Trace Metal Detection in Šibenik Bay, Croatia: Cadmium, Lead and Copper with Anodic Stripping Voltammetry and Manganese *via* Sonoelectrochemistry. A Case Study

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The vertical profiles of the concentration of reactive Mn and total concentrations of Cd, Pb, and Cu ions in the water column of the Šibenik Bay (Krka river estuary) were determined. The measured ranges of concentrations are: 60-1300 ng l^{-1} for Mn, 5-13 ng l^{-1} for Cd, 70-230 ng l^{-1} for Pb, and 375-840 ng l^{-1} for Cu. These values are comparable with the concentrations found in the unpolluted estuaries. The Krka river estuary is highly stratified, with the measured salinity gradient of 20% within a half meter of the freshwater-seawater interface (FSI). The main changes in the vertical profiles of the measured parameters occur in the FSI: the temperature increases for 1 °C and the pH decreases for 0.1 unit, whereas the metal concentrations show different behaviour. Generally, Mn, Pb, and Cd ions show the increase of concentrations in the FSI, while copper concentration profile indicates anthropogenic pollution in the brackish layer caused by agriculture activities and by the paint with copper basis used as an antifoulant biocide for the ships. UV-digested samples show an increase in manganese concentrations for at least 3.5 times comparing to non UV-digested. This suggests that in natural water manganese exists mainly in the form of inert complexes and as associated to particulate matter (about 70-80%). UV irradiation has no influence on the concentration of cadmium, while for lead an increase of 50% in the seawater layer is observed. The twofold increase of the copper concentration in the upper freshwater layer and at least the fourfold one in the seawater layer were measured in the UV-digested samples. These results show that copper is strongly bound to inert complexes, and that UV-digestion is necessary step in determination of the total metal concentrations in natural water samples. No significant increase of the metal concentrations in the deeper seawater layer was observed, indicating the absence of the processes of remobilization or dissolution of metals from the sediment. Presented results confirm that the new method for the determination of manganese by CSV on boron-doped diamond electrode with ultrasound enhanced accumulation can be successfully applied to natural waters.

Keywords: Sono-electrochemistry, Anodic stripping voltammetry, Manganese, Lead, Copper and Cadmium, Seawater, Estuary

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INTRODUCTION

Biogeochemistry of trace metals has important role in the ecological status of the natural waters. The total concentration is an indicator of the pollution of the system, but more important is the distribution of different forms and species of the metal. The uptake of the metal ions by living organisms, the association of metals with particulate matter and their removal form the water column as well as the rates of all these processes depend on the physico-chemical species/forms which are determined by the composition of the water system (pH, salinity, redox potential, etc.) [1-5].

In many studies, the trace metals in the rivers and estuaries are measured either as the dissolved ions (which is operationally defined as a content of the fraction of the water sample that passes through the 0.45 μm filter) or as the particulate metal forms (which remain on the filter) [6-12]. The distribution of trace metals in the water column between dissolved and particulate forms is a consequence of the change in the chemical and physical parameters, and there is no universal pattern of trace metal behaviour in estuaries [13,14]. The majority of perturbations are the result of the change of chemical species distribution with the salinity, and the change in the composition of the suspended matter. However, for better understanding of the biogeochemical cycle of trace metals in the particular natural system, the determination of as many as possible metal species/forms is a benefit [11]. The special attention in the ecological studies is devoted to toxic metals (*e.g.* Hg, Cd, Pb) and to essential metals (*e.g.* Cu, Fe, Mn, Zn).

In this work the concentrations of some trace metals (Mn, Cd, Pb, Cu) in the water column of the Krka river estuary (the Croatian Adriatic coast, see Fig. 1) are reported. Many studies were performed in this estuary, but there were no measurements of manganese in the water column, and only one paper [6] and few abstracts of the lectures presented on the symposia deal with the determination of other trace metals (except mercury). For the determination of manganese ions, a new method was applied that was based on the electro-deposition of MnO_2 on a bare boron-doped diamond electrode, combined with differential pulse cathodic stripping voltammetry [15]. Power ultrasound was used during the pre-concentration step to enhance the sensitivity [16].
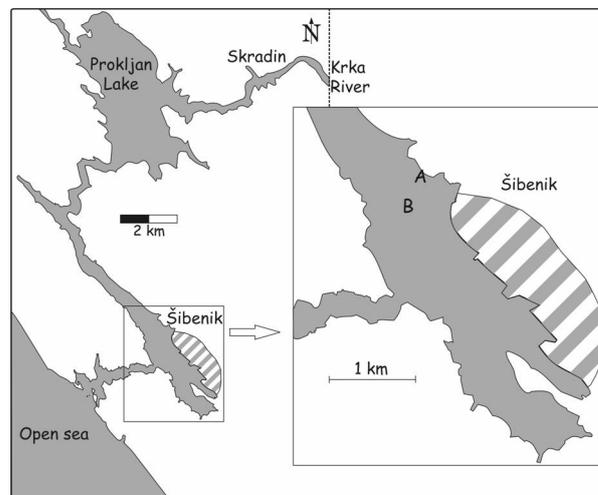
Fig. 1. The map of the Krka river estuary with the sampling sites A and B (the inset).

The aim of this work was to obtain the first reliable data for the manganese concentration level in water column in the vicinity of the former ferromanganese factory. Concentrations of manganese are compared with the concentrations of cadmium, lead and copper ions determined by the anodic stripping voltammetry.

Manganese is one of the most abundant transition metals in the earth's crust [17]. In natural waters, the biogeochemical cycling of manganese is essentially controlled by redox transformations between dissolved Mn(II) ions and insoluble oxide MnO_2 and oxyhydroxide MnOOH [1]. In oceans, the concentration of Mn(II) ions decreases from 2×10^{-9} M at the surface to 2×10^{-10} M in deep waters [18]. This distribution is caused by surface inputs and the scavenging to particulate phases. Due to the terrestrial inputs, the concentration of Mn(II) in the near-shore seawater increases to about 5×10^{-9} M [19,20]. Hydrothermal emanations may increase Mn(II) concentration near the ocean floor up to 5×10^{-8} M [21]. Also, the concentrations of Mn(II) as high as 10^{-5} M appear in anoxic waters [22]. Important sources of dissolved Mn(II) ions are tidal flushing from sediment pore waters [23] and estuaries [24,25]. In the Seine river estuary, the concentration of Mn(II) decreases from 3×10^{-7} M, at S = 10%, to 1×10^{-7} M for S > 30% [24]. In seawater, manganese is oxidized to insoluble oxide and oxyhydroxide which form colloidal suspensions,

amorphous solid particles mixed with iron oxides, and coatings on other minerals [26]. These particles are covered by a film of organic compounds, adsorbed metal ions and attached microorganisms [27]. Thus, they are efficient scavenging agents. Besides, dissolved Mn^{2+} ions form strong ion pairs with bicarbonate and carbonate ions and coprecipitate with calcite [28]. In surface waters, suspended particles of manganese oxides undergo bacterially mediated photoreduction and this process is believed to largely control the solubility of manganese in seawater [29].

There are several electroanalytical methods for the determination of dissolved manganese ions in natural waters. Anodic stripping voltammetry is based on the electroreduction of Mn(II) ions on a hanging mercury drop electrode at -1.7 V *vs*. Ag/AgCl/KCl (3 M), and on the subsequent electrooxidation of the metal deposit by differential pulse voltammetry [30,31]. In the method of electrochemical enrichment, the deposited metal is electrooxidized at -1.3 V for a short period and the reformed manganese(II) ions are determined by differential pulse cathodic polarization scan [32]. In the oxidative potentiometric stripping analysis, the metal deposit is oxidized by the mercury ions added to the solution, and the electrode potential is measured as a function of time [33]. In the reductive version of this technique, Mn(II) ions are electrooxidized on glassy carbon electrode at 0.8 V and deposited as MnO_2. Then, the deposit is reduced by hexacyanoferrate(II) ions added to the solution [34]. The same accumulation procedure is applied in the cathodic stripping voltammetry, but in this case the deposit is electro-reduced by the differential pulse voltammetry [35]. Alternatively, Mn(II) ions can be chelated by eriochrome black T and adsorptively accumulated on a hanging mercury drop electrode at -0.8 V, which is followed by the cathodic stripping voltammetric detection [36]. However, only differential pulse anodic stripping voltammetry at a mercury film electrode [37] and the method of electrochemical enrichment combined with square-wave voltammetry [38] can be used for the analysis of seawater samples.

EXPERIMENTAL

Reagents

All reagents were used as received without any further purification. These were as follows: HNO_3 suprapur, Pb^{2+}, Cd^{2+}, and Cu^{2+} standard solutions in 1 g l^{-1}, all by Merck, sodium acetate buffer solution (pH 5.2, Aldrich) and standard buffer solutions pH 4 and pH 8 (Kemika, Zagreb), manganese(II)-sulphate and ammonium nitrate (99%) (both Kemika, Zagreb) and hydrochloric acid (35%, Aldrich). All solutions were prepared with water deionised by ionic exchangers Millipore Milli Q until its specific resistance was 18.2 MΩ cm.

Standard for the trace metals in seawater was NASS-5 (National Research Council Canada). This is a seawater collected from the North Atlantic ocean at a depth of 10 m, 35 km southeast of Halifax, NS, Canada (salinity 30.4%, acidified to pH 1.6 with ultra trace nitric acid).

Instruments and Cells

Voltammetric measurements were carried out by using PGSTAT12 and PGSTAT30 AUTOLAB potentiostats (both Eco-Chemie, Utrecht, The Netherlands).

For manganese measurements a three electrode arrangement was used in the electrochemical cell with a boron-doped diamond disc (5 mm diameter, Windsor Scientific Ltd., UK) serving as the working electrode, and a large area bright platinum wire used as the counter electrode. A saturated calomel reference electrode (Radiometer, Copenhagen, Denmark) completed the circuit. The working electrode was polished in between the experiments using diamond-lapping compounds, and cleaned in 2 M HNO_3 in a sono-bath (Bandelin, Sonorex) for 2 min. The ultrasonic horn model CV 26 (Sonics and Materials Inc. USA) with operating frequency of 20 kHz was used. It was fitted with a 3 mm diameter titanium alloy microtip (Jencons, Leyton Buzzard, UK). The intensity of the ultrasound used in this work was 34 W cm^{-2}. The working electrode and the ultrasonic horn were placed in a face on arrangement. The distance between the tip of the horn and the electrode surface was 12 mm.

For Cd, Pb, and Cu measurements the working electrode was Static Mercury Drop Electrode (VA stand 663, Methrom, Herisau). An area of the working mercury drop electrode was 0.25 mm^2. The Pt wire was an auxiliary electrode and Ag/AgCl/NaCl/(sat.) was the reference electrode in these measurements.

Table 1. Results of Analysis of Standard Reference Material NASS-5

	[Mn] (nM)	[Cd] (nM)	[Pb] (nM)	[Cu] (nM)
Found	17.3 ± 0.5	0.15 ± 0.01	0.047 ± 0.017	4.74 ± 0.38
Certified	16.7 ± 1.0	0.20 ± 0.03	0.039 ± 0.024	4.67 ± 0.72

Sonoelectroanalysis of Mn

Acoustically assisted electrodeposition of MnO_2 on a boron-doped diamond electrode was performed at +0.85 V for 240 s. The samples were analysed as collected, without filtration and pH adjustment since the stripping peak is suitably positioned in the potential window at the pH of natural seawater. Furthermore, the ultrasound serves to keep the electrode active *via* an acoustic cavitation from any surface passivating material that may be present in the sample. The cathodic stripping of the accumulated MnO_2 was performed by differential pulse voltammetry with the following parameters: a pulse height 150 mV, a pulse duration 0.02 s, an interval between pulses 0.1 s, a step potential 10 mV and a standby potential 0 V. The concentration of Mn(II) ions was determined by the standard additions method. A typical example of the voltammograms is shown in Fig. 2.

The precision of the method was verified by a certified reference material NASS-5 (see Table 1). The nominal concentration of Mn(II) ions in this standard is $(1.67 \pm 0.10) \times 10^{-8}$ M. Before sonoelectroanalytical measurements, the pH of the standard was adjusted to pH 7 with sodium hydroxide. Using an insonated electrodeposition of MnO_2 in the duration of 300 s, and the parameters of the cathodic stripping scan as above, the concentration of Mn(II) ions was determined as $(1.73 \pm 0.05) \times 10^{-8}$ M, which corresponds to the recovery of $(104 \pm 9)\%$. This shows that the method can be used to accurately determine the concentration of dissolved manganese in seawater samples.

Anodic Stripping Voltammetry of Cd, Pb, and Cu

The determination is carried out simultaneously for all three metals. The reductive accumulation of metals in the mercury drop electrode was performed at -0.8 V for 600 s. After 15 s of equilibration, the anodic scan is applied from
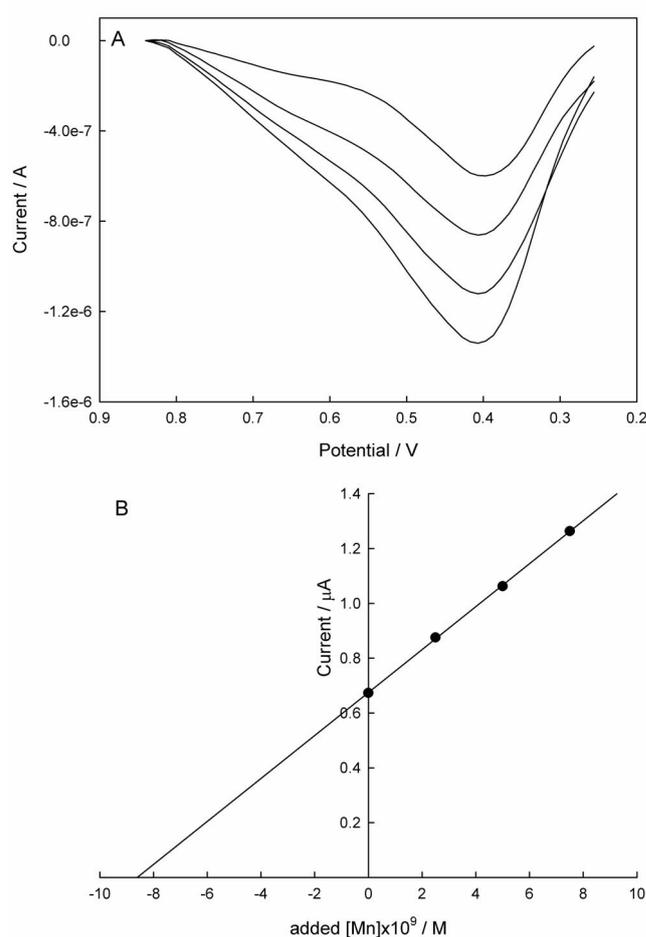
Fig. 2. An example of a typical standard addition plot (bottom) and voltammetric curves (Top) for the determination of manganese by ultrasound assisted accumulation in a seawater sample taken from the estuary. Parameters: $E_{acc} = 0.85$ V, $t_{acc} = 240$ s, $dE = 10$ mV, $t_{int} = 0.1$ s, $\Delta E = 150$ mV, $t_p = 0.02$ s.
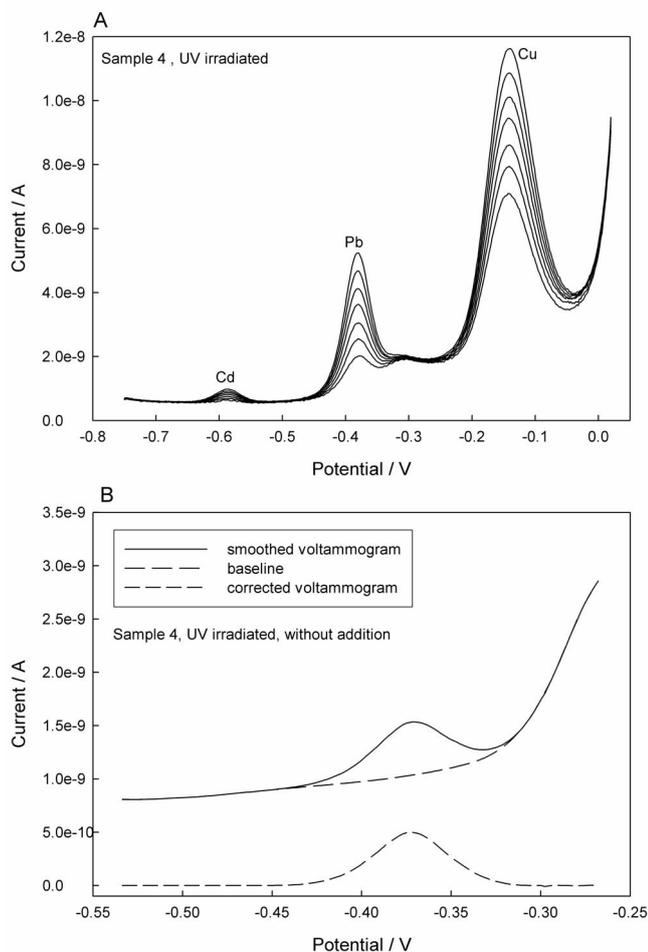
Fig. 3. (A) Typical voltammetric curves for the determination of Cd, Pb, and Cu by standard addition method using DPASV in a seawater sample (sample 4, UV-digested, additions by: Cd, 5×10^{-11} M; Pb, 5×10^{-10} M; and Cu, 2×10^{-9} M). Parameters: $E_{acc} = -0.8$ V, $t_{acc} = 00$ s, $dE = 2$ mV, $t_{int} = 0.1$ s, $\Delta E = 20$ mV, $t_p = 0.04$ s. (B) An example of baseline correction of lead peak using "smoothed spline" method.

-0.75 V to 0.02 V in differential pulse mode. A pulse amplitude 20 mV, a pulse duration 0.040 s, an interval between pulses 0.1 s and a step potential 2 mV (scan rate 20 mV s^{-1}) were used in all measurements. The enhanced accumulation of metals in the mercury drop is realized by

rotating Teflon stirrer at 3000 rpm.

The measurement is fully automated by using the Cavro XL 3000 Syringe Pump (which is driven by the home-made software) and by utilizing the features of the GPES software in project mode. The concentrations of the metals are determined by the standard addition method, by at least six additions of the standard solution (25 µl of the standard on 25 ml of the sample). The typical example of voltammetric responses for the natural water sample is shown in Fig. 3A. Recorded voltammograms are treated for reliable evaluation of peak height used for the construction of the calibration plots. Each peak is separately treated as follows (Fig. 3B)

Data are smoothed by "smoothed spline" method using the algorithm incorporated in the home-made software for data presentation, treatment and manipulation called ECDSOFT. The voltammograms are corrected by subtracting baseline current which is constructed by manually selected points on the voltammogram, and using "smoothed spline" method.

The precision of the ASV method was verified by a certified reference material NASS-5. The measured data are given in Table 1. A quite good agreement between measured and certified values was obtained, allowing us to use this method for accurately determination of the selected trace metals concentration in natural water samples. The voltammetric curves and the standard addition plots for the NASS-5 standard are presented in Fig. 4.

Sampling Area

The Krka river estuary, cut into the limestone erosionale plane, occupies the lowest part of an ancient river valley between the last active tufa barrier (the Skradinski buk waterfalls) and the Šibenik channel, in the total length of 22 km (Fig. 1). Its depth increases from 2 m, at the falls, to 42 m in the channel. An average freshwater inflow is 55 m^3 s^{-1}, with considerable seasonal and monthly variations ranging from 10 to 400 m^3 s^{-1}. The estuary is highly and permanently stratified because of its sheltered geography and a very low tidal range (0.2-0.5 m). The consequence of the stratification is a well-defined picnocline which can appear at various depths (0.5-7 m), depending on the position and freshwater inflow [39]. Due to a karstic drainage area and the lake above the waterfalls, the concentration of suspended matter in the estuary is in the range 1-5 mg l^{-1} [40], consisting primarily of seawater
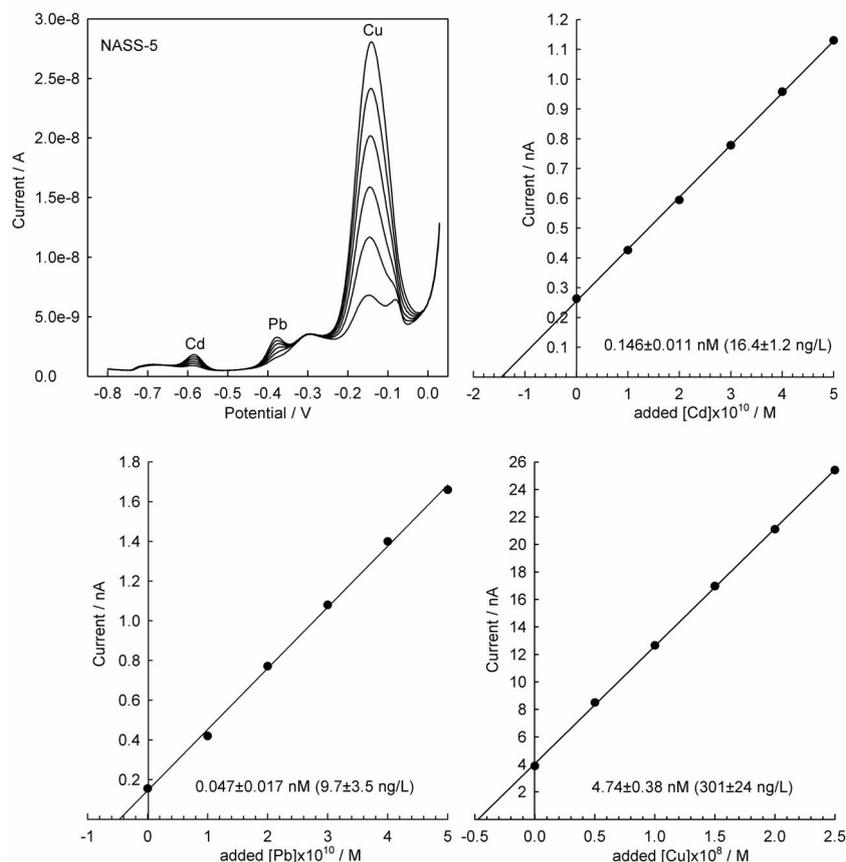
Fig. 4. DPAS voltammograms of increasing concentrations of Cd, Pb, and Cu obtained in sample of NASS-5 seawater reference standard and corresponding standard addition plots. Parameters: E_{acc} = -0.8 V, t_{acc} = 900 s, t_{int} = 0.1 s, t_p = 0.04 s, ΔE = 20 mV.

organisms, while the concentration of terrestrial particles is negligible. Carbonate sediments are covered with oxic seawater and consist predominantly of calcite with smaller amounts of aragonite, quartz, dolomite and clay minerals [41]. In the lower estuary, opposite to the Šibenik Harbor, the content of manganese in the sediment is 0.3%, but the values higher than 1% were found in the vicinity of the former ferromanganese factory. A manganese mineral kutnahorite calcian was identified as a minor component of the sediment on the latter position [42].

Sample Collections and Preparation

The samples were collected at the vertical profile on two selected stations on June 29 and 30, 2004. The sampling site A was ten meters away from the bank of the estuary, near the site of former ferromanganese factory in town Šibenik (Fig. 1, the inset). The sampling site B was in the middle of the estuary, three hundred meters from the first point. Water samples were directly collected in the preconditioned Teflon FEP bottles (Nalge Nunc International, Rochester, NY) with a total volume of 1 l, by scuba diving [43]. Before the sampling, the sample bottles were removed from the plastic bags and rinsed several times with the subsurface water. Sampling depths were controlled by a marked weighted line suspended from a service vessel. Three distinguishable water layers are clearly visible to the diver: upper brackish water, deeper saline water

Table 2. pH, Salinity (S/%), Temperature (T/°C), Depth (D/m) and the Concentrations of Measured Metals (in nM) without and with UV Digestion in the Water Column on Sampling Sites A and B

Sampling site A:

No.	pH	S	T	D	Mn	Cd	Pb	Cu
1	8.15	11	25.5	0.2	5.43/27.94	0.040/0.050	0.51/0.40	5.26/11.00
2	8.05	21	26.0	2.5	1.39/6.93	0.054/0.067	0.55/0.57	1.79/13.00
3	8.00	38	23.5	8.5	2.40/8.56	0.093/0.090	0.40/0.40	0.25/3.80

Sampling site B:

No.	pH	S	T	D	Mn	Cd	Pb	Cu
1	8.33	13	23.0	0.2	2.44	0.045/0.045	0.35/0.34	5.24/12.50
2	8.33	14	23.5	1.0	4.91	0.050/0.054	0.50/0.56	3.90/12.20
3	8.26	18	24.0	1.5	2.63	0.072/0.069	0.95/1.10	5.19/13.20
4	8.18	30	25.0	1.8	22.9	0.084/0.082	0.60/0.74	3.62/11.30
5	8.16	38	25.0	2.0	11.1	0.106/0.100	0.55/0.80	4.50/7.10
6	8.15	38	23.0	3.0	9.15	0.098/0.107	0.74/1.10	1.79/7.79
7	8.12	38	21.0	12.0	1.12	0.107/0.095	0.20/0.37	0.90/5.90
8	8.08	38	19.5	19.0	2.02	0.080/0.088	0.43/0.75	0.69/7.51

and the intermediate layer, which is of a veil-like appearance. The diver was facing the direction of the current, opening and closing the sampling bottle with outstretched hands. The temperature was measured *in situ*. Salinity was immediately determined in each sample using a refractometar (Atago, Japan). A Mettler Toledo model MP120 pH-meter was calibrated with buffer solutions 4 and 8 and was used for recording the pH of the samples.

Natural organic matter in the sample was destroyed by UV-irradiation using a 150 W UV lamp (Hanau, Germany). An aliquot of 300 ml of the sample was acidified with 1 ml of concentrated suprapur HNO_3, placed into a quartz bottle with a volume of 0.5 l and irradiated for 24 h.

RESULTS AND DISCUSSION

The concentrations of Mn(II), Cd(II), Pb(II), and Cu(II) ions along vertical profiles on the sampling sites A and B are reported in the Table 2. The samples were analysed both directly as collected (the first number) and after UV-digestion (the second number). Also, the pH, the salinity and the temperature of the samples are reported. On the site B the samples were collected in small intervals of depth, with the special attention to the freshwater-seawater interface (FSI). This is a half-metre thick layer within which the salinity increases from 18‰ to 38%, the temperature rises from 24 °C to 25 °C and pH is decreased from 8.26 to 8.16 (see Fig. 5).

The depth at which the FSI is formed is variable anddepends on the position in the estuary, on the inflow of freshwater and the weather conditions [44]. On the site B, the FSI appeared at the depth between 1.5 m and 2 m, but on the site A it was situated below 2.5 m. In the upper layer, consisting of the fresh water, the temperature, pH and salinity were variable, but in the lower seawater layer the variation of these parameters was smaller.

The concentration of Mn(II) ions in the brackish layer decreases from 5.4×10^{-9} M (298.3 ng l^{-1}) at the surface to 1.4×10^{-9} M (76.4 ng l^{-1}) near the FSI, but this distribution is not
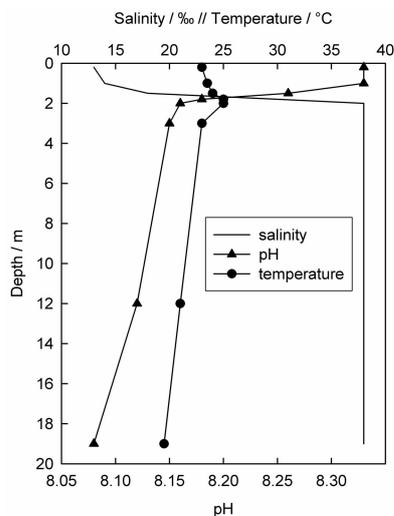
Fig. 5. Vertical profiles of pH, temperature and salinity in the water column on the sampling site B.

stable and the maximum concentration may appear at the depth of 1 m. In the seawater, well below the FSI, the concentration of Mn(II) ions is 1.1×10^{-9} M (61.5 ng l^{-1}), while near the bottom it is about 2.0×10^{-9} M (110 ng l^{-1}). These values are comparable with the concentrations reported for near-shore seawater [19,20]. but they are ten times lower than the concentrations reported for some other estuaries (*e.g.* Columbia river; [25]). This can be explained by the low terrestrial input and by the adsorption of Mn(II) ions on suspended calcite microparticles [45,46]. Calcite is the prevailing mineral in surface sediments and in the water column in the estuary [41]. It is believed that Ca^{2+} and Mn^{2+} ions are exchanged at the calcite surface [28]. After UV-digestion, the concentration of Mn(II) ions in the brackish water samples increased five times, while in the seawater sample it increased 3.6 times. This would indicate that between 70% and 80% of the dissolved manganese exists in the form of inert organic complexes and that Mn(II) ions bound to these complexes can not be electrooxidized at 0.85 V. However, there is a possibility that Mn(III) and Mn(IV) ions, present in unfiltered samples as suspended microparticles of oxides and oxyhydroxide, undergo reduction by organic matter during the UV-irradiation [29]. Also, Mn^{2+} ions bound to calcite microparticles could be dissolved in the acidic

medium. In this case, the results obtained after the UV-digestion of the samples would not depend only on the concentration of total dissolved manganese, but also on the presence of suspended manganese containing microparticles.

Within the FSI and immediately below it, the concentration of Mn(II) ions increases up to 2.3×10^{-8} M (1258.1 ng l^{-1}), as can be seen in Fig. 6A. Similar maxima of dissolved mercury ions were observed in the FSI [39,43,44]. The maximum can be connected to the accumulation of dissolved organic compounds, insoluble liquid organic aggregates and suspended mineral particles, including takanelite, $(Mn_{2/3}^{2+}, Ca_{1/3}^{2+})Mn_4O_9 \times 3H_2O$, which was observed in the FSI [39,47]. It was proposed that slag and dust from a former ferromanganese factory was partly dissolved in estuarine water and takanelite was formed by the oxidation of Mn^{2+} ions and disproportionation of manganite [42]. The organic matter accumulated in the FSI originates primarily from the decomposition of the freshwater phytoplankton [48]. This could be a main source of dissolved Mn(II) ions in the FSI because manganese is an essential micronutrient for all organism. Furthermore, it is possible that nanoparticles containing Mn(II) ions, with size smaller than 50 nm, can be partly electrooxidized during the insonated electrodeposition of MnO_2 because of relatively high mobility under these conditions. The reactivity of nanoparticles was observed during measurements of Mn(II) in anoxic lake water using anodic stripping voltammetry [49].

In the previous paper we reported that the sediment collected at the sampling site A contained 2.7% of manganese [15]. This is in agreement with earlier measurements on this position, in which kutnahorite calcian, $Ca_{0.74}(Mn, Mg)_{0.26}CO_3$, was determined as a main manganese mineral [42]. The present results do not indicate significant remobilization of Mn(II) ions from the sediment, as their concentration near bottom is much smaller than below the FSI. It appears that Mn(II) ions released inside the FSI are either oxidized, or scavenged by calcite microparticles and removed from the seawater layer.

The concentration of cadmium ions increases from 4.5×10^{-11} M at the surface (salinity ~12%) to 10.6×10^{-11} M at FSI (salinity between 30 and 38%) and slightly decreases in deeper seawater layers to 8.0×10^{-11} M, which is in the level of the reference point in the open sea (7×10^{-11} M). The typical peak
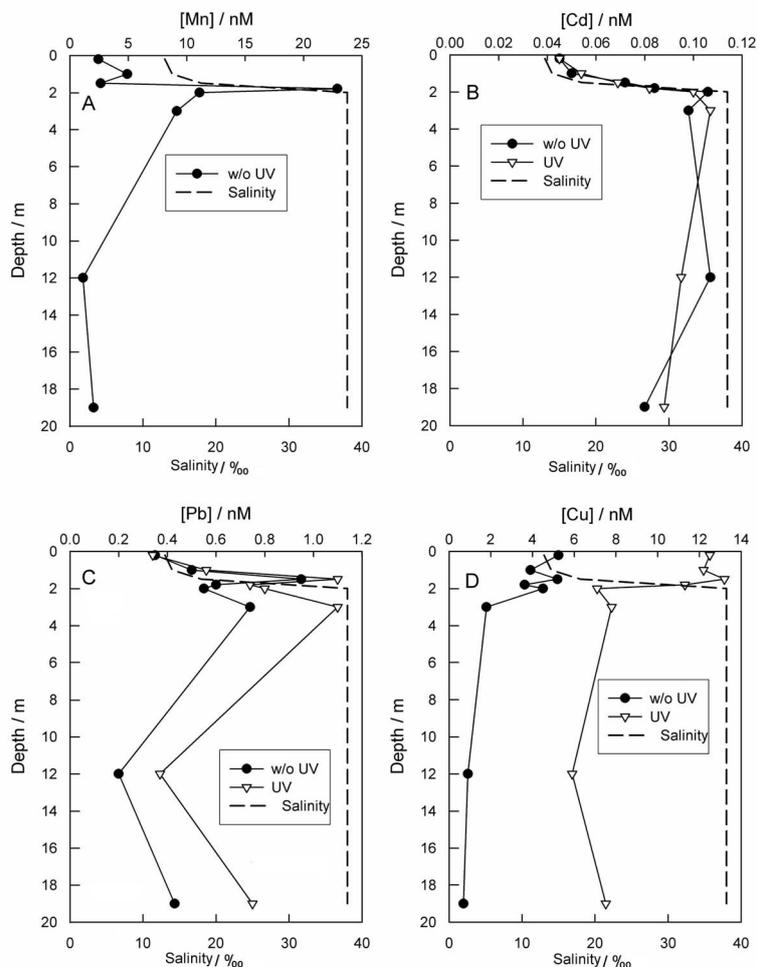
Fig. 6. Vertical profiles of Mn, Cd, Pb, Cu and salinity in the water column on the sampling site B. The concentrations are measured in unfiltered samples with or without UV digestion procedure.

of increased concentration in the FSI is not obtained, contrary to what was registered by Branica *et al.* [50] , but an increase of Cd concentrations towards FSI is noticeable (Fig. 6B). As was previously reported, the increase of dissolved organic compounds, insoluble liquid organic aggregates and suspended mineral particles appears in the range of FSI. All of these compounds can bind cadmium ions, which are liberated by the acidification of the sample. The concentration of Cd in the reference open seawater sample is almost twice of that in upper brackish layer, which is one of the reasons of the higher

concentrations of Cd in the mixed FSI layer. Fig. 7 shows the ratio of the concentration of metals in UV and non-UV digested samples (UVR). For the cadmium, this ratio varies from 0.86 to 1.10, which is in the range of experimental error. Only acidification of the sample is sufficient for complete release of the cadmium from its inert complexes and from the particles. The low concentration of cadmium ions in the upper layer is indicator of its small input in the estuary by the Krka River. Obtained values for the total cadmium concentrations agree well with the data obtained by Elbaz-Poulichet *et al.* [6]
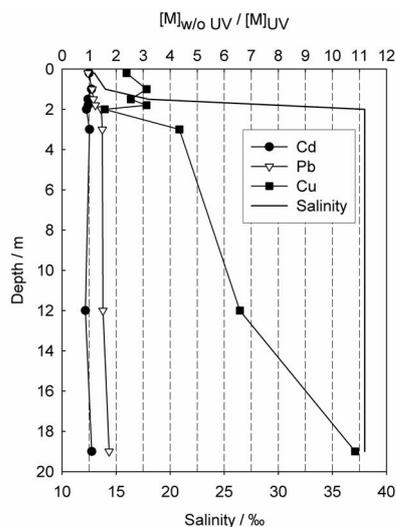
Fig. 7. Vertical profiles of the ratio of metal concentrations with and without UV digestion (UVR).

and Branica *et al.* [50] in the same estuary.

Figure 6C shows the profile of lead concentrations across the water column. The concentration of lead ions ranged from the 3.5×10^{-10} M (3.4×10^{-10} M -UV) in the upper low salinity layer, to 9.5×10^{-10} M (1.1×10^{-9} M -UV) just above the FSI. In and just below FSI, lead concentration is in the range of 6.0×10^{-10} M, while in deeper seawater layer it is 2.0×10^{-10} M (7.4×10^{-10} M and 3.7×10^{-10} M -UV, respectively). There is the second peak below FSI with increased concentration of lead ions (7.4×10^{-10} M, 1.1×10^{-9} M -UV). This peak can be attributed to the local microlayer equivalent to FSI or to the contamination of the sample. Comparing to UVR of cadmium, the UVR of lead shows an increase in the range of FSI (Fig. 7). In the upper brackish layer UVR ranged from 0.96 to 1.23, while below FSI it is in the range 1.46 to 1.74. These indicate that although the samples were acidified, there were still about 30-40% of total lead bound to strong complexes which were destroyed during UV irradiation. Although the increase of salinity favours the formation of labile lead chloro complexes, the concentration of ligands that form stable complexes with lead in the seawater layer is much higher than in the upper brackish layer.

The vertical profile of copper concentration shows different relationship with the salinity. The concentrations in the upper layers are higher than in the lower seawater layer, with the sharp decrease within the FSI (see Fig. 6D). This is in contrast with the data reported by Elbaz-Poulichet *et al.* [6] (for some sampling sites) and by Branica *et al.* [50]. However, it must be noted that sampling sites are not the same. These authors worked at sites which were several kilometres upstream, while our site is in the region of the Šibenik harbour. Elbaz-Poulichet *et al.* [6] reported some measurements for the region of our sampling site B with increased concentrations in the upper layers comparing to values obtained upstream. There are two possible sources of anthropogenic pollution: the first is agriculture in the area along the Krka river, and second is the paint on the basis of copper which is used as an antifouling biocide on the ships and boats. Namely, in the time of year when our samples were collected, the nautical tourism was increased because of the vicinity of the National park "Krka", which caused the enlargement of the traffic along estuary.

The large difference is obtained in the concentrations of copper with or without UV-digestion. The UVR ranges from 2.5 in the upper layers to 11 in the seawater layer (Fig. 7). Higher UVR in the lower seawater layers can be attributed as first to increase of the concentrations of stable metal complexes, and as second to the consequence of the smaller total copper concentration, available for complexation. The total copper concentrations (UV digested) are comparable with those reported by Elbaz-Poulichet *et al.* [6] for dissolved copper. The concentration range of copper in non-UV digested samples agrees with that obtained by Branica *et al.* [50], while the concentrations in UV-digested samples are several times higher. This suggests that the UV-digestions step is necessary for accurate determination of copper concentration in natural waters.

The comparison of UVR values of selected metals (Fig. 7) suggests that the fraction of copper destroyed by UV-digestion is much higher than the corresponding fractions of Pb and Cd. This is in agreement with the results of many authors which reported that more than 95% of Cu in seawater is complexed with ligands which form inert complexes. The stability constants with known organic ligands decrease in the following sequence Cu > Pb > Cd, which corresponds to UVR values obtained at the seawater layer.

The thickness of FSI may vary from only few centimetres

[40] to about 0.5 m, and the increase in salinity of 24% can be observed in this layer. This depends on weather conditions [44]. So, the variations in the composition of samples taken from day to day at the same site and depth can be expected. The great difficulty in obtaining reproducible samples inhibits the efforts to understand complex mechanisms that occur within the FSI.

The precise estimation of the distribution of metal ions in the water column can be obtained only by measuring different fractions of metals: total dissolved metal ions (UV-digested and acidified), their labile complexes (at natural pH) and particulate metal forms. Both electrochemical methods applied in this work have detection limits which enable such measurements.

CONCLUSIONS

The results of this study are the first measurements of manganese in water column of this estuary, while the measurements of cadmium, lead and copper are the first in the last 16 years. Although the sampling sites were situated in the part of the estuary in which we expected the higher metal concentrations (vicinity of the Šibenik Harbour), these preliminary results show that the concentrations of Mn(II), Cd(II), Pb(II), and Cu(II) ions in the brackish and seawater layers in the Krka river estuary are rather small and comparable with the concentrations in unpolluted near-shore seawaters.

As it was reported previously in several papers, the main variations of the measured parameters (salinity, pH, temperature, and metal concentrations) occur within the freshwater-seawater interface (FSI), in which the salinity increases from 18% to 38%. Within FSI, only the concentrations of Mn(II) and Pb(II) ions are significantly increased. This is explained by the release of metal ions from the decomposed freshwater phytoplankton and by the accumulation of manganese containing minerals, such as takanelite, in the FSI. The concentrations of cadmium are smaller in the upper brackish layer, than in the seawater layer, while only a small increase in FSI layer is observed. This is the consequence of the release of cadmium ions bound to suspended particles and organic complexes accumulated in this layer. The concentration profile of copper ions in water column shows the doubled values in the upper brackish layer comparing to the seawater layer. This is explained by the anthropogenic pollution, possibly caused by the agriculture activities along the Krka River and by the paint which is used as an antifouling biocide for the ships.

It is confirmed that UV-digestion step is necessary for the determination of total metal concentrations. As is expected, copper forms more stable inert complexes with the organic ligands than lead and cadmium.

Although rather high concentrations of manganese were found in the sediments, its remobilization/dissolution has no significant influence on the concentration of total dissolved manganese in the water column. The same applies for the other trace metals measured in this work.

These results confirm that calcareous Krka river has a high self-purification ability with respect to metal ions as a result of adsorption on the surface of suspended calcite microparticles.

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