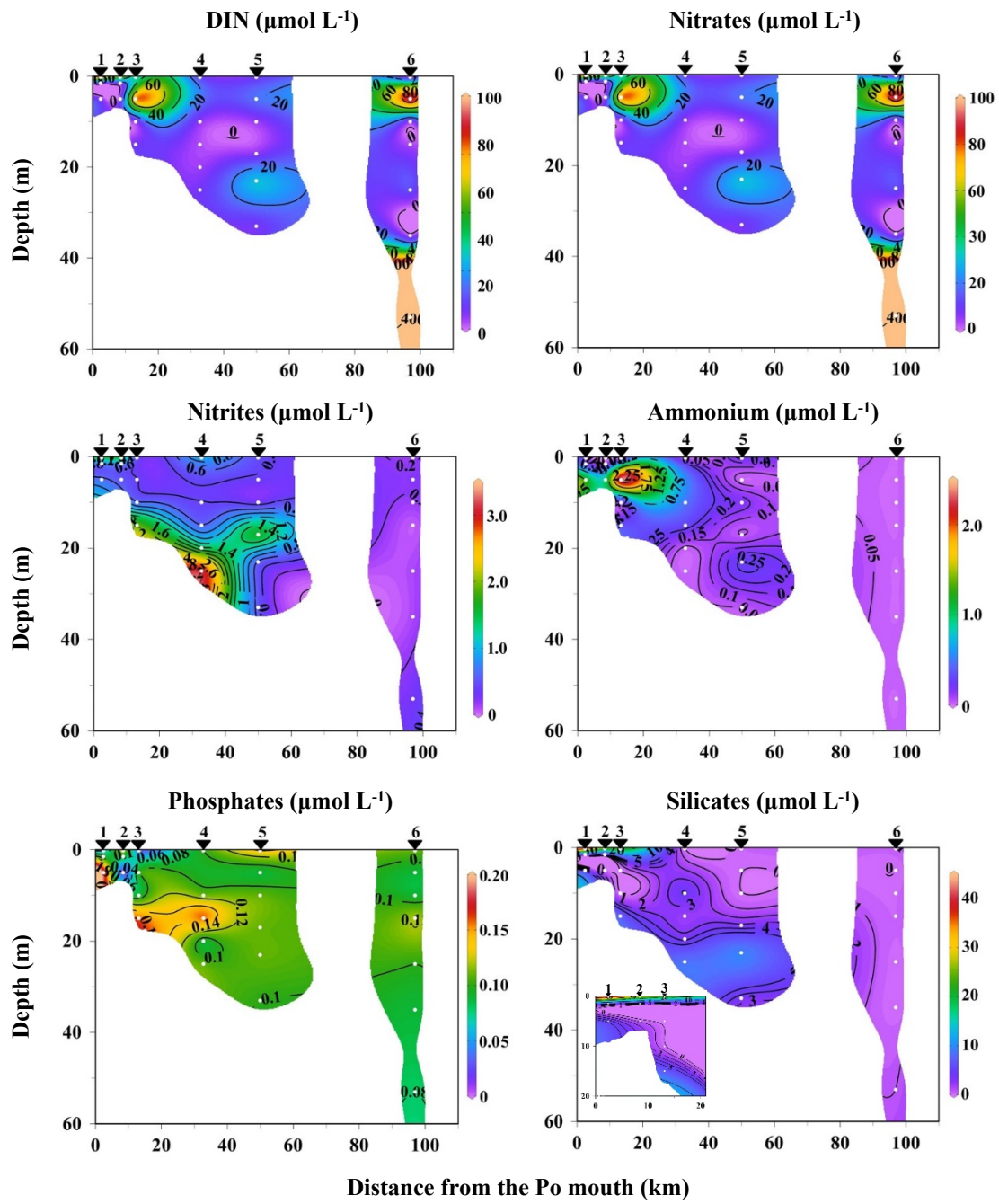


Fig. 4.



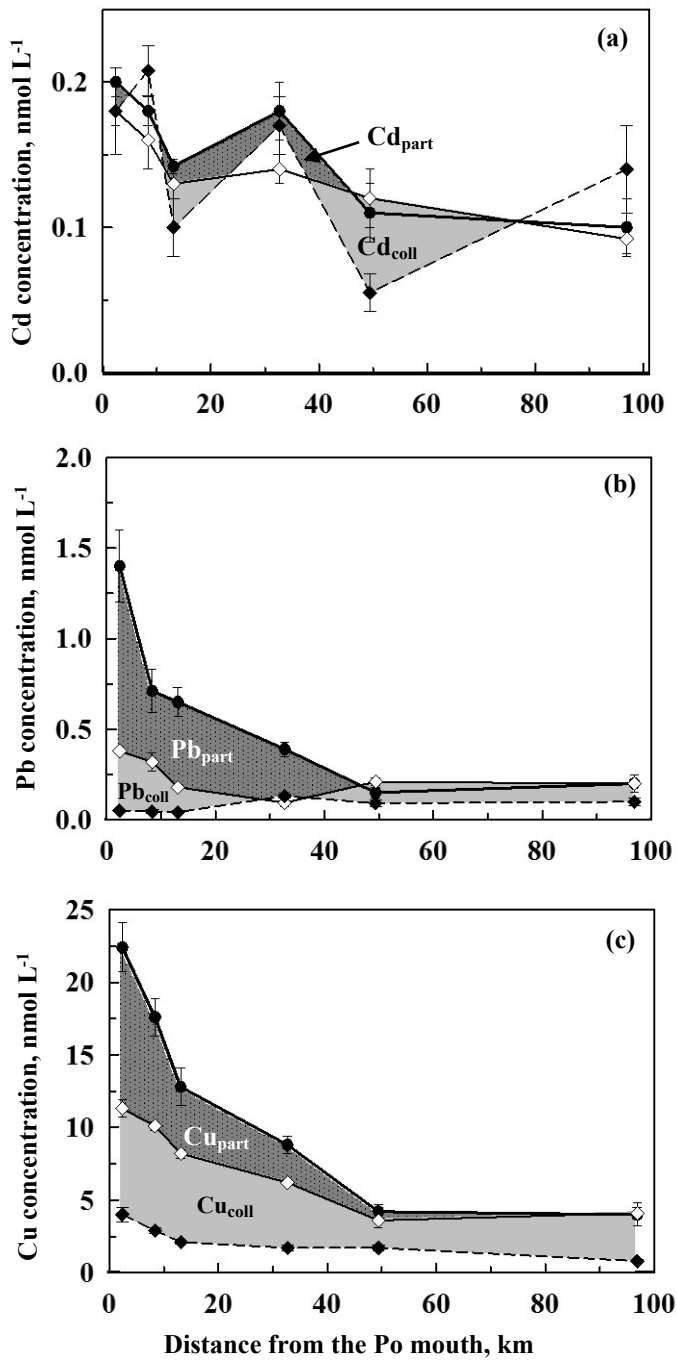


Fig. 5.

Cd concentration, nmol L<sup>-1</sup>

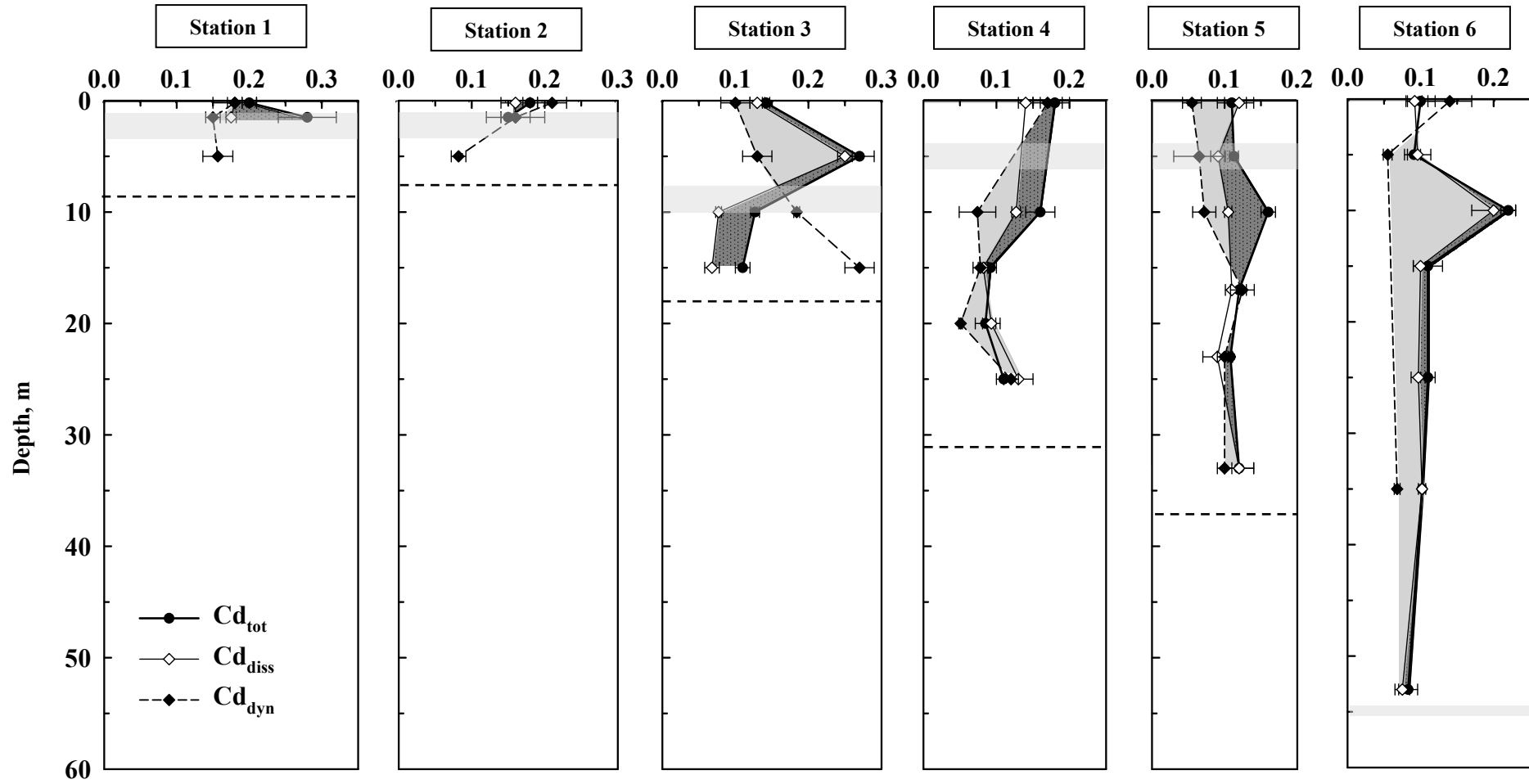
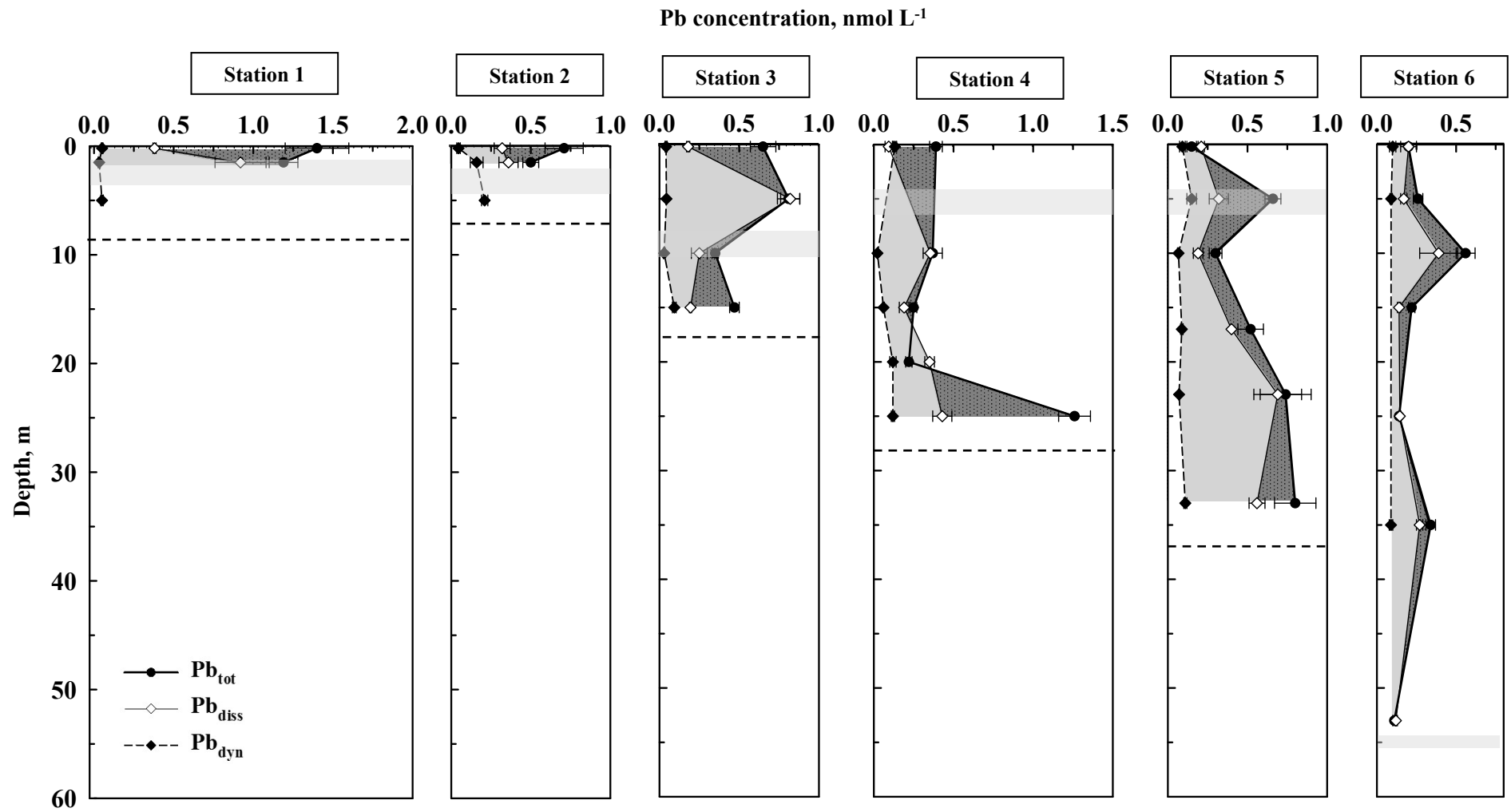


Fig. 6



**Fig. 7**

Cu concentration, nmol L<sup>-1</sup>

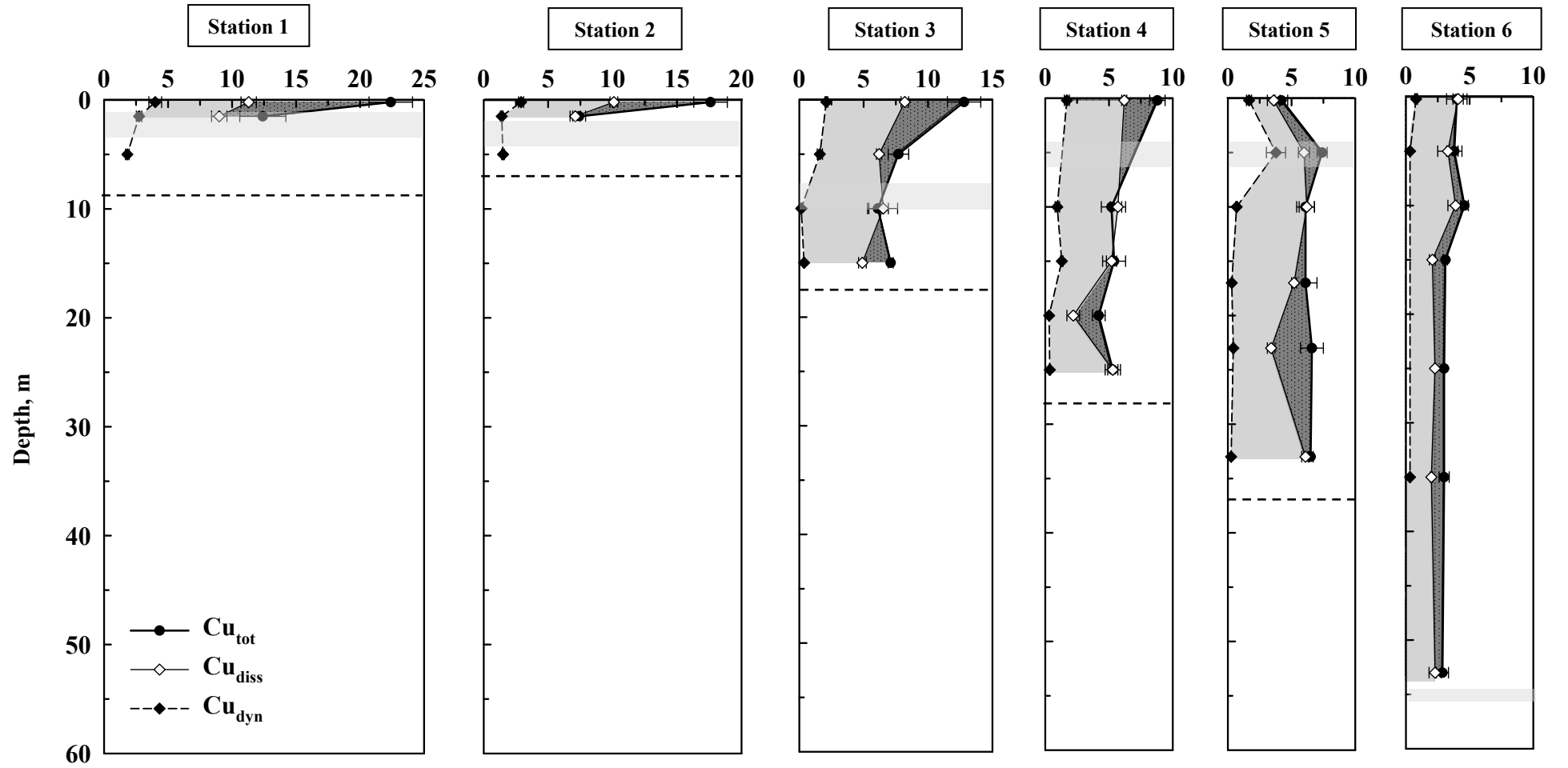


Fig. 8



# Supporting information for

## **In-situ trace metal speciation along the Po River plume (Northern Adriatic Sea) using submersible systems.**

Silvia Illuminati<sup>\*a</sup>, Anna Annibaldi<sup>a</sup>, Cristina Truzzi<sup>a</sup>, Mary-Lou Tercier-Waeber<sup>b</sup>, Stéphane Noël<sup>b</sup>, Jacques Buffle<sup>b</sup>, Charlotte B. Braungardt<sup>c</sup>, Eric P. Achterberg<sup>d</sup>, Kate A. Howell<sup>c,e</sup>, David Turner<sup>f</sup>, Mauro Marini<sup>g</sup>, Tiziana Romagnoli<sup>a</sup>, Cecilia Totti<sup>a</sup>, Fabio Confalonieri<sup>h</sup>, Flavio Graziottin<sup>h</sup>, Giuseppe Scarponi<sup>a</sup>

<sup>a</sup>*Department of Life and Environmental Sciences, Polytechnic University of Marche, Italy*

<sup>b</sup>*CABE, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland*

<sup>c</sup>*School of Earth, Ocean and Environmental, University of Plymouth, UK*

<sup>d</sup>*National Oceanography Centre, Southampton, School of Ocean and Earth Science, University of Southampton, UK*

<sup>e</sup>*Nanotecture Ltd. Epsilon House, Southampton Science Park, UK*

<sup>f</sup>*Department of Chemistry, University of Göteborg, Sweden*

<sup>g</sup>*Institute of Marine Science, National Research Council (CNR), Ancona, Italy*

<sup>h</sup>*Idronaut Srl, Brugherio (MI), Italy*

\*Corresponding Author

*e-mail address: s.illuminati@univpm.it*

## I. 2 Texts

Text S-1. Decontamination procedure

Text S-2. Intercomparison exercises

## II. 5 Tables

Table S1. Comparison of the results obtained by the different VIPs available during the cruise for the determination of the Cd, Pb and Cu dynamic fractions.

Table S2. Inter-comparison exercises for the determination of the total fractions and the free ion concentrations of Cd, Pb and Cu.

Table S3. Comparison between the Cd dynamic fraction measured by VIP system and the Cd HCl-extractable dissolved fraction measured by the conventional Metrohm instrumentation.

Table S4. Abundances and biomass of the main phytoplanktonic taxa along the Po river plume.

Table S5. Correlation matrix (Pearson's coefficients) among physical, biological and chemical variables.

## III. 1 Figures

Fig. S1. Contour plots of density, pH, and turbidity during the 2002-Adriatic Sea cruise.

## Text S-1. Decontamination procedure

The decontamination procedure used for polyethylene bottles, GO-FLO bottles, cellulose mixed ester filters, digester quartz vessels and all other plastic material has already been reported in Annibaldi et al. (2009), Illuminati et al. (2013) and Illuminati et al. (2015). Briefly, careful and prolonged acid washings were applied using, in sequence, detergent solution, 1:10 diluted analytical grade HNO<sub>3</sub>, 1:10 diluted superpure HCl and final storage in 1:1000 diluted superpure HCl until use. Similar acid washings were used for sampling equipment (GO-FLO bottle) and filtration apparatus. Membrane filters were cleaned by soaking twice in 1:10 diluted superpure HCl for one week and stored in 1:1000 diluted superpure HCl. Before used GO-FLO bottle, filtration apparatus and membrane filters were three-times washed with ultrapure water and in-situ conditioned with seawater before the contact with samples. As regards the digester quartz vessels, after the above decontamination procedure, these were repeatedly treated with digestion solution (10 mL of 2+1000 diluted ultrapure HCl plus 9 µL of ultrapure H<sub>2</sub>O<sub>2</sub>) and UV-irradiated for several 12-h cycles.

## Text S-2. Inter-comparison exercises

*Dynamic fractions.* Table S-1 reported the results on the metal dynamic fraction concentrations measured by the six voltammetric probes available on board (below reported as VIP-A from partner Ancona, VIP-B and VIP-C implemented on the MPCP of the Geneva partner; VIP-D and VIP-E from the partner Plymouth and VIP-F from the partner Göteborg). The dynamic inter-comparison here describe is complementary to that in Braungardt et al. (2009), who reported results only for the stations of the study transect closer to the Po river mouth.

Data showed that, excepting for the high values of Cd<sub>dyn</sub> measured by VIP-A on surface samples, probably due to contamination of the plastic containers in which samples are stored (note that the determination of the metal dynamic content in surface samples was carried out on board because of the inability of the VIPs to be used at depth below to 4 m), a general agreement can be observed between the results obtained for the in-situ measurements of the metal dynamic fractions carried out by all the voltammetric probes, when placed at the same depth.

*Total concentrations.* Table S-2 reported the results of the total metal concentration, the total HCl-extractable fraction and the *in-situ* total HCl-extractable fraction measured by the GIME-FIA system. Unfortunately, no values of the *in-situ* total acid extractable concentration measured by the GIME-FIA system are available for Stns. 5 and 6 of transect, due to pump rupture. The very few values of the in-situ Cd total HCl-extractable fraction (no GIME-FIA data available for Cd in surface samples) are significantly higher than both the total and the total HCl-extractable concentrations, measured by the conventional Metrohm instrumentations, and which showed similar values, within the experimental error. Concerning Pb, if we exclude the very high values obtained for the first two stations (probably resulting from sample contamination), the other two results available for Stn. 3 and 4 demonstrated that the GIME-FIA procedure allows to determine the “total” lead concentration. On the contrary, for Cu, it can be seen that the total extractable concentration obtained by the GIME-FIA system corresponded to the total acid extractable fraction (a few percent lower) and not to the actual total concentrations. It has to be noted that the GIME-FIA value amounts to about 70% of the total and apparently, this proportion tends to remain almost constant going toward the sea, as well as the acid extractable fraction that always represented ~75% of the total content. Details on the total HCl-extractable variations in the longitudinal profile were reported in Tercier et al. (2005).



*Free metal ion concentrations.* Data on the free metal ion concentration measured by the CGIME sensor and the HF-PLM technique are very few (Table S-2). The comparison between the two techniques is possible only for Cu. In fact, concerning Pb, only the values given by the HF-PLM are available, while for Cd no signals were obtained from both CGIME sensor and PLM system.  $\text{Cu}_{\text{free}}$  values obtained by the two techniques are very variable; generally, the HF-PLM results are systematically higher than CGIME (Tab. S-2).

**Tab. S1.** Comparison of the results obtained by the different VIPs available during the cruise for the determination of the Cd, Pb and Cu dynamic fractions. Means ( $\pm$  SD).

Metal	Stn.	Depth, m	Dynamic metal concentration, nmol L <sup>-1</sup> ( $\pm$ SD)						
			VIP A	VIP B	VIP C	VIP D	VIP E	VIP F	
Cd	1	0.2	0.54( $\pm$ 0.01)	0.16	0.19( $\pm$ 0.03)	0.34( $\pm$ 0.05)	–	–	
		1.5	0.14( $\pm$ 0.01)	0.21( $\pm$ 0.02)	0.16	–	–	–	
		5	0.16	0.18( $\pm$ 0.01)	0.15( $\pm$ 0.02)	–	0.14( $\pm$ 0.03)	–	
	2	0.2	0.21	0.22( $\pm$ 0.04)	0.20( $\pm$ 0.0)	–	–	0.21( $\pm$ 0.02)	
		1.5	0.15( $\pm$ 0.06)	0.23( $\pm$ 0.03)	0.17	–	–	–	
		5	0.08( $\pm$ 0.01)	0.23( $\pm$ 0.02)	0.085( $\pm$ 0.007)	0.08( $\pm$ 0.01)	–	–	
	3	0.2	0.40( $\pm$ 0.07)	0.10( $\pm$ 0.02)	0.10( $\pm$ 0.02)	–	0.25( $\pm$ 0.02)	–	
		5	–	0.12 ( $\pm$ 0.04)	0.13( $\pm$ 0.01)	0.12( $\pm$ 0.02)	0.17	–	
		10	0.184( $\pm$ 0.004)	–	–	–	–	–	
		15	–	0.26( $\pm$ 0.02)	0.28( $\pm$ 0.01)	–	–	–	
	4	0.2	0.17( $\pm$ 0.01)	0.17	0.17( $\pm$ 0.03)	–	–	–	
		10	0.07( $\pm$ 0.02)	–	–	–	–	–	
		15	0.08( $\pm$ 0.01)	–	–	–	–	–	
		20	–	0.053( $\pm$ 0.004)	0.05( $\pm$ 0.0)	–	–	–	
		25	–	0.12( $\pm$ 0.02)	0.13( $\pm$ 0.01)	–	–	–	
	5	0.2	0.43( $\pm$ 0.09)	0.06( $\pm$ 0.01)	0.14( $\pm$ 0.02)	–	–	–	
		4	–	–	–	0.06( $\pm$ 0.04)	0.29( $\pm$ 0.02)	–	
		10	0.07( $\pm$ 0.02)	–	–	–	–	–	
		18.6	–	0.145( $\pm$ 0.007)	0.12( $\pm$ 0.02)	–	0.125( $\pm$ 0.006)	–	
		23	0.10( $\pm$ 0.01)	–	–	–	–	–	
		33	–	0.11( $\pm$ 0.01)	0.10( $\pm$ 0.01)	–	–	–	
	6	0.2	0.14( $\pm$ 0.03)	0.055( $\pm$ 0.006)	–	–	–	–	
		5	–	–	–	–	–	–	
		10	–	–	–	–	–	–	
		15	–	–	–	–	–	–	
		25	0.068( $\pm$ 0.004)	–	–	–	–	–	
		35	–	–	–	–	–	–	
		53	–	–	–	–	–	–	
	Pb	1	0.2	0.051( $\pm$ 0.007)	0.23( $\pm$ 0.02)	0.16( $\pm$ 0.02)	–	–	0.051
			1.5	0.032( $\pm$ 0.009)	0.15( $\pm$ 0.02)	0.17( $\pm$ 0.02)	0.034( $\pm$ 0.007)	–	–
			5	0.054( $\pm$ 0.009)	0.11( $\pm$ 0.06)	0.14( $\pm$ 0.06)	0.045( $\pm$ 0.001)	0.12( $\pm$ 0.05)	–
		2	0.2	0.046( $\pm$ 0.011)	0.17( $\pm$ 0.03)	0.24( $\pm$ 0.06)	–	0.30( $\pm$ 0.14)	0.27( $\pm$ 0.07)
			1.5	0.87( $\pm$ 0.11)	0.155( $\pm$ 0.007)	0.183( $\pm$ 0.006)	–	–	–
5			0.21	0.22( $\pm$ 0.02)	0.185( $\pm$ 0.007)	0.022	0.24	–	
3		0.2	0.042( $\pm$ 0.007)	0.14( $\pm$ 0.01)	0.17( $\pm$ 0.03)	–	–	–	
		5	–	0.09( $\pm$ 0.02)	0.10( $\pm$ 0.03)	0.044( $\pm$ 0.002)	0.17( $\pm$ 0.06)	–	
		10	0.030( $\pm$ 0.006)	–	–	–	–	–	
		15	–	0.09( $\pm$ 0.01)	0.105( $\pm$ 0.007)	–	–	–	
4		0.2	0.13( $\pm$ 0.01)	0.13( $\pm$ 0.02)	0.14( $\pm$ 0.01)	–	–	–	
		10	0.024( $\pm$ 0.001)	–	–	–	–	–	
		15	0.061( $\pm$ 0.005)	–	–	–	–	–	
		20	–	0.14( $\pm$ 0.01)	0.11( $\pm$ 0.0)	–	–	–	
		25	–	0.12( $\pm$ 0.006)	0.12( $\pm$ 0.01)	–	–	–	
5		0.2	0.43( $\pm$ 0.09)	0.06( $\pm$ 0.01)	0.14( $\pm$ 0.02)	–	–	–	
		4	–	–	–	0.06( $\pm$ 0.04)	0.29( $\pm$ 0.02)	–	
		10	0.07( $\pm$ 0.02)	–	–	–	–	–	
		18.6	–	0.145( $\pm$ 0.007)	0.12( $\pm$ 0.02)	–	0.125( $\pm$ 0.006)	–	
		23	0.10( $\pm$ 0.01)	–	–	–	–	–	
		33	–	0.11( $\pm$ 0.01)	0.10( $\pm$ 0.01)	–	–	–	
6		0.2	0.14( $\pm$ 0.03)	0.06( $\pm$ 0.01)	0.14( $\pm$ 0.02)	–	–	–	
		5	–	0.055( $\pm$ 0.006)	–	–	–	–	
		10	–	–	–	–	–	–	
		15	–	–	–	–	–	–	
		25	0.068( $\pm$ 0.004)	–	–	–	–	–	
		35	–	–	–	–	–	–	
		53	–	–	–	–	–	–	

(to be continued)

(to be continued)

Metal	Stn.	Depth, m	Dynamic metal concentration, nmol L <sup>-1</sup> ( $\pm$ SD)					
			VIP A	VIP B	VIP C	VIP D	VIP E	VIP F
Cu	1	0.2	4.2( $\pm$ 0.20)	3.6( $\pm$ 0.0)	3.4( $\pm$ 0.07)	–	4.6( $\pm$ 0.07)	6.6( $\pm$ 0.92)
		1.5	2.5	2.7( $\pm$ 0.31)	2.7( $\pm$ 0.05)	3.0( $\pm$ 0.10)	–	–
		5	1.8( $\pm$ 0.04)	1.8( $\pm$ 0.18)	1.9( $\pm$ 0.15)	3.6( $\pm$ 0.18)	3.2( $\pm$ 0.83)	–
	2	0.2	2.6( $\pm$ 0.07)	3.1( $\pm$ 0.21)	2.9( $\pm$ 0.15)	–	4.2( $\pm$ 0.50)	7.0( $\pm$ 0.67)
		1.5	1.4( $\pm$ 0.19)	1.4( $\pm$ 0.10)	0.89( $\pm$ 0.06)	–	–	–
		5	0.68( $\pm$ 0.10)	1.5( $\pm$ 0.05)	–	2.7( $\pm$ 0.47)	–	–
	3	0.2	2.1( $\pm$ 0.18)	2.0( $\pm$ 0.12)	2.2( $\pm$ 0.05)	–	–	–
		5	–	0.88( $\pm$ 0.13)	1.6( $\pm$ 0.19)	2.6( $\pm$ 0.06)	1.6( $\pm$ 0.35)	–
		10	0.152( $\pm$ 0.002)	–	–	–	–	–
		15	–	0.40( $\pm$ 0.10)	0.36( $\pm$ 0.02)	–	–	–
	4	0.2	1.6( $\pm$ 0.10)	1.7( $\pm$ 0.21)	2.5( $\pm$ 0.10)	–	–	–
		10	0.95( $\pm$ 0.18)	–	–	–	–	–
		15	1.3( $\pm$ 0.12)	–	–	–	–	–
		20	–	0.34( $\pm$ 0.006)	0.28( $\pm$ 0.006)	–	–	–
		25	–	0.35( $\pm$ 0.05)	0.36( $\pm$ 0.06)	–	–	–
	5	0.2	1.8( $\pm$ 0.21)	1.5( $\pm$ 0.12)	1.6( $\pm$ 0.14)	–	–	–
		5	–	–	–	3.8( $\pm$ 0.75)	–	–
		10	0.69( $\pm$ 0.06)	–	–	3.8	–	–
		18.6	–	0.34( $\pm$ 0.0)	0.30( $\pm$ 0.04)	3.0( $\pm$ 0.68)	–	–
		23	0.43( $\pm$ 0.05)	–	–	–	–	–
		33	–	0.26( $\pm$ 0.02)	0.25( $\pm$ 0.01)	–	–	–
6	0.2	0.80( $\pm$ 0.11)	–	–	–	–	–	
	5	–	0.330( $\pm$ 0.008)	–	–	–	–	
	10	–	–	3.0( $\pm$ 0.38)	–	–	–	
	15	–	–	–	–	–	–	
	25	0.32( $\pm$ 0.04)	–	–	–	–	01.4( $\pm$ 0.11)	
	35	–	–	–	–	–	–	
	53	–	–	–	–	–	–	

**Tab. S2.** Inter-comparison exercises for the determination of the total fractions and of the free ion concentrations of Cd, Pb and Cu. Means ( $\pm$  SD).

Metal	Stn.	Depth, m	Total metal fractions, nmol L <sup>-1</sup>			Free metal ion conc, nmol L <sup>-1</sup>	
			GIME-FIA system	Total	HCl-extractable	CGIME sensor	HF-PLM technique
Cd	1	0.2	–	0.20( $\pm$ 0.01)	0.18( $\pm$ 0.02)	–	–
		1.5	–	0.28( $\pm$ 0.04)	0.27	–	–
		5	0.23	–	–	–	–
	2	0.2	–	0.18( $\pm$ 0.01)	0.14( $\pm$ 0.03)	–	–
		1.5	0.20( $\pm$ 0.05)	0.15( $\pm$ 0.01)	0.15( $\pm$ 0.01)	–	–
		5	0.33( $\pm$ 0.04)	–	–	–	–
	3	0.2	–	0.142( $\pm$ 0.005)	0.13( $\pm$ 0.01)	–	–
		5	–	0.27( $\pm$ 0.02)	0.25( $\pm$ 0.01)	–	–
		10	–	0.13( $\pm$ 0.006)	–	–	–
		15	0.40	0.11( $\pm$ 0.01)	0.09( $\pm$ 0.01)	–	–
	4	0.2	–	0.18( $\pm$ 0.02)	0.13( $\pm$ 0.03)	–	–
		10	–	0.16( $\pm$ 0.02)	–	–	–
		15	–	0.092( $\pm$ 0.008)	–	–	–
		20	0.135( $\pm$ 0.007)	0.08( $\pm$ 0.01)	0.08( $\pm$ 0.02)	–	–
		25	0.29( $\pm$ 0.08)	0.11( $\pm$ 0.01)	0.12( $\pm$ 0.01)	–	–
	5	0.2	–	0.11( $\pm$ 0.02)	0.10( $\pm$ 0.02)	–	–
		5	–	0.113( $\pm$ 0.006)	–	–	–
		10	–	0.16( $\pm$ 0.01)	–	–	–
		18.6	0.26( $\pm$ 0.02)	0.12( $\pm$ 0.01)	–	–	–
		23	–	0.108( $\pm$ 0.003)	–	–	–
		33	–	0.12( $\pm$ 0.02)	–	–	–
	6	0.2	–	0.10( $\pm$ 0.02)	0.084( $\pm$ 0.02)	–	–
		5	–	0.09( $\pm$ 0.01)	–	–	–
		10	–	0.22( $\pm$ 0.01)	–	–	–
15		–	0.11( $\pm$ 0.02)	–	–	–	
25		–	0.11( $\pm$ 0.01)	–	–	–	
53		–	0.102( $\pm$ 0.001)	–	–	–	
Pb	1	0.2	2.0( $\pm$ 0.1)	1.4( $\pm$ 0.2)	1.3( $\pm$ 0.09)	–	–
		1.5	1.4( $\pm$ 0.2)	1.2( $\pm$ 0.09)	0.96	–	0.002
		5	0.94( $\pm$ 0.06)	–	–	–	0.016
	2	0.2	2.5( $\pm$ 0.3)	0.71( $\pm$ 0.12)	0.70( $\pm$ 0.03)	–	0.009
		1.5	2.8( $\pm$ 0.2)	0.50( $\pm$ 0.05)	0.31( $\pm$ 0.05)	–	–
		5	0.84( $\pm$ 0.04)	–	–	–	–
	3	0.2	0.66 ( $\pm$ 0.08)	0.65( $\pm$ 0.08)	0.54( $\pm$ 0.05)	–	–
		5	0.70( $\pm$ 0.03)	0.81( $\pm$ 0.07)	0.28( $\pm$ 0.04)	–	0.014
		10	–	0.35( $\pm$ 0.02)	–	–	–
		15	0.80( $\pm$ 0.03)	0.47( $\pm$ 0.03)	0.38( $\pm$ 0.03)	–	0.005
	4	0.2	0.34( $\pm$ 0.04)	0.39( $\pm$ 0.04)	0.33( $\pm$ 0.07)	–	0.010
		10	–	0.37( $\pm$ 0.06)	–	–	–
		15	–	0.25( $\pm$ 0.02)	–	–	–
		20	0.56( $\pm$ 0.04)	0.22( $\pm$ 0.02)	0.14( $\pm$ 0.02)	–	0.011
		25	0.60( $\pm$ 0.04)	1.3( $\pm$ 0.1)	1.0( $\pm$ 0.07)	–	–
	5	0.2	–	0.15( $\pm$ 0.03)	0.16( $\pm$ 0.05)	–	0.009
		4	–	0.66( $\pm$ 0.05)	–	–	0.016
		10	–	0.30( $\pm$ 0.04)	–	–	0.002
		18.6	0.42( $\pm$ 0.11)	0.52( $\pm$ 0.08)	–	–	0.001
		23	–	0.74( $\pm$ 0.16)	–	–	0.001
		33	–	0.80( $\pm$ 0.13)	–	–	–

(to be continued)

(continuing)

Metal	Stn.	Depth, m	Total metal fractions, nmol L <sup>-1</sup>			Free metal ion conc, nmol L <sup>-1</sup>	
			GIME-FIA System	Total	HCl- extractable	CGIME sensor	HF-PLM technique
Pb	6	0.2	–	0.20(±0.05)	0.19(±0.05)	–	–
		5	–	0.26(±0.03)	–	–	–
		10	–	0.56(±0.06)	–	–	–
		15	–	0.22(±0.02)	–	–	0.001
		25	–	0.14(±0.0)	–	–	–
		35	–	0.34(±0.03)	–	–	–
		53	–	0.11(±0.01)	–	–	–
Cu	1	0.2	16.3(±0.2)	22.4(±1.7)	16.6(±1.7)	0.62	0.25
		1.5	6.4(±0.1)	12.4(±1.8)	33.2	0.26	0.29
		5	–	–	–	–	–
	2	0.2	11.4(±0.6)	17.6(±1.3)	12.4(±1.7)	0.06	0.17
		1.5	7.6(±0.5)	7.4(±0.5)	7.2(±0.6)	–	–
		5	6.3(±0.09)	–	–	–	–
	3	0.2	8.5(±1.9)	12.5(±1.3)	9.8(±2.4)	–	–
		5	3.3(±0.4)	7.7(±0.8)	7.4(±0.4)	–	0.13
		10	–	–	–	–	–
		15	3.0(±0.2)	7.1(±0.2)	4.5(±0.4)	0.027	0.31
	4	0.2	6.4(±0.04)	8.8(±0.6)	8.1(±0.3)	–	–
		10	–	5.2(±0.8)	–	–	–
		15	–	5.4(±0.9)	–	–	–
		20	1.3(±0.2)	4.2(±0.5)	2.7(±0.2)	0.12	0.18
		25	1.5(±0.1)	5.3(±0.4)	5.7(±0.4)	–	–
	5	0.2	–	4.2(±0.5)	3.4(±0.6)	–	0.24
		4	–	7.4(±0.4)	–	–	0.22
		10	–	6.1(±0.7)	–	–	0.27
		18.6	1.2(±0.01)	6.1(±0.9)	–	–	0.37
		23	–	6.6(±0.9)	–	–	0.30
		33	–	6.5(±0.2)	–	–	–
	6	0.2	–	4.0(±0.9)	2.8(±0.2)	–	–
		5	–	3.8(±0.6)	–	–	–
10		–	4.6(±0.3)	–	–	–	
15		–	3.1(±0.2)	–	–	0.41	
25		–	3.0(±0.06)	–	–	–	
35		–	3.0(±0.4)	–	–	–	
53		–	2.9(±0.5)	–	0.14	–	

**Tab. S3.** Comparison between the Cd dynamic fraction measured by VIP system and the Cd HCl-extractable dissolved fraction measured by the conventional Metrohm instrumentation. Only surface values. Means ( $\pm$  SD). In bold high Cd dynamic values due to contamination problems.

Stations	Cd concentration, nmol L <sup>-1</sup> ( $\pm$ SD)	
	dynamic fraction	HCl-extractable dissolved fraction
1	0.18( $\pm$ 0.03)	0.18( $\pm$ 0.01)
2	<b>0.21(<math>\pm</math>0.02)</b>	0.14( $\pm$ 0.03)
3	0.10( $\pm$ 0.02)	0.13( $\pm$ 0.04)
4	<b>0.17(<math>\pm</math>0.02)</b>	0.11( $\pm$ 0.03)
5	0.055( $\pm$ 0.013)	0.095( $\pm$ 0.041)
6	<b>0.14(<math>\pm</math>0.04)</b>	0.079( $\pm$ 0.012)

**Tab. S4.** Abundances ( $\times 10^6$  cells L<sup>-1</sup>) and biomass ( $\mu\text{g C L}^{-1}$ ) of the main phytoplanktonic taxa along the Po river plume during the October-November 2002 field campaign.. Data are reported as means  $\pm$  10% SD for all the phytoplankton taxa.

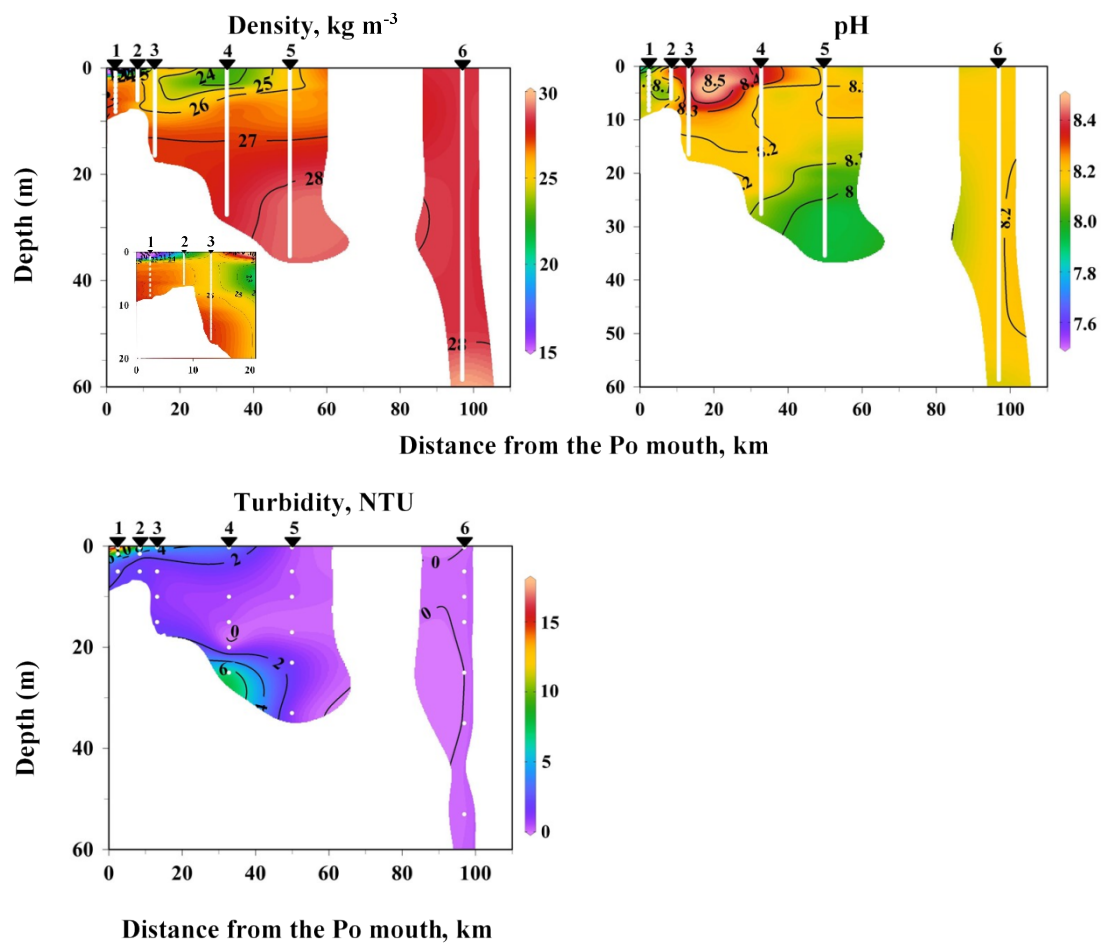
Sampling station and depth (m)	Diatoms		Dinoflagellates		Phytoflagellates		Coccolithophorids		Total	
	Abundance 10 <sup>6</sup> cells L <sup>-1</sup>	Biomass $\mu\text{g C L}^{-1}$	Abundance 10 <sup>4</sup> cells L <sup>-1</sup>	Biomass $\mu\text{g C L}^{-1}$	Abundance 10 <sup>6</sup> cells L <sup>-1</sup>	Biomass $\mu\text{g C L}^{-1}$	Abundance 10 <sup>4</sup> cells L <sup>-1</sup>	Biomass $\mu\text{g C L}^{-1}$	Abundance 10 <sup>6</sup> cells L <sup>-1</sup>	Biomass $\mu\text{g C L}^{-1}$
Stn. 1										
0.2	4.0	402	0.088	2.5	3.0	10	0	0	6.9	415
1.5	4.6	378	0.080	9.7	2.8	14	0.04	0.028	8.3	402
5	0.85	42	0.029	0.25	2.1	7.3	0	0	3.0	50
Stn. 2										
0.2	3.7	335	2.6	0.65	2.3	7.9	0	0	6.0	343
1.5	4.8	367	1.8	0.87	1.7	5.9	0.010	0.016	6.6	374
5	4.7	353	0.020	0.20	1.8	6.3	0.88	0.23	6.5	360
Stn. 3										
0.2	2.4	219	0.14	3.6	2.1	9.1	0.010	0.006	4.5	232
5	2.9	296	5.6	26	1.4	6.0	2.8	1.7	4.4	329
10	2.5	240	3.2	19	0.67	2.8	0.12	0.42	3.2	263
15	0.36	44	0.90	0.40	1.5	5.4	0.90	0.58	1.9	51
Stn. 4										
0.2	4.5	396	13	20	1.6	7.0	0.059	0.25	6.2	422
10	0.10	7.6	0.74	0.99	0.69	2.6	0.036	0.070	0.80	11
15	0.031	3.4	0.33	0.20	0.64	2.3	0.033	0.022	0.67	5.8
20	0.018	3.3	2.0	2.7	0.59	2.0	0.085	0.092	0.63	8.1
25	0.014	2.6	0.45	0.094	1.5	5.3	0.010	0.014	1.5	8.0
Stn. 5										
0.2	1.3	126	1.2	4.6	0.62	2.9	0.78	4.0	2.0	138
5	1.0	87	2.3	8.3	0.52	2.7	1.2	2.0	1.6	100
10	1.0	124	3.6	5.5	0.45	2.1	1.2	0.69	1.5	132
17	0.86	90	1.7	2.8	0.49	1.7	0.31	0.092	1.4	94
23	0.27	25	1.1	0.41	0.55	2.0	0.048	0.038	0.83	27
33	0.73	72	1.7	3.6	0.60	2.7	0.59	0.19	1.4	79
Stn. 6										
0.2	0.038	6.9	0.71	0.83	0.45	1.8	0.49	0.26	0.49	9.7
5	0.35	34	3.8	7.5	0.25	1.4	0.64	0.75	0.64	44
10	0.045	12	2.0	2.1	0.47	1.6	0.54	1.3	0.54	16
15	0.064	9.8	0.57	1.1	0.37	1.5	0.46	1.8	0.46	13
25	0.024	4.8	1.6	1.3	0.39	1.8	0.46	2.4	0.46	8.4
35	0.029	6.9	0.93	2.3	0.31	1.2	0.36	1.8	0.36	11
53	0.043	4.5	1.1	1.2	0.28	1.0	0.34	0.92	0.34	6.9











**Fig. S1.** Contour plots of density, pH, redox potential, and turbidity during the 2002-Adriatic Sea cruise. The white dots represent the sampling points. The transect position is plotted in Fig. 1.

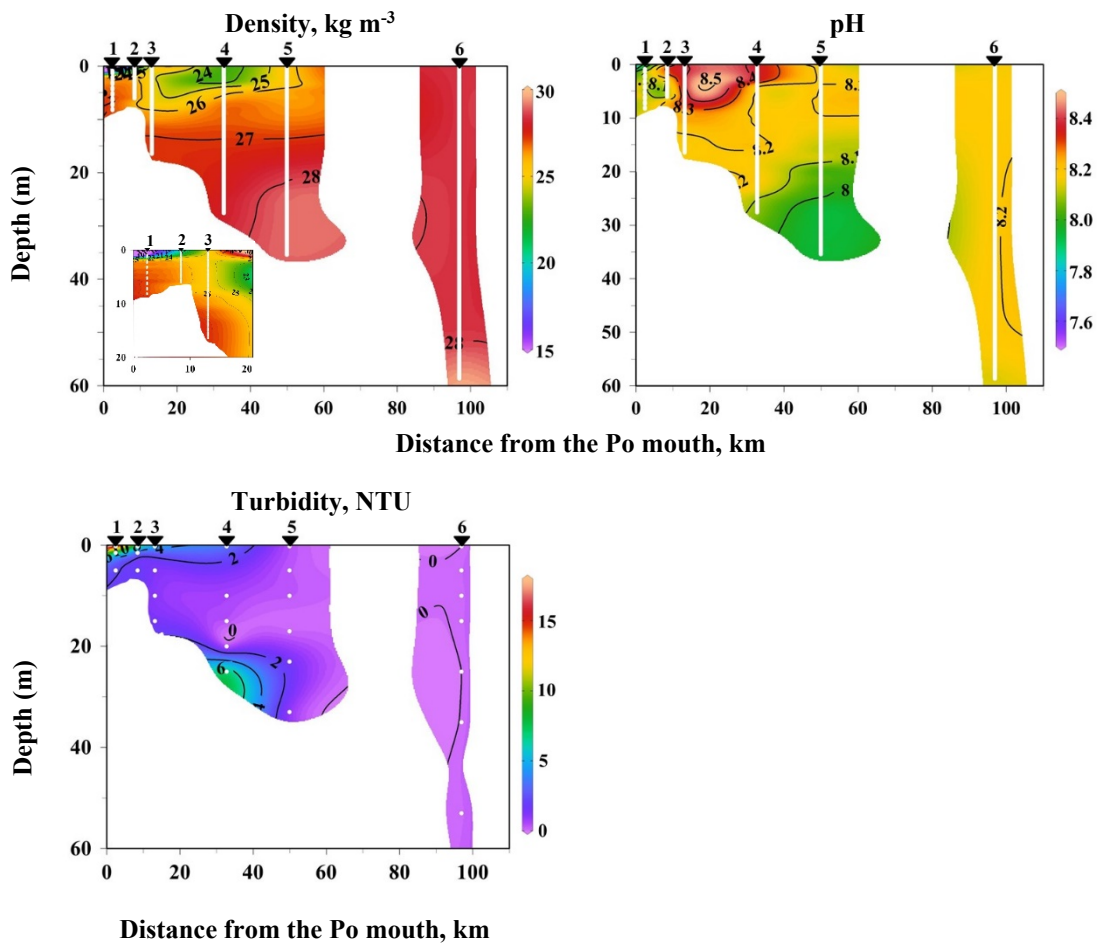


Fig. S1.