

## Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: the Krka River estuary (Adriatic, Croatia)

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### Abstract

Distributions of trace metals (TM), organic carbon, SPM and physico-chemical parameters were studied in the highly stratified Krka River estuary in winter/summer periods. The non-conservative behaviour of Zn, Cd, Pb and Cu in the brackish layer (plume), easily spotted due to very low inputs by the river, was mainly caused by their inputs from the pleasure boats, nautical marinas and harbour (e.g. release from antifouling paints). Contrarily, Ni and Co followed near-conservative behaviour. The extremely low SPM discharged by the river, resulted in a predominant dissolved fraction (>80%) of all TM, except Pb. Vertical scavenging, coupled with the long residence time, caused accumulation and progressive upstream increase of TM and SPM in the bottom seawater. Decrease of distribution coefficient ( $K_D$ ) in the brackish layer for winter period was ascribed to the change of SPM nature (terrestrial vs. biogenic), whereas a variable and increased biogenic component of SPM caused scattered  $K_D$ s in summer.

**Keywords:** stratified estuary; trace metals; organic carbon; distribution coefficient; estuarine mixing; Krka River estuary

### Highlights

- Pristine Krka levels of TM strongly influenced by anthropogenic inputs in estuary
- Landward increase of accumulated SPM and scavenged TM in bottom seawater layer
- Low river SPM, DOC and POC highlight summer biogenic organic carbon production
- TM behaviour in surface salinity gradient controlled by terrigenous vs. biogenic SPM
- Anoxia in area of organic rich SPM accumulation affects the levels and fate of TM

## 1. Introduction

Depending on their concentration levels and chemical speciation, some trace metals may act as micronutrients or toxic pollutants in aquatic ecosystems ([Donat and Dryden, 2001](#); [Sunda et al., 1987](#); [Tessier and Turner, 1996](#)). They could be released in the environment due to land weathering, aerosols, municipal and industrial wastes and also by maritime traffic. Coastal closed aquatic systems, especially those with reduced water exchange (e.g. microtidal estuaries) are especially vulnerable to those inputs. Aside of the hydrodynamic patterns, common processes for all estuaries which are considered in order to understand and explain the spatio-temporal distributions of trace metals primarily encompass adsorption/desorption, flocculation, biological uptake, sediment resuspension, as well as organic/inorganic complexation alongside with the competitive effects of major cations/anions ([Abril et al., 2002](#); [Cobelo-Garcia and Prego, 2004](#); [Dassenakis et al., 1995](#); [Elbaz-Poulichet et al., 1996](#); [Hatje et al., 2003b](#); [Louis et al., 2009](#); [Oursel et al., 2013](#); [Rigaud et al., 2013](#); [Waeles et al., 2009](#)).

The trace metal distributions and behaviour within the estuarine mixing zone were basically examined and interpreted in relation to salinity, i.e. if they behave conservatively or not. Pb is known as particles-reactive metal, and thus its reactivity is strongly dependent on the particles origin and concentration. Mn and Fe oxyhydroxides were found as most reactive particles towards Pb ([Elbaz-Poulichet et al., 1984](#); [Waeles et al., 2008b](#)). While a rapid release of dissolved Pb from suspended particulate matter (SPM) may occur upon contact with the salt water, a reverse process may occur with the time controlled by the kinetics of exchange with the major ions. Thus, the time of the filtration after the sample is taken, is found to be of great importance in interpretation of the dissolved/particulate partitioning of several trace metals ([Oursel et al., 2013](#)). Basically, conservative ([Dai et al., 1995](#); [Dai and Martin, 1995](#); [Elbaz-Poulichet et al., 1996](#)) and non-conservative ([Fu et al., 2013](#); [Waeles et al., 2007](#)) behaviour with declining trend was recorded for Pb in different estuaries. Behaviour of Cd in mixing zone is characterised by its release due to the competitive effect of chloride, i.e. formation of stable Cd chloro-complexes, causing consequently a non-conservative behaviour ([Comans and Vandijk, 1988](#); [Elbaz-Poulichet et al., 1996](#); [Elbaz-Poulichet et al., 1987](#); [Fu et al., 2013](#); [Oursel et al., 2013](#); [Waeles et al., 2009](#); [Waeles et al., 2005](#)). Cu in estuaries is well documented in the literature and generally exhibits contrasting behaviours, with no specific pattern. Formation of strong organic complexes is known to control Cu speciation and distribution in natural waters ([Bruland and Lohan, 2004](#); [Louis et al., 2009](#); [Waeles et al., 2008b](#)), and thus the concentration and the type of organic matter may be of great importance in the mixing zone in regulation of dissolved/particulate Cu fractionation.

Most of the studies performed in estuarine systems are primarily oriented toward understanding of trace metal distribution in relation to their partitioning between dissolved and particulate phase (fractionation), which aside of the chemical composition (e.g. salinity) is driven largely by the particles content and their nature ([Cobelo-Garcia et al., 2004](#); [Deycard et al., 2014](#); [Duarte et al., 2014](#); [Elbaz-Poulichet et al., 1996](#); [Hatje et al., 2003a](#); [Koshikawa et al., 2007](#); [Martino et al., 2002](#); [Ollivier et al., 2011](#); [Oursel et al., 2014](#); [Petit et al., 2013](#); [Robert et al., 2004](#); [Waeles et al., 2007](#)). Dissolved-particulate fractionation in natural waters is usually described and explained by the particle-water distribution coefficients ( $K_D$ ):

$$K_D = \frac{c_{part}}{SPM \times c_{diss}} (L\ kg^{-1}),$$

where  $c_{part}$  and  $c_{diss}$  are metal concentrations in particulate and dissolved form, respectively, while  $SPM$  is concentration of suspended material. As  $K_D$  depends on many factors (e.g. solution and particle composition, speciation, dissolved organic carbon (DOC) and SPM concentration, pH, temperature, salinity), a large variation could be expected in systems which undergo different physico-chemical changes, implying that the justification of  $K_D$  variation should be taken with caution ([Bourg, 1987](#); [Cobelo-Garcia et al., 2004](#); [Comans and Vandijk, 1988](#); [Elbaz-Poulichet et al., 1996](#); [Fu et al., 2013](#); [Hatje et al., 2003b](#); [Turner, 1996](#)) and thus the direct comparison of results within different studies is not always straightforward.

Characteristics of Mediterranean estuaries are weak tidal amplitude and almost negligible tidal currents. In a micro tidal type of an estuary, river energy is higher than energy produced by the sea, inducing a permanent vertical stratification. In this work behaviour and fate of trace metals were studied in such a type of an estuary, in the Krka River estuary located on the eastern coast of the Adriatic Sea. This estuary is characterized by three well-separate vertically stratified layers: an upper brackish layer, a thin freshwater–seawater interface (FSI) and a bottom seawater layer. Between the surface fresh/brackish and the underlying seawater layers, which flows in opposite directions, a velocity shear is formed which induces progressive salinity increase (and transport of elements) of the overlaying brackish layer and the movement of the upper edge of seawater toward the sea ([Legovic, 1991](#)). Note that, this hydrodynamic transport model was principally considered in explaining the distributions of measured parameters along the vertical and horizontal estuarine transect in this work. Many different studies in the Krka River estuary were performed in late 80's during which estuary fundamental physical and chemical properties were characterized (special issue of Marine Chemistry, 1991, volume 32) ([Cauwet, 1991](#); [Elbaz-Poulichet et al., 1991](#); [Legovic, 1991](#); [Legovic et al., 1991b](#); [Sempere and Cauwet, 1995](#); [Zutic and Legovic, 1987](#)). The most interesting feature of this estuary is a

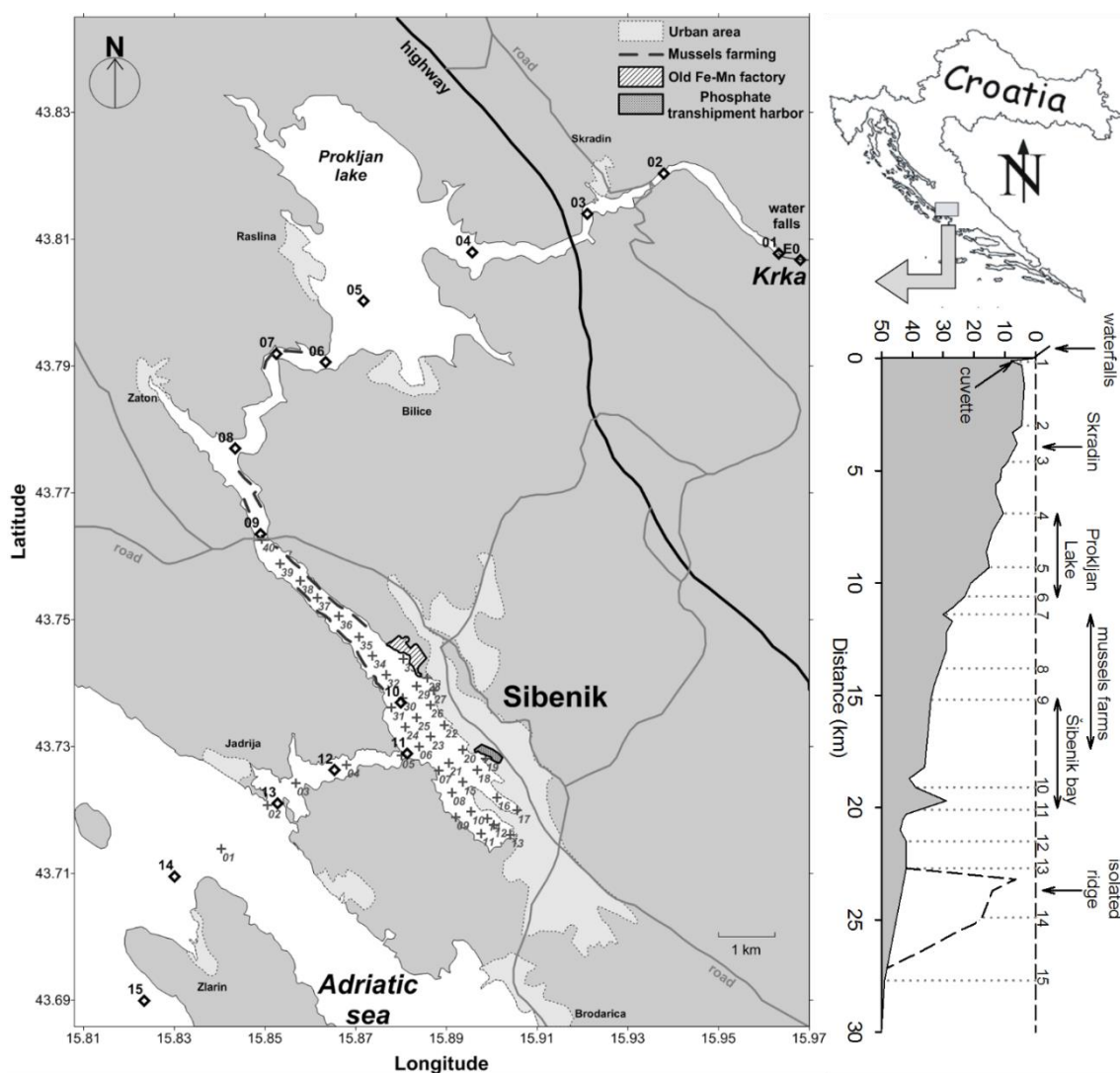
boundary FSI layer in which an enrichment of the organic matter and trace elements was found as a results of the specific physical and chemical properties ([Bilinski et al., 2000](#); [Mantoura, 1987](#); [Zutic and Legovic, 1987](#)). First studies of trace metals ([Elbaz-Poulichet et al., 1991](#)), organic matter and SPM ([Cauwet, 1991](#)) and biological activities ([Denant et al., 1991](#); [Grzetic et al., 1991](#); [Legovic et al., 1991a](#); [Legovic et al., 1991b](#); [Legovic et al., 1991c](#); [Legovic et al., 1994](#)) revealing the low pollution within the estuary were partially confirmed by more recent studies ([Bilinski et al., 1992](#); [Bilinski et al., 2000](#); [Cetinic et al., 2006](#); [Louis et al., 2009](#); [Omanović et al., 2006](#); [Supraha et al., 2014](#)). An anthropogenic influence noticed in the estuary was mainly related to the increased nautical tourism in ([Louis et al., 2009](#); [Omanović et al., 2006](#)).

Emphasizing mentioned specific features of the Krka river estuary, in the present study we focused on (1) the determination of spatial distributions of environmentally relevant trace metals (Zn, Cd, Pb, Cu, Ni and Co), organic carbon, SPM, as well as main physico-chemical parameters under summer and winter conditions, (2) identification of main sources of anthropogenic inputs (contamination state) and, (3) elucidation of processes regulating behaviour and fate of trace metals in different layers of this highly stratified estuary. Of particular interest was to examine an evolution of the trace metal “pollution” in the Krka river and its estuary since the previous studies conducted in 1980’s in relation to several key factors: the displacement of the sewage outfall of Šibenik, the increase of nautical tourism/traffic, the ban of Pb as an gasoline additive, the ban of Tributyltin-Tin (TBT) as active biocide compound and its replacement by Cu.

## 2. Study site particularities

The karstic Krka River, mainly encompassing the National Park Krka, is situated on the eastern coast of the Adriatic Sea (Croatia). The measured flow over the last 50 years is between 5 and 450 m<sup>3</sup> s<sup>-1</sup> with average annual flow between 40 and 60 m<sup>3</sup> s<sup>-1</sup> (Fig SI-1 provides information for period of 2001-2013). The river is characterized by numerous lakes formed by tufa barriers, each finishing with waterfalls. After the last and the largest waterfall (Skradinski buk) starts 23 km long the Krka River estuary. Few small villages are located along the foremost part of the estuary shoreline with approximately 4000 permanent inhabitants. Majority of the population is living in the lower part of the estuary in the Šibenik city (19<sup>th</sup> km) and surrounding area (around 35000 permanent inhabitants). However, in a summer season, total number of persons increases several times due to the touristic load, mainly because the Krka river estuary is a very attractive nautical destination. Thus, along with the permanent harbour activities in the Šibenik

town, the nautical tourism (more than 1000 berths) is considered as a serious periodic (seasonal) anthropogenic treat for the estuary ecosystem. Although the National Park Krka with over 700 000 visits during the year undergo high touristic pressure, it is not considered as a potential source of pollution for the estuary, because the activities and waste water management within the Park are strictly controlled and processed. For a long period the waste waters from Šibenik town were discharged directly into the estuary. Since 2007, the treated waste waters were discharged to the open sea about 5 km from the coast at the depth of 60 m. Numerous aquaculture plants (~20) for mussels farming (*mytilus galloprovincialis*) are mainly located in the lower part of the estuary (Fig. 1). A heavy industry along the estuary is not developed at a current time. The main treat for years was the factory of electrodes and ferroalloys close to the Šibenik town (Fig. 1). That zone was rehabilitated after production stopped in 1995.



**Figure 1.** Map of the Krka River estuary with marked transect (open diamonds) and high-resolution mapping (crosses) sampling sites. Right plot shows horizontal bottom depth transect with marked sites and specific regions.

According to Legović (1991) ([Legovic, 1991](#)), exchange time of both freshwater and seawater is mainly dependent on the Krka River inflow: for freshwater during winter it is between 6 and 20 days, and during summer up to 80 days, whereas for seawater it is between 50 to 100 days in winter and up to 250 days in summer.

### 3. Materials and method

#### 3.1. Sampling strategy and sampling

Two field scheme were surveyed: the first along the whole estuary transect, involving two contrasting periods of a year (19<sup>th</sup> July 2011 and 28<sup>th</sup> February 2012), covering 15 sites (KE1 to KE15) and the second, “high-resolution” mapping, within the Šibenik Bay (20<sup>th</sup> July, 2012) covering 40 sites (SB1 to SB 40). Sampling locations are presented in Fig. 1 (details about sampling locations are given in Tables SI-1 and SI-2). Fig SI-1 shows the Krka River flow for the period 2008-2013, with the extracted part covering the period of our sampling campaigns. During winter sampling, the Krka River flow was ( $44 \text{ m}^3 \text{ s}^{-1}$ ) which is at the average annual level, while in summer it was at the lower end ( $10 \text{ m}^3 \text{ s}^{-1}$ ). During the estuary transect campaigns, three samples were collected along the depth at each station, whereas one surface sample was collected at each station during the mapping campaign. Additional freshwater sample was collected in the Krka River during the first campaign, which together with surface samples at the first sampling site (KE-1/1) were considered as freshwater end-members. Seawater sample at the last site (KE-15/1) was considered as a seawater end-member.

All bottles for sampling and sample storage (FEP - fluorinated ethylene propylene or PFA – perfluoroalkoxy, Nalgene) were previously cleaned with 10%  $\text{HNO}_3$  (analytical reagent grade), rinsed several times with ASTM Type I water (labelled hereafter as MQ water, 18.2 M $\Omega$ , Millipore, USA) and finally filled with MQ water until use.

Vertical profiles of main physico-chemical parameters (salinity, temperature, pH, and dissolved oxygen) were measured *in-situ*, at each site by multiprobes (OTT Minisonde 4a, Hach Lange HQ40D), calibrated before each campaign. At the 15 sites of the estuary transect campaigns, samples were collected using a van Dorn type 2.2 L horizontal water sampler (Wildco) at three depths, covering three different vertical salinity sections: a brackish, a FSI and a seawater layer. The brackish layer was sampled at a depth of 0.5 m, while the sampling depths at the halocline (FSI) and in the seawater layer were selected according to measured vertical salinity profiles and the total water column depth, respectively. Due to the relatively thin layer of the FSI layer in the upper estuary (e.g. ~30 cm), sampling was repeated until representative sample with the salinity within  $\pm 5$  range comparing to average value between brackish and seawater layer was taken. The pre-cleaned FEP bottles were used for sample storage. Upon sampling, a FEP bottle



was thoroughly rinsed with the sample and 1 L of the sample was then immediately taken and stored in a portable refrigerator. Sample treatment (filtering and acidification) was performed immediately after arrival to the laboratory.

A grab sampling technique was used for high-resolution surface sampling at 40 sites in the Šibenik Bay: a clean 1L FEP bottle was mounted at the end of a ~ 3 m aluminium telescopic pole and after rinsing three times with the ambient water, the sample was taken from the boat at a depth about 0.5 m. When the wind was causing slight boat movement, samples were taken from the “front” side regarding the boat moving direction.

### 3.2. Preparation of samples

Estuary transect samples were filtered under the nitrogen pressure (~1 bar) through 0.45 µm cellulose-nitrate membrane filters (Sartorius) using Sartorius polycarbonate filter holder (model 16511). Before filtration of each sample, membrane filter was thoroughly rinsed (250 mL of MQ water and around 250 mL of sample) before collecting 250 mL of a filtered sample in FEP/PFA bottle. A subsample of unfiltered sample was taken also in 250 ml FEP/PFA bottle. Concentrations in unfiltered samples in this paper represent acid-leachable metal concentration, but within the text are referred as total metal concentrations.

During high-resolution surface sampling in the Šibenik Bay, around 250 mL of sample was immediately filtered on-board using 0.2 µm cellulose-nitrate syringe-mounted capsule filters (Minisart Sartorius) which were previously cleaned in the laboratory (0.1% HNO<sub>3</sub>; MQ) and then with sample before collection of the filtered sample. Both filtered and unfiltered samples were collected in 250 mL FEP/PFA bottles and stored in a portable cooled refrigerator.

All samples for trace metal analyses (filtered and unfiltered) were acidified with ultrapure concentrated HNO<sub>3</sub> (*suprapur* Merck or *TraceSelect* Fluka) to pH < 2 (500 µl of acid in 250 mL of sample) and irradiated directly in FEP/PFA bottles by UV light (150 W mercury lamp, Hanau, Germany) during at least 24 hours in order to decompose natural organic matter ([Omanović et al., 2006](#)).

All tubes, glass filters and glass filtering system used for dissolved organic/inorganic carbon (DOC/DIC) and particulate organic carbon (POC) analyses were previously cleaned with 10% HCl (pro analysis, Fisher Scientific) and rinsed with MQ water, then calcinated for 4 hours at 450 °C. Aliquot of estuary transect samples were filtered using a glass system filtration (Wheaton) using glass filters (Whatman GFF, 25 mm, 0.7 µm) ([Oursel et al., 2013](#)), while for high-resolution surface sampling an aliquot of on-board filtered sample was used. Filtered

samples were stored in 24 mL glass tubes (Wheaton, equipped with Teflon/silicone septa) and preserved with 25  $\mu$ L of 1 M  $\text{NaN}_3$  ( $\text{NaN}_3 > 99\%$ , Aldrich).

Suspended particulate matter (SPM) was quantified by the difference between glass filter mass after and before filtration, after filter drying ( $40^\circ\text{C}$ ) until constant mass.

### *3.3. Analysis of samples*

#### *3.3.1. Trace metals analysis*

Trace metal concentrations were determined by differential pulse stripping voltammetry: anodic (DPASV) for Zn, Cd, Pb, Cu and adsorptive cathodic (DPAdCSV) for Ni and Co. Measurements were carried out on Autolab (EcoChemie) potentiostats ( $\mu$ Autolab 2 or PGSTAT128N) controlled by GPES 4.9 software in a three-electrode cell (663 VA Stand, Metrohm).  $\text{Ag}|\text{AgCl}|\text{sat. NaCl}$  electrode was used as the reference electrode, a Pt wire as the auxiliary and a static mercury drop (SMDE) as the working electrode. Parameters used for measurement are adopted according to Omanović et al. ([Omanović et al., 2006](#)) for DPASV and Vega and van den Berg ([Vega and Van den Berg, 1997](#)) for DPAdCSV. Trace metal analyses were performed using a fully automated system assembled with the instrument, home-made sample-changer and five Cavo XE 1000 syringe pumps. The handling software managing preparation of the project file for the GPES software, as well as voltammograms treatment and final calculations was developed for that purpose.

Concentrations of trace metals were determined by means of standard addition method. A certified “Seawater Reference Material for Trace Metals” – NASS-5 (NRC CNRC) was used for validation of the analysis. All determined metal concentrations were within the certified limits.

#### *3.3.2. Carbon analysis*

TOC-VCSH analyzer (Shimadzu) was used for determination of DOC and DIC concentrations. Calibration was done with hydrogenophthalate (Shimadzu) and  $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$  standard solutions, respectively, with an accuracy of  $0.02 \text{ mg C L}^{-1}$  ([Louis et al., 2009](#); [Oursel et al., 2013](#)). A satisfactory accuracy of analyses was validated using certified reference material MISSIPPI-03 (Environment Canada).

TOC-VCSH analyzer coupled with an SSM-5000A module was used for determination of POC on GFF filters. The filters were dried to constant weight at  $60^\circ\text{C}$ , and exposed to HCl fumes for 4 h in order to remove all of the inorganic carbon ([Lorrain et al., 2003](#)). POC content was determined by the high-temperature ( $900^\circ\text{C}$ ) catalytic oxidation method with  $\text{CO}_2$  IR detection,

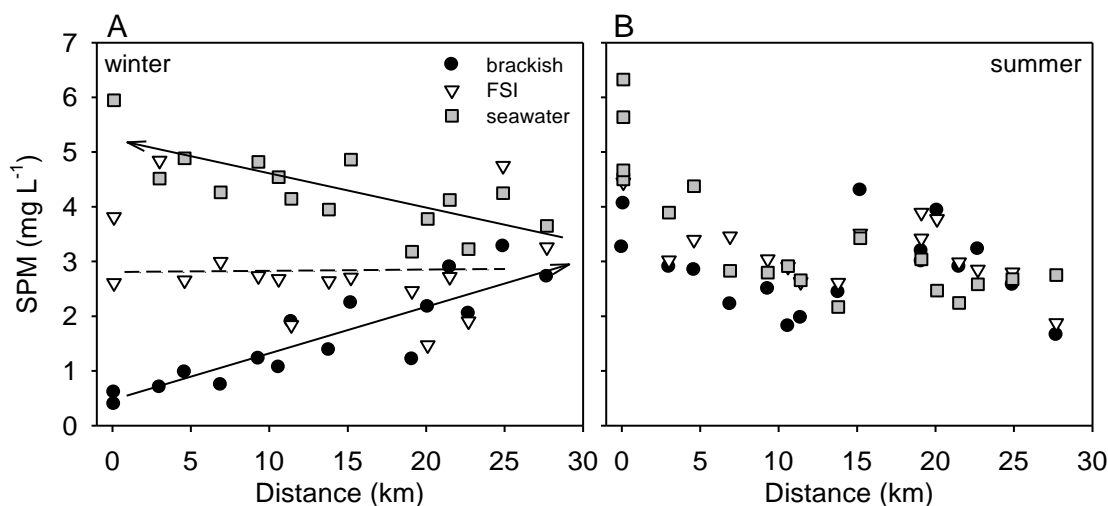


calibrated with glucose (Analytical reagent grade, Fisher Scientific), with an accuracy of 0.1 mgC.

## 4. Results and discussion

### 4.1. Estuarine major physico-chemical parameters

**SPM.** Due to the numerous tufa barriers and the big lake in the freshwater part preceding the estuary (serving as traps for the particles), the level of SPM introduced by the river is very low. Indeed, the measured level of SPM in the estuary ranged between  $\sim 0.4 \text{ mg L}^{-1}$  and  $\sim 6 \text{ mg L}^{-1}$  (Fig. 2). For winter period, well-separated trends for all three layers are evident (Fig. 2A): while SPM in surface layer is increasing seaward from  $\sim 0.4 \text{ mg L}^{-1}$  to  $\sim 3 \text{ mg L}^{-1}$ , the level of SPM in the seawater is progressively increasing in opposite, landward direction (from  $\sim 3 \text{ mg L}^{-1}$ , up to  $\sim 6 \text{ mg L}^{-1}$ ) as a consequence of its enrichment in the bottom seawater layer having longer residence time ([Legovic, 1991](#)). In the halocline (FSI layer), the SPM lies between these two trends, suggesting that the upward transport from the seawater layer is regulating vertical profile of the SPM along the estuary as projected by the described transport model (in Introduction). Less defined SPM relationships with salinity were obtained for summer campaign (Fig. 2B) probably due to the non-negligible contribution of biogenic particles (phytoplankton production) to the overall SPM concentration.



**Figure 2.** Distribution of SPM in (A) winter and (B) summer period in relation to distance for the three layers. Arrows indicate direction of water mass flow for brackish and seawater layers.

**Temperature, salinity and dissolved oxygen.** Figs. 3A-C shows contour plot profiles of temperature, salinity and dissolved oxygen along the estuarine transect at 15 sites recorded in winter 2012 and in summer 2011. As expected, due to the low atmospheric temperature in

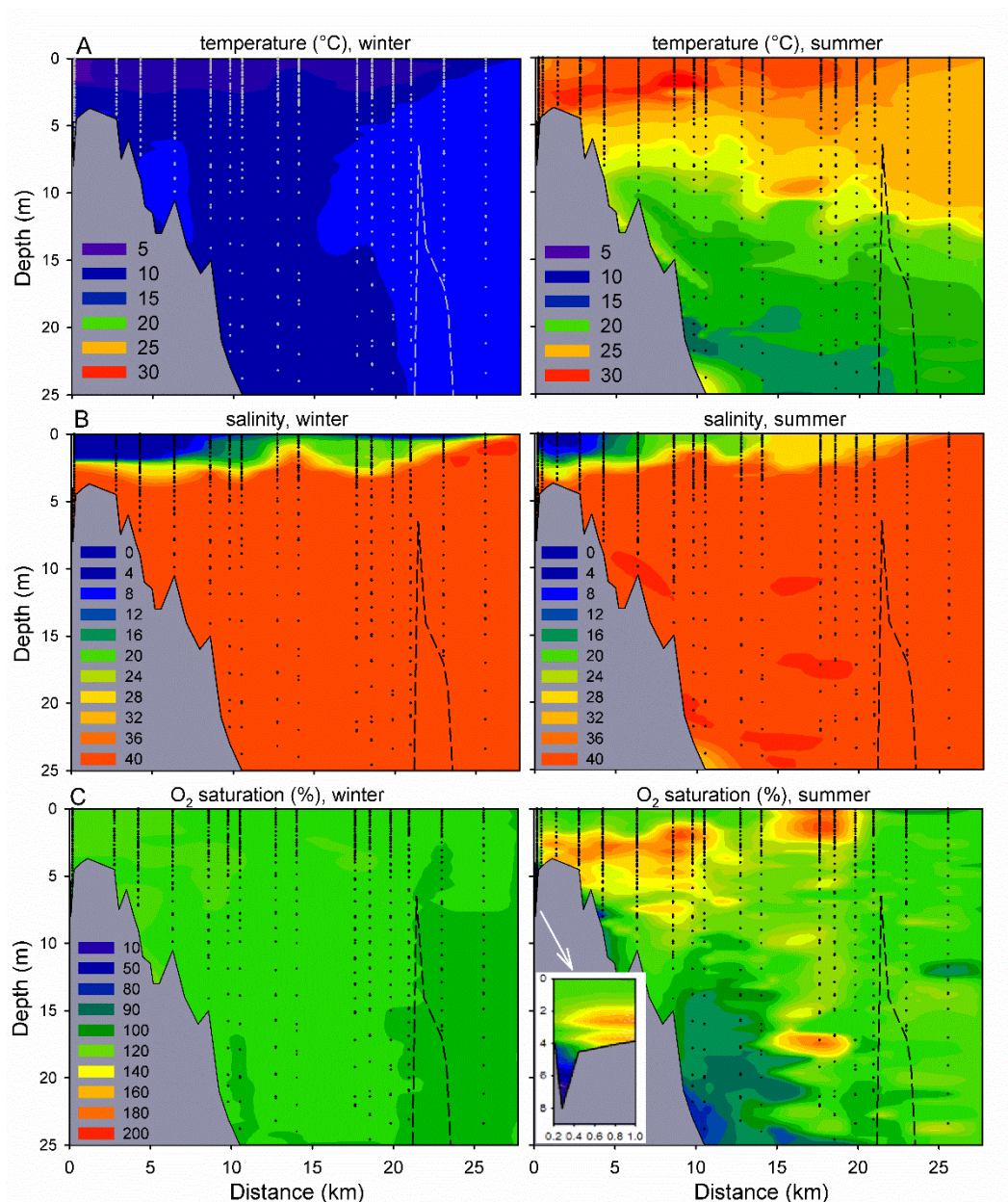
winter period lower temperature at the surface layer was registered than in deeper seawater layer. Contrary, an opposite temperature trend, with a well pronounced thermocline was registered in summer period. Depth profiles showed occurrence of the temperature maxima in the FSI layer in the upper estuary region and in the downstream estuary segment (the Šibenik bay) during the mapping campaign (July, 2012; data not shown). The occurrence and the intensity of the temperature maxima is regulated by the combination of atmosphere temperature (solar irradiation) and entrainment of seawater (mixing) and is a periodic behaviour in a scale of several days ([Legovic et al., 1991a](#)) and also within one day (our unpublished results).

Vertical profiles of salinity show a clear segmentation of the three separate layers with a sharp halocline (FSI layer) encompassing the whole estuary. Maintained at the depth between 2 and 3 m, the thickness of the halocline increased progressively in seaward direction (from ~0.4 m to ~2 m). Due to the larger Krka River flow in winter ( $44 \text{ m}^3 \text{ s}^{-1}$ ) comparing to summer ( $10 \text{ m}^3 \text{ s}^{-1}$ ), the length of the sharp halocline was extended more in winter (~10 km) than in summer (~5 km) period. Our profiles of salinity and temperature generally fit with the results and predictions of the previous studies performed at few selected locations in the Krka River estuary ([Legovic, 1991](#); [Legovic et al., 1991a](#)).

Relatively homogenous depth profiles of dissolved oxygen corresponding to 100-110% of saturation were observed along the estuary in the winter period. However, oversaturation by oxygen (140-180%) at a depth just below the FSI (Fig. 3C) was registered in summer campaigns for the most upstream estuary segment and for the Šibenik bay as a result of high biological activities ([Cetinic et al., 2006](#); [Legovic et al., 1991c](#); [Legovic et al., 1994](#)). Namely, freshwater phytoplankton produced in the Visovac Lake (the Krka River) sinks and decomposes at higher salinity, producing nutrients which favour marine phytoplankton bloom at the lower edge of the halocline ([Legovic et al., 1991c](#)). Observed temperature maxima additionally contribute to the high development of the bloom, which was found to induce a hypoxia in the autumn in the Prokljan Lake due to the decomposition of the organic matter ([Legovic et al., 1991b](#)). Although in our case such hypoxia event was not observed, a clear decrease of oxygen saturation down to 90% in the bottom seawater layer was measured, signifying a decreasing trend, potentially leading to hypoxia in autumn.

However, as an isolated case, localized hypoxic conditions (<2%  $\text{O}_2$  saturation) were found in summer in the bottom seawater layer at the upper most site (KE-1). This site is characterized by a specific cuvette (bottom depth ~7.5 m, Fig. 1-right and 3C), in which the residence time of the seawater is most probably increased comparing to adjacent seawater, enhancing the effect of bacterial mineralization of settled (and accumulated) biogenic material (e.g. lysed freshwater

phytoplankton), which caused oxygen decline. The effect of low oxygen at this location was found to influence vertical distribution of trace metal concentrations, which is discussed latter in the text.



**Figure 3.** Two-dimensional distributions of temperature (A), salinity (B) and dissolved oxygen (C) in the Krka River estuary in winter 2012 and in summer 2011.

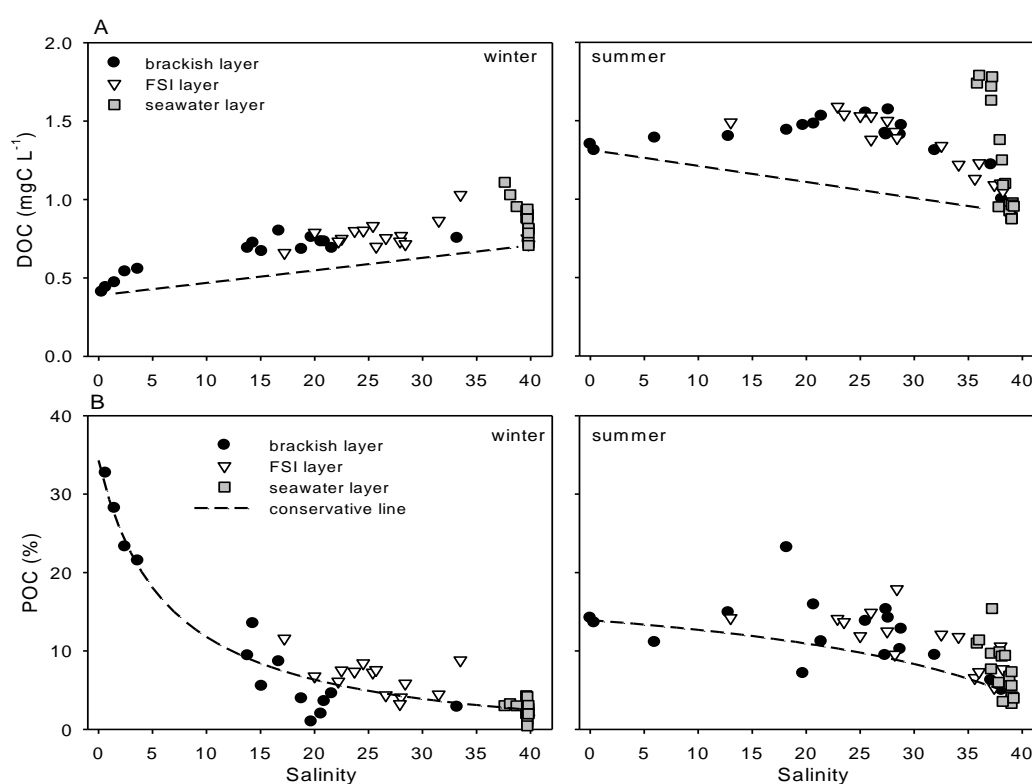
**DIC and pH.** DIC and pH distributions are more detailed in supporting information document (Figs. SI-2 and SI-3). Briefly, the range of DIC values along the estuary/salinity gradient agrees well with the previous study ([Cauwet, 1991](#)). Compared to that study in which linear relationship with salinity was found, our measurement showed existence of a peak of the DIC in a surface layer for both periods at the 5<sup>th</sup> km. This increase is most probably the consequence of reestablishment of the carbonate equilibria, i.e. uptake of CO<sub>2</sub>, which was previously degased

at the waterfalls. pH measured in the estuary transect in both seasons ranged between 8.7 in the surface layer at the head of the estuary and 8.1 at the seawater end member. Higher pH observed at the first site (KE-1) in the estuary is a consequence of CO<sub>2</sub> removal (degassing) from the water at preceding waterfalls located 500 m upstream ([Cukrov et al., 2008a](#); [Vukosav et al., 2014](#)). The mineralization of organic matter in the cuvette led to drop of pH down to 7.8.

**DOC and POC.** Although previous studies reported that DOC concentration in the Krka River is relatively constant throughout the year (mean value of 1.18 mgC L<sup>-1</sup>, ([Cauwet, 1991](#))), our measurements performed at contrasted periods clearly evidence a difference in DOC between winter (0.42 mgC L<sup>-1</sup>) and summer (1.35 mgC L<sup>-1</sup>). Compared to European rivers like Rhine (~ 2.9 mgC L<sup>-1</sup>), Loire (~3.9 mgC L<sup>-1</sup>), Elbe (~ 4.6 mgC L<sup>-1</sup>), Thames (~ 5.8 mgC L<sup>-1</sup>) ([Abril et al., 2002](#)) or Gironde (~ 3.1 mgC L<sup>-1</sup>) ([Veyssy et al., 1998](#)) concentrations of DOC measured in the Krka River are extremely low. Comparing the two end-members for winter and summer period, lower DOC were measured in freshwater in winter (0.4 mgC L<sup>-1</sup> vs. 0.72 mgC L<sup>-1</sup>) and higher in summer period (1.35 mgC L<sup>-1</sup> vs. 1.0 mgC L<sup>-1</sup>). Higher DOC during summer period in freshwater part is a consequence of the enhanced biological activity in the Visovac Lake (freshwater part) ([Petricioli et al., 1996](#); [Svensen et al., 2007](#)). Fig. 4A presents the distribution of DOC in function of salinity for all three layers (Fig. SI-5 in addition presents DOC in relation to distance) for winter and summer periods. Generally, higher DOC values were obtained in summer and are a consequence of the higher biological activity. Although it is obvious that no clear relationship was established for any sampling period (dashed line), the non-conservative behaviour is even more pronounced for the summer campaign. The range of DOC values and its distribution along the estuary is in agreement with studies performed more than 20 years earlier ([Cauwet, 1991](#); [Sempere and Cauwet, 1995](#)), showing a persistency of the pristine/oligotrophic nature of the Krka River and its estuary. Contrary to our records, a conservative behaviour was found in various freshwater/seawater mixing zones such as the Huveaune and the Jarret Rivers plume ([Oursel et al., 2013](#)), the Rhône Estuary ([Dai et al., 1995](#)), the Mississippi River plume ([Guo et al., 2009](#)), the bay of Saint Louis ([Cai et al., 2012](#)), or the Humber Estuary ([Alvarez-Salgado and Miller, 1998](#)).

Detailed surface mapping of the Šibenik bay showed slight change of DOC within the bay (Min/Max = 1.47/1.64 mgC L<sup>-1</sup>, N = 36) suggesting no evidence of urban input from the town or harbour/nautical marina (Fig. SI-4). At more downstream sites (>15 km, Fig. SI-5), DOC was maintained quasi-constant for all three layers in the winter period, whereas a linear (conservative) decrease was observed for the surface and the FSI layer in summer period as an

effect of dilution with seawater containing lower DOC concentration (Fig. 4A, summer). A landward increase of DOC in seawater (with the increase much more pronounced for summer than for winter) is a clear consequence of the organic detritus decomposition sank from the surface layers, combined with the slow entrainment of seawater (residence time of seawater is increasing in upstream direction). In the FSI layer (halocline) DOC exhibited variable trends for the summer and the winter period, but generally spanning the range between values in the surface and the seawater layers. A typical increase of DOC in FSI layer reported to be characteristic of the estuary ([Zutic and Legovic, 1987](#)) was not observed, however this could be due to only three samples collected along the vertical gradient in our work.



**Figure 4.** Distribution of (A) dissolved organic carbon (DOC) concentration and (B) particulate organic carbon (POC) content in relation to salinity for the three layers, in winter and summer period.

A general decreasing trends of POC with distance for winter and summer campaigns in all three layers, with higher average POC value in summer than in winter (0.33 mg L<sup>-1</sup> and 0.13mg L<sup>-1</sup>, respectively; Fig. SI-6) were obtained. These values are extremely low if compared to other estuaries: the Scheldt estuary (~18.3 mgC L<sup>-1</sup>), the Loire estuary (~6.3 mgC L<sup>-1</sup>), the Gironde estuary (~2.6 mgC L<sup>-1</sup>) or the Elbe estuary (~1.6 mgC L<sup>-1</sup>) (all values from ([Abril et al., 2002](#))). When normalized on SPM, a well-defined decreasing trend of POC content with separated segments for each layer for winter period in relation to salinity was obtained (Fig. 4B; in



addition, Fig. SI-7 presents POC content in relation to distance). The content of POC fraction decreased considerably in the surface layer at salinities up to ~15, following the conservative (dashed) line. Contrarily, points at salinities higher than ~15 are below this line. The release of organic matter (OM) associated with SPM due to the competitive effect at increased salinities (considering the prevailing terrigenous, inorganic nature of the SPM supplied by the Krka River) and/or the prevailing biogenic material in freshwater could explain the observed POC content trend. Slightly higher POC content in FSI layer than predicted by the conservative line could be explained by the accumulation of biogenic material ([Cauwet, 1991](#); [Sempere and Cauwet, 1995](#); [Zutic and Legovic, 1987](#)). Almost no change was obtained for the seawater layer. In the summer period, higher POC concentrations, but also POC content in majority of the estuary transect were found in all layers. It results from the increased phytoplankton productivity within the estuary, which caused higher POC content than expected from the theoretical conservative line. A similar increase of the POC content and also concentration at the lower edge of the halocline compared to overlaying and underlying water was already registered in previous studies in the Krka River ([Cauwet, 1991](#); [Sempere and Cauwet, 1995](#)).

## 4.2. Distribution of trace metals

### 4.2.1. End-members

Only two publications reporting concentrations of trace metals in a freshwater end-member, in the Krka River were published up to now (Table 1). Our measurements of a surface sample at the first estuary point (KE-1/1,  $S < 0.5$ ) showed systematically lower concentrations for all studied metals compared to site upstream of the waterfalls, and thus, this site was considered as the end-member. The decrease of concentrations of trace metals is probably caused by the “self-purification” process, i.e. their removal along the waterfalls preceding the estuary (46 m of total downfall) with 17 tufa barriers serving as traps ([Cukrov et al., 2008a](#)). Comparing to other Mediterranean rivers (Table 1), concentrations of trace metals in the Krka River are much lower ([Dorten et al., 1991](#); [Ollivier et al., 2011](#); [Oursel et al., 2013](#)).

For the seawater end-member (KE-15), there is no difference between winter and summer concentrations for Zn, Cd, Pb and Cu, while for Ni and Co, slightly higher values were measured in summer than in winter. Although this site is located inside of the coastal region, measured concentrations are very similar to those measured in the open parts of the Adriatic or the Mediterranean ([Annibaldi et al., 2011](#); [Tankere and Statham, 1996](#)). This suggests that the general contribution of metal inputs from the land to the sea is insufficient to cause a permanent increase of trace metal concentrations.



Table 1. Concentrations of trace metals in the Krka River, two end-members, selected Mediterranean rivers and open Mediterranean Sea, as well as ranges (all depths) across the estuary transect sites for the winter and summer periods and in the Šibenik bay (high resolution mapping; summer) obtained in this study.

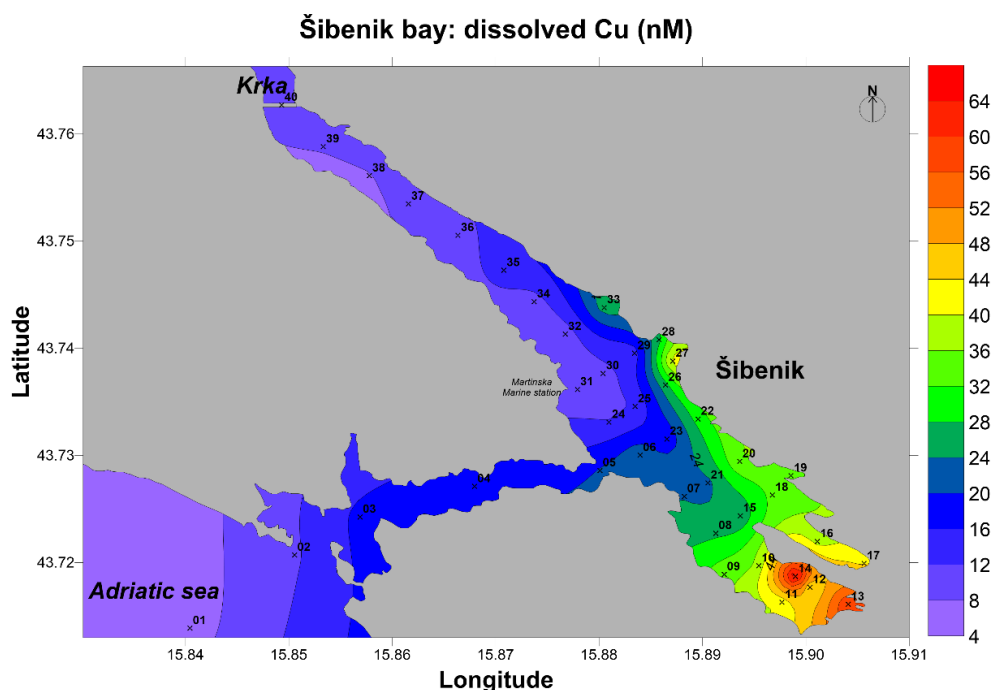
	Dissolved (total ) metal concentrations (in nM)					
	Zn	Cd	Pb	Cu	Ni	Co
Elbaz-Poulichet et al. (1991) – Krka	-	0.044	0.08	1.78	2.47	-
Cukrov et al. (2006) – Krka	4.51	0.018	0.037	1.47	-	-
Krka – this study	(4.63)	(0.025)	(0.166)	(2.52)	-	-
KE1-1 (S<0.5)	3.25	0.015	0.03	4.44	2.09	0.31
	(8.06)	(0.020)	(0.14)	(6.51)	(2.96)	(0.6)
KE-15 (S=38)	2.03	0.008	0.02	2.45	1.86	0.24
	(2.78)	(0.10)	(0.09)	(3.23)	(2.85)	(0.36)
	4.5	0.067	0.070	3.2	7.0	0.36
	(5.2)	(0.069)	(0.101)	(3.6)	(7.45)	(0.36)
Range winter	2.0-14.9	0.02-0.16	0.01-0.22	1.76-7.29	1.76-7.29	0.20-0.66
Range summer	2.0-15.3	0.01-0.10	0.02-0.29	2.45-20.2	1.86-10.3	0.24-1.22
Šibenik bay	7.1-101.0	0.05-0.21	0.04-1.11	6.42-68.7	5.11-10.70	0.26-0.51
River						
Rhone <sup>1</sup>	41.3		0.33	32.7	16.7	
Huveaunne <sup>2</sup>	52.0	0.07	0.64	27.0	43.0	6.9
Jarret <sup>2</sup>	73.0	0.07	0.37	28.0	29.0	4.1
Po <sup>3</sup>	57.7	0.58	0.72	25.7	-	-
Ebro <sup>3</sup>	42	1.07	0.75	15.3	-	-
Arno <sup>3</sup>	30.6	0.89	1.01	27.5	-	-
World average river <sup>2</sup>	9.2	0.71	0.38	23	14	2.5
South. Adriatic <sup>4</sup>	2.71	0.076	-	2.95	5.27	-
Central Adriatic <sup>5</sup>	-	0.12	0.19	6.3	-	-
Mediterranean <sup>4</sup>	2.7	0.062	-	1.7	2.4	-

<sup>1</sup>(Ollivier et al., 2011), <sup>2</sup>(Oursel et al., 2013), <sup>3</sup>(Dorten et al., 1991), <sup>4</sup>(Tankere and Statham, 1996), <sup>5</sup>(Annibaldi et al., 2011)

#### 4.2.2. Distribution of trace metals within the Šibenik bay

In order to identify locations and quantify contamination sources a detailed mapping of the Šibenik bay (40 sites, Fig. 1) was performed. Similar surface distributions as presented in Fig. 5 for dissolved Cu were obtained for Zn, Cd and Pb (Fig. SI-8a,b). All four metals correlate between each other with correlation coefficient higher than 0.8 (see Table SI-3 for details), pointing on same/similar sources. As expected, the harbour and the nautical marina exhibited the highest concentrations for most of the metals. Comparing to the lowest levels found within the bay (which are already increased comparing to end-members) an additional increase of all metals occurred at particular locations: ~10× for Zn, ~4× for Cu, ~3× for Cd, ~5× for Pb and

$\sim 2\times$  for Ni. No clear distribution/enhancement was observed for Co concentrations. If compared to seawater/freshwater end-members, the following “enrichment” factors were obtained: 22/50 for Zn, 3/20 for Cd, 16/55 for Pb, 22/27 for Cu, 1.5/6 for Ni and 1.4/2 for Co. It is likely that the levels of contamination fluctuate depending on the activities within the harbour and the nautical marina, and thus, the measured values could be used only as merits of contamination sources and general levels when considering the distribution of metals for the overall estuary transect performed at different periods.



**Figure 5.** Distribution of dissolved Cu in the surface layer of the Šibenik bay.

#### 4.2.3. Distribution and behaviour of trace metals along the salinity gradient

The main characteristic for all measured metals is that the concentration in freshwater end-member is lower than in seawater (Table 1), either as dissolved or total. This is opposite to many other estuaries, characterized by high input of metals to the sea by the river ([Cobelo-Garcia et al., 2004](#); [Elbaz-Poulichet et al., 1984](#); [Hatje et al., 2003a](#); [Kraepiel et al., 1997](#); [Oursel et al., 2013](#); [Turner and Millward, 2002](#); [Waeles et al., 2007](#); [Waeles et al., 2008a](#)). Due to the low SPM input, the impact of removal processes within estuarine mixing zone is diminished, making identification of this processes (by comparison of the theoretical dilution curve and the actual concentrations) much more complicated ([Dorten et al., 1991](#); [Elbaz-Poulichet et al., 1991](#); [Fu et al., 2013](#)). In line with this, as the particulate fraction is low, the contribution of potentially released metals to the dissolved pool is also probably minor. The subsurface maximum within FSI layer previously reported to occur for organic matter and trace metals

([Bilinski et al., 2000](#); [Kniewald et al., 1987](#); [Louis et al., 2009](#); [Zutic and Legovic, 1987](#)) was not registered in our study mainly due to insufficient number of points in the vertical profile (only three points, from which only one in FSI layer). In most cases, the concentrations in FSI layer for all metals were between those measured in two adjacent layers (Table 2). Generally, the average concentration of trace metals is increasing with depth, with the exception of Cu in summer. The highest difference along the vertical profile was observed for Pb, pointing on sediments as a potential source of Pb along with its accumulation in the seawater layer. In overall, average trace metal concentrations in all three layers were higher in summer samples than in winter. This is particularly evident for Cu, for which  $\sim 3\times$  higher average concentrations were found in surface layer in summer, indicating an additional input primarily as a consequence of the increased nautical activities ([Omanović et al., 2006](#)).

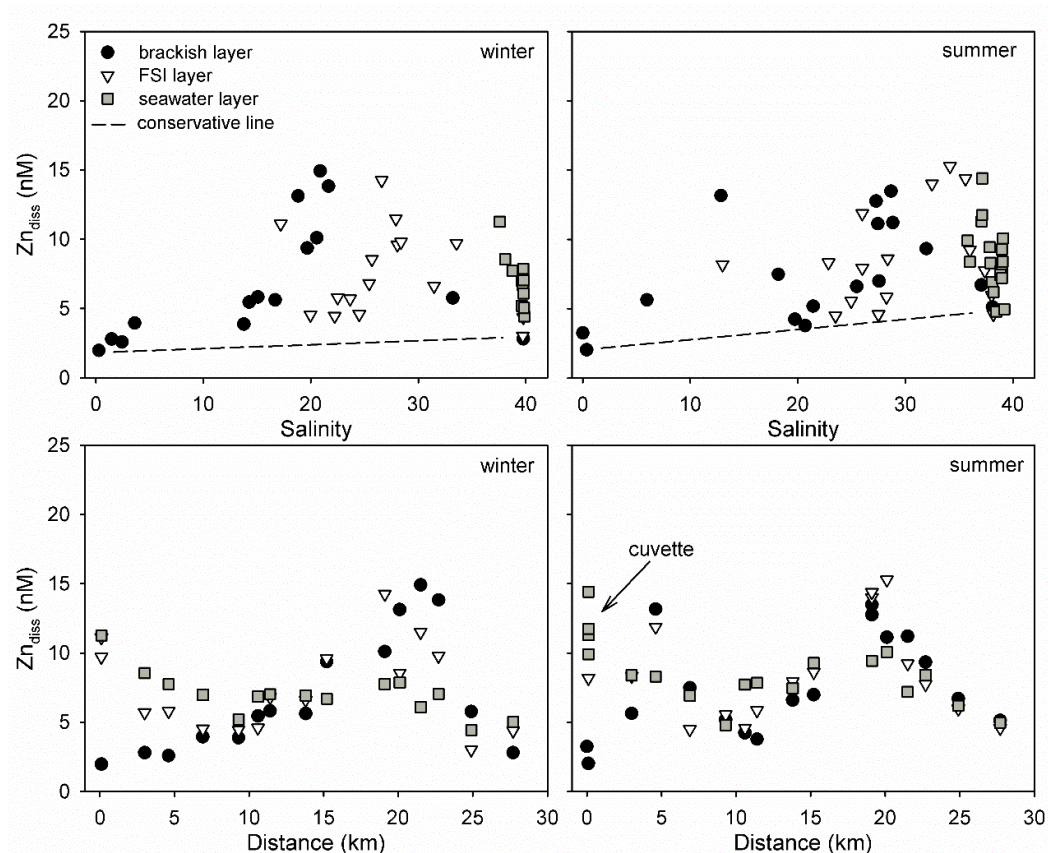
Shortly, with the exception of Pb, a general characteristic of all other metals is a very high average degree of dissolved fraction (DF; see Table 2 and Fig. SI-9). Average dissolved fraction was slightly smaller in summer than in winter period, which is probably more a consequence of the different type of SPM (more of biogenic origin), than its concentration.

Table 2. Average dissolved metal concentrations (in nM) in each layer for winter/summer period and average dissolved fraction (DF) (all samples).

Layer	Zn	Cd	Pb	Cu	Ni	Co
Surface - brackish	6.7/7.7	0.052/0.060	0.042/0.074	3.9/11.3	3.7/5.5	0.33/0.38
Middle - FSI	7.5/8.5	0.076/0.071	0.055/0.098	4.4/10.2	5.1/6.3	0.36/0.41
Bottom - Seawater	7.0/8.6	0.081/0.080	0.108/0.118	4.0/5.7	6.4/7.8	0.30/0.51
DF (%)	87/68	93/91	52/36	90/84	92/91	92/83

**Zinc (Zn)** Distributions of dissolved Zn in relation to salinity and distance along the estuary transect for the winter and the summer period and for each layer are presented in Fig. 6. In the surface brackish layer, Zn exhibited highly positive deviation from the theoretical dilution line for both periods, indicating an additional supply of Zn. As expected, for both sampling periods the highest concentrations were measured in samples collected in the Šibenik bay. However, unexpected was the same level of Zn in the winter as for the summer period ( $\sim 15$  nM), despite the fact of decreased activities (touristic) in the winter period. This implies on the “continuous” supply of Zn in the surface layer. While for the winter campaign (Fig. 6, left plots), Zn concentration increased progressively from  $\sim 2$  nM at the freshwater end-member up to 15 nM at the Šibenik bay, and then decreased seaward, reaching  $\sim 3$  nM at the seawater end-member, in summer period (Fig. 6, right plots) an additional increase of Zn was observed at the 5<sup>th</sup> km.

This increase is attributed to the intensive touristic activities in summer, especially related to a large number of boats anchored in front of the Skradin town and entrance of the National park.



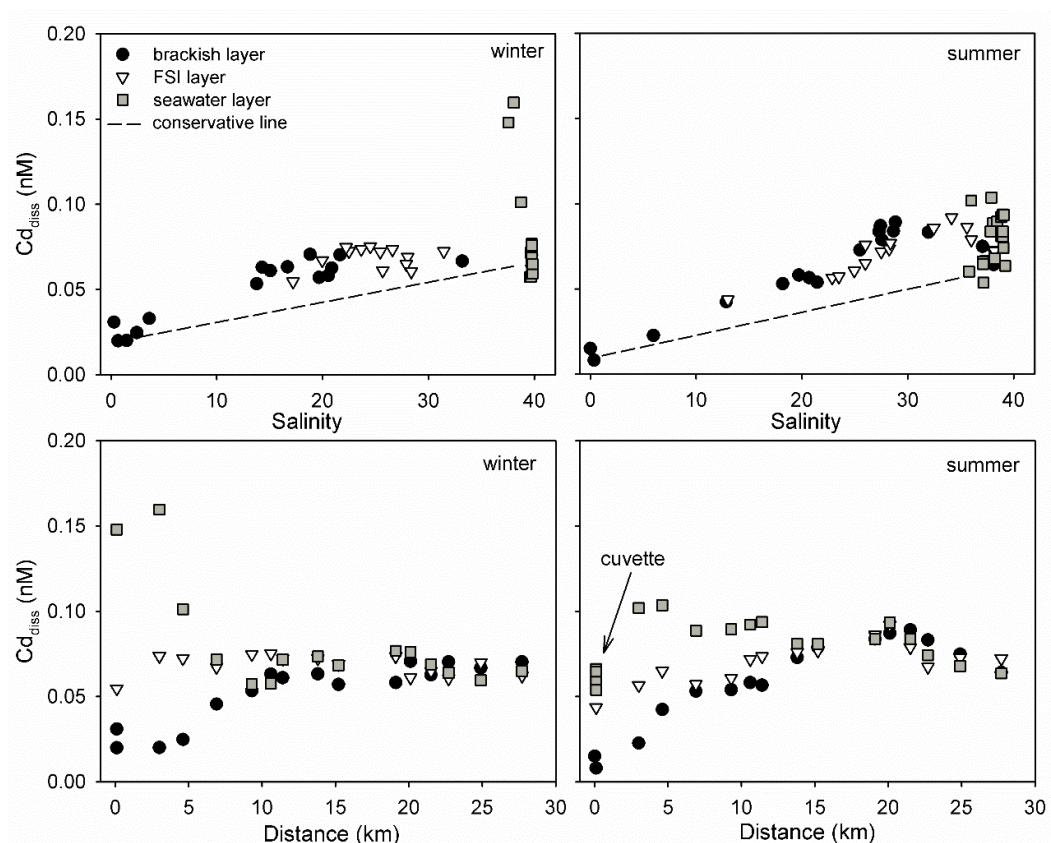
**Figure 6.** Distribution of dissolved Zn concentrations in relation to salinity and distance for the three layers, in winter and summer period. Dashed line represents theoretical dilution line.

Considering the magnitude of the estuary spatial scale, the mentioned Zn supplies in the surface layer could be considered as point-source inputs, causing “pyramidal” redistribution of Zn (downstream as well as upstream) in the surface layer as a result of the two-directional transport model (described in Introduction). In seaward direction, a near-linear decay with the salinity (Fig. 6), indicates a quasi-conservative behaviour (considering seawater end-member). The described hydrodynamic transport model assumes vertical downward transport, which in conjunction with the longer residence time of the seawater flowing upstream is causing a progressive accumulation of Zn (and other metals). Mixing of the “metal-enriched” seawater layer flowing upstream with the surface layer led to higher Zn concentrations upstream of the point-source location (“pyramidal” distribution). Concentration of Zn in the halocline is generally between these two layers, confirming this simple mixing model, as observed for SPM. Vertical transport of Zn supplied in the surface layer was reflected along the vertical column, yielding a “concentration peak” in other two layers at the source site (KE-10, KE-11).

As a general behaviour for almost all metals in both campaigns, an upstream increase of concentration (dissolved and total) was observed in the seawater layer (Fig. 6, grey squares). The intensity and the shape of these spatial distributions are a “net” result of several physico-chemical parameters and processes. The residence time of the seawater layer is increasing in upstream direction, which in combination with above mentioned continuous supply and a low removal from the water column (low Zn particulate fraction), produce a progressive accumulation and an upstream increase of Zn concentration, leading to  $\sim 5\times$  higher concentration of Zn in the seawater layer than in the freshwater layer at the first sampling point (KE-1). Comparing to seawater end-member the concentration in the seawater layer increased about 3 times for both, the winter and the summer period.

**Cadmium (Cd).** Dissolved Cd concentration in the surface brackish layer showed an increasing trend with increasing salinity for both periods: from  $\sim 0.010$  nM in the freshwater, to  $\sim 0.070$  nM in the seawater end-member, respectively (Fig. 7). Along the salinity gradient, most of the values are above the theoretical dilution line, indicating a non-conservative behaviour. Commonly, this behaviour is ascribed to the competitive effect of the chloride ions, i.e. release of Cd from the SPM into solution as a result of the formation of stable chloro-complexes ([Elbaz-Poulichet et al., 1996](#); [Elbaz-Poulichet et al., 1991](#); [Kraepiel et al., 1997](#); [Oursel et al., 2013](#); [Waeles et al., 2005](#)). Although such behaviour is consistent with other studies, in most of these studies which reported non-conservative Cd behaviour in an estuarine mixing, the concentration of SPM was much higher than in our case, and the distribution is characterised by the “concentration peak” appearing mainly at salinities between 10 and 20 ([Dabrin et al., 2013](#); [Elbaz-Poulichet et al., 1996](#); [Elbaz-Poulichet et al., 1987](#); [Fu et al., 2013](#); [Hatje et al., 2003a](#); [Kraepiel et al., 1997](#); [Oursel et al., 2013](#); [Oursel et al., 2014](#); [Paucot and Wollast, 1997](#); [Waeles et al., 2005](#); [Waeles et al., 2004](#)). As the particulate concentration of Cd measured in the freshwater end-member is very low ( $<10\%$ ), the release of Cd from SPM is excluded as a main contributor to the observed trends in the Krka River estuary (although it can contribute partly to the overall increase). Thus, the observed non-conservative behaviour of Cd is primarily ascribed to the point-source input in the Šibenik Bay, and could be explained by the seaward/landward redistribution by the transport model previously described for Zn. Similar trend of Cd with salinity obtained in the previous study with a smaller number of sites was defined as “near-conservative” ([Elbaz-Poulichet et al., 1991](#)).





**Figure 7.** Distribution of dissolved Cd concentrations in relation to salinity and distance for the three layers, in winter and summer period. Dashed line represents theoretical dilution line.

As for Zn, an upstream increase of Cd concentration in the seawater layer was registered. For the winter period, a very strong upstream increase ( $\sim 3\times$ ) of Cd, starting upstream of the  $\sim 10^{\text{th}}$  km (site KE-4) was measured. The same relative increase was found also for Co. Currently, we do not have reasonable physico-chemical explanation/scenario which led to such a strong concentration increase of these two metals, other than that the increase is a consequence of the progressive accumulation accompanied with the absence of removal processes which do not occur in “old” seawater (as is the case for Pb, described later in the text). This scenario might be partly supported by the observed trend in particulate fractions of metals, which is the lowest for Cd and Co. A more detailed study would be needed in order to fully explain this observation. Contrary to the winter period, a drop of Cd in the seawater layer in the summer period was recorded at the first sampling site (KE-1), characterised by the cuvette with anoxic conditions. Anoxic conditions favour reduction of sulphate to sulphide ([Rigaud et al., 2013](#)), leading to formation of strong metal-sulfide complexes and their precipitation (removal from the water column). The same concentration drop is also observed for Cu, while other metals showed either an increase (Zn, Co) or no significant (Pb, Ni) change in concentration. Another possibility which should not be excluded as a possible explanation of the observed

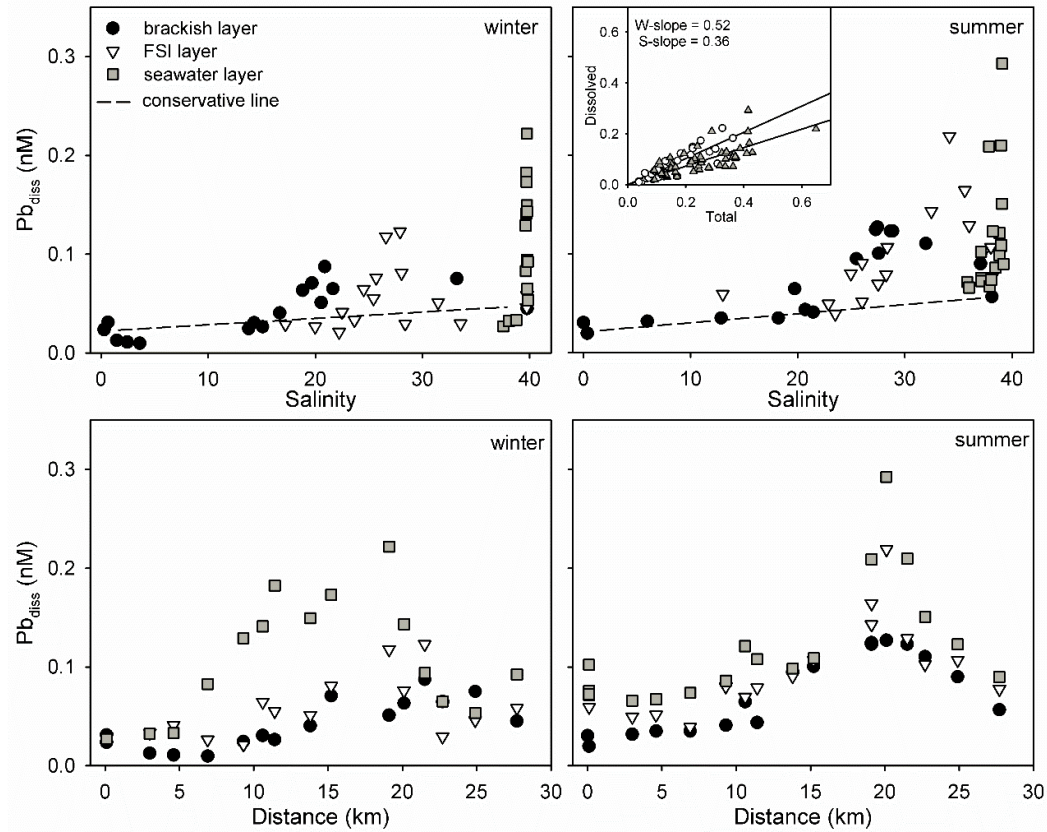


concentration drop is related to the analytical procedure of metal determination. Namely, the determination of dissolved/total metals include acidification to  $\text{pH} < 2$  and UV-light digestion of samples in order to destroy residual organic matter which could interfere with the measurement. There is a possibility that the formed CdS and CuS complexes, if not dissociated by the acidification, were not transformed to the measurable species by the applied electrochemical method and remained “invisible”. Among the measured metals, the highest sulphide stability constants in seawater were found for Cd and Cu ([Al-Farawati and van den Berg, 1999](#)), which could partly justify the second scenario ([Jiann et al., 2005](#)). A more detailed study is needed in order to explain this observation.

**Lead (Pb).** Similar trends of dissolved Pb in the surface brackish layer in relation to salinity and distance were observed for the winter and the summer period (Fig. 8). Both, positive and negative deviations from the theoretical dilution line were observed for dissolved Pb, with increasing concentrations in seaward direction from  $\sim 0.020$  to  $\sim 0.050$  nM. However, if examined by separate segments, almost conservative behaviour for Pb was maintained up to the  $\sim 15$  km for the summer period, whereas a negative decline from the dilution line is obvious for the first  $\sim 10$  km in the winter period. Although of a very small absolute intensity, this decline could be attributed to the removal of Pb, often explained by rapid sorption of dissolved Pb onto re-suspended particles and/or co-precipitation with iron and manganese oxides ([Elbaz-Poulichet et al., 1984](#); [Fu et al., 2013](#); [Ouseph, 1992](#); [Waeles et al., 2007](#); [Waeles et al., 2008b](#)). In the summer period this decline was not observed probably due to the different average nature of SPM and/or steady state equilibrium between Pb input and removal from the layer. Positive deviation from the dilution line at more downstream sites for both periods is primarily caused by the additional anthropogenic input of Pb within the Šibenik bay. Similar trend of Pb distribution in the surface layer was noticed in the previous study ([Elbaz-Poulichet et al., 1991](#)), however it should be highlighted that 2-3 times lower Pb concentrations were measured in our study, most probably as a results of absence of gasoline-Pb input.

The particularities of Pb distributions are systematic concentration increase with depth at almost all sites (much more expressed in the winter than in the summer period) and an absence of continuous upstream concentration increase in the seawater layer, the behaviour observed for all other metals. Compared to other metals, Pb is known as particle-reactive metal. While the dissolved fractions for most of the metals were quasi-constant along the estuary in all three layers (mainly  $>85\%$ , Fig. SI-9), the dissolved fraction of Pb varied from  $\sim 20\%$  at the most upstream site, to  $\sim 80\%$  at the seawater end-member (Fig. 9) for all three layers. The observed

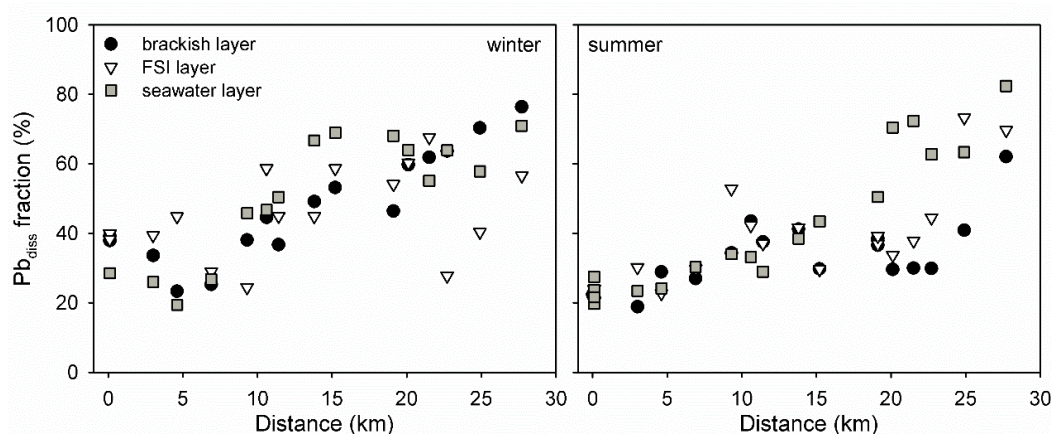
trends could not be ascribed exclusively to the change in SPM concentration, but also to the type of SPM. While similar dissolved fractions in brackish and seawater layers (~30-40%) were found at the most upstream sites in winter, a 10× difference in SPM concentrations were measured, pointing to stronger affinity of Pb to particles derived by the river, than those produced in the estuary.



**Figure 8.** Distribution of dissolved Pb concentrations in relation to salinity and distance for the three layers, in winter and summer period. Dashed line represents theoretical dilution line. Inset: Relationship of dissolved vs. total Pb for winter (W, empty circles) and summer (S, grey triangles) period.

In the study performed by Elbaz-Poulichet et al. ([Elbaz-Poulichet et al., 1991](#)), the concentration of Pb in the seawater layer was “continuously” increased in a landward direction (based on 4 locations), reaching the highest value at the most upstream site (KE-3 in our case, 5<sup>th</sup> km). Profiles obtained in our study showed completely different behaviour: while an increase of Pb was recorded starting from the seawater end-member with the maximum within the Šibenik bay, a decrease in Pb concentration was registered thereafter in the landward direction, reaching Pb concentration at the most upstream sites smaller than those found in the seawater end-member. This clearly indicates that removal processes control the content and distribution of Pb in more upstream parts, commonly explained by Pb scavenging by Mn and Fe oxyhydroxide ([Elbaz-Poulichet et al., 1984](#); [Waeles et al., 2008b](#)). As Mn and Fe

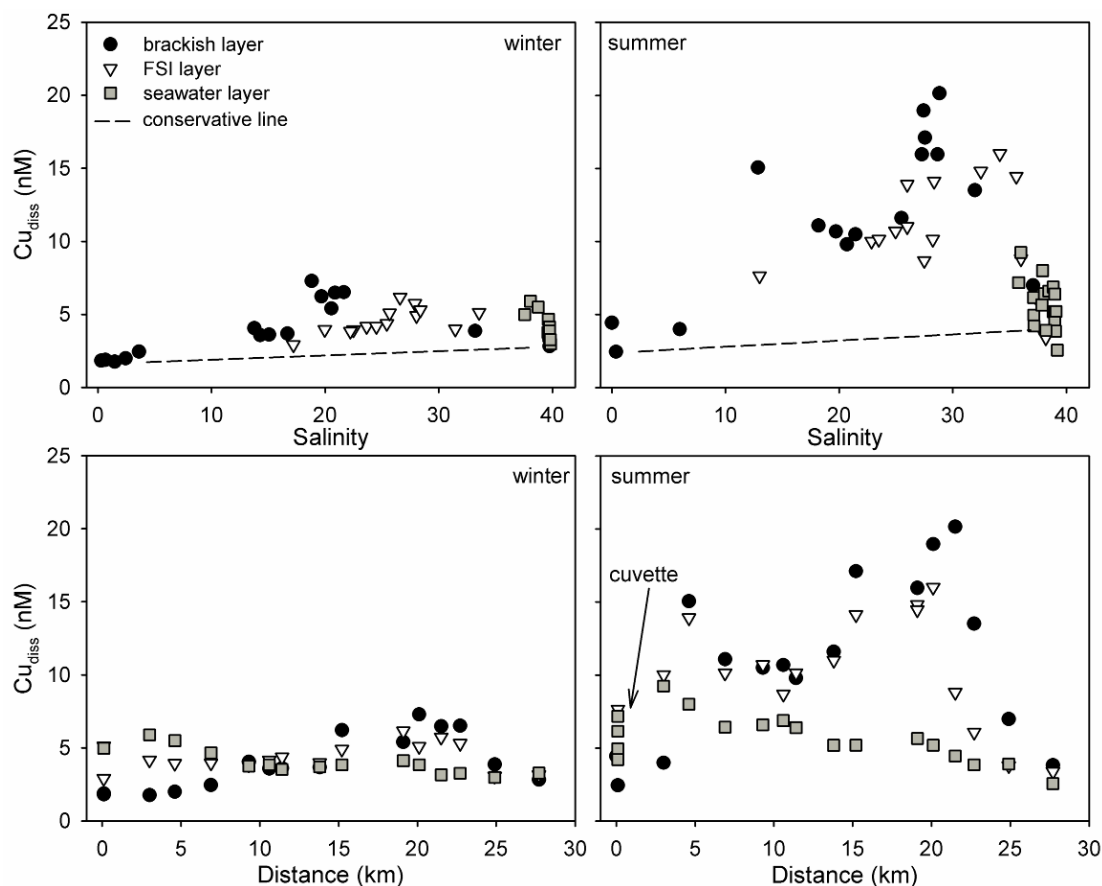
concentrations were not measured in our study, we cannot confirm that this removal process is dominating. However, taking into account that a very high correlation factor ( $CF = 0.941$ ,  $n = 13$ ,  $p = 95\%$ ) between Pb and Mn sediment contents was found across the estuary transect ([Cukrov et al., 2008b](#)) and that high concentration of Mn and Mn-particles were measured in the estuary zone in the vicinity of the former ferro-manganese factory ([Bilinski et al., 1996](#); [Omanović et al., 2006](#)), this assumption seems reasonable in our case as well. The increased Pb concentration in the seawater layer is more pronounced and also extended in winter (up to the 5<sup>th</sup> km) than in the summer period (up to the 10<sup>th</sup>/15<sup>th</sup> km), despite a higher average level of Pb in summer. This could be explained by the faster settling rate in summer linked to a different nature of suspended particles, which is supported also by the higher particulate fraction in summer (see inset in Fig. 8), as well as the “energy” of water related to a longer residence time ([Legovic, 1991](#)). An increase of Pb with depth is a consequence of the accumulation of Pb sank from the surface layer, while its spatial distribution within the seawater layer is a net result of above mentioned processes affecting distribution between particulate and dissolved phase. However, sediment should not be discarded as a possible additional source of Pb due to numerous biogeochemical processes within sediments and at the sediment/water interface (SWI) ([Cobelo-Garcia and Prego, 2004](#); [Dang et al., 2015](#); [Lourino-Cabana et al., 2011](#); [Martino et al., 2002](#); [Rigaud et al., 2013](#)). Most recent information from media (July 2014) is that there is an army shipwreck at the entrance of the Šibenik bay (site KE-11) sank at the end of 2<sup>nd</sup> world war. As in that time Pb was used as an antifouling paint and as such torpedo-boats were equipped by Pb-batteries, there is a possibility that some portion of increased Pb in seawater layer originate from that source. Moreover, an open question remains the contribution of Pb released from the lost Pb fishing-weights deposited at the sediment, which was documented by the diver to be heavily spread at the “fishing” locations within the bay.



**Figure 9.** Distribution of dissolved Pb fraction in relation to distance for the three layers, in winter and summer period.

**Copper (Cu).** Among the measured metals, the most variable spatio-temporal distributions along the estuary transect were found for both, dissolved and total Cu concentrations (Fig. 10). Considering the concentrations of end-members, only slightly higher concentrations (in absolute values) were measured in seawater samples (~2.2 nM vs. ~3.3 nM), similarly as previously measured by Louis et al. (2.7 nM vs. 4.0 nM) ([Louis et al., 2009](#)) and Elbaz-Poulichet et al. (1.8 nM vs. 4.4 nM) ([Elbaz-Poulichet et al., 1991](#)). In addition, the difference between winter and summer end-member samples was also minor. Contrary to these similarities, dissolved concentrations exhibited quite different inter-estuarine distributions for winter and summer campaigns, especially pronounced for the surface and the FSI layers. With increasing salinity a positive deviation from the conservative line was a general characteristic for both periods. The maximal level (~7 nM) and a common trend in the winter period agree with those measured by Elbaz-Poulichet in May 1988 ([Elbaz-Poulichet et al., 1991](#)). In both studies, the highest concentrations, ascribed to “regular” anthropogenic input, were measured in the Šibenik bay. However, while in 1988-study the main source of Cu was likely the contaminated untreated waste water discharged within the bay, in our study increased Cu originates primarily from the antifouling paints of the boats, located in the nautical marina, harbour and along the costal line of the Šibenik town. About 3-fold Cu concentration increase compared to end-members was registered (winter period). While both, conservative ([Abe et al., 2003](#); [Elbaz-Poulichet et al., 1996](#); [Fu et al., 2013](#); [Koshikawa et al., 2007](#); [Waeles et al., 2009](#)) and non-conservative ([Hatje et al., 2003a](#); [Waeles et al., 2008b](#)) behaviours were observed in estuaries worldwide, a non-conservative behaviour observed in our case is exclusively a consequence of a point-source anthropogenic inputs within the estuary, producing a characteristic “concentration peaks” in brackish and FSI layer (Fig. 10). The two “hot-spots” observed in summer, located at 5<sup>th</sup> km (Skradin village) and 20<sup>th</sup> km (Šibenik bay) can be easily distinguished. Compared to concentrations in winter period at these two sites, dissolved Cu increased for 3× (up to ~20 nM) in Šibenik bay, whereas at more upstream site (Skradin village, southern entrance to the National park) an increase of 7× (up to ~15 nM) occurred. Enhanced Cu concentrations in summer period are primarily the result of intensive touristic activities, i.e. nautical traffic of pleasure/recreational boats. As majority of boats is protected by antifouling paints containing Cu as an active biocide component, it is not surprising that such a high increase of its concentration occurred in the surface layer. The exact intensity of nautical traffic (number of boats passes) is unknown for the period of sampling, however our recent video monitoring survey conducted in 2014 (data not presented) showed more than exponential increase of boats in period from January to August (~10× increase). In the summer season peak

(July/August), between 500 and 1500 boats (depending on daily weather conditions) are passing every day across the estuary. Unfortunately, due to the unknown number of boats, their average size/area and the duration of their stay within the estuary, it is not possible to calculate the total budget of Cu released in that way.



**Figure 10.** Distribution of dissolved Cu concentrations in relation to salinity and distance for the three layers, in winter and summer period. Dashed line represents theoretical dilution line.

As observed for some other metals, dissolved Cu concentrations in the seawater layer increased continuously in upstream (landward) direction as a result of progressive accumulation of Cu. The higher increase observed in summer (~3-fold increase compared to seawater end-member) than in winter (2-fold) is expected due to the higher contribution of Cu from the surface layer by downward vertical transport. A decrease of Cu in the cuvette (Fig. 10) at the first site (KE-1) in summer, already discussed along with Cd behaviour, could be related to the occurrence of anoxic conditions and likely formation of sulphide precipitates.

Due to the known role of Cu in natural waters related to bioavailability/toxicity to aquatic organisms ([Fichet et al., 1998](#); [Santore et al., 2001](#); [Ytreberg et al., 2011](#)), it should be examined according to relevant ecotoxicologically based marine acute water quality criteria for metals intended for protection of coastal areas. Such criteria were recently proposed by Duran and

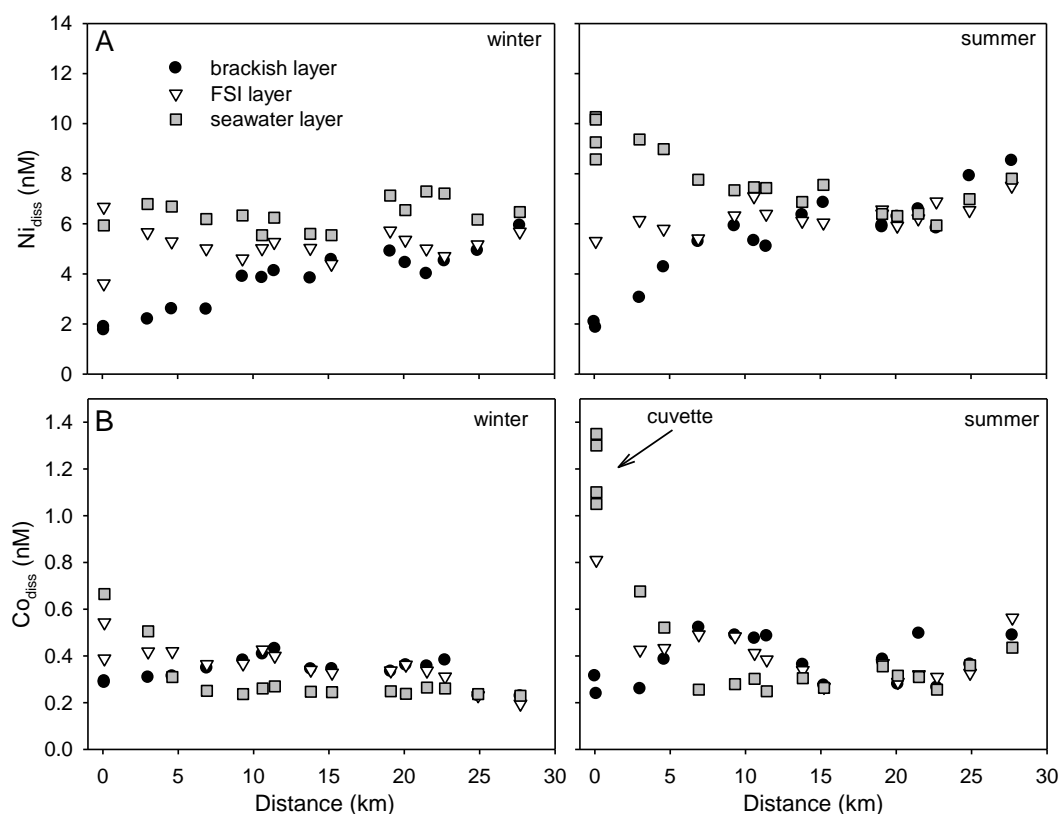
Beiras (2013) ([Duran and Beiras, 2013](#)) and quote 22 nM of dissolved Cu as the upper limit. This is equally to the highest concentrations we measured along the estuary transect (excluding highly elevated sites within the Šibenik bay already obtained during mapping survey), warning on caution if the intensity of nautical traffic will further increase. However, taking into account that dissolved Cu concentration within the Šibenik bay increased up to 68 nM and that the DOC concentration remained at level of  $\sim 1.5 \text{ mgC L}^{-1}$ , potential harmful effects are likely to occur for some sensitive plankton species, which are commonly adapted to “pristine” environmental conditions, as it was shown by Jean et al. (2012), who found that zooplankton diversity/abundance variation is linked to the pollution gradient ([Jean et al., 2012](#)).

**Nickel (Ni) and Cobalt (Co).** Among the examined metals, only dissolved Ni concentrations followed nearly-conservative behaviour with the salinity (data not shown). Starting with  $\sim 2 \text{ nM}$  at the freshwater end-member, dissolved Ni increased up to  $\sim 6 \text{ nM}$  in winter and up to  $\sim 8 \text{ nM}$  in summer at the seawater end-member (Fig. 11A). These concentration ranges fully agree with those measured by Elbaz-Poulichet in 1988 ([Elbaz-Poulichet et al., 1991](#)). A good agreement with the theoretical dilution line is consistent with the observed stable distribution of Ni concentrations within the Šibenik bay, i.e. an absence of the anthropogenic input. Both, conservative ([Elbaz-Poulichet et al., 1996](#); [Elbaz-Poulichet et al., 1991](#); [Fu et al., 2013](#); [Hatje et al., 2003a](#); [Paucot and Wollast, 1997](#)) and non-conservative behaviour of Ni in the salinity gradient was reported in the literature ([Koshikawa et al., 2007](#); [Oursel et al., 2013](#)), however different “nature” and composition (concentrations in end-members and SPM) of these systems prevent direct comparison of processes regulating Ni behaviour in relation to salinity.

Vertical concentration profiles of Ni follow the horizontal one, i.e. concentration increases with the depth/salinity. A typical upstream increase of concentrations in the seawater layer was also observed for Ni.

Horizontal profiles of dissolved Co showed less defined profiles compared to the other metals (Fig. 11B). While in winter concentration in the freshwater end-member ( $\sim 0.3 \text{ nM}$ ) was higher than in the seawater end-member ( $\sim 0.2 \text{ nM}$ ), higher dissolved Co ( $\sim 0.4 \text{ nM}$ ) was measured in summer in the seawater end-member. A slight positive deviation from the theoretical dilution line was observed in winter (data not shown). A release of dissolved Co was also observed in mixing zones in studies of other authors ([Oursel et al., 2013](#); [Takata et al., 2010](#)). However, a positive deviation up to salinity 25 in summer was followed by negative one ([Fu et al., 2013](#)), showing complex behaviour of Co. As for Ni, there is no additional anthropogenic input of Co in the Šibenik bay.





**Figure 11.** Distribution of dissolved (A) Ni and (B) Co concentrations in relation to distance for the three layers, in winter and summer period.

Similarly to other metals, horizontal profiles of dissolved Co in the seawater layer are characterized by a strong upstream increase (>3-fold increase). Contrary to Cd and Cu for which a decrease of concentration with depth in the “cuvette” at the first site (KE-1) was observed, dissolved Co strongly increased. The increase of Co concentration in “cuvette” is coupled also with an increase of its dissolved Co fraction (88% average in cuvette vs. 77% average for stations 2 to 5). Such behaviour could be related to the link between Co and Mn cycles, i.e. remobilisation of Co due to the reduction of settled  $MnO_2$ -containing particles as previously reported in hypoxic-anoxic waters ([Canavan et al., 2007](#); [Rigaud et al., 2013](#)). Additional study will be required in order to validate this observation.

#### 4.3. Partitioning of trace metals

With the exception of Pb which is described earlier in the text, concentrations of trace metals in particulate fraction are in most of the samples below 20% (see Fig. SI-9). Slightly higher average particulate fractions obtained in summer samples are mainly the consequence of the different SPM nature (more biogenic). Due to the relatively low total quantity, any release of metals from particles to dissolved phase in salinity gradient would be hardly noticeable if

sample treatment as well as the accuracy and precision of analytical technique are not “ideal”. At the level of metals measured in estuary, the uncertainty of applied analytical techniques is usually up to 10%. Thus it is reasonable to expect relatively high uncertainties in parameters related to particulate fraction, such as distribution coefficient  $K_D$ . As is already mentioned in the Introduction,  $K_D$  depends on various parameters. Consequently, taking into account that our data cover two contrasting periods of the year, a large salinity range and different physico-chemical characteristics of each separate layer, the wide range of  $\log K_D$  values obtained in our work was in a way expected. The variability of elements partitioning related to the fluctuating  $K_D$  could be perceived by plotting the relationship of particulate metal fraction (in %) on SPM concentration (Fig. SI-10) along the lines representing theoretically expected values for a defined  $\log K_D$  ([Deycard et al., 2014](#); [Oursel et al., 2014](#)). Relatively high dispersion of values shows complexity, but also “sensitivity” of the studied estuary, in which even small change in either SPM concentration or their nature can be strongly reflected on  $K_D$  value. As is mentioned above, the propagated uncertainty in the parameters estimate due to very low concentrations of metals and SPM contributed to the dispersion of data, as well.

If averaged for all samples, the order of  $\log K_D$  values ( $\text{Pb} > \text{Zn} > \text{Co} \sim \text{Cu} > \text{Ni} \sim \text{Cd}$ ) generally agrees with those of other authors ([Chiffoleau et al., 1994](#); [Fu et al., 2013](#); [Hatje et al., 2003a](#); [Munksgaard and Parry, 2001](#)). Comparing to results obtained by Fu et al. (2013) for the East Hainan estuary having similar levels of metal concentrations and SPM (Table SI-4), our values are slightly lower, indicating in average a different composition of SPM. However, rather than averaging data in systems with changing chemical composition, sources of elements supply and SPM, it is more interesting to examine data for possible existing trends in relation to other changing parameters (e.g. SPM, salinity). Among the six possible “independent” variations of  $\log K_D$  for each metal in relation to various parameters (salinity, distance, SPM), only data for winter campaign and brackish layer showed well-defined tendency for all metals: decrease of  $\log K_D$  with the distance, i.e. with salinity and/or SPM increase (see below).

#### 4.3.1. Distribution of $K_D$ in the surface (brackish) layer

In papers of Turner and Millward (2002) ([Turner and Millward, 2002](#)) and Benoit et al. (1994) ([Benoit et al., 1994](#)), authors provided empirical equations which describe the dependence of  $K_D$  on salinity ( $K_D = K_D^\circ (S+1)^{-b}$ ) (where  $K_D^\circ$  applies for the freshwater end-member) or on SPM ( $\log K_D = b + m \times \log \text{SPM}$ ) on which data could be fitted in order to extract optimized parameters. The decline in  $K_D$  with SPM increase observed primarily in systems with high SPM is known as a particle concentration effect (PCE) ([Benoit et al., 1994](#)), ([Benoit et al., 1994](#); [Jiann et al.,](#)

2005; Robert et al., 2004). It is not worthwhile to expect that only one parameter is controlling the observed behaviour, but rather the combination of different parameters (for each metal). The fact that salinity and SPM concentrations usually covariate makes the individual interpretation of the influence of each variable on the  $K_D$  distribution complicated (Hatje et al., 2003a). Thus, rather than to separately fit data to the above two equations, we calculated for each metal a theoretical relationship of  $\log K_D$  vs. SPM, presuming a conservative mixing within the salinity gradient, based on the following equation:

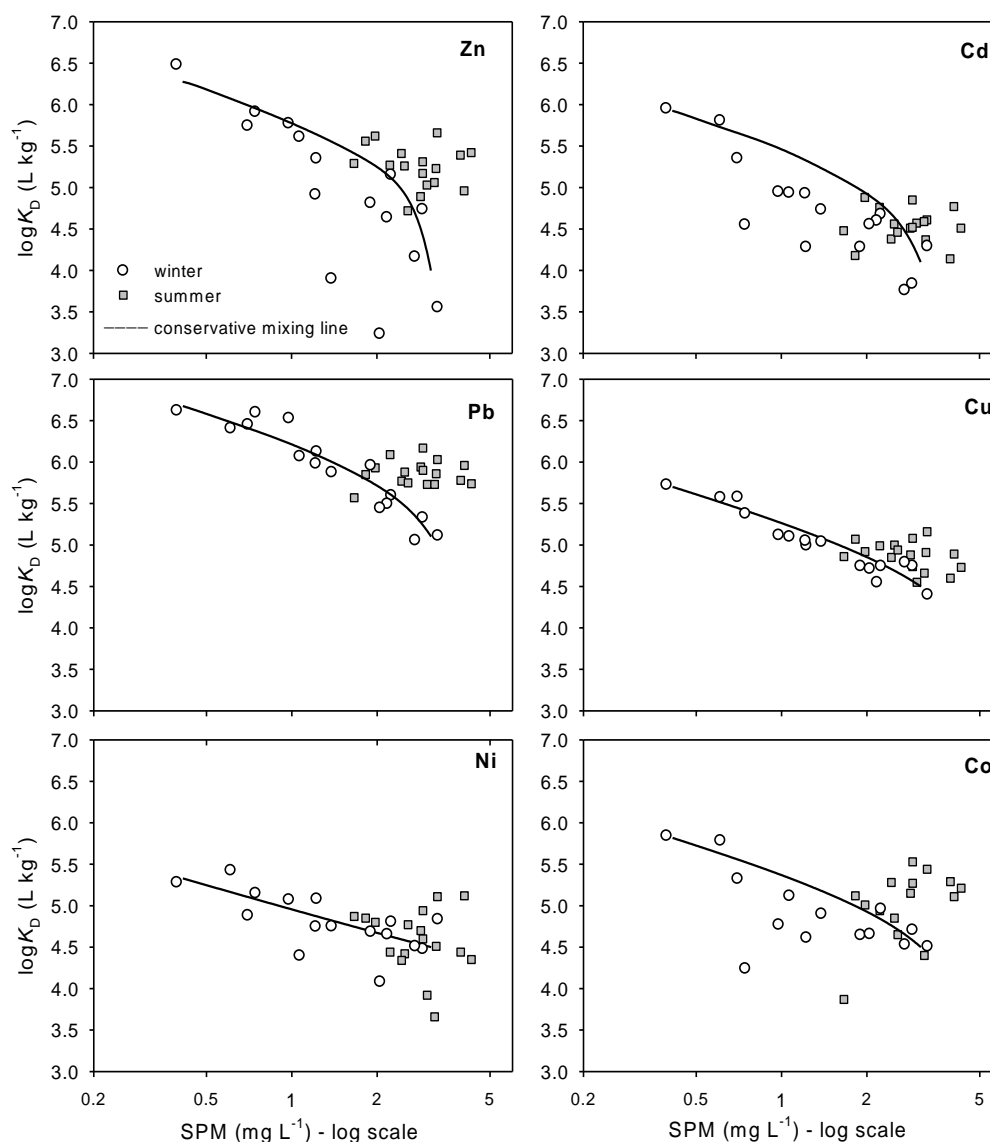
$$K_{D,i} = \left( SPM_F \cdot \frac{S_{SW} - S_i}{S_{SW}} \cdot K_{D,F} + SPM_{SW} \cdot \frac{S_i}{S_{SW}} \cdot K_{D,SW} \right) / \left( SPM_F + S_i \cdot \frac{SPM_{SW} - SPM_F}{S_{SW}} \right),$$

where  $S$  is salinity, while subscripts “F” and “SW” denote freshwater and seawater end-members, respectively.

Positive or negative deviation from the expected theoretical curve will allow identifying additional factors controlling the partitioning of TM within the salinity gradient, i.e. between the two end-members. Fig. 12 shows obtained values of  $\log K_D$  in relation to SPM (symbols) and calculated theoretical curves (full line). A good agreement between experimental and expected  $\log K_D$  values for Pb, Cu and Ni was obtained (Fig. 12), indicating that the decrease of the  $\log K_D$  with the SPM (e.g. salinity) is a simple result of the conservative mixing of the SPM from the two end-members, characterised by different properties towards these metals (i.e. different  $K_D$ ) due to their contrasted origin/nature. Consequently, even if Cu and Pb are submitted to significant anthropogenic inputs in the Krka estuary, their dissolved/particulate partitioning is mainly controlled by the proportion and affinity of riverine vs. marine particles and less by the salinity changes. However, for Cd, Co and Zn, majority of experimental points lie below the expected theoretical curve, pointing on additional physico-chemical processes influencing the redistribution of metals between dissolved and particulate phase. While for Cd and for Zn such deviation could be explained by the formation of chloro-complexes (salinity and SPM positively correlate) (Hatje et al., 2003a; Waeles et al., 2005) (Dabrin et al., 2009; Deycard et al., 2014; Elbaz-Poulichet et al., 1996; Fu et al., 2013), for Co this is less expected considering its inorganic speciation. Contrary, no evident trends for other metals, or even increasing trend with salinity (Fe and Pb) were found (Fu et al., 2013). Organic complexation ascribed for decrease of  $\log K_D$  of Cu (Paulson et al., 1994) is not assumed to play a significant role in our case due to low absolute change of DOC in the salinity gradient.

A clear distinction between freshwater and seawater derived SPM properties noticed in winter (independent of salinity effects) was not observed in summer samples which are characterized by scattered distribution of  $\log K_D$ . Slightly higher average  $\log K_D$  within the observed range of

SPM (Fig. 12, squares), imply that higher biogenic component of SPM in summer (due to higher phytoplankton productivity, as evidenced by POC content, Fig. 4B) increased affinity towards metals. However, a common influence of other factors/processes (e.g. analytical performance, biological uptake of metals, temperature, etc.) most probably contributed to the observed variability.



**Figure 12.** Distribution of partition coefficient ( $\log K_D$ ) in brackish layer for winter (circles) and summer (squares) periods in relation to SPM. Full lines represent theoretically expected relationship based on the conservative mixing (see the text for details).

#### 4.3.2. Distribution of $K_D$ in the bottom (seawater) layer

Besides mentioned winter trends in brackish layer, if examined in a view of PCE, a completely opposite trend in seawater layer in both seasons were observed for Zn and Pb (an increase of  $K_D$  with SPM increase; data not showed). If examined according to distance, particles in upper estuary region (longer residence time) are characterized by stronger affinities toward metals,

which is clearly reflected on the partitioning of Pb and Zn. Benoit et al. (1994) related the PCE effect to the presence of Fe and Al colloids, which also caused the presence of Pb and Zn largely in a colloidal fraction. A strong positive correlation of the particulate Pb and Zn fraction with the Fe colloids were found ([Benoit et al., 1994](#); [Dassenakis et al., 1997](#); [Fu et al., 2013](#)), while in addition, a positively correlation was found between particulate Pb and Mn ([Admiraal et al., 1995](#); [Dassenakis et al., 1995](#)). Fe and Mn were not measured in our study, but as high concentrations of Mn and Fe were found in the estuary sediments ([Bilinski et al., 1996](#); [Cukrov et al., 2008b](#)), formation of colloidal Fe and Mn is likely to occur. Progressive accumulation of particles of biogenic origin having higher specific surface could also explain an observed upstream increase of the  $K_D$  of Zn and Pb.

## 5. Concluding remarks

The Krka River estuary is an atypical microtidal estuary characterized by a very low input of trace metals (TM), suspended particulate matter (SPM) and organic carbon (OC) by the river. From the other side, the adjacent coastal sea features the environment of an open sea. These particular characteristics, coupled by the three vertically stratified layers, allowed identification of pathways and processes governing horizontal and vertical redistribution of TM across the whole estuary, whether they are added into the system by a human activity or by “in-situ” processes (e.g. scavenging). In addition, the “calm” nature and the “sensitivity” of the estuarine system allowed recognition of a few phenomena and events which influence the distribution, but also the biogeochemical cycling of TM within the estuary.

The obtained results could be summarized by a few final remarks:

- non-conservative behaviour of both dissolved and total Zn, Cd, Pb and Cu between the two end-members were mainly caused by the anthropogenic inputs (nautical marina, harbour), especially highlighted in summer period as a consequence of intensive nautical activities,
- due to the specific hydrodynamics of the vertically stratified layers, TM introduced at the “point source” locations were redistributed in downstream, but also in upstream direction causing pyramidal spatial distribution in the surface layer,
- TMs introduced in the surface layer were transported downward into the FSI and deeper layers and further more upstream resulting in their progressive accumulation coupled with the increased residence time of seawater,
- with the exception of Pb, for which dissolved fraction accounted from 20% in the upper, to 80% in the lower estuary region (in all three layers), the spatial distribution of other

dissolved metals was fairly uniform at the level of >80%, and independent on the season or salinity,

- it was presumed that dissolved/particulate fractionation of TM (expressed by the distribution coefficients) is driven by the variation of SPM nature along the salinity gradient (winter, surface) and the biogenic production of SPM (summer, surface),
- the specific TM behaviour identified in the cuvette, a consequence of anoxia conditions induced by long water accumulation and settling of organic-rich particles, is a situation not only limited to this restricted area but can occur at a larger scale in the estuary, and more generally, in many environments submitted to both, strong stratification and significant organic carbon load,
- the significant anthropogenic TM inputs (e.g. Cu leaching from antifouling paints) in such enclosed and relatively clean environment may induce harmful effects on sensitive species such as phytoplankton not “adapted” to such conditions.

A more focused and detailed studies would be needed in order to fully explore and understand each of the observed specific TM behaviours and their effect onto TM chemical speciation and fate. Based on the summarized observations and findings, it could be concluded that the Krka River estuary hosts several particularities which make it a model site for studies on behaviour and fate of TM as well as of their biogeochemical cycle, representative for much larger coastal unpolluted aquatic systems, but also for an open sea.

## **Acknowledgements**

AMC, IP and DO were supported by the Ministry of Science, Education and Sport of the Republic of Croatia, within the project “Interactions of Trace Metals in Aquatic Environment”. CG and BO were supported by ANR CES MARSECO and MERMEX-WP3-C3A projects. This joint work was strongly facilitated by a PHC COGITO project (founded by Croatian and French ministries of science) and by University of Toulon via invited professor positions (“invited months” for IP and DO). We acknowledge Neven Cukrov and Fred Bobek (Honda Marine Croatia) for support in organization of the sampling boat.

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