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## Improved voltammetric methodology for chromium redox speciation in estuarine waters

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### Abstract

Chromium is a toxic element naturally present in natural waters whose chemical speciation regulates its cycling, mobility and bioavailability. We present here: 1- an improved analytical method for chromium speciation (Cr(VI) vs Cr(III)) in estuarine samples by catalytic adsorptive cathodic stripping voltammetric (cat-AdCSV) and 2- a study highlighting a significant change of redox speciation during summer and winter. Initial measurements first revealed that surface-active substances (SAS) present in estuarine samples strongly influenced the analytical determination of Cr by partially masking the Cr peak through an increase of the background current. We found that the application of a low negative accumulation potential (-1.65 V) resulted in much better voltammograms compared to those obtained using the usual accumulation potential of -1.0 V. Using humic acid (HA) as a model SAS of natural origin, we show that this negative potential clearly prevents adsorption of SAS on the Hg-electrode surface, which in turns benefits the adsorption of the in-situ formed Cr(III)-DTPA complex and the resulting signal. The optimised method was applied to determine chromium redox speciation and distribution along the 23 km long salinity gradient, well oxygenated, Krka River estuary (Croatia). Cr(VI) was found to be the dominant redox species in both summer and winter, with Cr(III) contribution being lower in summer (up to ~30%, average of ~5%) than in winter (up to ~50%, average of ~30%). In summer, lower concentrations of Cr(VI) were found in the freshwater end-member (2.5 nM) than in the seawater end-member (4-5 nM), while the opposite trend was found in winter. Hexavalent chromium exhibited a non-conservative behaviour along the salinity gradient for both seasons. Chromium predominantly exists in dissolved phase, and contribution of particles reactive Cr(III) was minor.

**Keywords:** chromium redox speciation, estuary, organic matter, surface active substances

## 1. Introduction

Chromium (Cr) is a redox-sensitive element which, in natural waters, predominantly exists in two stable oxidation states: trivalent {Cr(III)} and hexavalent {Cr(VI)} [1, 2]. The major interest and concern for chromium redox speciation determination in natural waters (including drinking waters) is driven by the fact that in trivalent form (+3) it is an essential element (at trace levels), whereas hexavalent form (+6) is reported to be toxic for both humans and animals (being a possible human carcinogen and mutagen), as well as for other organisms living in natural waters [3, 4]. Natural sources of chromium are varied, from ore mineral, shales, river suspended matter and soils, particularly fine grain size soils. Anthropogenic sources are mainly from metallurgy, electroplating and leather tanning [5]. Cr(VI) is the major oxidation state in oxygenated waters, whereas Cr(III) predominates in anoxic conditions. Under typical conditions in natural waters, Cr(VI) is highly water soluble and mainly exists in forms of stable oxo-compounds  $\text{CrO}_4^{2-}$  and  $\text{Cr}_2\text{O}_7^{2-}$ , and the complexation with organic and inorganic ligands is thought to be insignificant. In freshwaters, predominant inorganic forms of Cr(III) are hydro complexes ( $\text{Cr}(\text{OH})^{2+}$ ,  $\text{Cr}(\text{OH})_3$ ), while in the presence of chloride (seawater) it forms hexaquo complex ( $[\text{Cr}(\text{H}_2\text{O})_6]\text{Cl}_3$ ). Unlike the hexavalent form, Cr(III) has affinity to form complexes with natural or anthropogenic organic substances and to adsorb on suspended particulate matter [6, 7]. The oxidation state primarily depends on the aeration status of the water body. In anaerobic conditions, chromium is reduced to Cr(III) by ferrous oxide at pH above 5.5, and by hydrogen sulphide ( $\text{H}_2\text{S}$ ) if pH is below that value [8].

The two major issues related to the redox speciation of chromium are identified as environmental [9] and methodological [10, 11]. The ratio of concentrations of Cr redox species in natural waters is highly variable depending on the specific physicochemical conditions of the water column (pH, redox potential, oxygen concentration, presence of appropriate reducers/oxidizers, photochemical redox transformations, mediators acting as ligands or catalysts). However, some experimental evidences show that their actual ratio could deviate from theoretical predictions [12].

Alike other metals, chromium speciation methodology usually involves the following steps: 1. sampling, 2. preservation/storage, 3. species preconcentration/separation, 4. species detection. Each of these steps can modify the natural speciation distribution; the goal is thus to minimize their influences. The first issue of concern for Cr speciation in natural water is sample storage, i.e. preservation of its original concentration and redox speciation. The typical storage conditions for metals are acidification to  $\text{pH} < 2$  (if only the dissolved concentration has to be

70 determined), or at natural pH (if speciation is of primary interest). However, this storage  
71 scheme is not adequate for Cr. On one hand, at natural pH of  $\sim 8$ , Cr(VI) is stable (especially  
72 under a CO<sub>2</sub> blanket [13]) but Cr(III) is rapidly (minutes to hours) removed from the solution  
73 due to adsorption on the container walls. On the other hand, in acidic conditions, Cr(VI)  
74 could be reduced to Cr(III) by the oxidation of organic matter. In addition to these storage  
75 issues, the relatively low Cr concentration encountered in natural waters (0.1 - 16 nM in  
76 seawater, 0.5 - 100 nM in freshwater) is also presenting an analytical challenge.

77 As a result, there are only scarce studies [3, 14-16] that describe the behaviour and actual  
78 distribution of Cr(III) and Cr(VI) in the aquatic environment. Thus, field studies and  
79 laboratory model experiments under well-controlled conditions are of importance to help  
80 improving our understanding of chromium behaviour and its environmental impact in natural  
81 aquatic systems.

82 From an analytical point of view, the most used techniques for chromium redox speciation  
83 measurements in natural waters are high performance liquid chromatography hyphenated to  
84 inductively coupled plasma mass spectrometry (HPLC/ICP-MS) [17] and the catalytic  
85 adsorptive cathodic stripping voltammetry (Cat-AdCSV) [10, 18-20]. Despite numerous  
86 variations of the latter, Cr speciation still remains a challenging task and there is a need for  
87 improvement of existing analytical procedures [20-24].

88 This work is aiming to: (i) develop an improved Cat-AdCSV procedure for Cr determination  
89 in samples having a high concentration of organic matter and surface active substances (SAS)  
90 and (ii) use this procedure to determine the distribution and behaviour of Cr redox species  
91 along the salinity gradient of an estuary (Krka, Croatia).

## 92 93 **2. Study site**

94 Krka River and its estuary are part of National Park Krka, which is situated on the eastern  
95 coast of Adriatic Sea (Croatia). The river is characterized by numerous lakes formed by tufa  
96 barriers, each finishing with waterfalls. Measured flow in Krka River over the last 50 years  
97 range from 5-450 m<sup>3</sup>s<sup>-1</sup>, while in the period from 2001 to 2013, the average annual flow spans  
98 from 40-60 m<sup>3</sup>s<sup>-1</sup> [25]. This highly stratified estuary is restricted between the last and largest  
99 waterfall (Skradinski buk) and Šibenik Channel, measuring in total of  $\sim 23$  km. The map of  
100 the estuary with marked sampling locations is presented in Fig.1. The Krka River estuary is a  
101 typical, highly stratified salt-wedge estuary. Its vertical gradient is characterized by three

102 layers: (1) surface fresh/brackish layer (FWL), (2) freshwater-seawater interface (FSI) and (3)  
103 seawater layer (SWL). While FWL flows downstream (seaward), the bottom SWL flows in  
104 opposite direction, upstream (landward). The halocline is usually positioned between 1.5 and  
105 3 m, and its "thickness" varies between few cm only to 1 m. Due to numerous tufa barriers  
106 preceding the estuary and the absence of significant anthropogenic sources, the terrigenous  
107 material, nutrients and trace metal river input [26] are very low.

### 3. Sampling and storage

110 Sampling was performed using FEP Nalgene bottles which were previously cleaned with 10%  
111 HNO<sub>3</sub> (suprapur) and thoroughly rinsed with MQ water (18.2 MΩ, Millipore, USA). Samples  
112 were collected using a van Dorn horizontal acrylic sampler or by using grab sampling with 1  
113 L FEP bottle at 16 sites along the whole estuary (Fig. 1). Three sampling campaigns were  
114 conducted: summer 2017 and 2018 and winter 2017. For the summer campaigns (summer  
115 2017/2018), both surface (~0.2 m below the surface) and bottom seawater samples were  
116 collected, whereas for the winter campaign, due to logistic difficulties, only surface samples  
117 were taken. Samples were filtered either immediately onboard or in the laboratory within few  
118 hours by using precleaned (MQ +sample) syringe filters 0.22 μm (cellulose-acetate, Minisart,  
119 Sartorius). All samples were stored at natural pH at +4°C until analysis in 125 mL FEP bottles  
120 which were previously washed using trace metals clean procedure. For total chromium  
121 determination, samples were UV-irradiated at 254 nm directly in the FEP bottle for 24h prior  
122 to measurement. Concentrations of Cr(VI) in estuarine samples were always determined  
123 within two days of sampling. Repeated analyses on the same filtered samples stored for up to  
124 5 days in the dark and at +4 °C did not show any significant differences (within experimental  
125 uncertainty, i.e. 10% [12]). This result indicates that adsorption of Cr(III) on the container  
126 walls did not occur, in contrast to previously reported [10], possibly because fluorinated  
127 (FEP) bottles were used in this study. Vertical profiles of physico-chemical parameters (S, T,  
128 O<sub>2</sub>, pH and Chl-a) were recorded using the EXO2 multiparameter CTD probe (YSI).

### 4. Equipment and chemicals

131 Voltammetric measurements were performed using a μAutolabIII (EcoChemie) potentiostat  
132 coupled with a three-electrode cell (663 VA Stand, Metrohm) with a static mercury drop  
133 (SMDE), Ag|AgCl|sat. NaCl and Pt wire as the working, reference and auxiliary electrodes

134 respectively. A home-made sample-changer, five Cavro XE 1000 syringe pumps and home-  
135 made software VoltAA were used in conjunction with the potentiostat allowing fully  
136 automated measurements to be performed.

137 Sodium nitrate (3 M stock solution) was prepared by mixing HNO<sub>3</sub> (*suprapur* Merck) and  
138 NaOH (*suprapur* Merck), Diethylenetriaminepentaacetic acid (DTPA; 0.25 M stock solution  
139 prepared) was purchased from Fluka (analytical grade) and 2-(N-morpholino)ethanesulfonic  
140 acid (MES; 1 M stock solution prepared) was purchased from VWR (ultrapure). Stock  
141 solutions of Cr(VI) were prepared by appropriate dissolution of K<sub>2</sub>CrO<sub>4</sub> (Fluka). Humic acid  
142 (HA) was from Sigma-Aldrich. All laboratory solutions were stored in polyethylene bottles,  
143 while all the samples were stored in acid-washed FEP (Nalgene) bottles.

## 145 **5. Basics of Cat-AdCSV method for Cr analysis**

146 The method for chromium determination in freshwater and seawater is based on the *in-situ*  
147 formation and adsorption of the Cr(III)-DTPA complex (from Cr(VI) reduction which exists  
148 in the solution at potentials more negative than -0.05 V) during the accumulation step. During  
149 the stripping step, this complex is further reduced to Cr(II) which is immediately oxidised  
150 back to Cr(III) by nitrate, resulting in an enhancement of the signal due to this catalytic effect  
151 [10, 18, 19, 27]. Since the original description of this method [28], numerous authors reported  
152 on using AdCSV to measure trace levels of chromium in different matrices and using  
153 different working electrodes [10, 18, 21, 22, 29-31]. Even though the method was widely  
154 used, its analytical application was limited until the mechanism of reaction, formation,  
155 adsorption and electrode reaction of Cr(III)-DTPA complex were fully studied [27, 30]. The  
156 critical step of the methodology is that the Cr(III) originally present in the solution slowly  
157 forms an electro-inactive complex with DTPA. At room temperature, the kinetics of this  
158 complexation is believed to take 30 min, allowing then the sole determination of Cr(VI)  
159 (increasing the temperature decreases this time [32]).

160 In this work, the determination of Cr concentrations was performed in buffered samples  
161 (MES, pH 5.5) using the following fully automated procedure: rinsing of the cell between  
162 samples by acidified MQ (pH 2, 10 mM HCl), sample exchange, addition of reagents (DTPA,  
163 NaNO<sub>3</sub>) and Cr(VI) standard using syringe burettes. Adequate volume of Cr standard was  
164 determined by using predefined sensitivity. Prepared samples were measured ~ 1 h after the  
165 addition of reagents when all originally present Cr(III) was transferred into an electroinactive

166 complex. Total Cr is determined after 24h UV-irradiation of the sample at neutral or slightly  
167 acidic pH (to convert the existing Cr(III) to Cr(VI)). Cr(III) concentration is calculated as the  
168 difference between total Cr and Cr(VI). Typical voltammetric conditions were: 3 min initial  
169 purging, accumulation at -1.65 V for 60 s, stripping from -0.95 to -1.35 V using differential  
170 pulse mode (2 mV potential step, 0.1 s interval time, 0.040 s pulse time, 10 or 40 mV  
171 amplitude).

## 6. Results and discussion

### 6.1. Optimization of the analytical procedure

175 Analytical parameters used for Cr voltammetric determination by various authors [10, 21, 30,  
176 31] are shown in Table 1. Except for the work of Korolczuk [21] who used a very negative  
177 deposition potential (-1.7 V) in conjunction with a matrix exchange procedure, all other work  
178 applied the usual deposition potential of -1.0 V. Solutions were always buffered at pH ranging  
179 from 5 to 6.5, depending on if freshwater or seawater is analysed. We found here that the  
180 addition of 5 mM MES buffer at pH 5.5 resulted in a similar sensitivity at all salinities (0 –  
181 38). While this is contrast to Li and Xue [30] who reported a low sensitivity when using MES  
182 as a buffer, it agrees with the approach proposed by Korolczuk [33]. Previous studies showed  
183 that deviations from the optimized pH may cause decrease in sensitivity [10, 30], but the  
184 optimized pH varies from study to study, ranging from 5 to 6.5 (Table 1). In this study pH =  
185 5.5 was used and the sensitivity were found to be adequate for the field study across the  
186 salinity range (Fig. S1), so no further tests on the pH was performed.

187 Initial tests in estuarine samples revealed that the sensitivity and the shape of voltammograms  
188 were changing from sample to sample when using a deposition potential of -1.0 V. In  
189 contrast, we obtained much better stability and better-shaped peaks when using a more  
190 negative deposition potential (e.g. -1.65 V). This is also corroborated by previous studies: a  
191 low deposition potential was found optimal by Korolczuk *et. al* [21, 22] when used with a  
192 medium exchange procedure and an increase of the Cr peak was also reported at low  
193 deposition potentials (-1.8 V) at a vibrating silver amalgam microwire electrode [20],  
194 although that potential was not suggested as the optimum one. The same study reported that  
195 Cr(VI) determination in samples without UV-irradiation step was not possible due to  
196 interference by dissolved organic matter (DOM), although no further study was performed by  
197 the authors. Surface active substances (SAS), naturally present in aqueous samples, were  
198 identified to have interference in the determination of chromium by AdCSV [23] and were

199 removed using a fumed silica column. It is thus likely that the application of a low deposition  
200 potential minimise the interference from SAS, similar to what was observed for Cu  
201 complexation studies [34] or for the determination of platinum [35].

202 Below, we show that the use of a low deposition potential removes SAS interferences from  
203 our estuarine samples, without the need of a medium exchange procedure such as that used by  
204 Korolczuk *et. al* [21]. Humic acid (HA) was used as a model of natural organic substance,  
205 which is common in coastal environment [36]. The concentration of HA was increased up to 1  
206 mg/L, which is equivalent to ~0.5 mg/L dissolved organic carbon (DOC). This DOC  
207 concentration is lower than that previously reported for the Krka River estuary (0.8-1.5 mg/L)  
208 [25], but humic substances (HS) are not the sole contributor to DOC. Voltammograms were  
209 recorded in UV-irradiated seawater spiked with 6 nM Cr(VI) at deposition potentials of -1.0  
210 and -1.65 V (Fig. 2). When using the former, HA visibly interferes: the baseline current is  
211 strongly increased at more negative potentials, while the Cr peak gradually diminished and  
212 practically disappeared at HA concentration of 1 mg/L. At HA concentrations above 0.5  
213 mg/L, the chromium peak is poorly expressed, even at such high Cr concentrations. When  
214 using a more negative accumulation potential of -1.65 V (Fig 2., inset), the Cr peak and  
215 background currents are clearly much less affected by addition of HA. A very small increase  
216 of the baseline current is still observed at more negative potentials (note the difference in the  
217 range of Y-axis for two plots), but the Cr peak remains well shaped, despite decreasing down  
218 to around 30% of the initial value (without HA addition). This decrease suggests that the  
219 interferences from SAS is not entirely removed or that Cr(VI) is complexed by HA.

220 To identify if the decrease of the signal is due to SAS interference on the voltammetric signal  
221 or is due to complexation by HA, we carried out analytical determinations of Cr by the  
222 method of standard addition at both potentials (-1.0 and -1.65 V) at each HA concentration  
223 (calibrations not shown). At low HA concentrations, below 0.7 mg/L, despite a strong  
224 decrease of signal intensity, the accuracy of Cr determination is not significantly impacted, at  
225 both potentials. At higher HA concentrations, analysis of Cr using -1.0 V deposition potential  
226 was not possible, whereas using deposition at -1.65 V a reproducible and accurate  
227 determination of Cr was always obtained (recovery ~100%), suggesting that complexation of  
228 Cr(VI) with HA did not occur within the time frame of the experiment. The only problem that  
229 was sometimes observed when using an accumulation potential of -1.65 V was the  
230 dislodgement of the Hg-drop. However, as the accumulation time for most of the samples  
231 measured in natural water is only 60 s, this problem did not impact Cr determination because



232 duplicate or triplicate measurements were always performed (for the sample and for each  
233 addition of Cr standard).

234 Figure 3 shows the variation of the peak intensity versus accumulation potential (so-called  
235 "pseudopolarograms") at different HA concentrations in UV digested seawater. An initial  
236 increase of peak intensities with decreasing accumulation potentials was first observed,  
237 reaching a maximum at -1.05 V, followed by a strong decrease to an almost complete loss of  
238 the signal (-1.2 to -1.5 V) before finally increasing again up to the lowest deposition potential  
239 tested here (-1.7 V). The maxima at -1.05 V disappears at the highest HA concentration of 1  
240 mg/L while the signal at -1.7 V is much less affected. Very similar U-shaped relationships  
241 was also found in our estuarine samples of different salinities (Fig. S2, SI), similar to  
242 previously reported at a vibrating amalgam micro-wire electrode [20]. In seawater, the loss of  
243 the Hg drop was observed at accumulation potentials more negative than -1.7 V, possibly due  
244 to partial reduction of major cations. In freshwater, that negative accumulation potential limit  
245 was shifted far more negative (down to -2.4 V) (Fig. S2) with a much-improved sensitivity.  
246 For instance, the maximum peak height obtained at -2.2 V was  $\sim 4\times$  higher than the one  
247 obtained at -1.0 V.

248 The Cr redox mechanism occurring at accumulation potentials more negative than -1.5 V has  
249 already been described [18, 22]: Cr(VI) is reduced to its metallic state Cr(0) and accumulated  
250 at the Hg surface. At the start of the stripping (-0.95 V), Cr(0) is oxidised to Cr(III) that  
251 immediately forms a complex with DTPA and the stripping is occurring along the catalytic  
252 pathway described above (section 4).

## 254 6.2. Voltammogram shape and baseline elimination

255 The most common way of expressing sensitivity is in terms of nA/nM. However, in cases  
256 where the signal is positioned at the steep part of the baseline (as for Cr), the signal to  
257 baseline shape is much more important than just pure (and high) sensitivity expressed in  
258 nA/nM. A typical example is given in Fig. 4: while the peaks obtained at -1.0 and -1.65 V are  
259 of the same absolute intensity ( $\sim 4.5$  nA; determined using curvature baseline), the shape of  
260 the latter peak is much better resolved than the former (see also voltammograms in Fig. 2).  
261 Thus, when optimising any voltammetric procedures, both the absolute intensity and the  
262 shape of the voltammogram should be improved. This is especially important at low signal  
263 amplitude, where the steep baseline could mask the analyte signal. In such cases, the

1 264 application of derivative transformations is beneficial: it eliminates the curvature of the  
2 265 baseline (see inset of Fig. 4) and consequently lower detection limits [35, 37]. We used here  
3  
4 266 the peak height of the 2<sup>nd</sup> derivative transformation for quantification of Cr concentration.  
5  
6 267

### 8 268 *6.3. Hydrography of the estuary*

10 269 Physico-chemical parameters for the sampling periods are presented in Fig. S3. The vertical  
11  
12 270 and horizontal profiles of salinity are typical of two contrasting sampling periods (summer  
13  
14 271 and winter): the halocline is deeper and the low salinity brackish layer extends more within  
15  
16 272 the estuary in winter than in summer. The pH of samples (not shown) was between 8.4 (at the  
17  
18 273 freshwater part) and 8.2 (at the seawater). Higher pH in freshwater part is related to CO<sub>2</sub>  
19  
20 274 removal at waterfalls which precede the estuarine transect [25]. Temperature profiles follow a  
21  
22 275 similar trend as those of salinity for both periods, whereas oxygen profiles were the most  
23  
24 276 variable. A clear increase of oxygen levels below the halocline (reaching value up to 140 % of  
25  
26 277 oxygen saturation) between 3<sup>rd</sup> and 20<sup>th</sup> kilometre were observed in summer, due to high  
27  
28 278 productivity occurring in this lacustrine part of the estuary (Prokljan lake) [38]. On the other  
29  
30 279 hand, hypoxic conditions were found in the deeper regions of the upstream part of the estuary  
31  
32 280 during winter. This is due to progressive degradation of organic matter produced during  
33  
34 281 summer period, associated with the high residence time of the seawater layer in that upper  
35  
36 282 part of the estuary. Fig. S4 presents typical profiles of dissolved organic carbon (DOC) for the  
37  
38 283 winter and summer periods. DOC concentrations were higher in summer (up to ~150 µM)  
39  
40 284 than in winter (up to ~80 µM). Typically, DOC was lower (~50 µM) in winter in the  
41  
42 285 freshwater end-member compared to the seawater end-member, whereas for the summer  
43  
44 286 period it could be the opposite, due to developed biological productivity in the freshwater  
45  
46 287 Visovac Lake, that is located before the waterfalls and the estuary [39].  
47

### 48 288 *6.4. Chromium distribution along the Krka River estuary*

49 290 The distributions of dissolved Cr(VI) and Cr(III) along the estuarine transect are presented in  
50  
51 291 Fig. 5 as a function of salinity. Due to the fully oxygenated samples, Cr(VI) predominates  
52  
53 292 both in summer and winter. Although the suspended particulate matter is generally low in the  
54  
55 293 Krka River estuary (< 5 mg/L) [25], some portion of Cr(III), which is particle-reactive in  
56  
57 294 contrast to Cr(VI), could be adsorbed on the particulate matter. Thus, higher concentrations of  
58  
59 295 Cr(III) in non-filtered samples were expected but these were not found. In unfiltered samples,  
60  
61 296 slightly higher (<5 %) concentration of Cr(III) were found compared to the dissolved ones  
62  
63  
64  
65

297 (Fig. S5). One of the known challenge in Cr determination is that Cr(III) is strongly adsorbed  
298 on the bottle walls [10]. In cases when UV-irradiation of sample to remove organic matter is  
299 performed in separate, usually quartz tubes [20], the adsorbed Cr(III) is not recoverable  
300 because the sample is transferred from storage bottle to the UV-digestion vessel. In our work,  
301 Teflon (FEP) bottles were used to store the samples and UV-irradiation was performed  
302 directly in these bottles (FEP is UV transparent), with adaptation of samples pH to around 5.  
303 In this way, any adsorbed Cr(III) is expected to be recovered and reliably quantified.

304 For both summer campaigns, very similar transects of both Cr redox species were obtained.  
305 Concentration of dissolved Cr(VI) slightly increased with the salinity (or distance), with a  
306 profile that could be characterised as non-conservative. The concentration level of Cr(VI) in  
307 the Krka River end-member was around 2.5 nM, while at the seawater end-member, it was  
308 between 4 and 5 nM (Fig. 5). In contrast, an opposite trend was found in winter with higher  
309 Cr(VI) found at the lower salinities. In winter, higher concentrations in the freshwater end-  
310 member can be explained by the higher river flow, leading to significant weathering processes  
311 while no significant differences were found in the seawater end member between winter and  
312 summer.

313 As for Cr(VI), no clear difference was found between dissolved and unfiltered Cr(III)  
314 concentrations, indicating that the fraction of Cr(III) associated to particles was minor.  
315 Hexavalent chromium can be photo-reduced to its trivalent state that can be then rapidly  
316 complexed with naturally occurring dissolved organic matter [40, 41]. However, according to  
317 our results, this process does not seem to occur, even in summer. In the work of Achterberg  
318 and van den Berg [41] in which Cr distribution in Mediterranean waters was studied, Cr(III)  
319 was found higher in surface layers for both winter and summer periods. It was suggested that  
320 this is possibly caused by photochemical conversion of Cr(VI) to Cr(III) during summer  
321 periods and by atmospheric inputs during winter periods. Atmospheric input of Cr(III) could  
322 be envisaged since it is abundant in atmospheric particulate matter [42]. On the other hand, in  
323 summer period, Cr(III) could be photo-oxidized to Cr(VI) while in the winter time, such  
324 conversion is limited due to lower solar irradiance. Additional seasonal sampling campaigns  
325 would be required to tests the hypothesis that atmospheric/aerosol inputs of Cr(III) is  
326 regulating its concentration in the surface layer of the estuary and that Cr(III) is photo-  
327 oxidised to Cr(VI).

328 The transect of chromium in the bottom water is unusual. While an upstream increase of trace  
329 metals was recorded for many metals in the bottom layer [25], Cr concentrations here follow

330 an opposite trend, similar to that observed in surface water. A lower concentration is detected  
331 on the freshwater side compared to those measured lower in the estuary (see Fig. S6),  
332 reaching a stable value of c.a. 4.4 nM from 6 km downstream. This unusual behaviour can be  
333 explained by a reduction of Cr(VI) to Cr(III) in the upstream part, followed by removal of  
334 Cr(III) through adsorption onto sinking particulate matter, which is known to be increased in  
335 that part of the estuary [25]. Reduction of Cr(VI) to Cr(III) can be favoured by the hypoxic  
336 conditions in that section of the estuary (Fig. S3) and/or through reduction by low molecular  
337 weight organic matter, as previously suggested [43].

## 7. Conclusions

340 We have developed here an optimised voltammetric procedure to measure Cr in presence of  
341 SAS, substances that are ubiquitous in natural waters. By applying a lower negative  
342 accumulation potential (-1.65 V) than the usual one (-1.0 V), the interfering effect of SAS  
343 adsorption on the mercury drop electrode is strongly minimised. Experiments performed in  
344 UV-digested seawater with addition of humic acid (HA) showed clear evidence of the benefits  
345 of using such low deposition potential. This optimised voltammetric procedure was  
346 successfully applied for the Cr speciation along the Krka River estuary in winter and summer.  
347 Cr(VI) was found to be the predominant redox species in all samples and higher Cr(III)  
348 concentrations were found in winter. While Cr concentration for summer samples increased  
349 towards the open sea, an opposite trend was found for winter campaign, probably related to  
350 weathering processes and higher Krka River flow, which increased Cr concentration in the  
351 freshwater part.

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**Table 1.** Parameters of the method for cat-AdCSV measurement of trace levels of chromium using DTPA.

	Boussemart [10]	Li and Xue [30]	deSouza [31]	Korolczuk [33]	This study
Deposition potential /V	-1.0	-1.0	-1.0	-1.7*	-1.65
Deposition time /s	60	60	60	60	60
DTPA / c(mM)	2.5	5	1.25	10	1.25
NO <sub>3</sub> <sup>-</sup> / c(mM)	500	500	1500	500	500
MES / c(mM)	-	-	-	10	5
pH seawater	5.0	5.7	5.0	-	5.5
pH freshwater	6.4	5.7	-	6.1	5.5

\* - with matrix exchange



## Figure Captions

Figure 1. Left: Map of the Krka River estuary with indicated locations of sampling sites (open diamonds). Right: horizontal bottom depth profile with positions of sampling sites and specific regions along the estuary.

Figure 2. Voltammograms recorded in UV-irradiated seawater spiked with 6 nM Cr(VI) at varying concentrations of HA using -1.0 V (main plot) and -1.65 V (inset) as accumulation potentials.

Figure 3. Variation of peak area on the accumulation potential without and with increasing concentration of HA in UV-irradiated seawater (UVSW) with total Cr concentration of 6 nM.

Figure 4. Voltammograms obtained at -1.0 V and -1.65 V accumulation potential in seawater sample ([Cr] = 4.5 nM). Inset: 2<sup>nd</sup> derivative vs potentials.

Figure 5. Distribution of dissolved chromium along the salinity gradient of the Krka River estuary in surface layer. Blue dotted line corresponds to conservative mixing line.

Figure 1  
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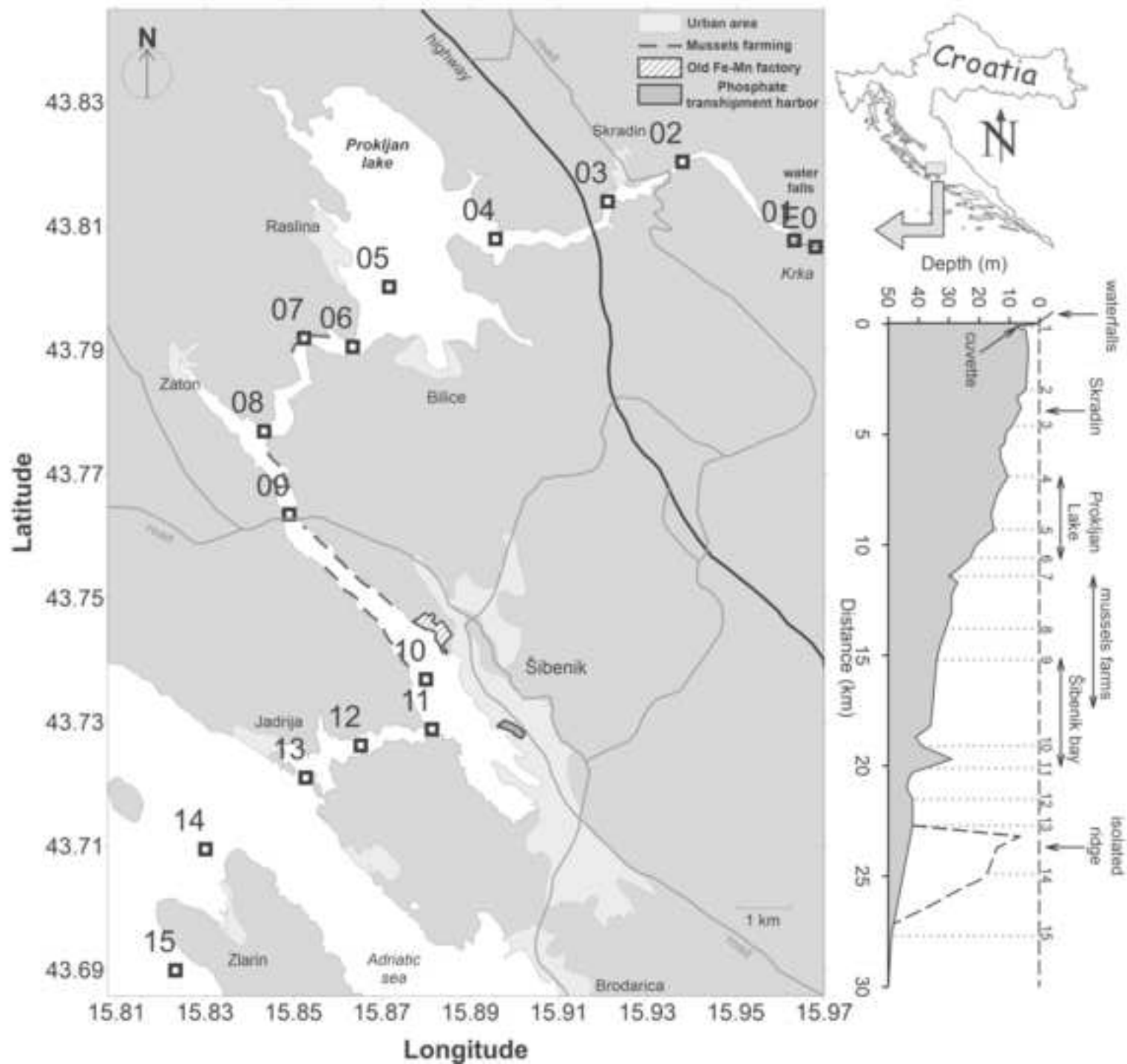


Figure 2  
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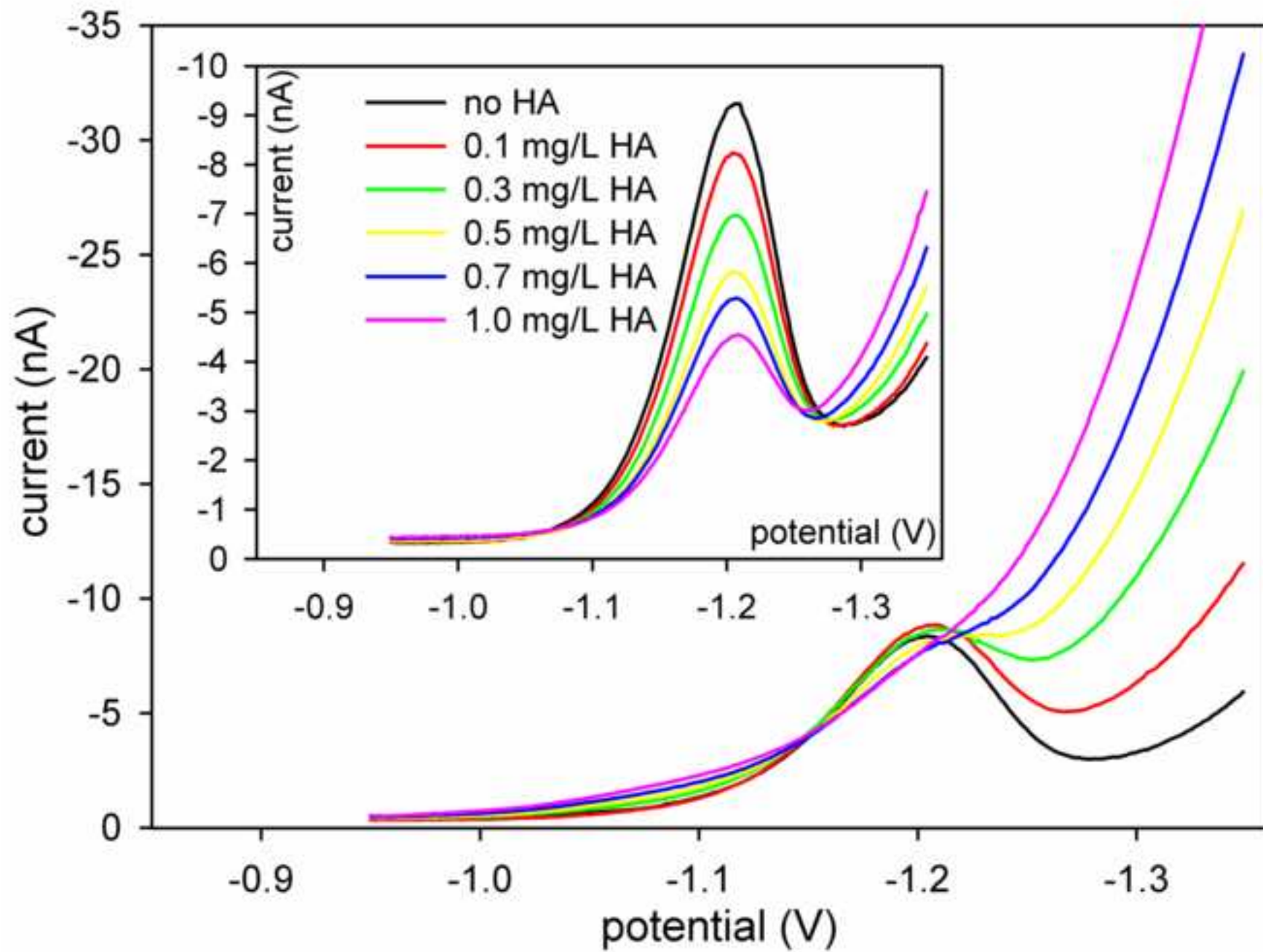


Figure 3

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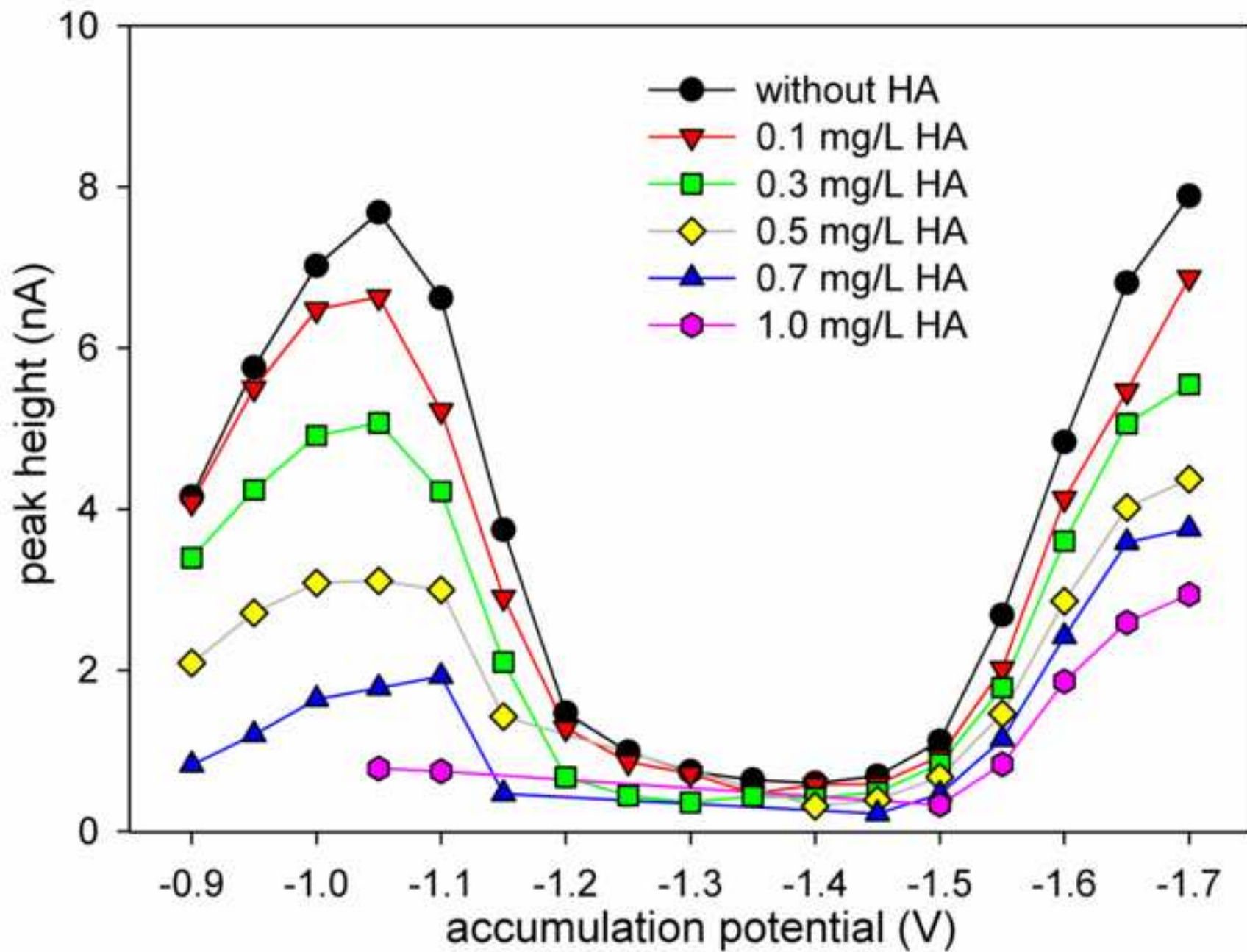


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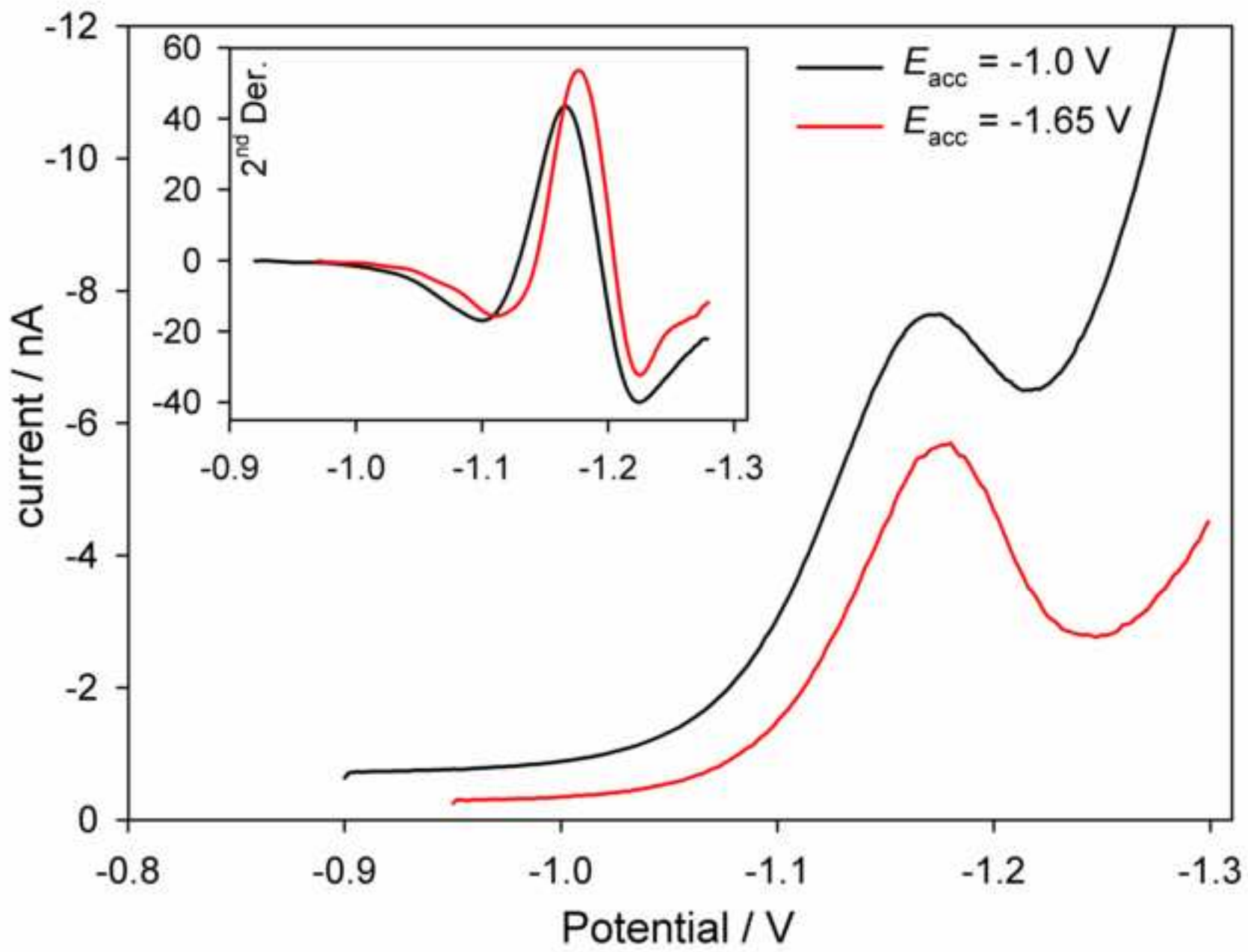
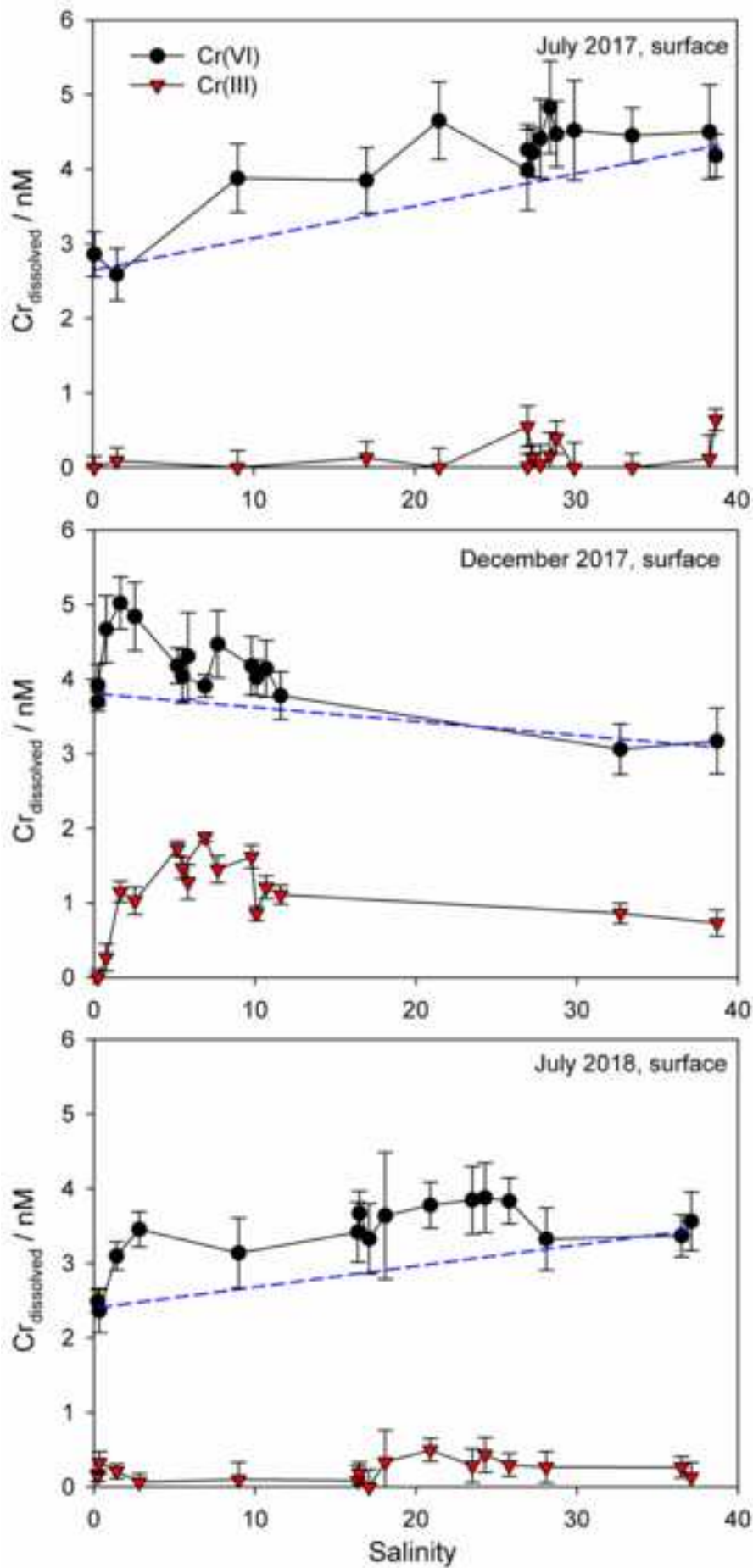


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